

Iron Release from Lucky Iron Fish® Across pH Levels and Acidification Treatments: Effects Relevant to Food Fortification

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Abstract Iron deficiency anemia (IDA) is a critical public health issue in populations living in resource-limited regions of the world. While iron-rich diets and iron supplementation can reverse IDA, settings characterized by economic hardship and limited access to nutritious foods may require more practical, sustainable approaches. The Lucky Iron Fish® (LIF), an iron ingot used in cooking, has been shown to improve iron status. Iron release from LIF is influenced by pH and boiling time, but the impact of different acidifying agents at varying pH levels remains unclear. Thus, optimum acidification and cooking methods to maximize iron enrichment of food have not been determined. This study evaluated the effects of water acidification treatments and pH levels on iron release from LIF and its ability to fortify black beans and white rice. Experiments, conducted in triplicate, used acetic acid, ascorbic acid, and lemon juice at pH levels 4.0, 4.5, 5.0, and 5.5. Foods were cooked with iron-fortified water using non-fortified tap water as a control. Total iron concentrations were measured for both aqueous and food samples. The results show aqueous iron concentrations significantly increased as pH decreased for all acid treatments, but only minor differences were found when acid types were compared. Using acetic acid (pH 4.5) as the acidification treatment, the iron content of both black beans and white rice doubled compared to the control. The iron content of black beans nearly tripled when pre-soaked in iron-fortified water prior to cooking in iron-fortified water. These results suggest that proper acidification and cooking preparation methods can enhance iron fortification of black beans and rice, both of which are staple foods in many regions of the world. Fortifying food with iron using the LIF provides a straightforward, affordable strategy that could have a significant global impact in preventing and treating IDA.

Keywords: Iron deficiency anemia, Lucky Iron Fish, Iron fortification of food

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1. Introduction

Iron deficiency anemia (IDA) remains one of the most widespread diet-related micronutrient deficiency disorders worldwide. This is particularly true in regions where poverty exacerbates the lack of access to food, healthcare, and effective interventions [1]. In children, IDA can lead to developmental delays and reduced learning capacity [2]. During pregnancy, IDA can increase the risk of premature birth and low birth weight [2]. The World Health Organization (WHO) has identified IDA as a global health priority, emphasizing the need for a comprehensive, multifaceted approach that addresses barriers related to screening and treatment [3]. While iron supplementation can effectively improve iron status, it is often a short-term solution to what is, in many areas of the world, a chronic

and endemic health issue.

One emerging approach for addressing IDA involves the use of the Lucky Iron Fish®, a fish-shaped ingot manufactured from electrolytic iron. The Lucky Iron Fish (LIF), which is reusable and lasts for up to five years, releases iron in a process comparable to cast iron cookware [4]. When boiled in acidified water, the LIF releases iron, which is then absorbed by food prepared using the iron-fortified water, thereby increasing the overall iron content of the meal. This approach is easily implemented in regions of the world where IDA is most prevalent and where access to affordable, nutritious food is limited.

Studies have shown that the regular use of LIF in meal preparation can significantly impact iron status across different age groups [5]. In rural Cambodia, for example, researchers reported a 46% improvement in the iron status of women after six months of regular LIF use [6,7]. Similarly, a year-long study in rural Guatemala found that

nearly 80% of participants showed significant improved hemoglobin and hematocrit levels, effectively reversing IDA when using the LIF [8]. A more recent study, also conducted in Guatemala, demonstrated that LIF can be used in large-scale meal preparation, such as school lunches [9]. After nine months, children with the lowest baseline iron levels showed significant improvements with daily LIF-fortified meals, while those with normal iron values showed no change. These studies demonstrate that regular LIF use contributes meaningfully to daily iron intake and helps improve iron status in vulnerable populations.

Two important factors influence iron release from LIF: pH and boiling time [10]. Using lemon juice for the purpose of acidification, researchers reported that boiling the LIF in acidified water ($\text{pH} \leq 6.5$) for 10 minutes significantly increased the iron content of the water to $70.5 \mu\text{g/mL}$ compared to controls ($<0.35 \mu\text{g/mL}$). The impact of acidification on iron solubility and subsequent bioavailability from LIF has also been demonstrated using an *in vitro* Caco-2 cell model [11]. Mean total iron concentrations in water (1 liter) acidified with ascorbic acid ($\text{pH} 2$) yielded 1.2 mM, resulting in greater iron uptake by intestinal cells (compared to controls). However, at $\text{pH} 7.0$, a 25% reduction in total iron concentration was noted, which coincided with reduced cellular iron uptake. Clearly, water acidification is an important variable when using the LIF for the purpose of food fortification.

