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(draft document for discussion)

**REPORT ON RESEARCH FINDINGS:**  
**FIELD TEST METHODS FOR THE DETERMINATION**  
**OF IRON IN FORTIFIED FOODS**

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## **RESEARCH FINDINGS:**

### **FIELD TEST METHODS FOR THE DETERMINATION OF IRON IN FORTIFIED FOODS**

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## RESEARCH FINDINGS:

### FIELD TEST METHODS FOR THE DETERMINATION OF IRON IN FORTIFIED FOODS

#### 1. INTRODUCTION AND PURPOSE

(brief description of the rationale for the development of rapid tests for iron in fortified foods, potential users, utility of information generated)

#### 2. METHODS

(very brief description)

#### 3. FINDINGS

##### 3.1 review of techniques of iron analysis

- (i) **phenanthroline method for ferrous iron** Ferrous ion ( $\text{Fe}^{2+}$ ) is brought into solution and treated at pH 3.2-3.3 with 1,10 phenanthroline. Three molecules of phenanthroline chelate each atom of ferrous ion to form an orange-red complex. The coloured solution obeys Beer's law; its intensity is independent of pH from 3 to 9. Ferric ion does not react. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of phenanthroline.
- (ii) **phenanthroline method for total iron** Both ferrous and ferric ions are brought into solution, and the ferric form is reduced to the ferrous state by boiling with acid and hydroxylamine. The solution is then treated with 1.10 phenanthroline at pH 3.2-3.3 as above.  
  
4,7 diphenyl 1,10 phenanthroline (bathophenanthroline) may also be considered as a reagent since it has a higher colour absorbance, but is unfortunately much more expensive.
- (iii) **ferrozine method for total iron** The sample is digested with nitric acid and hydrochloric acid, and all forms of iron are converted to the ferric form. The ferrozine reagent [3-(2-Pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine, monosodium salt, monohydrate] forms a purple coloured complex with ferric ion, and the colour is proportional to the iron content.
- (iv) **thiocyanate method for total iron** The iron content of the sample is oxidized to the ferric state. Thiocyanate is then reacted with the ferric ions forming to form an intense red complex. The colour fades rapidly especially in sunlight, due to the self-oxidation of thiocyanate and the subsequent reduction of the ferric ions to form a colourless complex. The fading is slower at low pH.
- (v) **TPTZ method for total iron** TPTZ (2,4,6-tripyridyl-s-triazine) reacts with ferrous ions to yield an intense violet colour over pH range 3.4-5.8. The indicator is combined with a reducing agent which converts precipitated or suspended iron to the ferrous state. The

ferrous iron test and the concentration of total iron.

(vi) **Other colourimetric reactions which may be considered**

- ferric ion      Deep blue colour with potassium ferrocyanide; violet colour with salicylic acid; or green colour with potassium ferricyanide;
- ferrous ion      Blue colour with potassium ferricyanide

(vii) **Other approaches**      We reviewed several other approaches to the analysis of iron based on gravimetric methods (AOAC), ion-selective electrodes for ferrous and ferric ions, fluorescence detection methods and HPLC techniques. However, we do not believe that these methods are adaptable to field testing, and they are not described in detail in this report.

### 3.2 methods for a field test for iron in salt

On theoretical and practical grounds we have selected, and tested the o-phenanthroline method as the basis of a rapid field testing system for iron in salt.

Initially, we tried to base the test on the iodate field test kit, where a reagent is directly applied to salt, and a colour is developed on the surface of the salt. Unfortunately the ortho-phenanthroline method is too sensitive, and coloured complexes formed with iron concentrations of 5 to 20  $\mu\text{g/g}$  are best distinguished visually. Using this approach, the test would be carried out as described in box X

**results of laboratory testing**      Initial tests in our laboratory produced reproducible results that readily distinguished different levels of iron in the salt in the range of 700 to 1300  $\mu\text{g/g}$ . Photographs of a series of tests are presented in Figure 4.

**alternative test for iron: results of laboratory testing**      Details of an alternative test method for iron are shown in box . Photographs of a series of tests are presented in Figure 5. Although the differences in colour are not so clearly distinguishable as in the solid phase dilution, with some practice or training iron concentrations can be determined to  $\pm 100 \mu\text{g/g}$  in the salt.

### 3.3 recommendations for further work

We have performed some preliminary tests for the measurement of total iron (ferrous and ferric) in salt by reducing the ferric ion to ferrous form with hydroxylamine solution and hydrochloric acid without applying water-bath and heat. Our results have shown that the reduction of the ferric ion to ferrous ion in salt matrix is quite easily achieved. However, further recovery data will be necessary to establish the range and reproducibility of this method under field environment.

