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Performance of iron spot test with Arabic bread made from fortified white wheat flour

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Abstract

Background.—The iron spot test (IST) is a simple qualitative technique for determining the presence of added iron in fortified flour. IST performance in bread has never been investigated. If found to perform well, the IST has the potential to provide a field-friendly method for testing bread and thus support the monitoring and evaluation of flour fortification programs.

Objective.—To assess the performance of the IST in Arabic bread made from white wheat flour.

Methods.—*Bread samples were collected from 1,737 households during a national micronutrient survey in Jordan. A subsample of Arabic bread (*n *= 44) was systematically selected for testing by both the IST and spectrophotometry (criterion reference). Performance measures (sensitivity, specificity, and positive and negative predictive values) were calculated using five cutoffs to define the presence of added iron, including ≥ 15.0 ppm (approximate level of natural iron in Arabic bread) and four additional cutoffs based on test performance.*

Results.—The iron contents of samples testing negative by IST ranged from 10.4 to 18.4 ppm, with one outlier at 41.0 ppm, which was excluded from subsequent analyses. The iron contents of

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samples testing positive by IST ranged from 16.1 to 38.4 ppm. With the exception of negative predictive values for the two lowest cutoffs (≥ 15.0 and ≥ 16.1 ppm), all performance measures exceeded 83.3%.

Conclusions.—These results show promise for the IST as an inexpensive, field-friendly method for testing bread that could have a useful role in the monitoring and evaluation process for flour fortification programs.

Keywords

Flour; fortification; iron; micronutrients

Introduction

Iron deficiency is the most common and widespread nutritional disorder, affecting approximately two billion people worldwide [1]. Iron deficiency is one of the primary causes of anemia and leads to impaired work performance, developmental delay, cognitive impairment, and adverse pregnancy outcomes [2]. Fortification of wheat flour with iron is a cost-effective strategy with the potential to reduce iron deficiency [3]. The cost of fortification is often passed on to consumers as a modest increase of approximately 0.06 to 0.15 international dollars (I$) per person per year [4]. Wheat flour fortification is currently mandatory in 68 countries [5]. To be effective, programs must be properly implemented and adhere to fortification guidelines. To ensure success, flour fortification programs require careful monitoring and evaluation, including market- and household-level assessment of the provision, utilization, and coverage of end products of fortified flour, including bread [6, 7]. In order to determine if fortified bread is reaching the target population, each of these market- and household-level assessments involves testing bread for fortification. Spectrophotometry, a method of quantifying the concentration of iron in flour or bread, is a validated method of testing bread for fortification. However, this method is expensive and time-consuming, and requires trained staff and specialized equipment in a laboratory setting. Thus, a field-friendly and inexpensive method of testing for the presence of extrinsic, or added, iron in bread would be useful for monitoring purposes.

The iron spot test (IST) is a qualitative technique for detecting the presence or absence of added iron in flour. The IST can be used with all types of cereal flours and is not affected by the extraction rate of wheat flours. With the IST, the appearance of red spots after the application of a solution indicates the presence of added iron. With the exception of sodium ferric ethylenediaminetetraacetate (NaFeEDTA), the IST detects all forms of iron currently used in fortification programs [8]. Because of its simplicity and low cost, the IST is commonly used at mills for quality control to ensure that iron, and thus the micronutrient premix, has been added to flour. Although the application of the simple and inexpensive IST for testing the presence or absence of added iron in baked bread would facilitate important market- and household-level monitoring and evaluation of flour fortification programs, the performance of the IST in bread has not yet been assessed.

A national micronutrient survey conducted in the Hashemite Kingdom of Jordan in 2010 provided an opportunity to assess the performance of the IST in Arabic bread made from *mowahad* wheat flour (73% to 78% extraction). In accordance with World Health Organization (WHO) recommendations [9], the government mandated that *mowahad* flour be fortified with 30 parts per million (ppm) of dried ferrous sulfate (iron). The objective of this investigation was to assess the performance of the IST in Arabic bread consumed in Jordanian households, as compared with spectrophotometry.