Although lemons have been used extensively to acidify water, their availability can vary depending on region, season, and cost. Other organic acids may serve the same purpose, although their effectiveness depends on their dissociation constant (pK_a). Additionally, organic acids can form complexes with metal ions, including iron, which may influence iron release from the LIF. Therefore, factors beyond achieving a critical pH must be considered.

This study aimed to measure ferrous (Fe^{2+}) and total iron release from LIF in response to three organic acids (ascorbic acid, acetic acid, or fresh lemon juice) at varying pH levels ($\text{pH} 4.0, 4.5, 5.0$, and 5.5). Alongside investigating the effect of acidification treatments and pH on iron release from LIF, experiments were also conducted to determine how food preparation methods can optimize iron fortification in black beans and white rice. These findings have the potential to optimize iron fortification of food using LIF in meal preparation and improve iron status, especially in populations where IDA is prevalent. As food becomes increasingly scarcer in many parts of the world, this study can make an important contribution in global health efforts in both the treatment and prevention of IDA.

1.1. Objectives

1. Quantify the amount of organic acid needed to achieve water pH values ranging from 4.0 to 5.5
2. Measure iron released from LIF at four pH values
3. Measure iron released from LIF using three organic acids
4. Measure iron fortification of black beans and white rice using LIF

2. Materials and Methods

2.1. Organic Acids and Acidification

Although distilled water is considered the most chemically pure form of water, it does not contain enough ions for electrodes to register a consistent pH value. For this reason, tap water was used to assess the effect of treatments (organic acids and pH) on the release of iron from LIF. The iron content of the tap water (TW) was verified to be negligible using the analytical methods described below. For each acidification treatment, tap water (1 L) was acidified to pH values of 4.0, 4.5, 5.0, and 5.5. Acidifying agents included reagent grade ascorbic acid, reagent grade acetic acid, or fresh lemon juice. Fresh lemons were purchased the same day and at the same grocery store to minimize variability. The choice to use reagent grade ascorbic and acetic acids in this research study, rather than food grade, was to utilize well characterized reagents to demonstrate proof of concept of LIF. The weights (g) or volumes (mL) of each acidifier added to 1 liter of TW to achieve the specified pH values are listed in Table 1. Experiments were conducted in triplicate, for a total of 12 samples per organic acid tested. The initial pH of the TW was also measured, which served as the control. Aqueous pH was determined using a Thermo Scientific Orion Star 211 pH meter with a combination electrode calibrated against three certified buffer solutions (4.0, 7.0, and 10.0).

To ensure that any detectable changes in iron content were associated with the LIF and no other potential sources, lab glassware that had been acid-washed and thoroughly rinsed with distilled water was used during sample preparation. Each liter of acidified water was brought to a boil. Once the boiling point was reached, 1 LIF was added to each beaker and boiled for ten minutes. All LIF used in this study were from the same manufacturer's lot. The LIF were removed from the beakers of boiling water, the water was cooled to room temperature, a subsample transferred to polypropylene bottles (50 mL) and analyzed for ferrous (Fe^{2+}) and total iron. Chemical analysis methods are detailed below.

2.2. Food Preparation

Food preparation was completed in a certified kitchen, using glass cookware and wooden/plastic utensils. Two different food samples (black beans and white rice) were prepared with tap water that had been iron-fortified using LIF as described above. The acetic acid/pH 4.5 treatment was selected for the food preparation studies. Acetic acid was selected for food preparation because it is the primary component of vinegar, which is commonly available. Although pH 4.0 released the greatest amount of iron, previous work in our laboratory has shown that excessive iron can lead to undesirable palatability in food. For this reason, pH 4.5 was used to prepare the black beans and white rice. A second set of food samples was prepared with tap water, which served as the control. Food

preparation adhered to protocols described by the United States Department of Agriculture (USDA) Food and Nutrition Service (<https://fns.usda.gov>) for cooking black beans and white rice.

