Iron content and its in vitro availability in two fish species; *Sardinella melanura* and *Carnax* spp.

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Abstract

Iron deficiency is one of the most common nutritional deficiencies in Sri Lanka and is associated with problems such as poor food supplies and inadequate amounts of available iron forms in the food consumed. A study was carried out using a modified *in vitro* iron availability method, therefore, to investigate the iron content and its availability in two fish species, namely *Sardinella melanura* (Salaya) and *Carnax* spp. (Para), commonly consumed in Sri Lanka.

The iron content was determined in cooked fish flesh, cooked fish bones and cooking water using atomic absorption spectroscopy and calculated on a dry weight basis. The *in vitro* availability of iron in cooked fish flesh was estimated using the method described by Svanberg *et al.*, (1993) with some modifications. The data were statistically analysed using MINITAB.

In cooked *Sardinella melanura*, the iron content in flesh was 335.3 ± 6.8 ìg/g whereas in bones, the iron content was 315.3 ± 6.3 ìg/g. In cooking water, the iron content retained was 25.0 ± 0.7 ìg/g. The *in vitro* available iron content was 66.2 ± 4.5 ìg/g. In cooked *Carnax* spp., the iron content in flesh was 164.8 ± 9.3 ìg/g whereas in bones, it was 101.1 ± 8.2 ìg/g. In cooking water, the iron content retained was 15.0 ± 2.1ìg/g. The *in vitro* available iron content in cooked fish flesh was 58.9 ± 4.4 ìg/g.

The content of iron in the fish flesh ($p<0.05$) and fish bones ($p<0.05$), cooking water medium ($p<0.05$) and the total available iron ($p<0.05$) were all significantly higher in *Sardinella melanura* than in those of *Carnax* spp.

Although there was a considerable amount of iron in these fish species, the iron availability was found to be relatively low. Both fish bones and fish flesh contain significantly high amounts of iron. As the cooking water also contains a substantial amount of iron, the consumption of gravy of the curry along with the fish flesh will increase the amount consumed.

Keywords: Cooking, *Sardinella melanura* (Salaya), *Carnax* spp. (Para), *in vitro* iron availability.

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Introduction

Iron deficiency is widespread with the highest prevalence in developing countries including Sri Lanka and is a result of the amount of dietary iron absorbed being insufficient to meet iron requirements. This in turn could be due to insufficient intake of dietary iron or low bioavailability of the iron present in the diet. According to the estimates of the FAO/ WHO (1988), 4-5 billion people may be iron deficient, corresponding to 66-80% of the world's population. In Sri Lanka, nearly one third of the pre-school and one fifth of the primary school children are reported to be anaemic (MRI, 2003). Iron deficiency is one of the most common nutritional deficiencies in Sri Lanka and is associated with problems such as poor food supplies and inadequate amounts of available iron forms in the food consumed.

There are two different forms of iron in the diet, namely, haem iron and non-haem iron. Haem iron originates from either haemoglobin or myoglobin found in animal tissues such as blood, meat and fish. Iron in plant foods such as cereals, legumes, vegetables and fruits are present in the non-haem form. The extent to which iron is absorbed from a meal depends on several factors such as the individual's iron status and requirements, nature of the source and iron content within the meal and the other constituents of the meal (Bothwell *et al.*, 1989).

As Sri Lanka is an island and also has a large number of inland tanks, the availability of fish as a food commodity is generally adequate. Since no studies on commonly consumed Sri Lankan fish species as a source of dietary iron has been carried out, the present study investigated the iron content and its availability in two fish species which are commonly consumed in Sri Lanka, namely; *Sardinella melanura (*Salaya) and *Carnax* spp (Para), using a modified *in vitro* iron availability method.

Materials and Methods

Preparation of the fish

The fish species *Sardinella melanura* and *Carnax* spp studied were purchased from local markets in and around Colombo District. The fish were identified by using their external morphological characters and total lengths of the fish were recorded. The gills, fins and the gut contents of the fish were removed and they were washed several times with water. Fish samples of about 50 g were placed into separate containers with 150 ml of distilled water and cooked under regulated heat for 15 minutes. Then the fish

were taken out and the volume of remaining water was measured. The flesh was removed from the bones, collected separately and their weights were recorded.

The separated cooked fish flesh and bones were dried at 105° C for 48 hrs until a constant weight was obtained. Then the dried fish flesh and bones were ground separately and used to prepare samples for the atomic absorption spectrometry.

Analysis by atomic absorption spectrometry

Samples of 1.0 g of powdered fish flesh and bones of each fish species were converted into char using a muffle furnace maintained at 550°C and left until white or grey ash resulted. The amount of ash in each crucible were weighed and dissolved in 1 ml of concentrated HCl to which 10 ml of distilled water was added. Then the samples were made up to 50ml in a volumetric flask and the resultant solution was nebulized into the atomic absorption spectrometer and measured at a wave length of 248.3 nm (GBC 932, Australia). The total iron content was expressed as mg/g of fish dry weight.

