Food Additives and Contaminants, 2001, Vol. 18, No. 1, 19–29

A comparison of techniques for preparing fish fillet for ICP–AES multielemental analysis and the microwave digestion of whole fish

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(Received 10 September 1999; revised 7 January 2000; accepted 30 August 2000)

Four catfish fillet homogenate treatments before multielemental metal analysis by simultaneous inductively coupled plasma/atomic emission spectroscopy were compared in triplicate. These treatments were: nitric acid wet-ashing by Parr bomb digestion; nitric acid wet-ashing by microwave digestion; tetramethylammonium hydroxide/nitric acid wet digestion; and dry-ashing. The tetramethylammonium hydroxide/nitric acid method was imprecise (coefficients of variation > 20%). The dry-ashing method was fast and sensitive but had low recoveries of 50% for spiked Pb and Al and was not as precise as the Parr bomb or microwave treatments. The Parr bomb method was the most precise method but was less sensitive than the microwave method which had nearly the same precision. The microwave method was then adapted to homogenates of small whole fish ≤3 cm in length. The whole fish homogenate required more vigorous digestion conditions, and addition of more acid after the evaporative step because of the presence of less oxidizable and acid-soluble components than fillet. The whole fish homogenate was also more heterogeneous than catfish fillet. A quality assurance protocol to demonstrate homogenate uniformity is essential. The use of a non-specialized microwave oven system allowed precise results for fillet and whole fish homogenates.

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Keywords: fish elements, multielemental analysis, ICP analysis, fish digestion, microwave digestion

Introduction

Multielemental analysis of the tissues of edible bony fish is important to assure human health relative to the toxic metals (Tsubaki and Irukuyama 1977, Tchounwou *et al.* 1996) antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), thallium (Tl), and zinc (Zn). Ecotoxicological studies also use metals in specific fish species at stated ages to indicate ecosystem health (Linde *et al.* 1996). Nutritional needs of fish are also important in fisheries involving the essential metals cobalt (Co), Cu, Fe, molybdenum (Mo), Se, vanadium (V), and Zn (Reichenbach-Klinke and Landolt 1973), and the macroelements calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P), to prevent fish kills (US EPA 1979) and fish morbidity (Reichenbach-Klinke and Landolt 1973).

The major methods of elemental analysis after fish tissue degradation are atomic absorption spectroscopy AAS (Cunniff 1996a), flame emission spectroscopy (Cunniff 1996b), inductively coupled plasma atomic emission spectroscopy ICP–AES (Martin *et al.* 1992), neutron activation analysis (Zeisler *et al.* 1988), X-ray fluorescence analysis XRF (Zeisler *et al.* 1988), prompt gamma activation analysis (Zeisler *et al.* 1988), and inductively coupled plasma/mass spectrometry ICP/MS (Beauchemin *et al.* 1988). During ICP–AES analyses, coprecipitation of non-aluminosilicaceous digests is prevented by using 11.6% HCl/2.8% nitric acid (Que Hee *et al.* 1985, Que Hee and Boyle 1988) or 4:1 HCl/HNO_3. Aspired HCl and H_2SO_4 in ICP/MS cause isobaric interferences (Holland and Tanner 1997).

Food Additives and Contaminants ISSN 0265–203X print/ISSN 1464–5122 online
http://www.tandf.co.uk/journals
DOI: 10.1080/0265203001000915 6

The Association of Official Analytical Chemists (AOAC) recommends that fish fillet be either digested under acid conditions ('wet-ashed') for Na and K (Cunniff 1996b), or pyrolysed dry ('dry-ashed') for Na and K (Cunniff 1996b) and Pb (Cunniff 1996a) before atomic spectroscopy. The US EPA multielemental ICP–AES method using tetramethyl ammonium hydroxide/nitric acid is for 'a fresh, not previously frozen, fish ... The method is not intended to be used for the analysis of dried fish tissue' (Martin *et al*. 1992). Samples have also been freeze-dried or frozen before digestion (Beauchemin *et al*. 1988). An interlaboratory study on Cd, Cu, Cr, Fe, Ni, Pb, and Zn in minced fish fillet using dry-ashing and AAS showed typical intrarun coefficients of variation (CV) of 8.1%, 11%, 14%, 7.7%, 39%, 6.8%, and 3.7%, respectively (Jorhem 1993). The interlaboratory interrun CVs were 19%, 35%, 16%, 7.0%, 64%, 20%, and 12%, respectively. Only the recoveries for Pb, Cd, and Cr were determined.

A standard reference material (SRM) is available for shellfish: SRM1566a oyster from the National Institute of Standards and Technology NIST (Trahey 1995, Negretti de Bratter *et al*. 1995), and certified RM (CRM) number 6 for mussel from the National Institute for Environmental Studies NIES of Japan (Okamoto and Fuwa 1984). A reference freeze-dried tissue from spiny dogfish (*Squalus acanthias*) is used for As (Beauchemin *et al*. 1988), as is NIST RM50 albacore tuna for Zn (Baldwin *et al*. 1994). The National Research Council of Canada has SRMs for dogfish muscle DORM-1, dogfish liver DOLT-1, and lobster hepatopancreas TORT-1 (Baldwin *et al*. 1994, Sheppard *et al*. 1994, Lamble and Hill 1998). The International Atomic Energy Agency IAEA supplies reference fish flesh homogenate MA-A-2 (Lamble and Hill 1998). The Community Bureau of Reference distributes CRM422 cod muscle for As, and CRM463 tuna fish muscle for Hg (Lamble and Hill 1998).