Dried black beans (33 g) were pre-soaked in water (tap water or iron-fortified water) for 8 hours and drained. The beans were then added to 165 mL of boiling water (tap water or iron-fortified water) and simmered for 60 minutes. Following cooking, the beans were drained, rinsed with tap water, cooled to room temperature, mashed, and frozen ($<-10^{\circ}\text{C}$) until chemical analysis. White rice (33 g) was added to 140 mL boiling water (tap water or iron-fortified water), covered, and simmered until the water was absorbed (approximately 20 minutes). Once cooled, the rice was homogenized and frozen ($<-10^{\circ}\text{C}$) until chemical analysis. Each food sample was prepared in triplicate. A summary of food preparation methods and iron treatments can be found in Table 2 and Table 3.

2.3. Total and Ferrous Iron Analysis

The ferrous iron content of aqueous samples associated with the organic acids and acidification study was determined by the phenanthroline colorimetric method (HACH, 2014) using a Thermo Scientific Genesys 10 UV-VIS spectrophotometer. Total iron content of both the aqueous and food samples was determined using concentrated nitric acid with an open vessel digestion system and a PerkinElmer 8300 ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer). Prior to analysis, the food samples were dried overnight at 50°C and homogenized using a ceramic mortar and pestle. Food samples were analyzed in triplicate and reported on a dry-weight basis. Analytical accuracy was documented using calibration verification standards and a certified reference material. Sample duplicates were used to document the precision of the analyses.

3. Statistical Analysis

Table 1. Ferrous and Total Iron Release from Lucky Iron Fish in Acidified Solutions (pH 4.0-5.5) with Ascorbic Acid, Lemon Juice and Acetic Acid. Means and Standard Deviations from Triplicates

Treatment	Acidifying Agent	Quantity Added	pH	Mean Ferrous Iron (mg/L)	Mean Total Iron (mg/L)
Control	Tap water	NA	7.2	<0.6	0.24
1	Ascorbic acid	1.2 g	4.0	27.32 ± 3.58	28.41 ± 2.93
2	Ascorbic acid	0.8 g	4.5	5.00 ± 0.45	10.42 ± 1.03
3	Ascorbic acid	0.6 g	5.0	$<0.6 \pm 0.00$	3.13 ± 0.87
4	Ascorbic acid	0.45 g	5.5	$<0.6 \pm 0.00$	0.60 ± 0.43
5	Lemon juice	8.0 mL	4.0	20.93 ± 6.25	33.81 ± 6.74
6	Lemon juice	6.0 mL	4.5	3.30 ± 4.57	18.91 ± 1.81
7	Lemon juice	4.5 mL	5.0	$<0.6 \pm 0.00$	10.30 ± 1.36
8	Lemon juice	2.5 mL	5.5	$<0.6 \pm 0.00$	4.72 ± 1.57
9	Acetic acid	18 mL	4.0	43.30 ± 2.47	47.34 ± 4.74
10	Acetic acid	9.0 mL	4.5	24.46 ± 1.93	24.23 ± 2.43
11	Acetic acid	5.0 mL	5.0	4.30 ± 1.45	7.31 ± 1.36
12	Acetic acid	3.5 mL	5.5	$<0.6 \pm 0.00$	1.32 ± 0.55

One-way ANOVA, $n=13$, $F_{4,69} = 58.567$, $p < 0.0001$

Descriptive data are presented as mean values and standard errors of the means (SEM). One-way analysis of variance (ANOVA) and pair-wise contrasts (t-tests) were used to compare the release of ferrous and total iron in response to all combinations of organic acids and pH. Two-way ANOVA followed by Fisher's Least Significant Difference test for pair-wise mean comparisons and pair-wise contrasts were used to determine the effect of food preparation methods on total iron content of black beans and rice, respectively. The R system was used for statistical computation, with significance set at $p < 0.05$.

