Rapid quantification of iron content in fish sauce and soy sauce: A promising tool for monitoring fortification programs

Arnaud Laillou, Christèle Icard-Vernière, Isabelle Rochette, Christian Picq, Jacques Berger, Pol Sambath, and Claire Mouquet-Rivier

Abstract

Background. *In a number of Southeast Asian countries and China, fish sauce and soy sauce produced at the industrial level are fortified with iron. Unfortunately, the food producers and regulatory agencies implementing fortification programs do not always have the capacity to monitor the programs on an ongoing basis.*

Objective. *To assess a new portable device for the quantitative measurement of iron content of fortified sauces that could be used to control fortification levels.*

Methods. *The linearity, detection limits, and inter- and intraassay variability of this device were assessed on fish sauce and soy sauce fortified with ferrous sulfate, ferrous fumarate, and sodium iron ethylenediaminetetraacetate (NaFeEDTA); the accuracy of the results was determined by comparing them with the results obtained by atomic absorption spectrophotometry.*

Results. *Measurements required a minimum incubation time of 1 hour for iron sulfate or iron fumarate and 24 hours for NaFeEDTA. Linearity of the results ranged from 2 to 10 mg iron/L for ferrous sulfate or ferrous fumarate and from 1 to 10 mg iron/L for NaFeEDTA, implying the need for proper dilution, as the iron contents of fortified sauce are usually in the range of 150 to 1,000 mg/L. Depending on incubation time, iron compounds, and sauces, the coefficient of variation (CV) of intraassay precision was between 1.5% and 7.6% and the CV of interassay precision was between 2.9% and 7.4%. Comparison with results from atomic absorption spectrophotometry showed high agreement between both methods, with* R = *0.926 and* R = *0.935 for incubation times of 1 hour and 24 hours, respectively. The Bland–Altman plots showed limits of agreement between the two methods of ± 70 mg/L in the range of fortification levels tested (100 to 500 mg/L).*

Conclusions. *This device offers a viable method for field monitoring of iron fortification of soy and fish sauces after incubation times of 1 hour for ferrous sulfate or ferrous fumarate and 24 hours for NaFeEDTA.*

Key words: Fish sauce, iron, monitoring, quality testing, rapid test kit, regulatory monitoring, soy sauce

Background

Fortification of staple foods and condiments with micronutrients has been used as a public health approach to reducing micronutrient deficiencies since the early 20th century [1] and has been shown to be cost-effective [2]. Condiments such as fish sauce and soy sauce are very commonly used in most Southeast Asian dishes, and therefore three countries (Vietnam, Cambodia, and China) in the region have decided to fortify those products with iron. There are important challenges in assessing micronutrient concentrations: current quantitative analysis methods are technically quite demanding, time-consuming, and expensive, and qualitative methods, such as the spot test for iron in wheat flour [3], do not measure the adequacy of fortification but merely provide information on the presence or absence of a fortificant. The limitations of this approach are demonstrated by the large discrepancy between coverage with fortified products and coverage with adequately fortified products that has been reported for several countries [4, 5]. To check the adequacy of fortification levels, simple-to-use quantitative methods that yield immediate and reliable results are needed at production sites for internal quality control, coverage surveys, and external and regulatory monitoring. In China and Vietnam, atomic absorption spectrophotometry has been used for the

Arnaud Laillou is affiliated with the Global Alliance for Improved Nutrition (GAIN), Geneva; Isabelle Rochette, Christian Picq, Jacques Berger, Christèle Icard-Vernière, and Claire Mouquet-Rivier, are affiliated with the Institut de Recherche pour le Développement, Montpellier, France; Pol Sambath is affiliated with the Reproductive and Child Health Alliance, Phnom Penh, Cambodia.

Please direct queries to the corresponding author: Arnaud Laillou, Global Alliance for Improved Nutrition (GAIN), P.O. Box 55, Geneva 1211, Switzerland; e-mail: alaillou@gainhealth.org.

determination of iron content in sauces [6, 7], but unfortunately no rapid quantitative test is available for ongoing monitoring at the production level to ensure adequate fortification. A simple, rapid, and efficient method of monitoring the content of iron in fortified fish or soy sauces would therefore be extremely useful. To meet this need, a small German enterprise, Bio-Analyt, has developed a portable device to simply and rapidly quantify iron concentration in fortified foods (iCheck-Iron, hereafter referred to as the "portable device"). The objective of the study reported here was to assess the ability and accuracy of the portable device for measuring iron contents in iron-fortified fish sauce and soy sauce using a four-step procedure: 1) determination of working and linear range, 2) intraassay precision, 3) day-to-day precision, and 4) person-to-person precision. Finally, measurements were performed on fish sauce and soy sauce fortified at two levels of iron and compared with results obtained by atomic absorption spectrophotometry, commonly regarded as the gold standard method for mineral analysis.

