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Analytical Methods

Arsenic speciation in fish sauce samples determined by HPLC coupled to inductively coupled plasma mass spectrometry

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ABSTRACT

Fish sauce is a condiment typically used in most East Asian cooking and has recently been considered as an effective route to iron fortification in countries where iron deficiency anaemia is widespread. Current consumption and the increasing awareness of the uniqueness of fish sauce as a condiment necessitate assessment of the health risk that it poses. This study focuses on the analysis of arsenic in fish sauce samples with special emphasis on identification of the species present as a consequence of the fermentation process. Total arsenic concentrations of six different fish sauces from Thailand and Vietnam were in the range of 0.69–2.75 mg l^{-1}. Speciation analyses done on the fish sauce samples showed that most of the arsenic present in the fish sauces were arsenobetaine (82–94%), arsenocholine (4.9–7.7%), trimethylarsine oxide (0.7–7.8%), and trimethylarsenopropionate (0.5–2.1%). Highly toxic arsenic compounds, such as arsenite, arsenate, methylarsonic acid (MA) and dimethylarsinic acid (DMA), were below the detection limit of 0.01 mg l^{-1}.

1. Introduction

In recent years, numerous studies have focused on the determination of arsenic in marine organisms, such as fish, marine algae, seagrass, oyster and other organisms, primarily because these are major sources of arsenic entering the human diet (Cullen, & Reimer, 1989; Edmonds, & Francesconi, 1988; Francesconi, & Edmonds, 1997). Though a lot of arsenic-containing compounds have been identified in marine organisms, arsenobetaine is almost always the major species present in marine animals with minor traces of the inorganic arsenicals and other organoarsenicals (Cullen & Reimer, 1989; Shibata, Yoshinaga, & Morita, 1994). While arsenic in marine organisms is mainly present as non-toxic arsenicals, other processes in food production may alter the species distribution; thus, the risk posed by processed foods derived from marine origins needs to be assessed. One such process of food production is fermentation of fish for the production of fish sauce.

Fish sauce is a brown liquid which is used in the same way as salt in most Western countries or as alternative to soy sauce in other Asian countries. It is prepared from shellfishes, such as oyster and shrimps, small fishes, such as anchovy, and the remaining parts of tuna or salmon after canning (Dissaraphong, Benjakul, Visessanguan, & Kishimura, 2006; Jiang, Zeng, Zhu, & Zhang, 2007; Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2006). Fish sauce is prepared by fermentation of the raw materials (fish/salt mixture, usually in 1:3 ratio) for a period of one year or more before collecting the liquid formed, bottling, and selling in the market. The fermentation process not only extends the shelf-life of the product but also enhances the flavour which makes it an indispensable condiment in most Asian kitchens. Fish sauce is also used, not only for flavour enhancement, but also as a source of nutrients (Jiang et al., 2007). Lately, fish sauce has been considered as a very convenient approach to iron fortification, to combat iron deficiency in most countries where its use is widespread (Fidler, Davidsson, Walczyk, & Hurrell, 2003; Mannar & Gallego, 2002; Thuy et al., 2003). Thus, the use of fish sauce is predicted to increase in coming years especially because its popularity has also spread to other countries. Several studies have even looked at the possibility of using capelin, a species common in the Atlantic Ocean, as a raw material for fish sauce production (Gildberg, 2001; Hjalmarsson, Park, & Kristbergsson, 2007).

The use of fish sauce in food preparation makes it important to determine the arsenic concentration and identify the species present in this condiment. There is only one study that has focused on the analysis of arsenic in fish sauces and which reported that dimethylarsinic acid (DMA) was the major component of most of the samples (Kato, Nagashima, & Shiomi, 2004). In this paper, we present our results for the analysis of six fish sauces by flow injection inductively coupled plasma mass spectrometry (ICPMS) and speciation analysis using high performance liquid chromatography (HPLC) coupled with ICPMS.

