Original Article

Iron release from the *Lucky Iron Fish®* : safety considerations

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Background and Objectives: The principal objective was to explore in greater detail safety issues with regard to the use of the *Lucky Iron Fish®* (fish) as a treatment for iron deficiency and iron deficiency anaemia in women in rural Cambodia. **Methods and Study Design:** Experiments were done to determine: (1) purity of the iron in the fish by mass spectroscopy; (2) release of iron and contaminants released during boiling in water using inductively-coupled plasma optical emission spectroscopy; (3) the impact of cooking time, acidity and number of fish in acidified water and two types of Khmer soups; and (4) drinkability of the water after boiling with different numbers of fish. **Results**: The fish is composed primarily of ferrous iron with less than 12% non-ferrous iron. Contaminants were either not detectable or levels were below the acceptable standards set by the World Health Organization. The length of time boiling the fish and the acidity of the water increased iron release but even with 5 fish boiled for 60 minutes, iron levels only approached levels where side effects are observed. Boiling one fish in water did not affect the perception of colour, smell or taste of the water but boiling in water with two or more fish resulted in the water being unpalatable which further limits the potential for iron toxicity from using the fish. **Conclusions**: The results suggest that the *Lucky Iron Fish*™ may be a safe treatment for iron deficiency.

Key Words: Lucky Iron Fish® , anaemia, iron deficiency, food safety, iron release

INTRODUCTION

Iron deficiency affects more than 3.5 billion people globally.[1] The majority of people living with this condition are women and children in the developing world where access to food diversity and conventional medicine is limited.[2,3] In 2008, the World Health Organization reported that 66.4% of pregnant and 57.3% of non-pregnant women of reproductive age suffer from anaemia primarily due to iron deficiency.[2] The health impacts can be severe and may be irreversible but anaemia also has a significant negative economic impact on the family and the community.[4-6] It is estimated that iron deficiency results in a $70 billion annual loss in the annual global gross domestic product.[7]

Despite significant international efforts to fortify food staples, such as flour, and to provide iron supplementation programs specifically aimed at vulnerable populations, iron deficiency continues to increase worldwide.[8] There is a critical need for a cost-effective solution to this problem.

Adventitious sources of iron, cooking in iron pots or with iron utensils effectively boost the intake of iron.[9-13] Iron leached from these sources is bioavailable,[14-18] however compliance with this approach is a significant challenge in some regions because iron pots are too heavy, too expensive, not readily available and not culturally acceptable.[13] Recently, it has been demonstrated that adding a small ingot of iron into the cooking pot releases enough iron to ameliorate iron deficiency – raising both circulating and stored iron and halving the incidence of iron deficiency anemia.[19,20] The positive effects of the iron ingot, known as the "*Happy Fish"*, were seen after continuous use in soup or boiling drinking water.[20] Compliance levels were high (greater than 90%) and the women accepted the fish shape. Further, Charles et al[21] showed that iron was released from the during cooking and reported preliminary data on the safety of the fish. The current report expands on the safety testing of the fish, explores optimum conditions for iron release, and provides an assessment of the impact of boiling the fish in water on the taste, colour, smell and drinkability of the water.

METHODS

The iron ingots used in the present study were modified from the original design used by Charles and colleagues.[19,20] After a number of years of use, the fish from the original study, became brittle, the surface markings wore away and the fins and tail broke when dropped (GR Armstrong, personal observation: January 2014). The shape of the fish was modified: this version of the fish branded and trademarked as the *Lucky Iron Fish* (fish). It is slightly heavier (~200 g compared with the *Happy*

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Fish that was ~175 g), slightly more convex (to increase surface area which increased the degree of "flutter" during the cooking process and thereby maximized the release of iron during the cooking process) and the surface markings were accentuated to increase the appeal to users (GR Armstrong, preliminary focus groups – data not included in the paper). A comparison of the two fish is shown in Figure 1.

