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Analytical Methods

Measurement of haem and total iron in fish, shrimp and prawn using ICP-MS: Implications for dietary iron intake calculations

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ABSTRACT

Twenty-five species of fish, shrimp and prawn from local markets in Bangladesh were analysed for concentrations of total Fe, haem Fe and non-haem Fe by ICP-MS. Total Fe and non-haem Fe concentrations were measured in nitric acid-digested samples and haem Fe was extracted using acidified 80% acetone for 60 min. Total Fe concentrations ranged from 0.55–14.43 mg/100 g FW, and haem Fe% ranged from 18%–93% of total Fe. Repeat extractions with 80% acetone recovered additional haem Fe, suggesting that previous measurement by this technique may have underestimated haem Fe content. Calculation of Fe balance (summing Fe in acetone extracts and Fe in the residue after haem Fe extraction) was not significantly different from total Fe, indicating the two processes recovered the different forms of Fe with similar effectiveness.

1. Introduction

Anaemia is estimated to affect 1.6–2 billion people, while iron (Fe) deficiency may affect up to 40% of the global population (de Benoist, McLean, Egli, & Cogswell, 2008; McLean, Cogswell, Egli, Woidyla, & de Benoist, 2009; WHO, 2007). Even in the absence of anaemia (<110–150 g haemoglobin/L blood, depending on age and sex; (McLean et al., 2009), Fe deficiency can adversely affect health, reducing cognitive function and physical work capacity and ultimately economic productivity (Graham, Knez, & Welch, 2012). While some causes of anaemia include diseases such as thalassaemia or malaria, around 50% of the prevalence of Fe deficiency is due to inadequate dietary Fe intake or poor absorption of Fe from the diet. This is particularly problematic in many low-income countries where consumption of animal-source foods is low and diets are often predominantly plant-based. Food-based approaches to alleviating inadequate nutrient intake are generally preferable to supplementation or fortification (Miller & Welch, 2013) and therefore, the identification of foods of high iron content and high bioavailability is of significant importance. Mammal, bird and fish muscle tissues (meat) are considered good sources of Fe for their high total Fe concentration, as well as presence of haem Fe (Fe protoporphyrin IX). Haem Fe is found only in meat and has greater bioavailability than non-haem Fe that is the only form of Fe found in plant tissue, but which is also present in meat. The difference in bioavailability (15–35% for haem Fe versus 2–20% for non-haem Fe; (Cook & Monsen, 1976; Hurrell & Egli, 2010), is due to the different physiological mechanisms of transport across intestinal membranes; haem Fe is absorbed as an intact molecule (Shayeghi et al., 2005), whereas non-haem Fe is digested in the stomach and reduced to Fe^{2+} before absorption (Waldvogel-Abramowski et al., 2014).

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The classical methods of haem quantification e.g. phosphate (Drabkin, 1950), acidified acetone (Hornsey, 1956), ammonium sulphate (Brown, 1961), including their recent modifications (Chaijan & Undeland, 2015; Cross et al., 2012; Gomez-Basauri & Regenstein 1992) all rely on UV/Vis spectrophotometry, as do HPLC-based methods (Sato, Ido, & Kimura, 1994). However, interactions between the extraction medium, storage conditions of the muscle tissue and delays between extraction and measurement can influence the efficiency of haem extraction, greatly influencing the accuracy of each method (Chaijan & Undeland, 2015). Similarly, the iron concentration of the different fractions of meat has been measured by multiple methods, including UV/Vis, AAS or inductively coupled plasma (ICP) spectrophotometry methods (e.g. Carpenter & Clark, 1995; Valenzuela, de Romana, Olivares, Morales, & Pizarro, 2009). However, the iron concentration of the haem fraction has rarely been measured directly (Cross et al., 2012), but has usually been inferred from the molecular weight ratio of haematin (approximately 8.8 mg Fe/g haem; e.g. Kongkachuichai, Napatthalung, & Charoensiri, 2002). Alternative methods, such as subtraction of non-haem iron from total iron, leads to highly varied values of iron concentration in haem, especially when different analytical methods are used for the separate analyses (Lombardi-Boccia, Martínez-Dominguez, Aguzzi, & Rincón-León, 2002).

Numerous equations (Beard, Murray-Kolb, Haas, & Lawrence, 2007) have been devised to estimate dietary Fe absorption from haem and non-haem Fe components, yet most use a single generic value of 40% for the amount of haem Fe present in meats. This is usually based on a truncated value from the range of 30–40% (Cook & Monsen, 1976) which is treated almost as a constant, despite the variation and clear evidence that haem Fe represents 25–70% of the total Fe of red meat (Hurrell & Egli, 2010; Schönfeldt & Hall, 2011; Valenzuela, de Romana, Olivares, Morales, & Pizarro, 2009). Variation within individual species and even individual cuts of meat from the same animal exist which will influence dietary Fe intake and absorption (Rangan, Ho, Blight, & Binns, 1997; Schönfeldt & Hall, 2011; Valenzuela et al., 2009), as does the source of meat: mammal, bird, or fish.