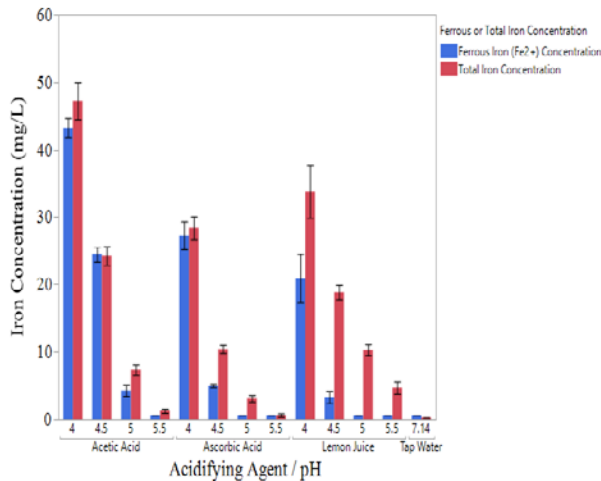
4. Results

4.1. Effect of Organic Acids and Acidification on Iron Release

Table 1 summarizes ferrous and total iron released (mg/L) from LIF at varying pH values. These values demonstrate that for each organic acid tested, iron release increased significantly as pH decreased, with all treatments yielding higher concentrations than the tap water control (One-way ANOVA, $n=13$, $F_{4,69} = 58.567$, $p < 0.0001$). The greatest concentration of ferrous and total iron was observed with Treatment 9 (acetic acid, pH 4.0) resulting in ferrous and total iron concentrations of 43.30 mg/L and 47.34 mg/L, respectively.

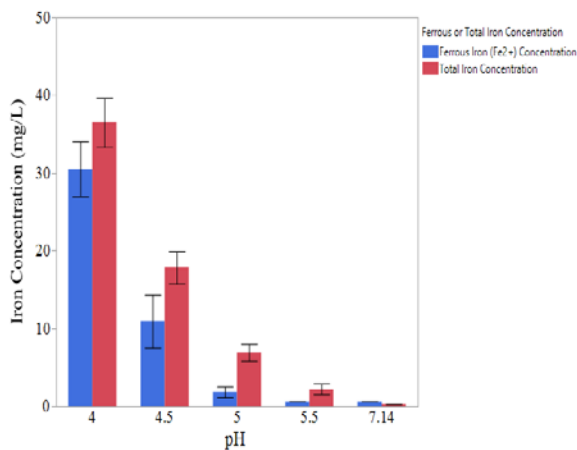
Figure 1 shows that both ferrous and total iron concentrations increase as pH decreases, regardless of the acidifying agent used. The two-way ANOVA indicates a significant interaction between pH and acid type ($F_{12, 61} = 2.65$, $***p < 0.0001$; $\text{SumSq}_{\text{pH} \times \text{acidifying agent}} = 8.79$, $**p < 0.001$) suggesting that while acidity is the primary driver of iron release, the specific acidifying agent also has a modest influence on the amount of iron release. Overall, the greatest iron release occurs at the lowest pH (pH 4.0) across all acid treatments.

Data pooled across all three acidifying agents to isolate the effect of pH on iron release is shown in Figure 2. A clear trend of decreasing iron concentrations with increasing pH, confirms that lower pH levels, with the strongest effect at pH 4.0 and 4.5, optimize iron release (LSD pH 4.5 = 1.95, $^{**}p = 0.02$; LSD pH 4.0 = 21.08, $^{***}p < 0.0001$). This trend again emphasizes that pH is the dominant factor influencing iron release, independent of the treatment used for acidification.



Two-way ANOVA, $F_{12, 61} = 2.65$, $^{***}p < 0.0001$; $\text{SumSq}_{\text{pH} \times \text{acidifying agent}} = 8.79$, $^{**}p < 0.001$.

Figure 1. Ferrous and total iron concentrations using three acidifying agents and four pH levels in the preparation of water with LIF. Data represent mean values of triplicate readings