Four different experiments were done on *Iron Fish*: (1) to determine the purity of both the raw iron samples and the ingot once it was cast into the shape of the fish; (2) to confirm the rate of iron and other potential contaminants release during cooking; (3) to explore the dynamics of iron released into the cooking medium; and (4) to establish whether or not the cooking with *Lucky Iron Fish*™ affected the taste, colour, smell and drinkability of the boiled water.

Experiment 1: purity of the raw iron samples and the Lucky Iron Fish™

To determine the purity of the iron in the raw metal before processing and in the fish after production, samples (approximately ~3 g) taken from the melted iron before casting or scraped from the interior and exterior surfaces of the fish after casting and exposed to mass spectroscopy using an Inductively Coupled Mass Spectrometer, ICPMS (Varian 820-MS), Laboratory Services Division, University of Guelph.

Experiment 2: confirming iron and no other contaminants are released in cooking

To determine that iron is released during the cooking period and that no other contaminants are released, seven fish were boiled for 30 minutes in either (1) 1 L distilled water (pH 7.4); or (2) 1 L distilled water acidified with lemon juice to pH 6.3. The fish were boiled in glass saucepans to eliminate any possibility of contaminants leaching from the cooking pot. At the end of the boiling period, three samples (15 mL) of water were collected from each pot and tested using inductively-coupled plasma optical emission spectroscopy ICP-OES, (Varian Vista Pro, Laboratory Services Division, University of Guelph) to determine the presence of a list of possible contaminants. The list of possible contaminants included in the analysis were: aluminum; antimony; arsenic; beryllium; boron; cadmium; chromium; cobalt; copper; iron; lead; manganese; mercury; molybdenum; nickel; selenium; tin; titanium; and zinc. The equipment was standardized daily using control samples for routine analysis. Statistical differences between the amount of iron and other minerals released between treatment groups were determined using a Students' *t* test with significance set at $p<0.05$.

Experiment 3: dynamics of iron released from Lucky Iron Fish™ during cooking

Experiment 3 was done to determine how the length of time boiled or the number of fish in the boiling water affected the release of iron. Iron release from one fish boiled for either 10, 30 or 60 minutes in 1 L of acidified distilled water (pH 6.5) was determined (7 fish were tested at each time point). Similarly, iron released after boiling 1, 3 or 5 fish in 1 L acidified water for 10 minutes was determined. Finally, a dose-response curve for the effects of acidification of the boiling water (range pH 3.5 to pH 8.0) was established for iron released from the fish during 10 minutes of boiling. After boiling and cooling, three samples of 15 mL of water were drawn from each experiment and tested for iron content using ICP-OES.

The release of iron from the fish was also tested in two soups commonly prepared in Cambodian households: lemon fish soup (sngor chuok trey) and sweet and sour pineapple and pork soup (salor macho manor nung s'ung chrouk). The soups were prepared using traditional Khmer recipes using fresh ingredients purchased in a local market in Kandal Province, Cambodia. The ingredients for the soups are shown in Table 1. The soups were prepared in aluminum pots using non-metals utensils over a charcoal fire for 20 minutes (the cooking time used in Cambodia). The women preparing the soups were asked to add citrus to the soups 10 minutes before serving similar to the treatment suggested for using the *Lucky Iron Fish*™ to ameliorate iron deficiency.[18] The soup was cooled and three samples of 15 mL of the liquid portion of the soup were extracted from each cooking pot using acid-washed glass pipettes. As a control, the two different

Happy Fish

Lucky Iron Fish

Figure 1. A comparison of the appearance of *Happy and the Lucky Iron Fish*. *Lucky Iron Fish* has more clearly defined markings, a larger smile, is embossed with the Khmer word meaning "good" and weighs ~200 g. On the obverse side (not shown in this image) there is a batch number to ensure that the source of iron and the safety assurance tests can be linked to a particular batch of fish. In contrast, *Happy Fish* has fewer surface features and weighs ~175 g.