A review on microwave digestions before elemental analysis (Lamble and Hill 1998) listed 56 studies on bony fish and shellfish for 1992–1997; 35 were for single elements. Of the 19 multielemental studies, eight were for bony fish, six for the certified dogfish elements only. The usual digestion medium for fish fillet is nitric acid HNO_3/hydrogen peroxide H_2O_2 (Quevauviller *et al.* 1993, Lamble and Hill 1998). Low Cd recoveries occur by electrothermal AAS. High As, Cr, and Ni and low Ag, Hg, and Zn result on ICP/MS. Digestion with nitric acid alone produced low recoveries of Cd in DORM-1, of Cu and Zn in NIST RM50 by furnace and electro-thermal AAS, and of Cd, Cu, Hg, Se, and Zn in IAEA fish flesh by ICP/MS (Lamble and Hill 1998). Other digestion media for bony fish tissue before ICP/MS are (Lamble and Hill 1998): $HNO_3/H_2SO_4/H_2O_2$ (low Hg), and $HCl/HNO_3/H_2O_2$ (high As and low Mn). Microwave digestion comparisons for canned tuna, canned salmon, frozen shrimp, fresh clams, frozen walleye, fresh lobster, and fresh lobster exist for As, Cd, and Pb (Baldwin *et al*. 1994, Sheppard *et al*. 1994, Lamble and Hill 1998), and for SRM 1566a Oyster tissue by ICP–AES (Negritti de Bratter *et al*. 1995, Trahey 1995, Lamble and Hill, 1998). Kuss (1992) has reviewed the literature before 1992.

The present study examines the tissue treatment step before aspiration in simultaneous ICP–AES by comparing four different methods of degradation for catfish fillet and then adapting the favoured method to the analysis of small whole fish. The utility of a non-specialized microwave system was also investigated.

Experimental

Equipment and chemicals

All equipment and chemicals were from Fisher Scientific, Pittsburgh PA, except where noted. Glassware including Pasteur pipettes for digest transfers and Teflonware were acid-washed before use as described elsewhere (Que Hee *et al*. 1985, Que Hee and Boyle 1988). The convection incubator (Isotemp Model 215G), furnace (Thermolyne 1300), and hotplate (Scientific Instruments Model SP-1025B) were calibrated against standard reference thermometers. All final solution shaking steps used 125 ml Teflon or polypropylene screw-capped wide-mouth bottles (Fisher Scientific, Tustin, CA) on a Jank and Karsen HS-500 mechanical shaker at 260 rpm. Dilution water was ASTM Type-I Milli-Q. Nitric acid (NA) for the concentration steps was doubly-distilled from Vycor (GFS Chemicals, Powell OH), otherwise Fisher Scientific A509 sufficed for dilutions and cleaning. Fisher Scientific A508 concentrated hydrochloric acid (HA) and A511 perchloric acid (PA) were used. Each triplicate digestion had parallel

method blanks. Washing down of the fume hood with water was done before and after ashings.

Fish selection, homogenization and storage

When this study was done in 1994, there was no SRM for edible fish muscle of a bottom dwelling bony fish or for small whole bony fish. Dogfish SRM tissues do not simulate the type of fish tissue likely to be encountered in freshwater fish. A house SRM was therefore prepared. Enough uniform samples of fish had to be available for all treatments in triplicate at least. Thus it was decided to homogenize and shake the fishes of a known species and length range (age) to provide a uniform homogenate, and to apportion out representative weights before freezing for storage. The latter procedure minimizes the number of freeze–thaw cycles that cause a single sample to deteriorate and become heterogeneous over time. The homogenate analysis results in an averaged elemental concentration. Homogenate uniformity may be an important factor, and has not been generally assessed in quality assurance protocols.

Supermarket freshwater catfish (*Ictalurus punctatus*) fillet was cut with Teflon-tipped scissors into 6 mm pieces on a clean polyethylene dissection board. The pieces were homogenized in a Pyrex blender bowl by a Servall Omni Mixer. The homogenates were combined in a Teflon wide-necked bottle and tumble-shaken for 60 min at 200 rpm on a Jank and Karsen HS-500 mechanical shaker. Weights of 10 g, 2 g, 1 g, and 100 mg homogenate were put into seal-tight polyethylene bags, and then frozen at -20°C. At least triplicate samples were placed into a vacuum desiccator for dry weight determinations.

