IRON SPOT TEST

Training Program on Wheat Flour Fortification –Chandigarh
December 6, 2010
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- **AACC Method 40-40**
- This method, approved by the AACC, is applicable for only qualitative determinations of iron in enriched flour.

Spot test method

- Ferric iron added to flour reacts with a thiocyanate (KSCN) reagent to form a red colored complex.
- A higher number of red spots and a deeper red color appear with enriched and fortified flour compared with untreated flour.

Principle

Advantages

1. It is a simple, fast, and easy technique requiring no sample pretreatment.
2. It is inexpensive; only two reagents, KSCN and HCl, are needed.
3. Personnel with minimal training can conduct this assay.
4. It does not require a laboratory; it can be conducted in the flour mill.

Limitations

- It is not quantitative, i.e., it does not determine the amount of iron present in the sample.
- It can not be used in case of NaFeEDTA . A special test ahs been developed by NaFeEDTA manufacturers

- This method shows only ferric iron.
- If iron is added in the ferrous form, the sample needs to be oxidized with hydrogen peroxide to convert the ferrous to ferric iron before analysis.

Important Note

Materials Required

- Flour and Fortified flour : standard flour and flour to be tested should be of approximately the same moisture content
- Rectangular glass or rigid galvanized iron plate, about 12 x 8 cm
- Flour trier (spatula)
- Fortifcant (premix)
- Reagents – KSCN : HCL : Hydrogen peroxide

Reagents Preparation

- Thiocyanate reagent - Dissolve 10 g KSCN in 100 ml water.
- HCL reagent : Prepare 2N HCl - To a 500 ml beaker, add 100 ml distilled water. Then pour slowly 17 ml of concentrated HCl, and finally 83 mL more of water.
- Hydrogen peroxide : Prepare 3% (only when iron is as elemental iron or as a ferrous salt). Add 5 ml concentrated H_2O_2 (30%) to 45 ml distilled water. Prepare daily.
- Discard after finishing the analysis

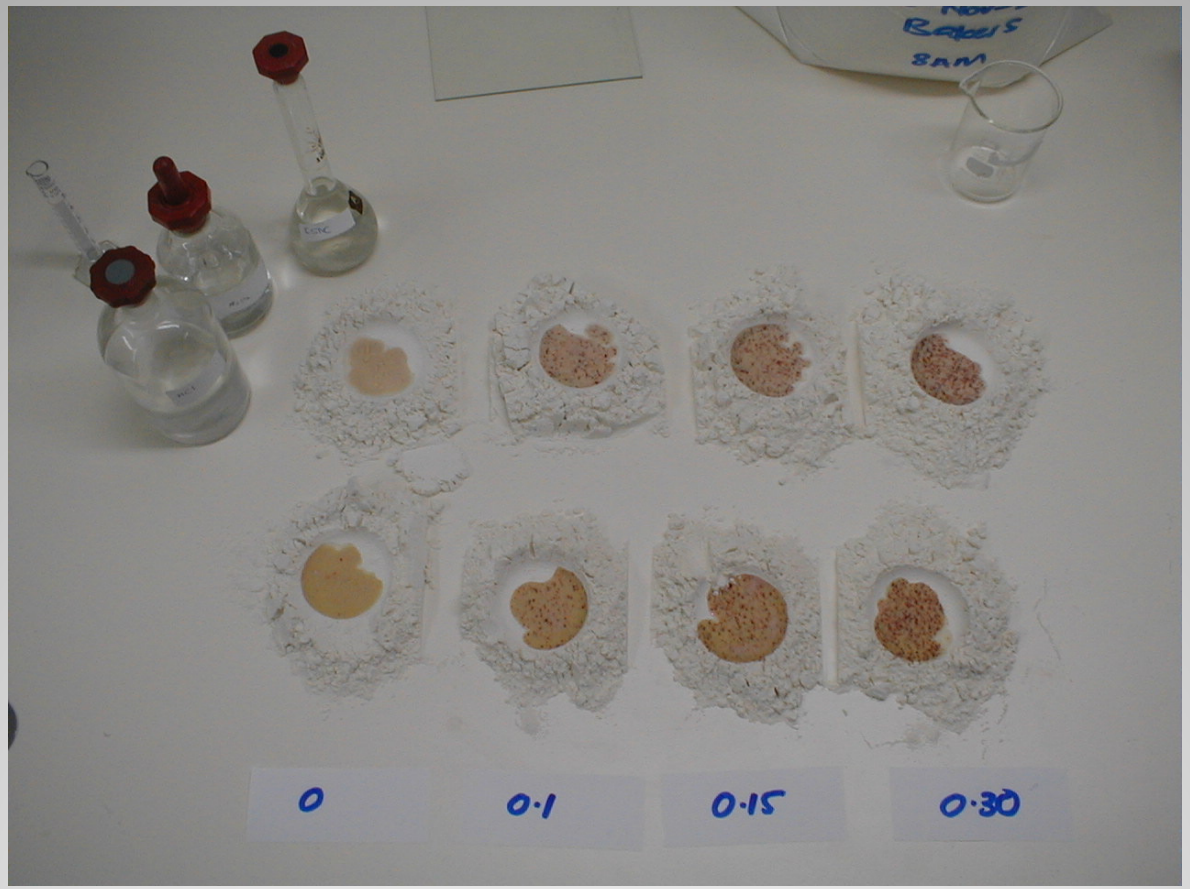
- Reagent 1 : , mix 10 mL of KSCN(potassium thiocyanate) solution with 10 mL of HCl-2 N.
- Reagent 2 : 3% Hydrogen peroxide

Reagent 1 & 2

Procedure for Determination of ferrous iron

- Slick untreated and enriched flour side by side in usual manner i. e , Place approximately 10-15 g flour on glass or iron plate. Pack one side in straight line by means of flour slick. Treat same quantity of standard flour used by comparison in same manner, so that straight edges of two flours are adjacent.
- Drop approximately 1 ml thiocyanate reagent at junction of the two flours, in amount sufficient to wet area approximately 1 inch in diameter.
- Drop approximately 1 ml of 3% hydrogen peroxide over same area wet by thiocyanate reagent
- Let stand at least 10 min. If added ferric compounds are present, deeper red color will be formed than in untreated flour. Small local areas of intense red show up after 20 min, indicating location of individual particles of iron compound. (This affords some estimation of uniformity of mixing.)
- The ferrous iron will have been oxidized to the ferric state by the hydrogen peroxide.

Iron Type	KSCN/1 N-HCl	$K_3Fe(CN)_6$ / 0.003 N HCl	KSCN/ HCl-1N + H_2O_2
Fe (+3) NaFeEDTA	Red diffused spots	Greenish or brownish spots	Red spots
Fe (+2) – Ferrous sulfate	-	Intense blue spots (1-2 minutes)	Red spots
Fe (+2) - Ferrous fumarate	-	Blue small spots (6-7 minutes)	Red spots
Fe (o) Electrolytic	-	Blue small spots (6-7 minutes)	Red spots
Fe (o) reduced iron	-	?	Red spots



Tests at different levels of iron dosages

Iron Spot Test for Enrichment: Appearances at different dosage levels

- Thank You

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