

Note on a Semiautomated Colorimetric Determination of Iron in Cereals

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Iron is routinely added to all enriched cereal products and determination of its level in such products is often necessary. Atomic absorption is commonly employed for this purpose, but colorimetric procedures are still used, especially when the only mineral requiring assay is iron. In such cases, automated chemistry can be used to increase the speed, accuracy, and production rate of colorimetric iron determinations.

The colorimetric procedure for iron involves three basic steps: 1) reduction of all iron in the sample extract to the ferrous form with hydroxylamine HCl or ascorbic acid, 2) adjustment of the acidic extract to pH 4.6 with an acetate buffer, and 3) formation of a colored iron complex, usually with *o*-phenanthroline or α, α' -dipyridyl. In automated chemistry, a buffered system such as this normally uses the buffer solution for both adjusting the pH and diluting the sample stream. This flow scheme, used in one such automated system (Technicon 1972), gives erroneously low iron values for some cereal products. An improved automated iron flow scheme that corrects this problem has been devised.

MATERIALS AND METHODS

Various types of cereal and oilseed products were tested (Table I.) These included selected AACC National Check Samples (series A), 28 enriched and 68 unenriched internationally milled wheat flours, and the U.S. National Bureau of Standards reference flour 1567, which has a certified iron content.

All samples were extracted by AOAC dry ashing method 14.011 (AOAC 1975). Iron content was determined by atomic absorption spectrophotometry using AACC method 40-42 (AACC 1974) on Instrumentation Laboratory's AA spectrophotometer model 251 and by two automated colorimetric methods using Technicon's AutoAnalyzer II. The first was Technicon's method 139-71A (Technicon 1972), in which the sample stream (0.23 ml/min) is added to the buffer stream (1.20 ml/min) before 10% hydroxylamine HCl (0.16 ml/min) is added. The second method is shown in Fig. 1. In both cases, iron standards containing 1, 2, 3, and 4 $\mu\text{g}/\text{ml}$ were run after every 20 samples.

RESULTS AND DISCUSSION

Evidence that Technicon's automated iron method 139-71A underestimates iron content came from comparison with the atomic absorption method when both procedures were used on the same extracts. The former gave values 2% low on the 28 enriched samples and 5% low on the 68 unenriched flour samples. An iron recovery test was run, in which six different levels of iron were added to an unenriched flour. Only 89% of the theoretical level was recovered by Technicon's procedure. This method appeared to give low values for most, but not all, wheat flours and was particularly prone to underestimate the iron level in certain types of products, such as self-rising flour (Tables I and II).

The pH of the extracts from six different types of cereal products showed little difference (1.1-1.3), and the pH of all samples was maintained at 4.57 ± 0.05 during the analysis. The pH of the iron standard was not significantly different to account for the difference in values. No noticeable precipitate or cloudiness occurred in the sample stream. The fact that Technicon's method gave depressed iron levels was therefore hard to understand; it merely had to compare the sample stream with the standard, and the two appeared to be identical.

The one difference between this method and the manual colorimetric procedures, such as AACC 40-41A (1974) and AOAC