Methods

A total of 1,992 Jordanian households from 166 nationally representative clusters were invited to participate in a 2010 micronutrient survey coordinated by the Jordan Ministry of Health to assess the impact of Jordan's flour fortification program. Samples of the most commonly consumed bread were collected from 1,737 households (87.2% of invited households) [10]. Approximately 200 g of bread, the amount in one large Arabic loaf, was collected from each participating household. A subsample of all collected bread samples (n = 50) was systematically selected for spectrophotometric testing from the first household that provided a sufficient amount of bread (≥ 200 g) in every third cluster (for 50 clusters). Each bread sample was divided in half. One half was tested for the presence or absence of iron by the IST, and the other half was tested by spectrophotometry for the quantification of iron in the bread. For the purpose of calculating sensitivity, specificity, and predictive values, the spectrophotometric analysis was the criterion reference [11]. Because of variations in the intrinsic iron content of the flour, only Arabic bread samples made from white wheat flour were included in the present analysis (n = 44). The samples not analyzed include *tabon* (n = 2) and *mashrooh* (n = 3) (other types of flat bread) and one unknown type.

The Central Public Health Laboratory in Jordan conducted the IST following a modified version of the AACC International IST for wheat flour (AACC International, Method 40–40, 1999; modification: Nicolas Tsikhlakis, Modern Mills, Amman, Jordan, 2002) [12]. Five drops of a pre-prepared solution of 10% potassium thiocyanate and 2 M HCl was added directly to the inner portion of the bread sample with a dropper. Afterwards five drops of 3% hydrogen peroxide was added, and the sample was left to stand for 1 to 2 minutes. A positive IST result was recorded if red spots indicating the presence of added iron appeared on the surface of the bread.

The Royal Scientific Society of Jordan tested the bread samples using the AACC International Method 40–70: Elements by atomic absorption spectrophotometry for the quantification of iron content in cereals and cereal products [13]. This method quantifies both added and natural iron content by reaction with ortho-phenanthroline and spectrophotometric measurement. The laboratory technicians conducting the IST and the spectrophotometric analyses were blinded to the results of the other laboratory test.

It was confirmed that a level of ≥ 15.0 ppm to define bread with added iron was appropriate for use in the present analysis by conducting duplicate spectrophotometric measures of an unfortified bread sample, which yielded results of 15.0 and 15.2 ppm of iron. These results are consistent with other investigations that have found that Arabic bread made from 72% to 78% extraction white wheat flour contains approximately 15.0 ppm of natural iron [14]. Thus, using ≥ 15.0 ppm, the percentage of samples with added iron based on spectrophotometric testing was calculated, in addition to the percentage of samples that tested positive by the IST. A dot plot of spectrophotometric results against IST results was constructed using Stata 10.1 SE. Sensitivity, specificity, and positive and negative predictive values of the IST were calculated for a cutoff of 15.0 ppm and for four additional cutoffs based on test performance: the level at which the IST yielded 100% sensitivity, the level at which the IST yielded 100% specificity, the level halfway between the first two levels, and the level at which the percentage of samples considered positive by the IST equaled the percentage positive by spectrophotometry. Performance measure calculations and two-sided 95% confidence intervals for a single proportion were calculated in Open Epi (http://openepi.com/OE2.3/Menu/OpenEpiMenu.htm). The Mid-P Exact method for a single proportion was used to calculate confidence intervals [15].

Results

Figure 1 shows a dot plot of spectrophotometric results against IST results. Samples testing positive by IST ranged from 16.1 ppm to 38.4 ppm iron by spectrophotometry, while samples testing negative by IST ranged from 10.4 to 18.4 ppm iron by spectrophotometry, with the exception of one outlier (41.0 ppm). Subsequent analyses were performed without this outlier.

For the 43 remaining bread samples selected for this investigation, spectrophotometric testing yielded a range of 10.4 to 38.4 ppm iron (mean, 23.6; SD, 7.4; median, 23.7; interquartile range, 17.0 – 28.9); 86.0% of bread samples ($n = 37$) had ≥ 15.0 ppm iron and thus were considered to have added iron. The IST detected 74.4% positive samples ($n = 32$) and 25.6% negative samples ($n = 11$).

Table 1 shows performance measures for five different cutoff levels: ≥ 15.0 ppm, the approximate level of natural iron in Arabic bread; ≥ 18.5 ppm, the level at which the IST yielded a sensitivity of 100%; ≥ 16.1 ppm, the level at which the IST yielded a specificity of 100%; ≥ 17.3 ppm, the level halfway between 18.5 ppm (100% sensitivity cutoff) and 16.1 ppm (100% specificity cutoff); and ≥ 17.1 ppm, the level at which the percentage of positive samples by IST equaled the percentage of positive samples by spectrophotometry (74.4%). With the exception of negative predictive value for cutoffs of ≥ 15.0 ppm (54.5%) and ≥ 16.1 ppm (72.7%), all performance measures were above 83.3%. The relationship of the test results in comparison to each of the cutoff levels is depicted in figure 1.