Materials and methods

Fortification of the fish sauce and soy sauce

In order to test the adequacy and accuracy of the portable device when used to measure iron levels in sauces, the Reproductive and Child Health Alliance (RACHA), which manages a national fortification program in Cambodia, collected samples of soy sauces and fish sauces from Cambodian manufacturers; 26 bottles of soy sauce were collected from three manufacturers and 20 bottles of fish sauce from four manufacturers. The intrinsic iron content of these sauces was measured by atomic absorption spectrophotometry; the results varied from 7.4 to 102.9 mg iron/L in soy sauces and from 0.8 to 40.7 mg iron/L in fish sauces. The iron compounds, ferrous sulfate ($FeSO_4$, H_2O), ferrous fumarate ($C_4H_2FeO_4$), and sodium iron ethylenediaminetetraacetate (NaFeEDTA) ($C_{10}H_{12}N_2O_8FeNa$, $3H_2O$), were provided by DSM, a chemical company certified by the Global Alliance for Improved Nutrition (GAIN), and added to the sauces in the laboratories of the Institut de Recherche pour le Développement (IRD). Stock solutions at 250 mg iron/L were prepared using each iron compound (ferrous sulfate, ferrous fumarate, and NaFeEDTA) by dissolving appropriate quantities of the compound either in ultrapure water (milli-Q) or in fish or soy sauces using volumetric flasks. This concentration (250 mg iron/L) was chosen as representative of those usually used in programs for the fortification of fish sauce and soy sauce. These stock solutions were then diluted according to the method described to reach concentrations in the portable device measurement range, i.e., 1 to 10 mg iron/L.

Portable device for measurement of iron concentration

The portable device consists of two units, the portable device (iCheck-Iron) and the disposable reagent vial (iEx IRON) in which the reaction is performed (**fig. 1**). The disposable reagent vials contain 2 mL of reagents, distributed in two phases, a water phase and an organic solvent phase; both are needed for completion of the reaction. Both the measuring unit and the reagent vials are commercially available (www.bioanalyt.com). The portable device determines the concentration of iron (intrinsic iron from the food matrix and extrinsic iron from fortification) in sauces by a photometric procedure. For quality control purposes, the device conducts an auto-control to verify that the emitter and receptor are working correctly.

During the study, the reagent vials and measuring units were stored at room temperature (20° to 30°C) prior to analysis. The recommendations and instructions of BioAnalyt were followed except with respect to the incubation times. For analysis, 0.4 mL of each properly diluted prepared solution was injected into the iEx IRON vial. To ensure that no air bubble was in the syringe, the lab technician released any air bubbles by holding the syringe upwards and flipping the syringe with his fingers. Once the injection was complete, the lab technician vigorously shook the iEx IRON vial up and down for 10 seconds and repeated this several times during the incubation period (0.3 hour, 1 hour,

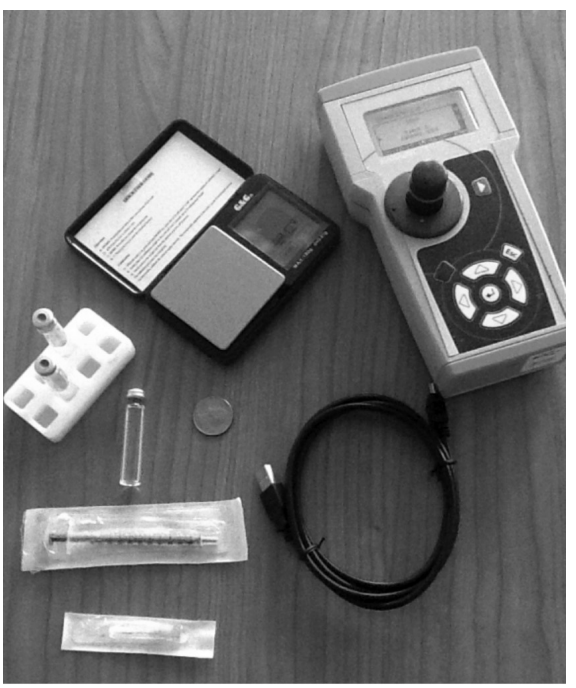

FIG. 1. Prototype of the portable device that was assessed as described in this paper. As an indication of size, a US 25-cent coin (quarter) has been placed in the picture

and 24 hours for ferrous sulfate and ferrous fumarate and 24 hours for NaFeEDTA).