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2. Materials and methods

2.1. Chemicals and samples

All reagents used in this work were of analytical grade. The arsenic and germanium stock standards (1000 mg l^{-1}) were purchased from CPI (Santa Rosa, USA). Nitric acid was purchased from Merck (Germany) and methanol was from Carl Roth GmbH (Karlsruhe, Germany). Working standard solutions and samples were prepared using Milli-Q water (18.2 MΩ cm; Millipore, Bedford, MA, USA). For quality control, extracts of the reference material DORM-2 (dogfish muscle) obtained from the National Research Council, Canada (Ontario, Canada) was analysed together with the samples. Extraction was carried out by weighing a representative sample (250 mg) in polyethylene vials and 10 ml of water/methanol (1 + 1, v/v) were added, shaken (top over bottom) for 18 h and centrifuged at 4500 rpm for 15 min. Aliquots (1 ml) of the extract were then digested together with the fish sauce samples and analysed by the conventional ICPMS method that we employ routinely in our laboratory. Another set of aliquots was analysed using the flow injection ICPMS method. Results from both conventional ICPMS (17.2 ± 0.2 mg kg^{-1}) and flow injection ICPMS analyses (17.2 ± 0.3 mg kg^{-1}) on these extracts were in good agreement with each other. Speciation analysis of the extracts returned the values of 16.6 ± 0.4 mg kg^{-1} for arsenobetaine (certified = 16.4 ± 1.1 mg kg^{-1}) and 0.24 ± 0.09 mg kg^{-1} for tetramethylarsonium ion (certified = 0.248 ± 0.054 mg kg^{-1}). The fish sauce samples (four different fish sauce brands from Thailand and two from Vietnam) were obtained from local stores in Graz, Austria. The fish sauce samples were diluted with Milli-Q water (1 + 99) prior to flow injection ICPMS analysis and speciation analysis.

2.2. Flow injection-ICPMS analysis

Flow injection ICPMS analysis was carried out using a method that we have developed (Rodriguez, Francesconi, & Goessler, 2008). Briefly, a Hewlett–Packard 1100 series system (Hewlett–Packard, Waldbronn, Germany), equipped with a binary pump, a vacuum degasser, and a thermostatted autosampler with a 100 mm^3 injection loop, was used as the injector system and was connected by PEEK (polyetheretherketone) capillary tubing directly to the nebuliser of an Agilent 7500ce ICPMS (Agilent, Waldbronn, Germany) equipped with a PFA microconcentric nebulizer, a Scott double pass spray chamber and an octopole reaction cell. Helium (3.0 ml min^{-1}) was used as a collision gas to reduce interferences on the signal of arsenic. The eluent used was 0.3% HNO_3 with 10% methanol (v/v) and the volume of injection was 20 μl. Quantification was done both by normalization against ^{74}Ge (spike level = 400 μg l^{-1} in standards and samples) and against an external calibration prepared using the arsenic stock solution (1.0–500 μg As l^{-1} prepared in 1% HNO_3).

2.3. HPLC/ICPMS analysis

Separations of the arsenicals were carried out under both anion- and cation-exchange conditions, using the same HPLC system coupled with ICPMS. Anion-exchange chromatography was carried out using a PRP-X100 column (4.1 × 250 mm, 10 μm particle size; Hamilton, Reno, Nevada, USA) maintained at a temperature of 40 °C. The mobile phase was 20 mM phosphate buffer (pH 6.0, adjusted with aqueous NH_3) at a flow rate of 1.5 ml min^{-1}. Cation-exchange chromatography was carried out using a Zorbax 300 SCX column (4.6 × 250 mm; Hewlett–Packard, Waldbronn, Germany) maintained at a temperature of 30 °C. The mobile phase used was 10 mM pyridine (pH 2.3, adjusted with formic acid) at a flow rate of 1.5 ml min^{-1}. The volume of injection used for both chromatographic conditions was 20 μl. The ion intensities at *m/z* 75 and 77 were monitored during the analyses and quantification was done using peak area.

2.4. HPLC/ESI–MS analysis

Species identification was also ascertained using HPLC with electrospray ionization mass spectrometry (HPLC/ESI–MS) performed on three of the samples. The analyses were carried out in positive mode using an Agilent LC/MSD 1100 series system with single quadruple MS of the SL type. The separation was done on a Shodex RSpak NN-614 column (6 × 150 mm), using a 5 mM ammonium formate buffer (pH = 3.0) supplied at 0.4 ml min^{-1} and the temperature was maintained at 30 °C. The volume of injection used was 5 μl.