LSD pH 4.5 = 1.95, $^{**}p = 0.02$; LSD pH 4.0 = 21.08, $^{***}p < 0.0001$

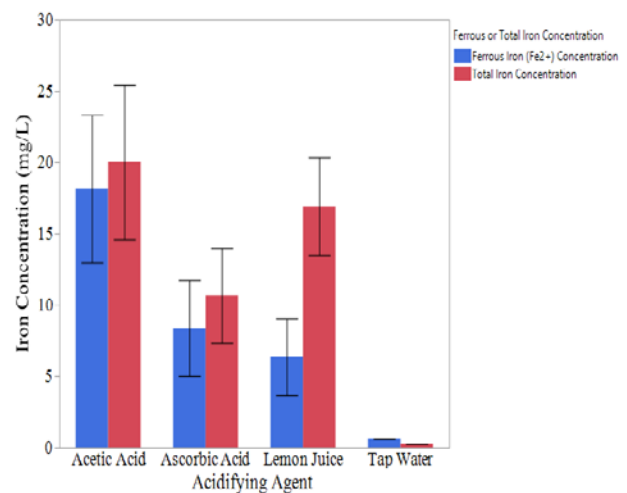
Figure 2. Ferrous and total iron concentrations at four pH values after treatment with LIF. Data are mean pooled across three acidifiers, across the three acidification agents

Pooled data (Figure 3) across all pH levels focus on differences between the three acidifying agents. Results show that while all acids enhance iron release compared to tap water, there were no statistically significant differences between acetic acid, ascorbic acid, and lemon juice. This reinforces the conclusion that the degree of

acidification is more important than the specific acidifying agent in maximizing iron release (One-Way ANOVA, $F_{3, 70} = 2.65$, $^{*}p = 0.05$; $\text{LSD}_{\text{AcA}} = -3.92$, $p < 0.11$).

4.2. Iron Concentration of Black Beans and White Rice

As reported in Table 2, black beans prepared with LIF-fortified water showed significantly higher total iron concentrations compared to the control (One-Way ANOVA, $n=13$, $F_{3,8} = 14.40$, $p = 0.001$; $\text{LSD}_{\text{Treatment3}} = 18.86$, $p = 0.001$). The greatest effect was observed when beans were both presoaked and cooked in LIF-fortified water (Treatment 3), which nearly tripled the iron concentration ($67.95 \pm 5.26 \mu\text{g}$) relative to the control ($30.09 \pm 13.70 \mu\text{g}$).



One-Way ANOVA, $F_{3, 70} = 2.65$, $^{*}p = 0.05$; $\text{LSD}_{\text{AcA}} = -3.92$, $p < 0.11$

Figure 3. Ferrous and total iron concentrations with three acidifiers in LIF-treated water. Data are means pooled across pH levels

Presoaking alone in LIF-fortified water (Treatment 1) also produced a meaningful increase ($49.28 \mu\text{g}$), while cooking alone in iron-fortified water (Treatment 2) resulted in a modest, non-significant increase ($33.93 \pm 4.61 \mu\text{g}$). Although there was no statistical significance detected in total iron concentration with Treatment 1 or Treatment 2 (iron-fortified water used for pre-soaking or cooking only), it is evident that these treatments did increase total iron uptake of the black beans compared to the control. Together, these findings indicate that maximum iron uptake occurs when beans are exposed to iron-fortified water during both soaking and cooking, highlighting the importance of integrating LIF into multiple stages of food preparation.

As was observed with the black beans, white rice prepared in water fortified with LIF exhibited significantly higher iron concentration compared to the control (Table 3; One-way t-test, $n=6$, $t=2.40$, $p = 0.06$). The LIF treatment resulted in a total iron concentration of $23.2 \mu\text{g/g}$, more than double that of the control ($9.9 \mu\text{g/g}$).

Table 2. Total Iron Content of Black Beans Prepared with LIF-Fortified Water Using Acetic Acid at pH 4.5. Total Iron Concentrations Summarized as Means and Standard Deviations from Triplicate Readings

Treatment	Food	Preparation	Total Iron (µg/g)
Control	Beans	Presoaked and cooked with tap water	30.09 ± 13.70
1	Beans	Presoaked in LIF-fortified water Cooked with tap water	49.28 ± 6.45
2	Beans	Presoaked in tap water Cooked with LIF-fortified water	33.93 ± 4.61
3	Beans	Presoaked in LIF-fortified water Cooked with LIF-fortified water	67.95 ± 5.26

One-Way ANOVA, $n=13$, $F_{3,8} = 14.40$, $**p = 0.001$; $LSD_{Treatment3} = 18.86$, $**p = 0.001$