*(to be developed)*

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## BOX 1. FIELD TEST FOR IRON IN SALT

### O-PHENANTHROLINE METHOD

1. **principle** Ferrous ion ( $\text{Fe}^{2+}$ ) in the salt reacts with 1,10 phenanthroline in an appropriate reaction mixture. Three molecules of phenanthroline chelate each atom of ferrous ion to form an orange-red complex. Ferric ion does not react. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of phenanthroline. There are no known interferences with salt or its common impurities.
2. **field test apparatus**
  - a. metal pan (8 cm o.d.)
  - b. 25 mL plastic flask containing the prepared reaction mixture
  - c. colour standard chart ; 500-1500 ppm iron in salt
  - d. 500 mL plastic sample container
  - e. sampling cap (to hold approximately 2 g salt))
3. **reagents**
  - a. **1,10 phenanthroline solution:** weigh 200 mg 1,10 phenanthroline monohydrate (ACS grade) in a 100 mL volumetric flask. Add 40 mL distilled water and 2 drops of concentrated hydrochloric acid. Stir and dilute to volume with distilled water. (Note: one mL of this reagent is sufficient for no more than 60 microgram iron)
  - b. **ammonium acetate buffer solution** in a 150 mL Erlenmeyer flask dissolve 25 gram ammonium-acetate (ACS grade) in 15 mL distilled water. Add 70 mL concentrated (glacial) acetic acid (ACS grade)
  - c. **reaction mixture:** Mix the above reagents in the following proportion: 2 volume 1,10 Phenanthroline solution and 1 volume Ammonium acetate buffer solution. Mix thoroughly and dispense into 25 mL plastic dropper flasks.
  - d. salt sample fortified with ferrous form of iron
  - e. iron free salt
4. **procedure**
  - a. Transfer a measuring cap ( 2 grams) of salt sample fortified with ferrous form of iron into a 500 mL plastic sample container containing 198 g iron free salt (1 to 100 "solid phase dilution" ) Wet the mixture with 3-4 drops of distilled water. Close the cap and shake and mix the content thoroughly approximately 15 minutes (the wetting helps to evenly distribute the fortified salt). Transfer a small sample of the salt mixture into a metal pan.
  - b. Apply several drops (10-20) of reagent solution to the surface of the salt.
  - c. Compare the developed orange colour of phenanthroline-ferrous complex colour with the similarly prepared colour standards and determine the iron concentration of the salt sample.

## BOX X. ALTERNATIVE FIELD TEST FOR IRON IN SALT

1. **principle:** Ferrous ion ( $\text{Fe}^{2+}$ ) in the salt is reacted with 1,10 phenanthroline in an appropriate reaction mixture. Three molecules of phenanthroline chelate each atom of ferrous ion to form an orange-red complex. Ferric ion does not react. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of phenanthroline. In a field situation, the large amount of iodine-free salt required would restrict the usefulness of the test. A liquid state dilution step is likely more accurate, and water should be available locally, especially, as the test would not likely require water of high purity, although iron would, of course interfere.

The salt must be present in a dilute form since the test is linear only in the 0 to 5  $\mu\text{g}/\text{mL}$  iron concentration range. A large sample is not feasible, since the phenanthroline concentration must be approximately 30 times higher than that of the ferrous ion concentration for optimum colour development. (56 milligram iron (atomic weight 55.85) requires 9 molecules (1783.8 mg) of phenanthroline monohydrate. One gram 1000 ppm iron -fortified salt contains 1 mg ferrous ion which would require 24 mL reagent solution.)

2. **field test apparatus**

- a. sampling cap (to hold approximately 5 g salt)
- b. 25 mL plastic flask containing the prepared reaction mixture
- c. colour standard chart ; 500-1500 ppm iron in salt
- d. 25 mL flat bottom tube (e.g. Nessler tube)
- d. 500 mL volumetric flask
- e. 1 mL and 5 mL pipettes (preferably Eppendorf or similar)

3. **reagents**

- a. **1,10 Phenanthroline solution:** weigh 200 mg 1,10 phenanthroline monohydrate (ACS grade) in a 100 mL volumetric flask. Add 40 mL distilled water and 2 drops of concentrated hydrochloric acid. Stir and dilute to volume with distilled water. (Note: one mL of this reagent is sufficient for no more than 60 microgram iron)
- b. **ammonium acetate buffer solution** in a 150 mL Erlenmeyer flask dissolve 25 gram ammonium-acetate (ACS grade) in 15 mL distilled water. Add 70 mL concentrated (glacial) acetic acid (ACS grade)
- c. **reaction mixture:** Mix the above reagents in the following proportion: 2 volume 1,10 Phenanthroline solution and 1 volume Ammonium acetate buffer solution. Mix thoroughly and dispense into 25 mL plastic dropper flasks.