3. Results and discussion

The total arsenic concentration in the fish sauce samples were determined by flow injection ICPMS. The results, together with the sum of species, are summarized in Table 1. Speciation analysis of the fish sauce samples, using HPLC/ICPMS, was done under both anionic and cationic conditions, and possible presence of thio-arsenicals was also checked using the chromatographic conditions reported by Raml, Goessler, and Francesconi (2006). The check for the presence of thio-arsenicals did not reveal the presence of these sulphur-containing arsenic species although the odour of the samples would suggest the probable presence of these compounds. The characteristic odour of fish sauce is attributed to the contributions of various amino acids, nucleotides, peptides, ammonia and urea present in the liquid (Jiang et al., 2007; Klomklao et al., 2006). Anion-exchange chromatography showed the absence of anionic arsenic species, such as arsenite and arsenate, in the fish sauce samples. Cation-exchange chromatography revealed that the arsenic present in the fish sauces studied was mainly arsenobetaine, arsenocholine, with traces of trimethylarsine oxide (TMAO), and trimethylarsoniopropionate (TMAP). Species distributions in the fish sauce samples are summarized in Table 2. Comparisons of the results from flow injection ICPMS on the fish sauce samples show that results correlate well with the sum of species accounted for after chromatographic separation.

Fig. 1 shows a typical cation-exchange chromatogram of a mixture of standards (5.0 μg l^{-1} each of AB, TMAO, AC and Tetra) overlaid with a chromatogram obtained from the analysis of fish sauce sample 4. TMAP was detected in most samples in trace amounts and was quantified using the correlation equation from AB. The signals at around two minutes are polyatomic interferences ($^{40}Ar^{35}Cl$) due to the high sodium chloride concentrations in the fish sauce samples. Sodium chloride content of fish sauce varies from 20% to 30% (Hjalmarsson et al., 2007). We also conducted HPLC/ESI–MS analysis for confirmation. Analysis of fish sauce samples 4–6 showed that the major component was arsenobetaine.

Table 1
Determined arsenic concentration in various fish sauce samples using different methods (means ± SD, *n* = 3)

Fish sauce sample	Concentration of arsenic (mg l^{-1})	
	Flow injection – ICPMS	Sum of species
Fish sauce 1	0.69 ± 0.03	0.67 ± 0.03
Fish sauce 2	0.92 ± 0.06	0.91 ± 0.03
Fish sauce 3	1.06 ± 0.03	1.04 ± 0.09
Fish sauce 4	2.13 ± 0.06	2.19 ± 0.16
Fish sauce 5	1.66 ± 0.03	1.58 ± 0.08
Fish sauce 6	2.75 ± 0.03	2.71 ± 0.09

Table 2
Determined arsenic species in fish sauce samples using HPLC/ICPMS (means ± SD, $n = 3$)

Fish sauce sample	Concentration of arsenic species (mg l^{-1})			
	Arsenobetaine	Arsenocholine	TMAO	TMAP
Fish sauce 1	0.55 ± 0.05	0.046 ± 0.009	0.052 ± 0.013	0.014 ± 0.004
Fish sauce 2	0.80 ± 0.07	0.062 ± 0.012	0.038 ± 0.012	0.012 ± 0.006
Fish sauce 3	0.91 ± 0.08	0.080 ± 0.009	0.030 ± 0.016	0.019 ± 0.007
Fish sauce 4	1.98 ± 0.17	0.16 ± 0.02	0.043 ± 0.011	0.011 ± 0.004
Fish sauce 5	1.44 ± 0.12	0.11 ± 0.01	0.011 ± 0.006	0.016 ± 0.007
Fish sauce 6	2.55 ± 0.06	0.13 ± 0.02	0.028 ± 0.012	<0.01

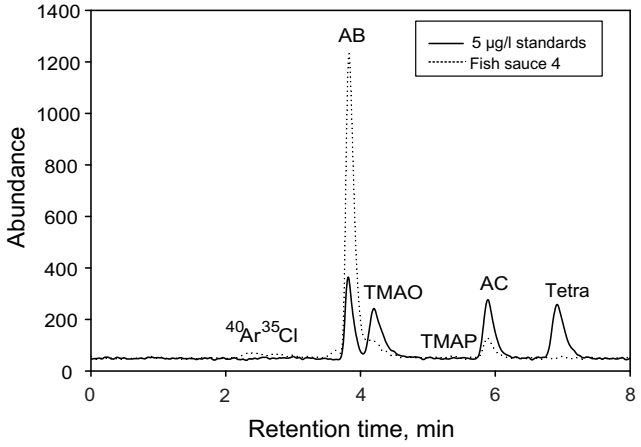

Fig. 1. Overlaid chromatograms of a standard mix of 5.0 µg l^{-1} each of AB, TMAO, AC and Tetra (solid line) and fish sauce sample 4 (dotted line). (Zorbax 300 SCX column, 4.6 × 250 mm; 10 mM pyridine, pH 2.3; flow rate: 1.5 ml min^{-1}; column temperature: 30 °C; volume of injection: 20 µl.)