Table 3. Total iron content of rice prepared with LIF-fortified water using acetic acid at pH 4.5. Total iron concentrations summarized as means and standard deviations from triplicate readings

Treatment	Food	Preparation	Total Iron (µg/g)
Control	Rice	Cooked in tap water	9.92 ± 1.26
1	Rice	Cooked in LIF-fortified water	23.19 ± 5.41

One-way t-test, $n=6$, $t=2.40$, $p = 0.06$

5. Discussion

The purpose of this study was to assess the impact of various acidification treatments and pH levels on iron release from LIF and to examine its effectiveness in fortifying black beans and white rice with iron. The experiments were designed to determine the extent to which different acidification treatments and varying pH levels impacted iron release. Table 1 provides mean comparisons across individual treatments, whereas Figures 1-3 use the same dataset to clarify which factor(s) (pH vs. acid type) explain most of the variation. Figure 1 confirms that both ferrous and total iron concentrations increase as pH decreases whereas Figure 2 demonstrates that acidification level is the dominant factor influencing iron release, independent of acidifying agents. Last, Figure 3 shows that differences among acids are not significant once pH is considered. Together, these analyses indicate that lower pH (≤ 4.5) strongly enhances iron release from LIF, while the specific acidifying agent plays a minor role.

Although not statistically significant, the magnitude of iron release did differ amount the acidification treatments. For example, at pH 4.0, iron values were 27.3, 20.9, and 43.3 (mg/L) for ascorbic acid, lemon juice, and acetic acid, respectively. A likely explanation for this lies in the tendency of organic acids to form strong organo-metallic complexes. The acidifiers used in this study are carboxylic acids which have the capability to adhere to solid surfaces and bind metals. The enhanced dissolution of metallic solids by organic acids has been reported previously and appears to play an important role in iron release from LIF [13,14]. To further explore this observation, a LIF was boiled in water acidified to pH 4 using a mineral acid (hydrochloric acid), which yielded no detectable iron release.

The greatest iron release occurred at $pH \leq 4.5$, regardless of the acidifying agent. These findings partly align with Armstrong et al., who reported an iron concentration of 7 mg/L in water acidified to pH 6.5 with lemon juice [10]. However, they observed no significant differences between pH values of 3.5 and 6.5, whereas our study demonstrated significant differences at pH 5.0, 4.5, and 4.0 across all treatments.

The exact reason for these discrepancies is unknown,

but it is worth noting that lemon juice is not a clearly defined and reproducible reagent. Not only do lemons contain varying amounts and multiple types of organic acids, differences in fruit maturity and variety may further influence acidification properties relevant to LIF use. This reasoning is supported by in vitro studies showing that LIF releases a substantial quantity of iron (about 1.2 mM) at pH 2 but at pH 7 iron was only slightly soluble and not taken up by cells [11].

Reported inconsistencies related to the efficacy of LIF in reversing IDA may in part be explained by poor acidification methods. For example, a randomized controlled trial reported that iron levels in Cambodian women with mild to moderate anemia did not improve in response to consuming food prepared with LIF [12]. The author attributed this finding to the possibility that IDA may have been caused by factors other than diet, such as a parasitic infection. Although analytical testing of acidifying agents during the distribution of LIF in communities with high prevalence of IDA is not feasible, the results of this study can serve as a guide for the necessary amounts of organic acids needed to achieve sufficient iron release. Consistent methodological approaches are essential to accurately and consistently assess LIF functionality, especially in clinical studies.

When organic acid data were pooled across treatments, iron release was consistently higher at lower pH values, especially at pH 4.5 and 4.0, underscoring the critical role of acidification in maximizing iron release. Although all three organic acids increased iron release, there were no statistically significant differences among them at equivalent pH values, which suggests that the degree of acidification is more influential than the specific acidifying agent. This is an important finding as it underscores a practical advantage that iron release from LIF relies more on achieving adequate acidification than on the specific acid used. This is especially critical in low-resource or culturally diverse settings, where the availability and affordability of acidifying agents differ.