Table 1. Ingredients of the two Khmer soups used in experiment 3

Ingredient	Fish soup	Pork soup
White fish	680 g	-
Pork belly	-	455 g
Jasmine rice	30 g	-
Fresh pineapple	-	355 g
Green onion	2 stalks	-
Bean sprouts	-	475 g
Tomato	-	240 g
Carrot	1 medium sized	-
Sweet basil	120 g	-
Asian coriander	120 g	-
Non-iodized salt	-	2.5 g
Fish sauce	15 mL	30 g
Fresh tamarind paste	-	15 mL
Lemon grass	1 stalk	-
Garlic	-	2 cloves
Fresh lemon juice	1 lemon	1 lemon
Black pepper	2.0 g	1.25 g
Sugar	7.5 g	30 g
Palm sugar	5.0 mL	30 mL
Strong chili pepper	1 small	2 small

types of soups were also made without the iron fish and samples prepared in the same way as described above. Samples from both the experimental and control soups were stored in 20 mL acid-washed glass vials and shipped to Canada for analysis at Laboratory Services Division, University of Guelph, Canada. The samples were subjected to microwave-assisted digestion (CEM Corporation Microwave Digestion Unit, MARSX press) using nitric acid, brought to volume with Nanopure water and the clear extract removed and analyzed using ICP-OES. Deionized water, water acidified with lemon juice (pH ≈ similar to fish soup) and saline solution (pH ≈ similar to pork soup) prepared with and without a fish were used as controls.

Statistical differences between the amounts of iron released between treatment groups were determined using a Students' *t* test with significance set at $p<0.05$.

Experiment 4: to determine whether or not the fish affects drinkability of the water

Experiments were carried out to determine whether or not the number of fish in the boiling water affected the taste, smell, colour and overall drinkability of the water. Six experimental groups were established: control (no fish) and acid-washed glass cooking pots with one and up to a total of five fish in 1 L acidified (pH 6.5) distilled water. After boiling for 10 minutes, the fish were removed and the water cooled. The water was bottled and sealed and stored at $4^{o}C$ until used in a series of blind taste tests (the maximum length of time that the water was stored was four days). The blind taste test and assessment was set up for 35 women recruited from Guelph. Ethical approval for the blind taste tests was obtained from the Research Ethics Board of the University of Guelph. Each woman was asked to examine six different samples of water. They were asked four questions: first, they were asked to assess the colour of the sample and asked to rank it on a four-point Lickert scale from clear to strongly coloured. Next, they were asked to assess the smell of each sample. Again, they were asked to rank their assessment on a four-point scale from no smell to strong odour. They were asked to taste the water samples and comment on whether the sample tasted of iron using the four-point scale: ranking the samples from no taste to a strong taste of iron. Finally, they were asked to determine whether or not they would be prepared to drink the water in the sample bottles. In this case they were asked simply to state whether they would drink the water or not.

RESULTS

Experiment 1: composition of the raw iron and the manufactured fish

An example of the results from the microanalysis of the internal surface of a fish is shown in Figure 2. The microprobe revealed that the interior surface of the fish was composed primarily of iron with smaller amount of carbon, silicon, manganese and sulfur. This composition is typical of plain steel. The iron was predominantly elemental iron in its reduced form with a small proportion of

