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chapter

Iron Determination by Ferrozine Method

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S.S. Nielsen, *Food Analysis Laboratory Manual*, Food Science Text Series,
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18.1 INTRODUCTION

18.1.1 Background

Chromogens are chemicals that react with compounds of interest and form colored products that can be quantified using spectroscopy. Several chromogens that selectively react with minerals are available. In this lab ferrozine is used to measure ferrous iron in an ashed food sample. The relationship between the absorbance of the chromogen-mineral complex is described by Beer's Law. In this procedure, a standard curve is generated with a stock iron solution to quantify the mineral in beef samples.

In this experiment, meat samples are first ashed to dissociate the iron bound to proteins, and the ash residue is solubilized in dilute HCl. The acid is necessary to keep the mineral in solution. Ferrozine complexes only with ferrous iron and not with ferric iron. Prior to the reaction with ferrozine, the solubilized ash is first treated with ascorbic acid to reduce iron to the ferrous form. This step is necessary with ashed samples, as this procedure would be expected to oxidize all the iron present in the meat. However, when other treatments are used to liberate iron, for example, trichloroacetic acid precipitation, comparison of samples treated with ascorbic acid and untreated samples could be done to determine the ratio of ferrous to ferric iron in foods.

18.1.2 Reading Assignment

Ward, R.E., and Legako, J.F. 2017. Traditional methods for mineral analysis. Ch. 21, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

18.1.3 Objective

Determine the iron content of food samples using the ferrozine method.

18.1.4 Principle of Method

Ferrous iron in extracts or ashed samples reacts with ferrozine reagent to form a stable colored product which is measured spectrophotometrically at 562 nm. Iron is quantified by converting absorbance to concentration using a standard curve.

18.1.5 Chemicals

	CAS no.	*Hazard(s)*
3-(2-Pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,-triazine (ferrozine; Sigma P-9762)	69898-45-9	
Ascorbic acid (Sigma 255564)	50-81-7	
Ammonium acetate (Aldrich 372331)	631-61-8	
Iron stock solution (e.g., 200 ppm) (Aldrich 372331)	4200-4205	

18.1.6 Reagents

- Ferrozine reagent, 1 m*M* in water
- Dissolve 0.493 g ferrozine reagent in water and dilute to 1 l in a volumetric flask.
- Ascorbic acid; 0.02% in 0.2*N* HCl, made fresh daily
- Ammonium acetate, 30% w/v
- Iron stock solution (10 µg iron/mL/0.1 N HCl)
- Solutions of 1.0*N*, 0.1*N*, and 0.2*N* HCl

18.1.7 Hazards, Precautions, and Waste Disposal

Adhere to normal laboratory safety procedures. Wear safety glasses at all times! Waste may be put down the drain using water rinse.

18.1.8 Supplies

- 16 Test tubes, 18 × 150 mm
- Meat sample
- Pipettes
- Porcelain crucible
- Volumetric flask

18.1.9 Equipment

- Analytical balance
- Hot plate
- Muffle furnace
- Spectrophotometer

18.2 PROCEDURE

(Instructions are given for analysis in duplicate.)

18.2.1 Ashing

1. In duplicate, place a ~5 g sample into the crucible and weigh accurately.
2. Heat on the hot plate until the sample is well charred and has stopped smoking.
3. Ash in muffle furnace at ca 550 °C until the ash is white.

18.2.2 Iron Measurement

1. Prepare standards of 10, 8, 6, 4, 2, and 0 µg iron/mL from a stock solution of 10 µg iron/mL. Make dilutions using ca 0.1*N* HCl.
2. Dissolve ash in small amount of 1*N* HCl, and dilute to 50 mL in volumetric flask with 0.1*N* HCl.
3. In duplicate, put 0.500 mL of appropriately diluted samples and standards into 10 mL test tubes.
4. Add 1.250 mL ascorbic acid (0.02% in 0.2*N* HCl, made fresh daily). Vortex and let set for 10 min.
5. Add 2.000 mL 30% ammonium acetate. Vortex (pH needs to be >3 for color development).
6. Add 1.250 mL ferrozine (1 m*M* in water). Vortex and let set in dark for 15 min.

7. Use water to zero the spectrophotometer at 562 nm (single-beam instrument) or place in the reference position (dual-beam instrument). Take two readings (repeated measures, msmt) for each tube at 562 nm.

18.3 DATA AND CALCULATIONS

Weight of original samples:
(1)_____g (2)_____g

Absorbance of standards and samples:

	Absorbance		
Standards (µg iron/ml)	*Rep 1*	*Rep 2*	Mean
0			
2			
4			
6			
8			
10			
Meat sample			
1			
2			

Calculation of total iron in sample:

1. Plot absorbance of standards on the y-axis versus µg iron/mL on the x-axis.
2. Calculate the iron concentration in the ash solution from the standard curve: (abs – y intercept)/slope = µg iron/mL ash solution.
3. Calculate iron in the sample using measured iron value from standard curve and meat sample:

C_{sample} = (µg iron/mL ash solution) x (50 mL ash solution/g meat) = µg iron/g meat

18.4 QUESTION

1. How else could iron be determined using the ash digest? What would be the advantages and disadvantages of the ferrozine method versus the other method you identified?

RESOURCE MATERIALS

Ward RE and Legako, JF (2017) Traditional methods for mineral analysis. Ch. 21. In: Nielsen SS (ed) Food analysis, 5th edn. Springer, New York