Whole small California mosquitofish (*Gambusia affinis*) $\leqslant$ 3.0 cm in length were also to be analysed. They were specimens from a 1993/1994 ecological environmental study of the Malibu Creek near Los Angeles, California (Ambrose *et al*. 1995). Whole small fish are frequent specimens in ecological studies. Unlike deboned fillet, whole fish samples also contain bones, scales, mucous layers, skin, fat, oils, fins, gills, and internal organs. The contributions of each extra factor may vary widely across species and with fish age. Each individual fish was washed with distilled water as soon as the fish sample of specific species and length in Malibu Creek water was received at the laboratory on catch day. The cutting, homogenization, shaking, apportioning, and freezing procedures for fillet were applied to all whole fish captured.

Tissue disruption methods

Triplicate samples of 10 g thawed frozen fillet homogenate were analyzed by each matrix disruption technique (except 2 g amounts for the US EPA Method) to determine intrarun and interrun means, standard deviations (SDs), and coefficients of variation (CVs) for each element. Thawed 10 g frozen homogenates were subjected to the favoured matrix disruption treatment for fillet as a starting point for method optimization for whole fish homogenates where scales, bones, and cartilage were expected to cause differences in the digestion requirements.

The reference method was the Parr Bomb wet-ashing method to minimize volatile element losses during high temperature digestion (Que Hee and Boyle 1988). The AOAC wet-ashing (Cunniff 1996a) and dry-ashing (Jorhem 1993, Cunniff 1996a,b) methods, the US EPA method (Martin *et al*. 1992), and a microwave wet-ashing method (Que Hee and Boyle 1988) were investigated. All multielemental quantitations were by simultaneous ICP–AES.

The least sensitive method and element, and the highest interrun CV for the dry weights of the wet weights investigated limited the final wet mass chosen for digestion. Relative to elemental sensitivity, the AOAC dry-ashing method recommended a starting wet weight of 25 g with flame AAS of Pb at 283.3 nm to detect 200 ng/ml of Pb in 25 ml final solution at the lowest concentration of the linear range (Cunniff 1996b). For a least quantifiable limit of about 50 ng/ml for Pb as the least sensitive element of interest in our ICP–AES multielemental array, a wet weight of about 6.25 g was needed to be equivalent. Thus 10 g was chosen as compromise for all techniques except for the EPA method where the recommended 2 g was used in five lots.

All solutions for ICP–AES final analysis except for the US EPA method utilized 11.6% HCl/2.8% HNO_3 ('acid cocktail', AC) to minimize metal coprecipitation (Que Hee and Boyle 1985). The minimum volume to dissolve the final residue had also to be found. All acid digestion methods also featured entrainment into 0.100 or 0.200 ml PA during the last concentration. This step was done in a fume hood. The analyst wore a laboratory coat with long sleeves, 6 mil nitrile

gloves, safety glasses, quantitatively-fitted negative pressure acid-mist respirator, long trousers, and enclosed shoes.

Parr bomb method for fillet

The Parr 4748 stainless steel bomb with 125 ml Teflon cup accommodated maximum dry weights of 5 g for inorganic analytical samples and 0.5 g for organic ones, and had a bomb relief pressure of 1900 pounds per square inch (psi) (Parr Instrument Company 1990).

A thawed 10 g fillet homogenate was digested in 25 ml GFS NA in a 50 ml beaker topped with a watchglass by holding at room temperature overnight (16 h) in a fume hood. The solution was then heated at 50°C on a hot plate for 1 h or until no solid was visible. The colour typically was clear yellow. The solution was transferred to the Teflon cup of the Parr bomb, as were three GFS NA beaker washings of 2.0 ml each. The cup was slid into the bomb, the bottom plate raised slightly to allow air release, and the bomb assembled following directions (Parr Instrument Company 1990). A torque wrench was used to apply 5 ft-lb to each lid screw. The digestion time and temperature conditions were then optimized without causing explosions. The optimized conditions follow.

The bomb was placed in the cold convection oven which was heated gradually to 140°C over 15 min. Every 150 min the bomb was taken from the oven using heat-resistant gloves and left at room temperature for 30 min. The cap was then loosened to relieve the pressure. The total digestion time at 140°C was 450 min to have no excess pressure after cap loosening. Thus the heating and cooling cycle was done three times, the whole procedure requiring 540 min.

The solution was then poured into a tall 100 ml pyrex beaker, and the Teflon cup then washed three times each with 5 ml of GFS NA, each washing being transferred into the Pyrex beaker. One hundred µl PA was then added, and the NA evaporated on a hotplate at 50°C to about 0.100 ml until white fumes were just observed. The beaker was then taken off the hotplate. A volume of 40 ml of AC was added gradually to the cooled but still warm beaker by irrigating its insides with a Pasteur pipette. The solution was swirled manually, and then poured into a 125 ml polypropylene or Teflon screw-capped wide-mouth bottle precalibrated to 80 ml. An AC volume of 20 ml was similarly applied down the beaker sides, and this beaker washing transferred into the bottle. The process was repeated with another 20 ml AC. The bottle solution was made up to its 80 ml mark with AC if necessary. The bottle was capped securely, and shaken on the mechanical shaker until there was no evident precipitate or floating solid when the mixer was stopped and the cap removed. A 20 ml volume was then transferred into a 30 ml polyethylene screw-capped test tube for ICP–AES analysis if there was no precipitate.