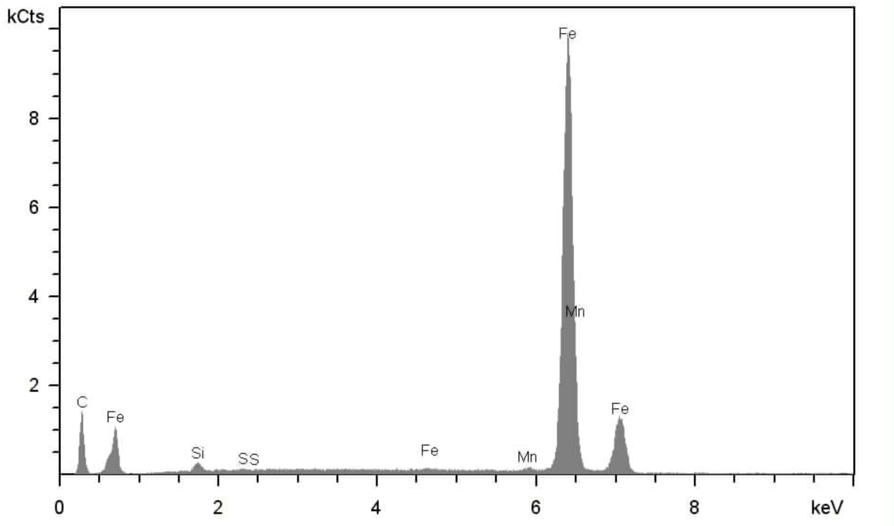

Figure 2. Energy dispersive spectroscope of the exposed surface of the *Lucky Iron Fish*.. Note: the predominant form of metal present in the same is ferrous iron (A) with small amount of ferric iron (B) and iron complexed with other minerals (C). In all samples the proportion of non-ferrous iron was always <12%.

Table 2. Release of non-iron metals into the cooking fluid from the *Lucky Iron Fish* (μg/mL)

	Aluminum	Antimony	Boron	Manganese	Molybdenum
Distilled water alone	<MDL	<MDL	<MDL	<MDL	<MDL
Water pH 7.5 plus a fish	0.054±0.001	0.003±0.0001	0.256±0.07	0.321±0.09	0.023±0.002
Water pH 6.5 plus a fish	0.055±0.002[†]	0.003±0.0003[†]	0.259±0.08[†]	0.342±0.08[†]	0.025±0.003[†]

MDL: minimum detection level.
[†]No significant difference (p>0.05) in values between water at pH 7.5 and water at pH 6.5.

Table 3. Dynamics of the release of iron from the *Lucky Iron Fish* (μg/mL)

Time (minutes)	10	30	60
Treatment group (fish)	70.5±4.33[a]	72.2±2.81[a]	86.1±3.92[b]
Distilled water only	<MDL	<MDL	<MDL
Number of fish (30 minutes)	1	2	5
Treatment group (fish)	73.4±2.11[c]	97.8±5.87[d]	120±12.9[e]
Distilled water only	<MDL	<MDL	<MDL

[ab]Values within a row with different superscripts are significantly different (p<0.01).
[cde]Values within a row with different superscripts are significantly different (p<0.02).

ferric iron and iron complexed with other metals. Less than 12% of the iron present in the samples was non-ferrous iron. The proportion of ferric to ferrous iron on the surface of the fish was higher than the internal surface, likely due to oxidation. The surface samples also contained small amount of sodium, calcium, potassium, chlorine, magnesium, aluminum and phosphorous, likely due to contaminants from handling.

Experiment 2: confirming iron and no other contaminants are released in cooking the fish

Boiling one fish in water at pH 7.5 or pH 6.5 for 30 minutes did not result in any detectable levels of a number of potential contaminants including: arsenic; beryllium; cadmium; chromium copper; cobalt; copper; lead; magnesium; mercury; nickel; selenium; tin; titanium; or zinc. The levels of these potential contaminants were below the minimum level of detectability of the assay in all samples (<0.001 μg/g water). Low levels of aluminum, antimony, boron, manganese and molybdenum were detected after boiling in distilled water at pH 7.5 and acidified distilled water at pH 6.5 (Table 2) but there was no significant (p>0.05) difference between the results of the two levels of pH (Table 1). In contrast, iron was released from the iron fish during cooking. Iron was below the level of detectability in the distilled only control group, but elevated in the distilled water (pH 7.5) group cooked with the fish (5.94±0.55 μg/g), which was significantly less than the iron levels in the acidified (pH 6.5) distilled water (72.2±3.92 μg/g).