Reference method

Measurement by the reference method, atomic absorption spectrophotometry, was performed using a Perkin-Elmer Atomic Analyst 800 (SpectrAA) with a deuterium background corrector. Iron was extracted with a closed-vessel microwave digestion system (ETHOS-1, Milestone, Italy) from about 1 mL of sample (stock solutions or sauces) in a 7:1 nitric acid/hydrogen peroxide mixture. The closed vessels were placed in a microwave oven and digested at 1,200 W power for 30 minutes. The elements were identified by air–acetylene flame. Standard reference materials, BCR-679 White Cabbage and BCR-191 Brown Bread (from IRMM, Institute for Reference Materials and Measurements, European Commission), were used as controls with iron SpectrAA measurements.

The coefficients of variation obtained with these two reference materials were 5.74% and 5.62%, respectively, with distances from the reference value of –1.24% in the case of white cabbage and –4.62% in the case of brown bread.

Procedure for assessment of the portable device

The procedure used to assess the performance of the portable device consisted of the three steps described below.

Linearity of the portable device

Assessment of a potential iron compound effect. Linearity was determined by measuring in triplicate five standard aqueous solutions with iron concentrations of 1, 2.5, 5, 7.5, and 10 mg iron/L (according to the portable device range); the five standard solutions were prepared for the three different iron compounds (ferrous sulfate, ferrous fumarate, and NaFeEDTA) by appropriate dilution of the aqueous stock solutions at 250 mg iron/L. The portable device measurements were carried out after 0.3 hour (20 minutes) of incubation for ferrous sulfate, 0.3 and 1 hour for ferrous fumarate, and 24 hours for NaFeEDTA.

Assessment of a potential food matrix effect. One fish sauce and one soy sauce were selected to test the effect of the food matrix. The concentration of intrinsic iron in these sauces was estimated by atomic absorption spectrophotometry. Each sauce fortified with 250 mg iron/L using one of the three iron compounds (ferrous sulfate, ferrous fumarate, or NaFeEDTA) was diluted to reach the expected extrinsic iron concentrations of 1.5 (instead of 1 mg/L due to the limit of detection), 2.5, 5, 7.5, and 10 mg iron/L. **Table 1** presents the final expected iron concentrations taking into account intrinsic and extrinsic iron from each food matrix. The portable device measurements were carried out after 0.3 hour, 1 hour, 17 hours, and/or 24 hours of incubation.

Variability of the measurements in laboratory conditions

To estimate intraassay, interassay, and interperson variation, the iron concentration of the diluted fish sauce (containing the three different iron compounds) and soy sauce (containing only NaFeEDTA) at 5.0 mg of extrinsic iron/L (corresponding to 5.50 mg/L in soy sauce and 5.56 mg/L in fish sauce) (**table 1**) was measured in triplicate by the same technician eight times on the same day (intraassay), by one technician on three different days (interassay), and by three different technicians on the same day (interperson), with different incubation times (1 hour and 24 hours). The mean and coefficient of variation (CV) of the results and the mean percentage of expected value were calculated.

Comparison of the portable device with the reference method

To compare the results obtained with the portable device with those obtained with the atomic absorption spectrophotometer (SpectrAA), four unfortified fish sauces and three unfortified soy sauces from Cambodia were fortified with ferrous fumarate, ferrous sulfate, and NaFeEDTA at 200 and 400 mg iron/L (the range of fortification levels used in Vietnam, Cambodia, and China) by adding 32 and 64 mg, respectively, in the case of ferrous fumarate and ferrous sulfate, and 80 and 160 mg, respectively, in the case of NaFeEDTA, to 50 mL of sauce (soy sauce only with NaFeEDTA). Then the sauce samples were diluted 50 times to reach iron concentrations in the linear range of the portable device between 2 and 10 mg iron/L.

TABLE 1. Expected iron concentration in iron-fortified soy and fish sauces (including intrinsic and extrinsic iron)

		Fortification level (mg iron/L)				
		1.5	2.5	5	7.5	10
Total iron concentration (mg iron/L)	Soy sauce	1.65	2.75	5.50	8.24	10.99
	Fish sauce	1.67	2.78	5.56	8.34	11.12

Statistical analysis

For the laboratory, standard protocols for measurement were followed, unless otherwise described. Data processing and statistics were conducted using Microsoft Excel 2007. The standard deviation (SD) and CV were used to assess the variability. When necessary, data were subjected to analysis of variance (ANOVA) and Fisher's LSD tests were used to compare means at the 5% significance level, using Statgraphics Plus, version 5.1, software. To compare the methods, besides plotting the two data sets and calculating the Pearson coefficient and the linear regression equation and paired Student's *t*-tests, the Bland–Altman plot was used [8]. This method consists of plotting the differences between results obtained by the new method (portable device) and the reference method (SpectrAA) against the mean of results obtained by both methods. The limits of agreement (LOA) were calculated using

$$\Delta - 2s = LOA_{low}$$

$$\Delta + 2s = LOA_{high}$$

where

- Δ is the mean of the difference between the two methods, and
- s is the SD of this difference.