Dry-ashing method for fillet

The AOAC dry-ashing method accommodated 25 g of fish sample and an analysis volume of 25 ml for Pb by flame AAS (Cunniff 1996a), and 4 g of sample and an analysis volume of 100 ml for Na and K by flame emission analysis (Cunniff 1996b). AC replaced 2% NA for ICP–AES. The optimized method follows.

A thawed 10 g fish sample in a 30 ml glazed porcelain crucible was placed inside the convection oven, which was then set at 150°C for 120 min. The dried sample was transferred to a Thermolyne 1300 furnace. The temperature was raised to 200°C for 5 min, 300°C for 5 min, 400°C for 5 min, and then 500°C for 16 h (overnight). The ramp prevented mechanical sample loss to the upper inner furnace roof. After cooling on a crucible rack at room temperature, 2 ml concentrated GFS NA was added slowly drop by drop down the sides, and the solution gently swirled. The crucible was heated at 80°C and the NA evaporated just to dryness. The crucible was replaced into the furnace whose temperature was slowly raised to 500°C as before, and held there for 60 min. The crucible was cooled again at room temperature in its crucible rack, and the GFS NA trituration/furnace heating steps repeated until the ash showed no charcoal. Usually three such treatments were necessary. A 10 ml volume of AC was added, and the ash was dissolved at 80°C on a hotplate. The solution was transferred by Pasteur pipette to a 25 ml flask. The crucible contents were again treated similarly with two more 5 ml aliquots of AC, each being transferred into the flask. The flask volume was then adjusted to 25 ml with AC, and shaken manually for 1 min. The solution was clear and colourless with no precipitate.

US EPA method for fillet

The US EPA method (Martin *et al*. 1992) was designed for a maximum sample weight of 2 g. A 2 g sample of thawed fish was placed in each of five preweighed labelled graduated polysulphone centrifuge tubes. The US EPA method was performed exactly as described using 25% tetramethyl ammonium hydroxide, NA, and centrifugations. The final centrifuge supernatants were combined via a Pasteur pipette in the same 100 ml volumetric flask with the final volume being made up to 100.0 ml with 5% NA.

Microwave method for fillet

The AOAC wet-ashing method accommodated 1 g of fish sample and a final analysis volume of 25 ml for analysis of Na and K by flame emission (Cunniff 1996b). For a 10 g sample, overnight digestion at room temperature as in the initial part of the Parr bomb method was used to process the increased fish weight. AC replaced 8% NA for ICP–AES analysis. The method is modified from Que Hee and Boyle (1988). The optimized method now follows.

A 10 g mass of fish was reacted overnight (16 h) in 150 ml GFS NA in a 250 ml polypropylene screw-capped container with a wide mouth. Heating of the solutions in the containers (cap just movable relative to finger tightness) was performed at 2450 MHz in a Sharp Model R-3A85 Carousel microwave oven (Sharp Electronics Corporation, Mahwah NJ), spray-coated internally with FluoKem or X64D (Laboratory Supplies Inc., New York) dry lubricant. Non-laboratory grade microwave units are not designed to contain explosions, and care must be exercised by the analyst. The oven was operated at lowest power (Setting 1, equivalent to 10% full power) as higher settings caused the contents to boil over. The exhaust of the oven was vented into a fume-hood through an internal ring-supported polyethylene 6-inch diameter ducting mated to the exhaust vent after removing the oven metal exterior grille using a hacksaw. The oven interior and vent tubing were washed down after each digestion, with Teflon-coating being carried before the next analysis. Heating was for 30 min periods followed by taking off the cap for 5 min at room temperature (oven door open) to relieve pressure and to cool the microwave oven. The heating time was 180 min with a total time of 210 min, or until no brown nitrogen tetroxide was evolved. The solution was transferred into a 400 ml beaker, 0.100 ml PA added, and the NA evaporated to approximately 80 ml at 80°C. The solution was then transferred to a tall 100 ml beaker, with the larger beaker being washed three times each with 5 ml of GFS NA, each washing being put into the 100 ml beaker. The solution was further evaporated to about 0.1 ml or when white fumes just appeared. The residue was dissolved in 80 ml AC as for the Parr bomb end step, and a 20 ml aliquot analysed. The rest of the AC solution was stored in a Teflon or polypropylene screw-capped bottle.

Microwave method for whole small fish

The microwave method was applied to a composite thawed wet weight homogenate of 10 g of small California mosquitofish (*Gambusia affinis*) $\leqslant 3$ cm in length in triplicate.

The optimized method had the following differences from the fillet microwave method: (1) 100 ml of GFS NA instead of 150 ml initially; (2) 0.2 ml PA, and heating to 200°C near the residual volume of 0.2 ml in the end evaporative step, with the beaker removed from the hotplate when the first copious white fumes appeared; (3) medium microwave power (50% full power) for a turntable loaded with triplicate samples; (4) a microwave heating time of 600 min for a fully loaded turntable, and a total method time of 700 min; (5) >80 ml but <120 ml of AC in a 250 ml beaker for final residue dissolution. The high temperature step with PA was essential for complete digestions. A PA fumehood allowing washing down with water should be used.