TABLE I
Iron Assays of Selected Processed Cereals^a

Sample	Fe (mg/100 g \pm SD)		
	Proposed Method	Technicon Method 139-71A	Atomic Absorption
NBS standard reference flour 1567 ^b	1.83 \pm 0.07	1.81 \pm 0.31	1.78 \pm 0.07
AACC National Check Samples			
1978 A-11	5.20 (5.00 \pm 0.64) ^c	4.76 \pm 0.04	...
A-12	5.35 (5.37 \pm 0.59)	4.78 \pm 0.33	...
1979 A-2	5.95 (5.75 \pm 0.79)	5.42 \pm 0.26	...
A-3	3.50 (3.19 \pm 0.90)	2.51 \pm 0.20	...
A-4	3.77 \pm 0.09	3.37 \pm 0.26	...
A-5	3.57 \pm 0.20 (3.55 \pm 0.62)	3.30 \pm 0.13	...
Oat ready-to-eat cereal	15.4 \pm 2.0	...	14.1 \pm 0.5
Wheat ready-to-eat cereal	77 \pm 9	...	83 \pm 2
Oat flour	4.34 \pm 0.22	...	4.62 \pm 0.20
Corn flour	1.17 \pm 0.11	...	1.15 \pm 0.04
Soy flour	8.41 \pm 0.55	...	9.39 \pm 0.20
Wheat flour, unenriched	1.10 \pm 0.02	0.77 \pm 0.07	...
Wheat flour, enriched	3.70 \pm 0.90	2.97 \pm 0.31	...
Wheat flour, enriched, self-rising	3.37 \pm 0.07	1.98 \pm 0.44	...
Corn meal, unenriched	2.20	1.92	...
Corn meal, enriched, self-rising	5.13 \pm 0.35	3.61 \pm 0.51	...
Semolina, enriched	3.74 \pm 0.48	3.50 \pm 0.46	...

^a As-is moisture basis unless otherwise stated.

^b Moisture-free basis; the NBS certified value is 1.83 \pm 0.11.

^c Figures in parentheses are the AACC check sample collaborative averages.

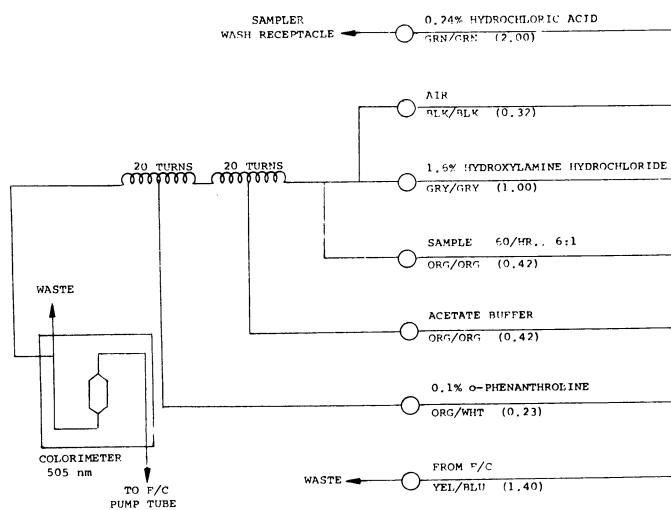


Fig. 1. Flow diagram for semiautomated iron assay. Figures in parentheses refer to flow rates in milliliters per minute.

14.011 (1975), was that the latter performed the reduction step before pH buffering. When the test was run using this sequence, according to the flow scheme shown in Fig. 1, the results were more in line with expected values (Table II).

Two reasons might explain why the reduction step needs to be performed at the low pH of the extract rather than at the higher pH of the buffered extract. One is that hydroxylamine HCl, which is a reducing agent at low pH, becomes an oxidizing agent at high pH. At pH 4.6, however, it should still have reducing activity. No

improvement was obtained by using higher concentrations of this reagent or by using another reducing agent, such as ascorbic acid. A more likely cause of the problem is the interference of phosphate salts, which could precipitate some of the ferric iron at pH 4.6. Such a precipitate would not be noticeable. After all the iron has been reduced to the ferrous form, phosphate precipitation at pH 4.6 cannot occur. This would account for the significant reduction in iron recovery in self-rising flour, which contains a higher level of phosphates.

In summary, an automated version of the AACC colorimetric iron method for cereal products is proposed, as shown in Fig. 1. This method requires no blanks, gives highly readable curves, and allows up to 50 determinations per hour, including standards.

TABLE II
Recovery of Iron Added to Unenriched Flour

Added Iron (mg/100 g Flour)	Added Iron Recovery (% ± SD)	
	Proposed Method	Technicon Method 139-714
1	105 ± 3	80 ± 9
2	93 ± 9	89 ± 2
3	103 ± 2	119 ± 9
4	96 ± 3	95 ± 9
5	93 ± 1	71 ± 31
6	97 ± 0	86 ± 19
Average Recovery (6 samples)	98 ± 6	89 ± 19

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