Experiment 3: dynamics of iron released from the fish during cooking

Boiling the fish for 10 or 30 minutes released 70.5±4.33 μg/g or 72.2±2.81 μg/g iron, respectively, into the acidified water (Table 3). There was no significant difference between iron levels in these two time periods. In contrast, boiling for 60 minutes released significantly (p<0.01) more iron 86.1±3.92 μg/g. As the number of fish increased from one, to two, to five, the amount of iron released was 73.4±2.11 μg/g, 97.8±5.87 μg/g and 120±12.9 μg/g, respectively. Iron levels were significantly higher in the pot with five fish than with either one or two fish (p<0.05).

Increasing acidity of the boiling water resulted in a higher release of iron (Figure 3) from the fish. At pH 7.5 or 8.0, low levels of iron were detected in the boiled water (<10 μg/g) but rose sharply to 70.5±4.52 μg/g at pH 6.5 or when the levels of acidity were higher. However, there were no significant differences (p>0.5) between the levels of iron detected when the fish was boiled in water between pH 3.5 and 6.5.

Boiling the fish in two different types of soup commonly prepared in Cambodia resulted in a significant (p<0.001) release of iron (43.9±6.8 and 48.1±9.4 μg/g) for fish and pork soup, respectively) compared with controls (<3.5 μg/g). However these levels were significantly (p<0.001) lower compared with the effects of boiling the fish in water alone (Table 4).

Experiment 4: to determine whether or not boiling with a fish affects taste of the water

The results of the focus group responses to the appearance (colour), smell and taste of the water samples after boiling with different numbers of fish are shown in Figure 4. There was an increase in the perception of the colour of the water as the number of fish in the pot increased. Almost all of the members of the focus group (95%) considered that the water boiled with either no fish or one fish was completely clear. However, only 57% of the participants determined that the water was clear when boiled with two fish and less than 15% with three or four fish. All participants detected that the water was coloured with five fish. A similar trend was observed with smell. Most (>80%) participants detected no smell from the water with either no or one fish. Increasing the number of fish, increased the noticeable smell. Likewise, increasing the number of fish affected perceptions of taste of iron in the water. The majority (>75%) reported no taste of iron with either no or one fish but that number fell to 20% or less with more than two fish.