Quality control and quality assurance

ICP–AES user positive controls were ICP-7 (a mixture of 1 ppm each of Ag, Al, B, and Ba plus 0.89 ppm Na, and 10 ppm K) and ICP-19 (a mixture of 1 ppm each of As, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Ti, V, and Zn) in 5% NA from Lot WP 988 from Spex Industries, Metuchen, NJ. These evaluated instrumental standardization, analytical spectral lines of the elements, interelemental correction, and background correction procedures (Que Hee and Boyle 1988) in an ICP–AES operator blind manner. A triplicate set of homogenates was also spiked in triplicate with known amounts of a mixture of the toxic

elements Al, As, Ba, Be, Cd, Co, Cr, Cu, Mo, Ni, Pb, and Se (Spex 1000 ppm standards, 99.999% pure, appropriately diluted) at nominal final analysis concentrations of 1 ppm above unspiked values for recovery and background correction evaluation purposes once each method was optimized. Method blank arithmetic mean values were subtracted from all fish sample values. Fish elemental concentrations were reported in terms of wet and dry weights.

ICP–AES analyses

The ICP–AES instrument was from Applied Research Laboratories, Valencia CA. It was 1.5 m in focal length, and was an air instrument with multiple filter/slit/photomultiplier ensembles allowing simultaneous multielemental analysis. The quartz argon-plasma torch used liquid argon of 99.999% purity as argon gas source (Alphagaz Specialty Gases, Los Angeles CA).

An aliquot of 20–25 ml was transferred into a 30 ml polyethylene screw cap tube from the final acid solution product of each treatment. Each individual ICP–AES analytical run for a nebulized sample utilized four estimations and a volume of about 7 ml to produce an arithmetic mean, its SD, and its CV, defining the homogeneity of each 7 ml nebulized sample. This CV is defined hereafter as the nebulization CV. The arithmetic mean, SD, and CV of three such analyses of the same AC solution define the homogeneity of the AC solution of each digest residue, and this CV is defined hereafter as the intrarun CV. The arithmetic mean, SD, and CV of AC solutions of three separate 10 g homogenate digest residues of the same type defined the interrun parameters, and this CV is defined hereafter as the interrun CV. The interrun CV measures the uniformity of the homogenate plus the instrumental precision and the homogeneity of the nebulized subsample.

Analytical spectral lines, standardizations, interelemental corrections, and background corrections were utilized as described elsewhere (Que Hee and Boyle 1988). Detection limits were calculated using the method of the International Union of Pure and Applied Chemistry. The ultrasonic nebulizer had the following detection limits in ppb (ng/ml) for 28 elements: Ag, 0.1; Al, 20; As, 6.; B, 20; Ba, 0.2; Be, 0.06; Ca, 5; Cd, 1; Co, 3; Cr, 1, Cu, 1; Fe, 0.8; K, 100; Li, 0.1; Mg, 0.4; Mn, 0.1; Mo, 2; Na, 30; Ni, 2; P, 50; Pb, 5; Se, 5; Si, 7; Sn, 7; Sr, 0.1; Ti, 0.3; V, 1; and Zn, 0.5. An Advanced Logic Research 3300 computer (Microflex, San Francisco CA) acquired the data and reported results. The interrun elemental mass balance, its SD, and CV were also calculated.

Results and discussion

Dry weight interrun CVs

Interrun CVs for the dry weights of all fillet wet weights examined (100 mg, 1 g, 2 g, and 10 g) were $< 10\%$. In contrast, 100 mg, 1 g and 2 g wet weights of whole fish homogenate had interrun CVs of $> 20\%$ for their dry weights. Only 10 g wet weight samples produced interrun CVs dry weights of $< 20\%$ for the whole fish homogenate. The heterogeneity and resistance to digestion of whole fish are expected to be greater than for fillet. A larger sample has to be taken in order for it to be representative. Thus, to provide an adequate comparison of matrix disruption techniques, 10 g homogenate samples were used for both fillet and whole fish homogenate treatments. About the same weight was also necessary from the perspective of Pb sensitivity as discussed above. Explicit demonstration of homogenate uniformity should be part of quality assurance protocols.

The usual procedure to digest wet organic matrixes is to take 100 mg samples for digestion, and then solubilize the digest residue in 7–10 ml of AC (Que Hee and Boyle 1988). This is adequate for many matrices including fish fillet homogenate. Whole fish homogenates produced in this study required a larger subsample for representative sampling using dry weight interrun CVs $< 20\%$ as the criterion. This effect is part of the typically large variations usually attributed to 'biological variation' in the same species, at least for elemental analyses for whole fish.