Results

Linearity of the portable device

Assessment of a potential iron compound effect

The linear range of the portable device was assessed between 1.0 and 10.0 mg iron/L as recommended by BioAnalyt. Over this range, the coefficient of linear regression (R) and the regression equations for the different iron compounds were as follows:

Ferrous sulfate with 0.3 hour of incubation:
$R = 0.9987$ and $y = 1.0267x + 0.05$

Ferrous fumarate with 0.3 hour of incubation:
$R = 0.9660$ and $y = 0.6956x + 0.465$

Ferrous fumarate with 1 hour of incubation:
$R = 0.9941$ and $y = 0.8424x + 0.5663$

NaFeEDTA with 24 hours of incubation:
$R = 0.9974$ and $y = 0.8425x + 0.9018$

As shown in **figure 2,** for ferrous sulfate, with 0.3 hour of incubation, all the portable device values were very close to the expected values in the range of 2.5 to 10 mg iron/L, although slightly higher. For ferrous fumarate, with 0.3 hour of incubation, the portable device results were around 20% lower than expected in the range of 2.5 to 7.5 mg iron/L and 40% lower at

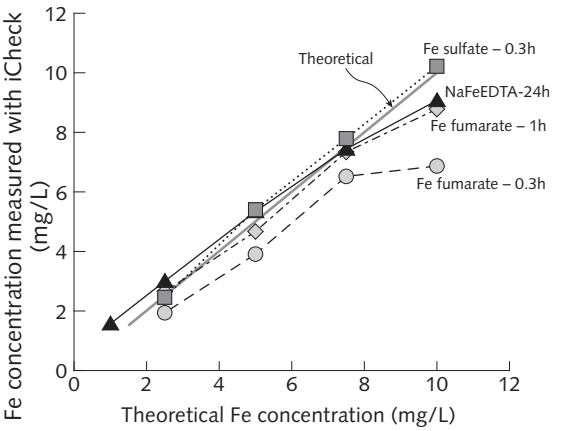

FIG. 2. Iron concentrations measured by iCheck-Iron in standard aqueous solutions of different iron fortificants compared with theoretical iron concentrations (calculated using the percentage of iron provided by the fortificant)

10 mg iron/L. If the incubation time was increased to 1 hour, the results were significantly improved and were close to the expected values in the range of 2.5 to 7.5 mg iron/L. For NaFeEDTA, all values obtained with the portable device after 0.3 and 1 hour of incubation were much below the expected values. After 24 hours of incubation, however, the portable device measurements were relatively good in the whole range of 1 to 10 mg iron/L; the results were slightly above expected values for the low concentrations and slightly below the expected values for the highest concentrations. The best results were obtained with the solution of iron at 5 mg/L. In general, with ferrous fumarate and NaFeEDTA, best results were obtained for the iron concentration of 5 mg iron/L, although at 10 mg iron/L, the results started to move away from the expected values, thus showing the upper limit of the portable device. These results show that the iron compound used for fortification has only a small impact on the measurements provided by the portable device iCheck-Iron. In all cases, the CV of the measurements was below 6.5%. At the lowest iron concentration tested (1 mg iron/L), the portable device was not capable of providing iron measurements. The effective range of measurement therefore appeared to be above 1 mg iron/L.

Assessment of a potential food matrix effect

For both sauces, at the lowest iron concentration tested (1.67 mg/L), the portable device was not capable of providing iron measurements in numerous cases, thus hindering the calculation of SD. From these experiments, the effective range of measurement of the portable device appeared to start around 2 mg iron/L. For NaFeEDTA, fish sauce and soy sauce showed similar results (**fig. 3A and A′**). It appeared clear that a minimum incubation time of 24 hours was needed, as the slope of the linear regression improved from 0.34 to