Determination of *In vitro* availability of iron in fish

In vitro iron availability was estimated as iron solubility under physiological conditions according to the method of Svanberg *et al.*, (1993) with some minor modifications. Samples of 0.5 g finely mixed cooked fish flesh of each fish species were mixed with 5ml of distilled water. 5 ml of 0.3% pepsin in 0.1 MHCl also containing physiological levels of Na^+ (49 mM as NaCl), K^+ (12 mM as KCl), Ca^{2+} (10 mM as $CaCl_2$. $2H_2O$), Mg^{2+} (2.4 mM as $MgCl_2$. $6H_2O$) and phosphate (3.5 mM as KH_2PO_4) (Diem and Lenther 1975) was added. The pH was adjusted to 2.0 with 2 M NaOH and the mixture was incubated in a shaking water bath at 37°C for 90 minutes. After incubation, 1.5 ml of a pancreatin (6mg, Sigma) and bile solution, (37.5mg, Sigma) in 0.1 M$NaHCO_3$ was added. The pH was adjusted to 5.0 with 2 M NaOH, and the mixture was incubated for a further 30 minutes. After adjustment of the pH to 6.0 with NaOH, the mixture was centrifuged at 4300 x g for 20 minutes. The supernatant was filtered through a 45 ìm filter. The pH in the filtrate was reduced by the addition of 200 μl of 0.5 M HCl to 1600 μl of sample, followed by an addition of 200 μl of ascorbic acid solution (20 g/l).

After 10 minutes, the samples were centrifuged (11000 x g, 10 minutes) and the clear solution obtained was analyzed for soluble iron, including free soluble complexes of iron, with atomic absorption spectrometer at 248.3 nm (GBC 932 spectrometer). The amount of soluble iron in the filtrate was expressed by using the dry weight (DW) in mg/g as the total amount of iron in the sample.

Statistical analysis

Differences in mean values for total iron in cooked fish flesh, cooked fish bones and *in vitro* available iron contents in triplicate samples were tested for significance ($p<0.05$) by analysis of variance (ANOVA) using Tukey's HSD multiple range test (Wilkinson,1990). Simple regression analysis was carried out for *in vitro* available iron content.

Results and Discussion

Table 1: Iron content and *in vitro* iron availability of cooked fish of *Sardinella melanura* and *Carnax* spp.

Fish species	Fe in Cooked fish flesh (µg/g DM)	Fe in Cooked fish bones (µg/g DM)	Fe in Cooking water (µg/ml)	Available Fe in cooked fish flesh (µg/g DM)
S. melanura	335.3 ± 6.8^a	315.3 ± 6.3^a	25.0 ± 0.7^a	66.2 ± 4.5^a
Carnax spp	164.8 ± 9.3^b	101.1 ± 8.2^b	15.0 ± 2.1^a	58.9 ± 4.4^b

Mean values ± Standard error of Mean n = 3; a,b; Significant differences are denoted by different superscripts ($p<0.05$). DM- on dry matter basis

In cooked fish flesh and in fish bones, the mean iron content was higher in *Sardinella melanura* than in *Carnax* spp. Although the reasons for the difference in iron contents can be many, it can be assumed that due to difficulties in removing the smaller bones in the flesh of *Sardinella melanura,* its iron content in cooked fish flesh may have been higher.

Within the two fish species there was a significant difference ($p<0.05$) between the mean iron contents of fish flesh and in fish bones.

It is evident from the previous studies that; the nutritional composition of fish muscle varies both from species to species and within species, from one season to another (Burgess, 1975). In addition, environmental factors too contribute to the concentration of metal in fish. This view is supported by the findings of Window *et al.*, (1987) and Khan *et al.*, (1987), which showed that variation in the concentrations of elements from one sample of fish to another was due to the chemical forms of the elements and their varied concentrations in the habitat.

In general, the fish samples show a higher mean iron content in bones. We can increase the availability of the iron in bones by different cooking methods and therefore, further studies need to be carried out to investigate the effect of cooking on iron availability. In the cooking water medium of these fish species, the iron content retained ranged from 15.0 ± 2.1 to 25.0 ± 0.7 μg/ml in *Carnax* spp. and in *Sardinella melanura,* respectively. Also the total iron content retained in the cooking water medium was higher in *Sardinella melanura* than in *Carnax* spp. The studies revealed that the amounts of iron leached in to the medium were not high and therefore substantial amounts of total iron were retained in fish flesh. However, the *in vitro* iron availability of these species was found to be lower. The studies also found that, the *in vitro* available iron content of *Sardinella melanura* was significantly higher than that of *Carnax* spp.

Conclusion

In *Sardinella melanura,* substantially higher amounts of iron were observed in both fish flesh and fish bones than in those of *Carnax* spp. Although there was a considerable amount of iron in these fish species, the iron availability is relatively low in cooked fish flesh (In *Sardinella melanura*, 20% and in *Carnax* spp. 36%). The amounts of iron leached in to the medium were not high and therefore substantial amounts of total iron were retained in fish flesh. However, it can be recommended the gravy in a fish curry along with the fish flesh be consumed in order to increase the amount of the available iron in the portion consumed.

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