Discussion

The performance measures in this analysis show promise for the IST as a qualitative means for determining the presence of added iron in Arabic bread made from white wheat flour. Our analysis looked at performance measures at a cutoff of 15.0 ppm (expected levels of naturally occurring iron in Arabic bread, as published in the literature) in addition to four cutoffs based on the performance of the IST against spectrophotometric testing. Consistently with expected levels of naturally occurring iron [14], the cutoffs based on test performance ranged from 16.1 to 18.5 ppm. These findings suggest the possibility that Arabic bread in Jordan generally contains higher levels of natural iron. This observation may explain the low negative predictive value observed at the two lowest cutoffs, ≥ 15.0 and ≥ 16.1 ppm. Since natural iron levels vary in wheat flour and thus in wheat flour bread, it is possible that some unfortified bread samples may have contained ≥ 15.0 ppm of natural iron and were thus classified as false negatives, leading to the low negative predictive values observed for the two lowest cutoffs (≥ 15.0 and ≥ 16.1 ppm).

Although the overall performance measures were promising, the outlying sample, whose iron content was measured as 41.0 ppm by spectrophotometry but which tested negative by IST, highlights the importance of equal dispersion of fortification particles in bread. This bread sample was retested to confirm the initial test results. Retesting yielded the appearance of a single red spot on two different tested portions of the bread. In the event that a single red spot was observed upon initial testing, the modified IST protocol called for another portion of the bread to be tested. If the testers observed at least one red spot on the second portion of bread, the sample was considered positive, as was the case in the retesting of the outlying sample. In this event, the testers concluded that the fortificant (i.e., added iron) was present, though poorly dispersed throughout the bread. The inconsistent results for this sample call attention to the potential limitations in the performance of the IST for samples with poorly dispersed fortificant.

The performance of the IST can be likened to that of the iodine rapid test kit for determining the presence of iodine in salt. Iodine rapid test kits are used widely in field settings and for household surveys because of their ability to provide immediate results and their valuable educational role for consumers at the point of use [16]. With the advantages provided by an inexpensive procedure (amounting to the minimal cost of the solutions, approximately US$1 per sample plus laboratory staff costs) that yields immediate results in a field setting, both the IST and the iodine rapid test kit provide ideal alternatives to the time-consuming titration and time-consuming and expensive (approximately US$70 per sample) spectrophotometry, which require trained staff and specialized equipment. However, the decreased performance observed when multiple individuals performed the iodine rapid test emphasizes the importance of training [17]. Furthermore, just as the iodine rapid test kits do not give a reliable estimate of iodine content [18], the IST cannot be used as a quantitative indicator of iron content in bread.

When interpreting these results, it is also important to consider that predictive values, particularly the positive predictive value, are affected by the percentage of bread that is fortified (contains added iron). In this investigation, 86% of the 43 Arabic bread samples included in this analysis had ≥ 15.0 ppm iron and were considered to be fortified. It is expected that the positive predictive value would decrease and the negative predictive value would increase in settings with a lower percentage of fortified bread. Another point to be taken into consideration is that this analysis only involved Arabic bread made from white wheat flour, which has no additional ingredients containing iron (eggs, nuts, other flours, etc.). The performance may vary with different types of bread baked under different conditions with different ingredients. Testing the IST on various bread types, including breads with variations in intrinsic and added iron, would provide more generalizable results.

The limitations of this investigation include the small sample size and the fact that the IST was conducted in a laboratory setting rather than in the field, where the benefit of the application of the IST on bread is optimized. Additionally, the criterion reference method used (spectrophotometry) could not distinguish between added and natural iron in bread and thus was unable to account for variation in natural iron contents in bread. Finally, the type of bread assessed in this study is most commonly consumed in the Middle East and North Africa (MENA) region; as mentioned above, performance may vary by bread type.

To our knowledge, there are no published studies assessing the performance of the IST for testing bread. In the present assessment, the IST performed acceptably as a general indicator of the presence or absence of added iron in Arabic bread made from white wheat flour. This type of field-friendly test may support market- and household-level monitoring and evaluation efforts required to ensure the successful provision, utilization, and coverage of end products of fortified flour, including bread. Although additional testing is needed for other bread types, these results show promise for the IST as an inexpensive, field-friendly method for testing bread that could have a useful role in the monitoring and evaluation process for fortification programs.