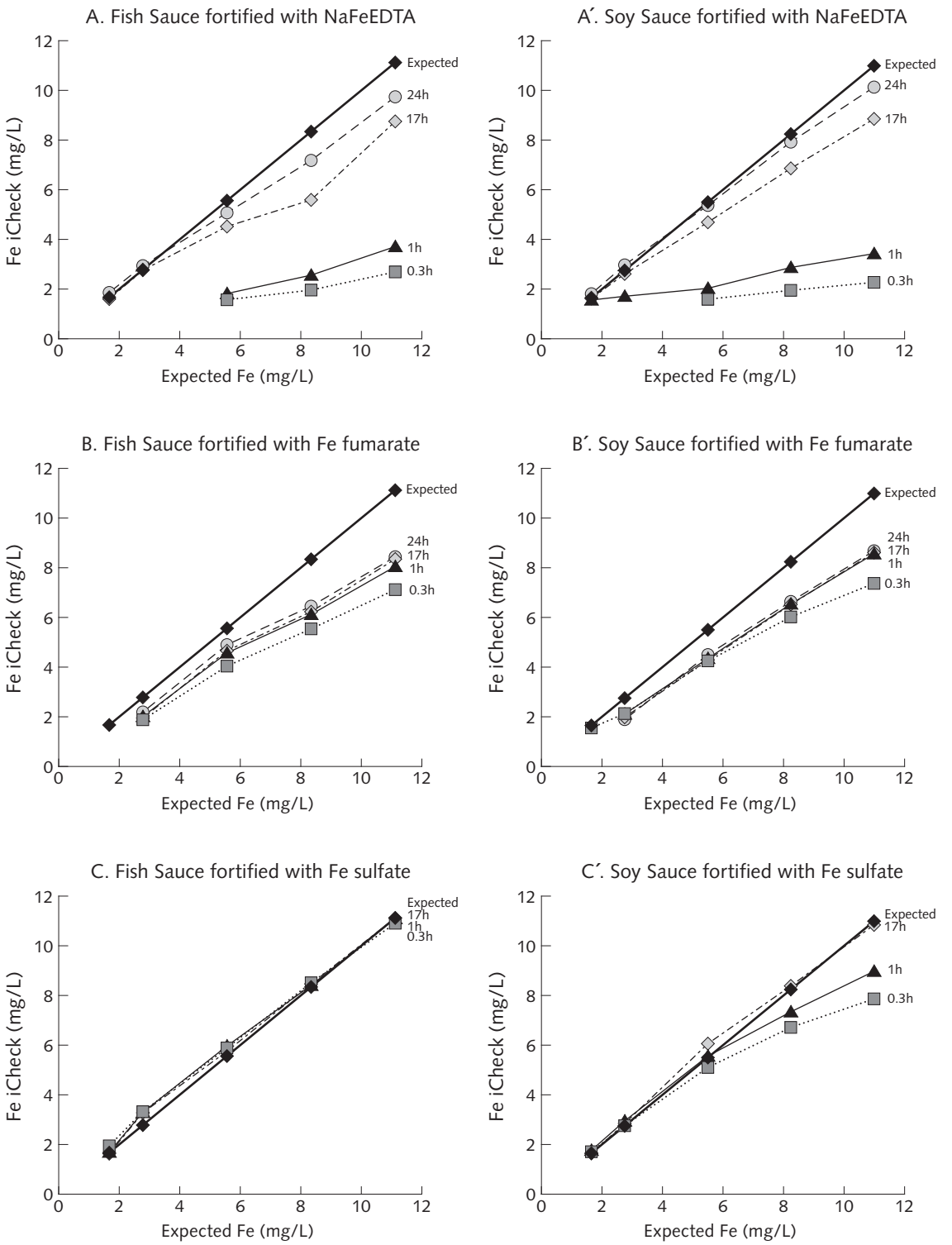

FIG. 3. Iron measurements with iCheck-Iron in fish and soy sauces fortified with NaFeEDTA (A and A´), ferrous fumarate (B and B´), or ferrous sulfate (C and C´) with different incubation times

0.82 in fish sauce and from 0.20 to 0.89 in soy sauce when the incubation time was increased from 1 hour to 24 hours. Therefore, an incubation time of 24 hours was used for any further measurement of sauce fortified with NaFeEDTA. For ferrous fumarate (**fig. 3B and B´**), except for the lowest iron concentration, similar results were obtained with both sauces. The gap between measured and expected values appeared to increase with iron concentration, which can be attributed to the poor solubility of ferrous fumarate. For ferrous sulfate, the results were different in soy and fish sauces, showing a food matrix effect. In fish sauce, iron concentrations measured with the portable device were close to the expected values independently of concentration and incubation time. Conversely, in soy sauce, the results showed an effect of incubation time for iron concentrations higher than 4 mg/L; the values were lower than expected for 0.3 and 1 hour of incubation. After 17 hours, in both types of sauce, the portable device values were very close to expected values, whatever the concentration (**fig. 3C and C´**).