User quality assurance and quality control

The user positive control samples showed relative errors $< 10\%$ for the following elements in ICP-7: Ag, B, Ba, K, Na, and Si. Al had a positive bias of 13%. Similarly, the elements in ICP-19 with relative errors $< 10\%$ were As, Be, and Pb. Cd, Co, Cr, Mg,

Mo, Ni, Se, Ti, V, and Zn had relative errors between 10 to 20%. For ICP-19, Ca, Cu, and Fe showed positive biases of 27%, 45%, and 24%, respectively. The only serious bias was for Cu since the US EPA error threshold is 30% for real samples (US EPA 1996). All the data in tables 1 and 2 are uncorrected for these user quality control bias results.

Fillet

Intrarun and nebulization CVs and spiking recoveries. All intrarun CVs of the elements of the same AC solution that were above their least quantifiable (practical) limits were $\leqslant 10\%$, as required by US. EPA precision guidelines (US EPA 1996). This result shows that individual AC solutions of the digest residues of the Parr bomb, microwave, and dry-ashing methods had acceptable homogeneity and stable instrumental operating conditions. Similarly, nebulization CVs were $\leqslant 10\%$. Hence, AC subsamples remained homogeneous during nebulization.

Nebulization and intrarun CVs for the end solutions of the US EPA method usually exceeded 10% signifying nebulization difficulties and end solution heterogeneity. The end solutions were supposed to be heterogeneous, however.

Table 1. Interrun elemental comparison of different sample treatments (arithmetic mean μg element/g wet weight with standard deviation in parentheses) for triplicate 10 g catfish fillet homogenates after ICP-AES analysis (10 g wet weight = 2.6±0.44 g dry weight).

		Elemental composition using different sample treatments				
E	DL	PARR	MICRO	DA	EPA	EPAR
Ag	< 0.001	< 0.001	< 0.001	< 0.0003	< 0.001	
Al	< 0.2	< 0.2	< 0.2	8.0(1.6)	< 0.2	< 0.3[a]
As	< 0.05	< 0.05	1.7(0.1)	< 0.02	< 0.06	0.45(0.10)
B	< 0.2	< 0.2	< 0.2	21(3)	< 0.2	
Ba	< 0.002	< 0.002	0.015(0.005)	0.060(0.030)	0.033(0.015)	
Be	< 0.0005	< 0.0005	< 0.0005	< 0.0002	< 0.0006	< 0.02[a]
Ca	< 0.04	73(6)	88(17)	68(11)	90(16)	110(5)
Cd	< 0.009	< 0.009	< 0.009	< 0.003	< 0.01	< 0.02[a]
Co	< 0.02	< 0.02	< 0.02	< 0.007	< 0.03	
Cr	< 0.008	< 0.008	0.094(0.033)	< 0.002	0.090(0.050)	< 0.05[a]
Cu	< 0.01	< 0.01	< 0.01	0.43(0.10)	1.2(0.70)	0.33(0.09)
Fe	< 0.006	2.4(0.2)	3.6(0.7)	2.7(0.4)	4.0(2.8)	2.01(0.30)
K	< 1	3 080(100)	2 800(180)	3 500(350)	2 400(520)	3 400(240)
Li	< 0.0008	< 0.0008	< 0.0008	4.8(1.6)	< 0.001	
Mg	< 0.003	171(6)	164(9)	198(4)	180(40)	244(16)
Mn	< 0.001	0.060(0.020)	0.090(0.010)	0.085(0.010)	0.100(0.030)	
Mo	< 0.02	< 0.02	0.23(0.03)	< 0.006	< 0.02	
Na	< 0.2	270(10)	260(20)	370(30)	220(50)	460(17)
Ni	< 0.01	< 0.01	0.64(0.22)	0.170(0.060)	< 0.02	< 0.08[a]
P	< 0.4	1 630(50)	1 540(50)	2 040(150)	1 400(320)	1 840(90)
Pb	< 0.04	< 0.04	< 0.04	< 0.01	< 0.05	< 0.2[a]
Se	< 0.04	< 0.04	0.31(0.10)	< 0.01	< 0.05	< 0.6[a]
Si	< 0.06	13(2)	< 0.06	6.0(1.0)	8(2)	
Sr	< 0.001	0.060(0.040)	0.110(0.020)	0.100(0.010)	0.100(0.030)	
Ti	< 0.003	< 0.003	< 0.003	0.38(0.13)	< 0.003	
V	< 0.008	< 0.008	< 0.008	< 0.002	< 0.01	
Zn	< 0.004	4.80(0.20)	5.40(0.40)	4.20(0.10)	7.0(2.0)	5.68(0.58)
ATE		5 244(174)	4 864(278)	6 224(553)	4 311(954)	6 062(369)
ATE CV		3.32	5.72	8.88	22.1	6.09

E, element; DL, detection limit for Parr bomb and microwave techniques in μg element/g wet weight; PARR, Parr Bomb wet-ashing method; MICRO, microwave method wet-ashing method; DA, AOAC dry-ashing method; EPA, US EPA alkaline/acid wet ashing technique; EPAR, US EPA method development value in μg element/g wet weight for fresh catfish fillet using US EPA method 200.11 (Martin *et al*. 1992, table 4 Version 2.0 data). ATE, average total element content in μg with standard deviation in parentheses; ATE CV, 100 × standard deviation of ATE/ATE. [a] EPA method detection limit at 99% confidence level for seven replicates of blank.