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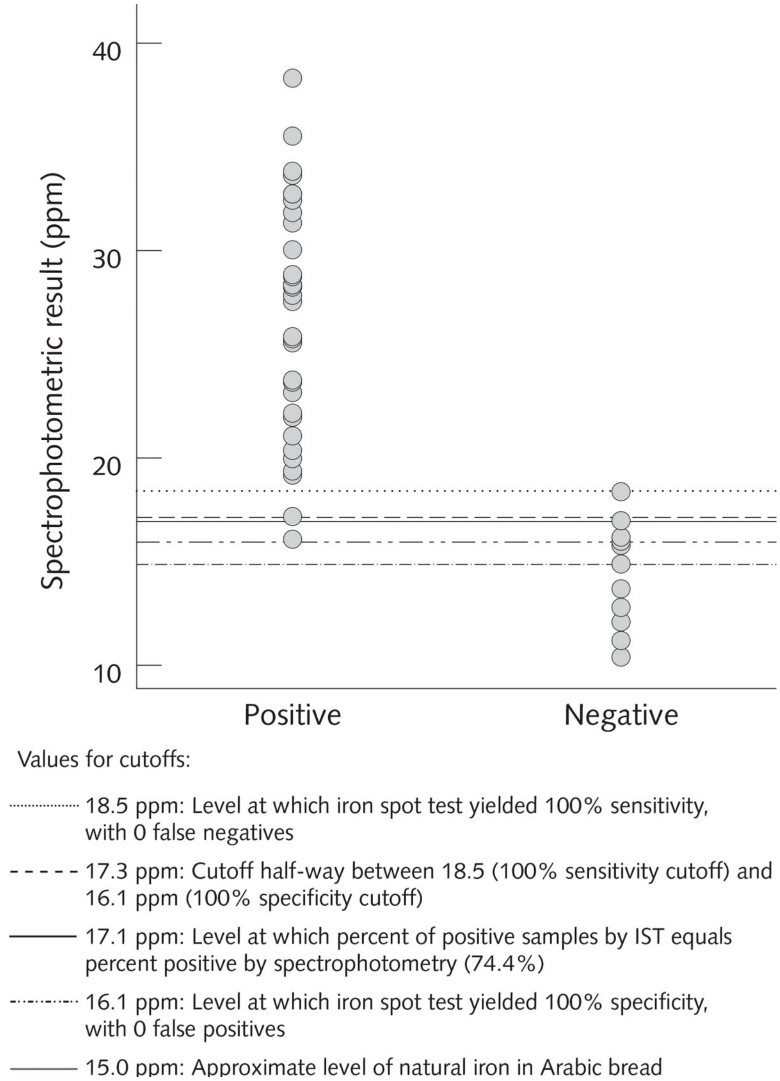

FIG. 1.

Dot plot of spectrophotometric results by iron spot test (IST) results for presence of added iron in Arabic bread, Jordan, 2010.

TABLE 1.

Performance measures at various cutoffs for the iron spot test (IST) compared with the spectrophotometric method for detecting iron in samples of Arabic bread ($n = 43$), Jordan, 2010

Characteristic	Cutoff (ppm)[a]	Performance measure — % (95% CI)			
		Sensitivity	Specificity	Positive predictive value	Negative predictive value
Level of natural iron in Arabic bread	≥ 15.0[b]	86.5 (72.6–94.9)	100.0 (60.7–100.0)	100.0 (91.1–100.0)	54.5 (25.9–81.0)
100% sensitivity for IST	≥ 18.5	100.0 (90.5–100.0)	84.6 (57.8–97.3)	93.8 (80.9–98.9)	100.0 (76.2–100.0)
100% specificity for IST	≥ 16.1	91.4 (78.4–97.8)	100.0 (68.8–100.0)	100.0 (91.1–100.0)	72.7 (42.2–92.6)
Halfway between 18.5 and 16.1 ppm	≥ 17.3	96.8 (85.1–99.8)	83.3 (54.9–97.1)	93.8 (80.9–98.9)	90.9 (62.7–99.6)
% positive by IST = % positive by spectrophotometry	≥ 17.1	96.9 (85.5–99.8)	90.9 (62.7–99.6)	96.9 (85.5–99.8)	90.9 (62.7–99.6)

[a.] Cutoff is defined as the concentration, in ppm, at which bread samples were considered positive for added iron.

[b.] Quail [14].