Worldwide, fish provided 158×10^6 tons of food in 2012 from >400 species (FAO, 2014), yet the nutrient composition of only a fraction of these species is reported in the FAO/INFOODS database (173 entries; FAO, 2013) and the USDA National Nutrient Database (48 entries; USDA, 2014). In contrast, 21 and 29 analyses of Fe in various cuts of meat from a single livestock species (cattle, *Bos taurus*), are reported in these databases. Fish species diversity is high in Bangladesh, with over 267 freshwater fish species and other aquatic animals, contributing to a large proportion of the population intake of haem Fe (Thilsted, 2013). Few studies of haem Fe concentrations from different fish species have been published (Kongkachuichai et al., 2002; Turhan, Ustun, & Altunkaynak, 2004; Turhan, Sule Ustun, & Bank, 2006; Roos et al., 2007). Because different species were tested by a range of different analytical methods and Fe pool calculations, haem Fe concentrations in these papers are varied and inconsistent.

In order to improve procedures for haem Fe analysis in fish, and consequently, dietary recommendations of fish intake, we have developed a method for the analysis and calculation of total Fe, haem Fe and non-haem Fe in fish by ICP-mass spectrometry (ICP-MS) based on an ICP-optical emission spectroscopy (ICP-OES) analysis of beef (Cross et al., 2012). This paper reports on the total Fe, haem Fe and non-haem Fe in a number of small indigenous fish species (SIS; <25 cm in length), shrimp and prawn species commonly consumed in Bangladesh.

2. Materials and methods

2.1. Fish preparation

Samples of SIS were obtained from local markets and fish landing sites in Mymensingh, Sylhet, Khulna, Dinajpur and Cox's Bazar districts of Bangladesh in July–August 2014. Four replicates of 26 samples, comprised of 23 species of fish, one species of shrimp and one of prawn were all from capture fisheries, plus one fish species (*Amblypharyngodon mola*) was taken from both capture and a household culture pond (Table 1). All samples were cleaned, using non-metal equipment to obtain raw, edible parts, according to traditional practice. Samples were frozen, air-freighted on dry ice to Adelaide, South Australia, and re-frozen at −80 °C, then freeze-dried until all water was removed (>48 h). Samples were ground with an IKA 11 stainless steel mill (IKA, Staufen, Germany) until uniform particle size was achieved. Variations between species in terms of bone density and skin thickness contributed to between-species heterogeneity.

The reference material Dogfish protein DORM4 (National Research Council of Canada, Ottawa, Canada) was digested in duplicate with each batch to estimate the recovery of variability of the digestion efficiency. No certified reference material for haem Fe content is currently commercially available so locally purchased, imported salted dried prawn (*Metapenaeus ensis*) and anchovy (*Engraulis* ssp.) were dried and ground and used as additional check samples for between-batch repeatability.

2.2. Sample digestion for total Fe

Sub-samples of each SIS were digested in 15 mL polypropylene tubes (SC415, Environmental Express, Chapel Hill, USA) in a 96 well Hotblock® acid digestion block (Environmental Express). Approximately 0.1 g of each freeze-dried, ground sample was weighed into a digestion tube to ±0.0001 g on a Kern ABJ balance (Balingen-Frommern, Germany), and 2 mL of Baseline grade

Table 1
Fish, shrimp and prawn species names.

Scientific name	Local name	Common name
Fish		
Ailia coila	Kajuli, Bashpata	Gangetic ailia
Amblypharyngodon mola	Mola	Mola carplet
Amblypharyngodon mola (cultured)	Mola (cultured)	"
Botia dario	Rani	Queen loach
Chela cachius	Chela	Silver hatchet chela
Colisa fasciata	Boro Kholisha	Banded gourami
Corica soborna	Kachki	Ganges river sprat
Eleotris fusca	Kuli, Bhut Bailla	Dusky sleeper
Esomus danricus	Darkina	Flying barb
Glossogobius giuris	Bele, Bailla	Tank goby
Gudusia chapra	Chapila	Indian river shad
Heteropneustes fossilis	Shing	Stinging catfish
Hyporhamphus limbatus	Ekthute	Congaturi halfbeak
Lepidocephalichthys guntea	Gutum	Guntea loach
Macrognathus aculeatus	Tara Baim	Lesser spiny eel
Mastacembelus pancalus	Guchi	Barred spiny eel
Mystus cavasius	Golsha	Gangetic mystus
Mystus vittatus	Tengra	Striped dwarf catfish
Osteobrama cotio cotio	Dhela	Dhela
Pseudambassis ranga	Chanda	Indian glassy fish
Puntius sophore	Jat Punti	Pool barb
Puntius ticto	Tit Punti	Ticto barb
Stolephorus tri	Kata Phasa	Spined anchovy
Xenentedon cancila	Kakila	Asian needlefish
Prawn/Shrimp		
Macrobrachium malcolmsonii	Najari Icha	Monsoon river prawn
Metapenaeus monoceros	Harina Chingri	Speckled shrimp