The fourth test used in this series of experiments was

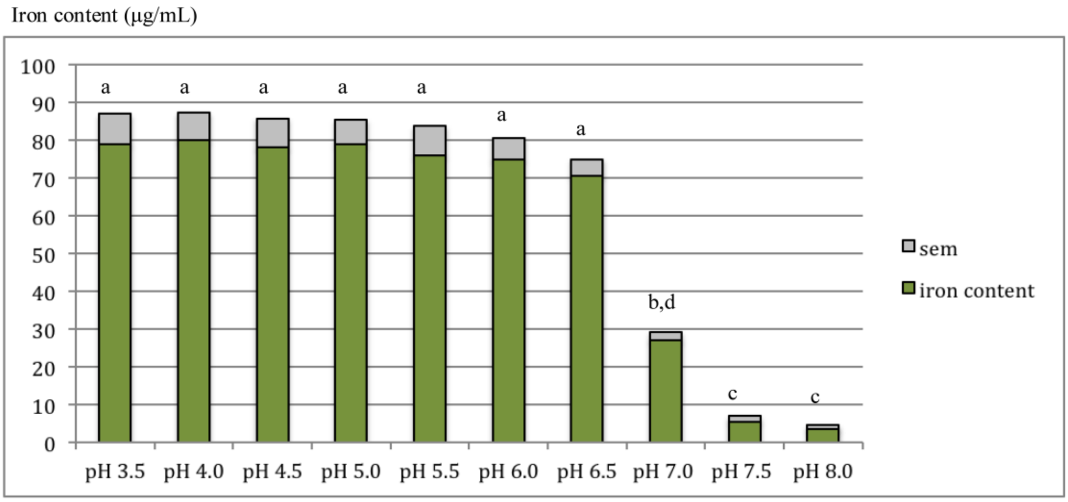

Figure 3. Iron released into distilled water boiled for 10 minutes with the *Lucky Iron Fish* at various levels of acidification. [a & c] indicates no significant difference (p>0.05) between iron levels. [c] indicates significant difference (p<0.001) from [a]. [d] indicates significant difference (p<0.01) from both [a & c]. sem: standard error of the mean.

Table 4. Iron content μg/mL (mean±SD) of food and water samples prepared with and without the fish

Treatment group	Cooking without the fish	Cooking with the fish
Distilled water (pH 7.4)	<0.35 (0)	<0.35 (0)
Saline (pH 4.5)	<0.35 (0)	<0.35 (0)
Lemon water (pH 4.7)	<0.35 (0)^{x}	72.4±16.5^{a,y}
Fish soup (pH 4.5)	<0.35 (0)^{x}	43.9±6.8^{b,y}
Pork soup (pH 4.4)	<0.35 (0)^{x}	48.1±9.4^{b,y}

[ab] Values within a column with different superscripts are significantly different (p<0.001).
[xy] Values within a row with different superscripts are significantly different (p<0.01).

designed to combine all the factors (colour, smell and taste) in a subjective assessment of the overall drinkability of the water that had been boiled with different numbers of fish. In this case, the women were simply asked to record whether the water was drinkable or not. More than 95% of the women reported that the water that had been boiled with no or one fish was drinkable. In contrast, less than 10% reported water was drinkable after it has been boiled with two or more fish: the remaining suggesting that the water was considered undrinkable.

DISCUSSION

Raw iron used to produce the fish and the manufactured fish was comprised primarily of ferrous iron with a small amount of ferric iron, and iron complexed with other minerals. This suggests that iron could be available for absorption if it is successfully leached during the boiling process.[18,22] No other significant contaminants, in any of the samples tested, were identified either before or after production, suggesting that the product is safe for distribution and use. Whilst the levels of potential contaminants were either undetectable or below the minimum acceptable standards for food contamination suggested by the WHO, it does not completely exclude the possibility that people who are vulnerable for a number of reasons could still be at risk. However, the slight risk posed by this fact is largely outweighed by the significant health benefits of using the fish on a regular basis.[18]

The fish released elemental iron in significant quantities in acidified boiling water but levels of other compounds that might be potentially dangerous to human health were either below the level of sensitivity of the assay (<0.0001 μg/g) or were lower than the minimal acceptable levels for potentially harmful contaminants set by the World Health Organization (<0.001 μg/g).[23] Similar to previous reports,[15,19,20] there was a significant difference between boiling fish in water at pH 7.5 (27.0±2.2 μg/g) compared with pH 6.5 (70.5±4.5 μg/g) confirming that acidification of the water was important to release sufficient amounts of iron. Although the levels of iron release in the liquid of the soup were less than boiling water alone, they were none-the-less significantly elevated 43.9±6.8 and 48.1±9.4 μg/g for fish and pork soup, respectively.

The daily iron requirements of individuals vary by gender, age, menstrual status and pregnancy.[22] Charles et al[19] suggested that by drinking one litre of acidified water prepared by boiling with the *Happy Fish* for at least 10 minutes would provide approximately 75% of the daily iron required for women of reproductive age. This estimate is based on the assumption that ~10% of total dietary iron is able to be absorbed[10,22] and that the daily required intake is 10 mg. While the daily-required intake for iron for women who are not pregnant, but are of reproductive age is generally accepted to be 10 mg, the daily requirement for women who are pregnant is estimated to be 18 mg/day.[22] Therefore, the *Lucky Iron Fish* is likely providing 40% of the daily-required intake of