Variability of the measurements in laboratory conditions

Intraassay precision

The means and CVs of the eight measurements conducted within 1 day by one technician on diluted sauces with total iron concentrations of 5.5 mg of iron/L for soy sauce and 5.56 mg of iron/L for fish sauce are presented in **table 2**. The CV varied from 1.5% to 7.6%, depending mainly on the iron compounds, and the mean of the measurements was relatively close to the expected concentration.

Interassay precision

The CV for the results of one person measuring fortified soy sauce with NaFeEDTA after 24 hours of incubation on three different days was 4.7%. For fish sauce fortified with ferrous sulfate, ferrous fumarate, and NaFeEDTA, the CVs were 4.3%, 2.9%, and 18.2%, respectively, after 1 hour of incubation and 6.0%, 2.7%, and 7.4%, respectively, after 24 hours of incubation. Performing the measurements on different days led to a small increase in the CVs. According to the ANOVA tests, in both types of sauce, whatever the iron compound, there was a slight difference between days. This shows that results obtained from iCheck-Iron may differ slightly from one day to another.

Interperson precision

There was no significant effect when different operators performed the measurement with the portable device, as long as they followed the measurement protocol rigorously, including strong agitation 10 minutes before reading the result ($p > .05$).

Comparison of the portable device with the reference methods

The results obtained with SpectrAA on fortified sauces at 250 mg iron/L were all very close to expected values and showed low variability, with CVs from 0.3% to 2.2%. The mean results obtained with the portable device on the different soy sauces (fortified with NaFeEDTA) and fish sauces (fortified with one of the three iron compounds) were plotted against those obtained with SpectrAA (**fig. 4**). In **figure 4A**, only the data obtained after 1 hour of incubation of fish sauces fortified with ferrous sulfate and ferrous fumarate were used. In **figure 4B**, all data obtained after 24 hours of incubation were used. Paired Student's *t*-tests performed on the differences between the results obtained with the two methods showed no significant difference between iCheck-Iron and SpectrAA ($p > .05$) after either a 1-hour or a 24-hour incubation. The equation of the correlation for 1 hour of incubation is $y = 0.989x + 5.112$ (with ferrous sulfate and ferrous fumarate), with y being the result obtained with the iCheck-Iron. The corresponding Pearson coefficient for the relationship is $R^2 = 0.874$, and the correlation coefficient is $R = 0.935$. The Bland–Altman plots were drawn to further

TABLE 2. Intraassay precision (mean, SD, and CV) of iron concentration measured with the iCheck-Iron device in fortified fish sauce and soy sauce[a]

Sauce and fortificant	Incubation time (h)	Expected conc. (mg/L)	Mean ± SD conc. (mg/L)	Recovery rate (%)		CV (%)
				Mean	Min–max	
Soy sauce		5.50				
NaFeEDTA	24		5.65 ± 0.23	103	99–112	4.1
Fish sauce		5.56				
Ferrous fumarate	1		4.90 ± 0.37	88	82–102	7.6
Ferrous fumarate	24		4.78 ± 0.34	86	80–98	7.1
Ferrous sulfate	1		5.84 ± 0.09	105	103–107	1.5
Ferrous sulfate	24		5.82 ± 0.15	105	102–109	2.6
NaFeEDTA	24		5.26 ± 0.25	95	89–101	4.8

a. Eight tests were performed within one day by one technician.

assess the agreement between the two methods (**fig. 5**). The mean of the differences between the two methods (the mean lines on **fig. 5**) corresponds to the bias. The Bland–Altman plot for 1 hour of incubation (**fig. 5A**) indicates that there are no data points outside the 2SD line. The limits of agreement are as follows: LOA_{low} is –63.2 mg/L and LOA_{high} is + 66.9 mg/L, the mean difference between the methods being + 1.8 mg/L.

For 24 hours of incubation, the equation of the correlation (with ferrous sulfate, ferrous fumarate, and NaFeEDTA) is $y = 0.8909x + 17.974$, with y being the result obtained with iCheck-Iron. The corresponding Pearson coefficient for the relationship is $R^2 = 0.8559$, and the correlation coefficient is $R = 0.926$. The Bland–Altman plot (**fig. 5B**) indicates that there are data points (two soy sauces) that are outside the 2SD line. The limits of agreement are as follows: LOA_{low} is –73.7 mg/L and LOA_{high} is + 68.9 mg/L, the mean difference between the methods being –2.3 mg/L.

When all iron fortificants are considered together, the mean values of the differences (+1.8 on **fig. 5A** and –2.4 on **fig. 5B**) are low compared with the order of magnitude of the studied parameter (the range of measurement was between 200 and 500 mg iron/L). This means that there is no significant bias with iCheck-Iron measurements compared with SpectrAA measurements.