4. **procedure**

- a. Transfer a measuring cap ( 5 grams) of salt sample fortified with ferrous form of iron into a 500 mL volumetric flask and dilute to volume with water. This should result in a solution containing 10 microgram ferrous ion per milliliter at a fortification level of 1000 ppm.
- b. Transfer 1 mL of the prepared solution into a 25 mL flat bottom tube (e.g. Nessler tube) and add 5 mL of reagent solution and dilute to mark with distilled water. Compare the orange colour of phenanthroline-ferrous complex with standard and determine the ferrous iron concentration of the salt sample.

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**Figure 1. Field test for iron based on o-phenantrolene .  
Liquid-phase dilution Salt with 700 to 1300 ppm  $\text{Fe}_2\text{SO}_4$  added**

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Figure 2. Field test for iron based on o-phenantrolene  
Liquid-phase dilution - Salt with 700 to 1300 ppm  $\text{Fe}_2\text{SO}_4$  added

## 5. DETAILS OF METHODOLOGIES FOR MEASURING IRON

### PHENANTHROLINE METHOD FOR FERROUS IRON

Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER -  
17th edition 1989

To determine ferrous iron, acidify aqueous sample with 2 mL concentrated hydrochloric acid to prevent oxidation. Withdraw a 50 mL portion of the acidified sample into a 100 mL volumetric flask and add 20 mL phenanthroline solution and 10 mL ammonium acetate solution (buffer) with vigorous stirring. Dilute to volume and measure the colour intensity within 5 min.

### PHENANTHROLINE METHOD FOR TOTAL IRON

Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER -  
17th edition 1989

Measure 50 mL of the aqueous sample containing ferrous and ferric ion into a 125 mL Erlenmeyer flask. Add 2 mL concentrated hydrochloric acid and 1 mL hydroxylamine solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until the volume is reduced to 15 to 20 mL. Cool to room temperature and transfer to a 100 mL volumetric flask or Nessler tube. Add 10 mL ammonium acetate buffer solution and 2 mL phenanthroline solution and dilute to the mark with distilled water. Mix thoroughly and allow at least 10 min. for maximum colour development

### FERROZINE METHOD FOR TOTAL IRON

Procedure for aqueous samples: HACH WATER ANALYSIS HANDBOOK 1992 Hach Co. Loveland,  
Colorado U.S.A.

The procedure is based on a commercial portable kit including a spectrophotometer: Collect samples in acid washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2.0 or less with nitric acid (about 2 mL per liter). Before testing digest samples in nitric/hydrochloric acid, then adjust the sample pH to 3 to 5 with ammonium hydroxide, ACS. Adjust wavelength of spectrophotometer to 562 nm. Fill a sample cell to the 25 mL mark with sample. Add the contents of one Ferrozine iron reagent pillow to the cell (the prepared sample). Swirl to mix. Let stand for five minutes. Read the display in mg/L Fe ferrozine.

### THIOCYANATE METHOD FOR TOTAL IRON

Procedure: E.B. Sandell. - COLOURIMETRIC DETERMINATION OF TRACES OF METALS. Interscience  
Publishers, Inc., N.Y. 1959

To a 30 mL of sample add 20 mL of 0.4 N hydrochloric acid and heat in water bath just below the boiling point for 20 minutes. Cool to room temperature and add a drop of 0.5 per cent potassium permanganate or enough to maintain a colour in the solution for one minute. Add 5.0 mL of potassium thiocyanate solution (30 gram in 100 mL) and compare the colour at once with a suitable series of standards. If less than 0.4 ppm of iron is present use 50 mL Nessler tubes for the comparison.

### TPTZ METHOD FOR TOTAL IRON

Procedure: HACH WATER ANALYSIS HANDBOOK, Hach Co. Loveland, Colorado U.S.A. 1992

The procedure is based on a commercial portable kit including a spectrophotometer:  
Collect samples in acid washed glass or plastic bottles. To preserve samples, adjust the sample pH to

2.0 or less with nitric acid (about 2 mL per liter). Adjust the sample pH to 2 or less with nitric acid (about 2 mL/L). Before testing, adjust the pH of the stored sample to between 3 to 4 with 5.0 N sodium hydroxide solution. Do not exceed pH 5 as iron may precipitate. Rotate the wavelength dial to 590 nm. Fill a sample cell to the 25 mL mark with sample. Add the contents of one TPTZ iron reagent powder pillow (which also contains the reducing agent) to the cell (the prepared sample). Cap and shake for 30 seconds. Compare reading with blank Read the display in mg/L total iron.

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