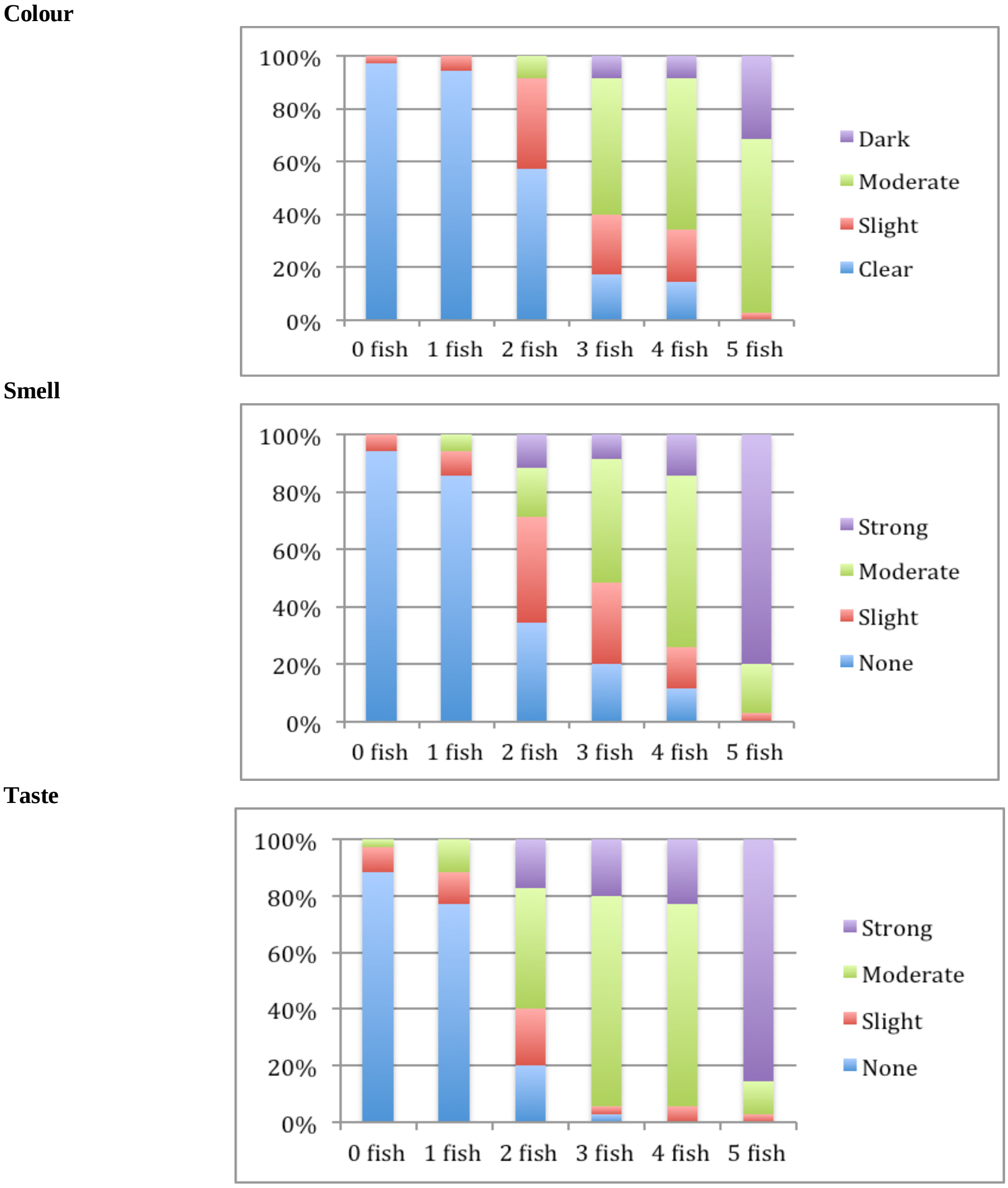

Figure 4. Perceived differences in colour, smell and taste of water boiled with fish Colour. Women (n=35) ranked colour, smell and taste on a four-point scale of samples of water boiled for 10 minutes with different numbers of fish. More than 80% of the participants could not detect changes in the water with one fish but that number decreased with two or more fish.

iron for pregnant women.