Table 2. Average elemental concentrations and standard deviations in parentheses (n = 3 homogenate samples) in μg/g relative to wet and dry weights for 10 g triplicate homogenates of California mosquitofish < 3 cm in length digested by the modified wet ashing microwave method.

		Dry	
E	Wet Sample	DL	Sample
Ag	0.0028 (0.02)	0.001	0.0039(0.02)
Al	23(2)	0.3	31(3)
As	0.11(0.1)	0.07	0.15(0.1)
B	4.7(0.9)	0.3	6.4(1.0)
Ba	< 0.02	0.003	< 0.003
Ca	4 300(2 000)	0.05	5 900(3 000)
Cd	0.03(0.01)	0.01	0.040(0.02)
Co	0.3(0.2)	0.03	0.4(0.2)
Cr	0.25(0.02)	0.01	0.34(0.03)
Cu	1.5(0.5)	0.01	2.0(0.6)
Fe	27(2)	0.008	36(2)
K	39(5)	1	54(7)
Li	0.11(0.020)	0.001	0.15(0.030)
Mg	100(4)	0.004	140(5)
Mn	3.9(0.2)	0.001	5.4(0.3)
Mo	0.22(0.03)	0.03	0.30(0.04)
Na	59(4)	0.3	81(6)
Ni	0.31(0.9)	0.01	0.43(1.0)
P	1 900(100)	0.5	2 600(200)
Pb	0.10(0.09)	0.05	0.13(0.10)
Se	0.21(0.20)	0.06	0.28(0.20)
Si	68(10)	0.08	93(20)
Sr	7.4(0.4)	0.001	10(1)
Ti	1.0(0.1)	0.004	1.4(0.1)
V	0.11(0.01)	0.01	0.15(0.03)
Zn	14(1)	0.006	19(1)
ATE	6 550(2 132)		8 982(3 249)
ATE CV (%)	33		36

E, Element; WET, wet weight basis; DRY, dry weight basis; DL, detection limit in μg element/g dry weight (the ratio of dry/wet weights was 0.73 ± 0.04). ATE, average total elemental content in μg with standard deviation in brackets; ATE CV is 100 × standard deviation of ATE/ATE.

Recoveries of all spiked toxic elements were >95% and < 115% for the Parr bomb, microwave, and dry-ashing methods, except for recoveries below 50% for Al and Pb in the last method. The recovery guideline of the US EPA (1996) is 85–115% with intrarun CVs ⩽ 10%. The Parr bomb and microwave methods are thus better than the dry-ashing method relative to Al and Pb.

Interrun investigation. The arithmetic mean concentration for each element, its SD, and CVs for the interrun data for the fillet homogenate are compared in table 1 for each treatment technique. The concentrations for fresh catfish fillet reported in US EPA Method 200.11 quality assurance are also provided (Martin *et al*. 1992). The average total elemental weights (table 1) varied between 0.43% and 0.62% of the total dry weight compared with 0.61% determined by the US EPA on fresh fillet. The order of decreasing average total elemental weight by treatment method was: dry-ashing >Parr bomb >microwave=US EPA. The order of increasing total elemental weight CVs was: Parr bomb < microwave < dry-ashing≪US EPA. The

microwave method was about equivalent in accuracy and precision to the reference Parr bomb method. Both were about twice the sensitivity of the general method using 100 mg (Que Hee and Boyle 1988).

The data analysis for specific elements now follows. Ag, Be, Cd, Co, Pb, and V were below all method detection limits. The arithmetic means for Ca, Fe, K, Mg, Mn, Na, P, Sr, and Zn were greater than ten times the reagent blank of all methods. The microwave method allowed the detection of eight elements with the highest arithmetic mean concentrations, followed by dry-ashing with seven, the US EPA method with four, and then the reference Parr bomb technique with one. Thus element volatilization is not an issue here. The dry-ashing method ranks high since its final solution is 3.2–4 times more concentrated than the others. The lowest interrun CVs were: Parr bomb (Ca, Fe, K, Mg, Na, P, and Zn were $\leqslant 10\%$ and Si was 15%); dry-ashing (K, Mg, Na, P, Sr, and Zn were $\leqslant 10\%$, and B, Ca, Fe, Mn and Si were between 10 and 20%); microwave (As, K, Mg, Na, P, and Zn were $\leqslant 10\%$, and Ca, Fe, Mn, Mo, and Sr were between 10 and 20%); and US EPA (Ca was $< 20\%$, and none were $\leqslant 10\%$). Thus while the Parr bomb method had interrun CVs $\leqslant 10\%$ for seven elements, the microwave and dry-ashing methods both produced six such elements, with none for the US EPA method. Using an interrun CV of $\leqslant 20\%$ as criterion resulted in the following number of elements that were adequate: 11 for both microwave and dry-ashing; eight for the Parr bomb; and one for the US EPA method. The US EPA method had the highest interrun CV (18–56%) for every element, except for Sr in the Parr bomb method (67%).