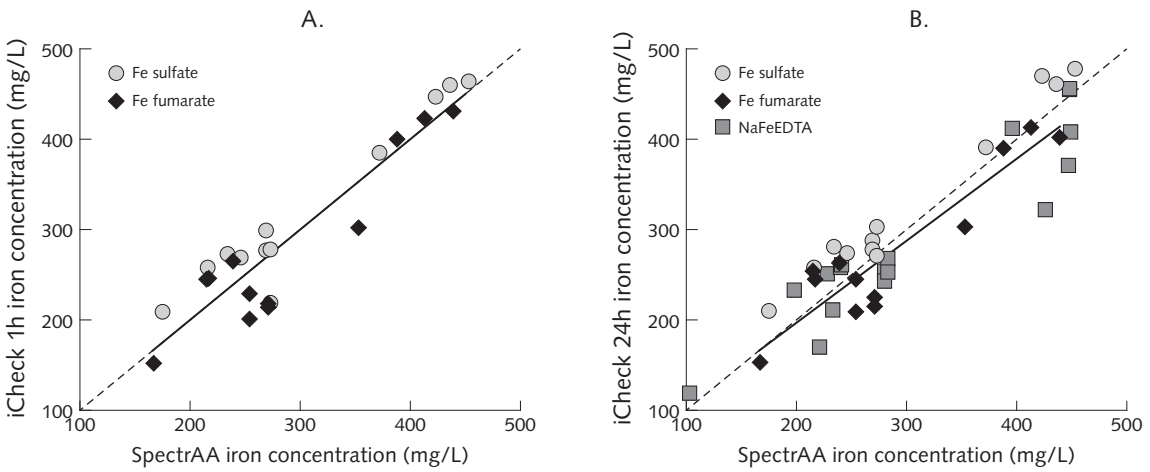

FIG. 4. Linear regression between results obtained by iCheck-Iron and SpectrAA conducted in the laboratory with 1 hour (A) or 24 hours (B) of incubation. The dashed line is the line of equality

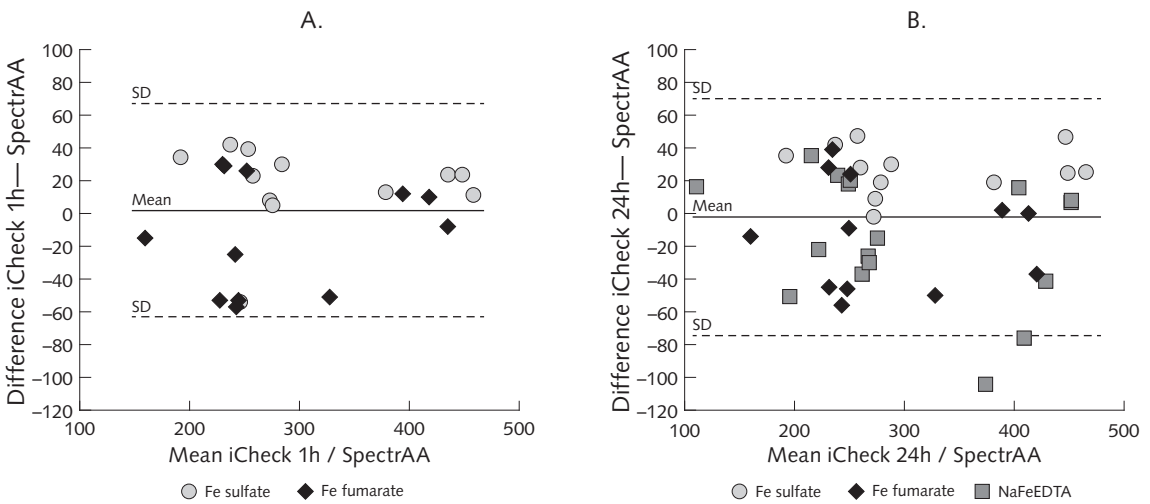

FIG. 5. Bland–Altman plots: difference versus average of values obtained by iCheck-Iron and SpectrAA conducted in the laboratory with 1 hour (A) or 24 hours (B) of incubation. The ± 1.96 SD lines represent the 95% limits of agreement