At the opposite end of the spectrum, it is possible that the fish could be providing too much iron and cause iron toxicity. Iron toxicity has been recognized as problem in some areas of sub-Saharan Africa.[24]

Leaching of iron from the fish was sensitive to the acidity of the boiling water but the levels of the other potentials contaminants did not appear to be so sensitive. Primarily this is due to the levels of a number of contaminants were below the level of sensitivity of the assays but iron is particularly sensitive to leaching under acid conditions. The experiments were carried out using deionized water and glass cooking to ensure no extraneous ions were introduced into the analysis from the equipment. The situation may be different in rural villages where the water consumed is normally drawn from the river or from wells and cooking is done primarily in aluminum pots.[11,15] There is a suggestion that tube well water in particular in Kandal province is contaminated with arsenic which may chelate available iron.[11] Finally, it would have been useful to measure the release of ferric and ferrous ion release to estimate the bioavailability of the iron but this was not done.

The current experiments demonstrate that *Lucky Iron Fish* releases most iron when cooked in water acidified with lemon juice. Although cooking the fish in soups was also associated with significant release of iron, the levels of release were lowered in soup compared with water. These findings are similar to other reports.[15,20]

It was important to carry out the tests on the drinkability of the water after boiling with different numbers of fish to establish whether or not increasing the number of fish during the cooking period affected the colour, taste, smell of the samples and then to assess the overall drinkability of the water. The women were aware of the possible treatment with iron but were blinded about the identity of the water samples. Most of the women clearly identified the control (no fish) or one fish as being without colour, smell or taste of iron and that these samples were drinkable. In contrast, cooking with more than one fish affected all three of these parameters and more than 90% of the women thought that the water cooked with two fish or more was undrinkable. These data suggest that including two or more iron fish in the pot when boiling water, which might risk iron overload, would affect the perception of colour, smell and taste of the water and that the women would be unlikely to drink such water. It is likely, but not shown by the current set of experiments, that similar results would be obtained by cooking soup (the staple diet of most Cambodian families) with two or more fish and therefore limit any possibility of iron toxicity. A drawback with the experimental design might be that women were asked to comment on appearance, smell and taste before they were asked about drinkability, which might influence their overall opinion. Nevertheless, they clearly identified samples boiled with more than one fish were different and less palatable.

Notwithstanding the observations on the impact on drinkability, prolonged iron intake at 60 mg ferrous iron per day results in side effects such as constipation, darkened stools, diarrhea, loss of appetite, nausea and stomach cramps[26] but these are the levels recommended for the treatment of severe anaemia (hemoglobin <70 mg/L) and far higher than the release of iron when cooking with five fish (120 ± 12.9 μg/g). Serious clinical signs (including death) vary with age and gender but generally intakes of more than 120 mg/day are considered toxic,[26] however, clinical signs (including death) vary with age and gender.

These findings confirm that cooking with a *Lucky Iron Fish* in acidified, distilled water releases sufficient iron to provide at least 40% and up to 75% of the daily iron requirement of a woman of reproductive age depending on her reproductive state. The levels of iron released from the *Lucky Iron Fish*™ are substantially lower than those provided in iron pills which are the conventional treatment for iron deficiency.[26] Moreover, the data imply that deviating from the recommended protocol of cooking with one *Lucky Iron Fish* for 10-30 minutes, will make the cooking medium unpalatable which would limit the prospect of iron overload.

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AUTHOR DISCLOSURES

None of the authors have any conflicts of interest to declare.

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