The reported US EPA Method 200.11 values for fresh fillet agreed within 20% of our US EPA method arithmetic means for Ca and Zn, and within an order of magnitude for our observed values for Fe, K, Mg, Na, and P. The EPA method was not further considered because of high intrarun, nebulization, and interrun CVs. The technique is not precise enough for fish that are not fresh, as concluded by the US EPA (Martin *et al.* 1992).

The dry-ashing method was the fastest (three person-h) and most sensitive (Al, B, Li and Ti were not detected by the other methods), and produced no coloured solutions or precipitates. An interlaboratory study (Jorhem 1993) utilized 450°C as the high temperature rather than the 500°C recommended in the AOAC method (Cunniff 1996a,b) and in the present study. The interrun CVs for the common elements of the present study/Jorhem 1993 study are, respectively: Cu, 23%/11%; Fe, 15%/7.7%; Ni, 35%/39%; and Zn, 2.4%/3.7%. The results for Ni and Zn are not significantly different at $p \leqslant 0.05$, but our interrun CVs for Cu and Fe are about two-fold higher. The dry-ashing technique was not considered further because the interrun CV for average total element weight (table 1) was higher than those for the Parr bomb and microwave methods, and spiked Al and Pb showed low recoveries, even though the method had superior sensitivity.

There were no significant statistical differences at $p \leqslant 0.05$ for the concentrations of elements that both the microwave and Parr bomb methods quantified with interrun CVs < 20% (Ca, K, Mg, Na, P, and Zn) except for Fe. The agreement for both acid wet-ashing techniques for their common well quantified elements also indicates that the homogenate was uniform. The microwave method took longer than the Parr bomb method, but allowed detection of more elements above detection, and did not require expensive specialized equipment like bombs, Teflonware, and specialized microwave ovens. The microwave method was therefore chosen to optimize the digestion of whole fish homogenate (table 2).

Whole fish

Table 2 gives the interrun results of the optimized microwave digestion for homogenized whole fish. Al, B, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, P, Si, Sr, Ti, V, and Zn were the 17 elements that had interrun wet weight CVs $\leqslant 20\%$ with Al, Cr, Fe, Mg, Mn, Na, P, Sr, Ti, V, and Zn being the 11 elements with CVs $\leqslant 10\%$. The 17 elements with CVs $\leqslant 20\%$ all had nebulization and interrun CVs of $\leqslant 10\%$. Ag, Ba and Ni had CVs $>100\%$ or arithmetic means less than the detection limit and are therefore not detectable. The elements with CVs 20% to 100% (As, Cd, Co, Pb, and Se) are present but are trace. Both Ca (CV, 47%) and Cu (CV, 33%) were above their least quantifiable limits, but were not reproducible for different samples of the same homogenate even though their intrarun and nebulization CVs for the individual acid cocktail solutions were acceptable. About 0.66% of the wet weight and 0.90% of the dry weight are accounted for by these elements in whole fish. The average weight CV of 33–36% is caused mostly by Ca. No values for California mosquitofish exist for comparison. The use

of a non-specialized microwave system did not cause an across-the-board imprecision because of poorer control of rate of delivery of microwave power relative to more specialized systems. Frequent relief of pressure buildup, interior oven spray teflon-coating, modification of the oven exhaust, and the initial overnight room temperature digestion in addition to careful sample handling allow acceptable precision. Use of specialized microwave ovens may produce even better results.

Micromethods using microwave wet-ashing techniques for fish < 100 mg in weight are available for more sensitive detection techniques like furnace AAS for Cd, Cu, and Zn (Baldwin *et al*. 1994), and ICP/MS (Sheppard *et al*. 1994, Mizushima *et al*. 1996). Interrun CV data were not provided, although spiking recovery data are available for the ICP/MS studies. There are many literature methods for shellfish (for example, Zeisler *et al*. 1988, McCarthy and Ellis 1991, Casper and Yess 1996). The microwave method for Cd, Cr, Cu, Pb, and Zn in shellfish was comparable to the conventional wet-ashing method, but dry-ashing caused significantly lower recoveries (McCarthy and Ellis 1991). Low recoveries for Al and Pb were found for dry-ashing in the present fish fillet study.

Conclusions

While the microwave method generally gave equivalent results to the reference Parr bomb method for fish fillet even with a non-specialized microwave system, dry-ashing caused low recoveries of spiked Al and Pb, and the US EPA method is applicable only for fresh fish fillet. Interrun elemental analysis is necessary to detect homogenate uniformity problems in large fish samples, since all nebulization and intrarun CVs were $\leqslant 10\%$. A starting wet weight of 10 g was necessary for analysis of homogenates of whole fish, though catfish fillet wet weights between 100 mg to 10 g produced dry weights with CVs $\leqslant 10\%$. Such a dry weight analysis procedure should be part of the quality assurance protocol to test representativeness and homogeneity of homogenates, as should at least triplicate digestions of the same homogenate weight for multielemental analysis to show homogeneity relative to elements.

Acknowledgements

The Las Virgenes Municipal Water District and the UCLA Center for Occupational and Environmental Health are thanked for financial support. Bret Holguin is thanked for his ICP–AES analysis support.

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