Only fish sauce samples 4–6 were subjected to HPLC/ESI–MS analysis because of the higher arsenic content in these samples. It should be noted, however, that the chromatographic profiles of all fish sauce samples, using both anionic and cationic chromatographic conditions, as described with ICPMS as detector, were all similar. These results show that the non-toxic species, arsenobetaine and arsenocholine, the major arsenic species in most marine animals, are not transformed into other species during the fermentation process.

Our results differ from previous work which looked at the presence of arsenic in fish sauce (Kato et al., 2004). Kato et al., 2004 used HPLC/ESI–MS in the analysis of fish sauce samples and its corresponding fish raw material and they reported that DMA was the major component of the fish sauces studied. Contrary to the report of Kato et al. (2004), we did not find DMA in the fish sauce samples studied. They stated that the HPLC/ESI–MS method that they used eliminated the possibility that the DMA peak may be misidentified as arsenobetaine. But the HPLC/ESI–MS analysis that we performed did not detect presence of DMA. Moreover, the use of ICPMS, which is more sensitive and more robust when it comes to complex matrices, provided a clear identification of arsenobetaine as the major arsenical in the samples studied (shown in Fig. 1). Furthermore, we spiked 5.0 µg/l in fish sauce sample 4 and analysed both (chromatograms shown in Fig. 2), and the results clearly support absence of DMA in the sample. Possibly, the instrumentation Kato et al., 2004 employed was influenced by the complex matrix; thus detection of arsenobetaine may have been shrouded. Also, our results may have differed from their results because of differences in the fish sauce samples; their samples were mainly from Japan, while ours were from Thailand and Vietnam. In any case, the significant difference between these two reports warrants broadening this study to properly assess whether fish sauce poses a health risk to populations using it. Moreover, clarification of this difference would also show whether this condiment is suitable for use as a medium in iron fortification programmes or to combat other nutrient-deficiency health problems.

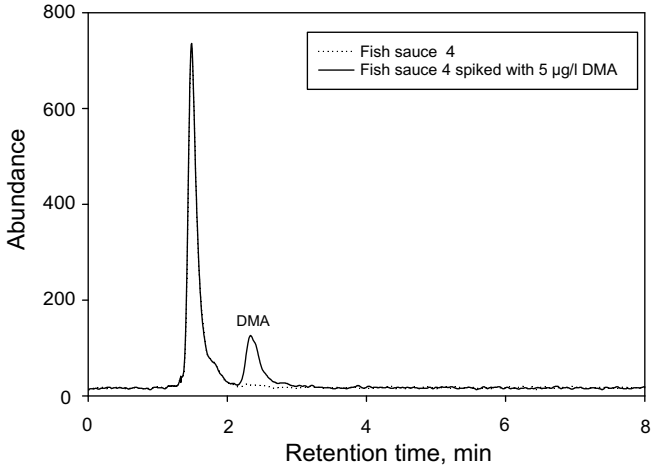

Fig. 2. Overlaid chromatograms of fish sauce sample 4 spiked with 5.0 µg l^{-1} of DMA (solid line) and fish sauce sample 4 (dotted line). (PRP-X100 column, 4.1 × 250 mm, 10 µm particle size; 20 mM phosphate buffer, pH 6.0; flow rate: 1.5 ml min^{-1}; column temperature: 40 °C; volume of injection: 20 µl.)

4. Conclusion

Analysis of the fish sauce samples showed that this condiment has a relatively high concentration of arsenic present mainly as arsenobetaine. It is comforting to know that there was no species transformation during the fermentation process of the raw materials (fish, oyster or other marine sources). It is important to stress that the species present in this condiment, as shown in our results, do not pose risk to the population regularly using it, and that it does not counteract the benefits of using it as a medium for iron fortification. But, as there are two differing reports on which species are the main components in fish sauce, there is a need to resolve the differing results to properly assess the health risk to consumers or populations being treated for iron deficiency anaemia using iron-fortified fish sauce.

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