Discussion

Lack of a field-friendly tool to assess the iron level in sauces at the production site or at national borders is a common problem. In many Southeast Asian countries, the absence of ongoing monitoring of national programs that fortify soy and fish sauces with iron jeopardizes the impact of the program. The linear range of the portable device was determined to be from around 2.5 to 10 mg iron/L for ferrous sulfate and ferrous fumarate and starting from 1 mg iron/L with NaFeEDTA. However, the lower limit of linearity was not identified in this study. Within the tested range, after an incubation of fish sauce and soy sauce for 1 hour with ferrous sulfate and ferrous fumarate and 24 hours with NaFeEDTA, the device shows a high linearity. The longer incubation required for NaFeEDTA could be explained by the fact that the portable device is conceptualized to analyze only ferrous ions. NaFeEDTA contains only small quantities of ferrous ions, as the majority of its structure is composed of ferric ions. Thus the ferric ions have to be converted to ferrous ions in order to be measurable by this tool. This happens over time either due to the reagents in the vial or due to light reduction (photoreduction of NaFeEDTA in aqueous solutions) or through both.

According to guidelines recently published by the World Health Organization/Food and Agriculture Organization (WHO/FAO), the levels of iron currently added to sauces are 250 mg/L globally [9], 230 to 270 mg/L in China [10], around 100 mg/L in some trials in Cambodia [11], and 503 mg/L in Vietnam [6]. Thus, accurate dilution of the fortified sauces to get approximately 5 mg of iron/L (i.e., with a dilution factor of 20 to 50) is required in order for the portable device to perform consistent measurements. No significant interperson or intraassay variations were observed. Although we were not able to identify the reason for the small interassay variation (ambient temperature in the laboratory, different light exposure or something else, or chance), it is important to highlight that this day-to-day variability was relatively low and remained acceptable. With intraassay, interassay, and interperson variations below 11.3%, the precision can be considered satisfactory for field applications [12].

However, the portable device might have more limited application in a regulatory environment. Although there is a low mean difference (+ 1.8 mg iron/L with 1 hour of incubation and –2.4 mg iron/L with 24 hours of incubation) between the two methods (the portable device vs. reference method), and the limits of agreement calculated from Bland–Altman tests show that 97.5% of the results do not differ by more than ± 70 mg/L between the two methods, regardless of the iron concentration (within 100 and 500 mg/L), even this level of disparity calls into question the possibility of using this device to assess levels of fortification with enough accuracy for use by regulatory agencies for commercial monitoring. For this kind of monitoring, strict governmental enforcement of regulations and stiff penalties for noncompliance are needed, and therefore the more precise SpectrAA method is required. In addition, attention should be paid to the fact that with sauces fortified with ferrous sulfate, although they were very close to the line of equality, all values (except one point after 1 hour of incubation) were above the line of equality, which means that the portable device values are always above SpectrAA values with this iron fortificant (which corresponds to an overestimation). This could be explained by a specific coloration development due to ferrous sulfate use. This is consistent with the fact that with aqueous iron sulfate solutions, the portable device values were slightly above expected values.

Even if it is not adequate to regulatory requirements, the device could be used for internal monitoring, quality control and quality assurance (QC/QA) practices conducted by producers, importers, and/or packers. In this environment, where results are needed quickly (so that corrective actions can be implemented promptly), quality control procedures demand fast and simple analytical assays [9]. These assays do not necessarily require high analytical resolution [9] (i.e., be able to discriminate between small concentration ranges), but they must be able to determine whether fortification standards are being met. This portable device could play an important role in this context in countries that are fortifying their sauces with iron.

A limitation of the portable device to date is that the actual instruction guide does not adequately reflect the incubation times required (at least 1 hour for ferrous sulfate and ferrous fumarate but at least 24 hours for NaFeEDTA). In the recent instruction guide (available on the internet), the detection limit has been increased from 1 to 1.5 mg iron/L. It is necessary that an independent laboratory test these new guidelines for ferrous sulfate and ferrous fumarate, since our study did not test the linearity range starting at 1.5 mg/L. After appropriate dilution, the iron concentration must be in the linear range of 2 to 10 mg iron/L, otherwise the "out of range" message will appear. It is important to recognize that appropriate dilution is time-consuming. In addition, a small food matrix effect was shown, and it might be that different food matrices would lead to stronger differences in the responses of the portable device. Finally, even if the hazardous reagents are reduced with this device, once the vials have been used they still must be handled by a company specialized in chemical waste due to their solvent composition; unfortunately, such facilities are not available in most developing countries.

Conclusions

Agencies or producers implementing fortification of sauces are requesting support for ongoing monitoring. The iCheck-Iron device offers a viable solution for ongoing internal monitoring but cannot replace the use of an atomic absorption spectrophotometer for commercial monitoring implemented by government agencies. Further research and use in the field is needed to extend the validation using a wider number of fish sauce and soy sauce brands, as the food matrix seems to have some effect on the variability of the results.

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