Study results suggest that any accessible and culturally acceptable acid source—whether vinegar or vitamin C-rich fruits—can be used to acidify water without compromising iron release from LIF, provided the proper pH is achieved. This flexibility enhances the potential for sustainable and continual use across diverse food

preparation practices. For example, unpublished data by Beerman et al. found that small amounts of tamarind (5 g/L water) released 5.68 mg of iron following acidification to pH 4.5 and boiling for 10 minutes, a release comparable to organic acids tested in this study. Similarly, increasing the amount of tamarind used for water acidification (9g/L) lowered the pH value (4.0 ± 0.15) and increased iron release ($28.3 \text{ mg} \pm 16.39$) from LIF. Tamarind, a leguminous tree bearing fruit that grows abundantly in tropical and subtropical regions of the world, is commonly used in culinary practices, making it a culturally appropriate and a readily accessible acidifying agent for LIF.

This study not only confirms the efficacy of LIF in iron fortification of food, but also demonstrates that food preparation methods influence subsequent iron uptake from acidified, iron-fortified water. All LIF treatments increased the iron content of black beans, with soaking and cooking in iron-fortified water more than tripling iron levels compared to the control. Thus, the optimal preparation method is to both soak and then cook black beans in iron-fortified water, rather than using tap water for soaking followed by cooking in iron-fortified water. Similarly, rice prepared with iron-fortified, water resulted in twice the amount of iron compared to the rice control approaching significance. Collectively, these findings suggest that consuming meals composed of both black beans and white rice prepared with LIF can make a substantial contribution to meeting daily iron requirements.

Although there are known health concerns associated with an excess of dietary iron, no reported safety issues have been attributed to using the LIF in food preparation [6,7] [9]. In this study, the maximum total iron content in the black beans and white rice was 62 $\mu\text{g/g}$ and 23 $\mu\text{g/g}$, respectively. While excessive iron supplementation can lead to serious health issues in children, research suggests that dietary iron, consumed through regular meals, does not pose the same risk. Notably, in a study where children received daily school lunches prepared with LIF for nine months, those with healthy baseline iron levels maintained stable levels throughout the study. This finding demonstrates the safety of dietary iron fortification using LIF [9].

For those consuming primarily plant-based sources of dietary iron (nonheme iron), it has been estimated that only 5-12% of consumed iron is absorbed [14]. As this is typically the case in many rural, impoverished regions of the world, preparing food with LIF can substantially increase daily iron intake, but remain well below the Tolerable Upper Intake Level (children and adults 40 and 45 mg/d, respectively) as established by the Dietary Reference Intakes [15]. Although daily use of LIF does not totally satisfy the Recommended Dietary Allowance for iron [15], it can improve a low-iron diet when used on a regular basis, while not increasing risk of consuming amounts that could potentially cause harm.

Although effective, iron supplements often fail to foster long-term continued use, likely due to cost or side-effects. In contrast, meals prepared with iron-fortified water provide a dietary source of iron for the entire family, making LIF an affordable option that benefits all household members. Regular use of the LIF in food preparation can substantially and safely contribute to daily

iron intake, helping to reduce IDA at both individual and community level [16]. However, successful adoption and long-term use of LIF in meal preparation requires further study to ascertain factors that influence commitment. Successful strategies will depend on the nature of local acidification agents and understanding regional food customs and dietary practices that can facilitate the integration of LIF into local food preparation traditions.

5.1. Conclusion

This study provides practical guidance for adequate acidification to promote iron release from LIF. To maximize iron release from LIF, the pH of cooking water should be ≤ 4.5 , using an organic acid such as lemon juice, acetic acid, ascorbic acid, or other fruits rich in vitamin C. This study also demonstrates that cooking methodology influences the amount of iron incorporated into food. Best practices suggest boiling the LIF for 10 minutes in one liter of water acidified to $\text{pH} \leq 4.5$ and using that water for both soaking and cooking black beans or directly cooking white rice in iron-fortified water. For the LIF to be established as a sustainable, global strategy to reduce the prevalence of IDA, dissemination of culturally tailored food preparation guidelines is needed.

This study provided valuable insights that demonstrated important guidelines for using LIF to improve iron status in vulnerable populations. However, it is acknowledged that iron fortification of food was not tested for all acidification treatments at the varying pH values. Furthermore, iron fortification was tested in only a small variety of food – black beans and white rice. As such, studies are needed to test the effect of iron fortification of foods that represent a wider variety of culinary practices.

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