The State of Iron in Flour, Dough, and Bread[1]

J. LEICHTER and **M. A. JOSLYN,** Department of Nutritional Sciences,
University of California, Berkeley

ABSTRACT

The available (extractable) iron in three different types of bread was determined chemically by the modified 2,2-dipyridyl method. In the breads examined, the total and the extractable iron values were identical. The iron contents of white enriched bread, rye bread, and French enriched bread were 33, 21, and 35 p.p.m. respectively (moisture-free basis). Changes in the available iron from flour to dough to bread were examined. It was found that the available iron in flour and in dough is approximately 80% of the total iron, which is approximately 20% lower than the available iron value in bread. No differences in results were noted whether the bread was made from enriched or unenriched flour. Aqueous extraction of bread does not liberate bread iron into the solution. When a reducing agent (sodium hydrosulfite) is added, all of the iron found in the bread is released into the solution. Further investigations of iron-binding capacity have shown that more of the added ferric than ferrous iron forms insoluble complexes with bread. The iron present in bread was found largely in the ferric state.

The value of a food as a dietary source of iron is affected more by the chemical state of iron in the food than by its total iron content. The absorption of the ingested iron depends more on the iron-deficiency state of the patient, the presence of food in the stomach, and particularly on the presence of ascorbic acid than on the state of iron added to flour (1). Solubility, ease of ionization, and the ferrous valence state are properties assumed to lead to the greatest assimilation of iron (1,2,3).

Nutritional availability of iron has been investigated by both chemical and biological methods (1,4–8).

Several studies have been made of the comparative biological values of the various forms of iron commonly used in bread enrichment (1,9). None of these studies have dealt with the actual chemical state of iron present in different types of bread, in both crumb and crust, as well as with the changes in the state of iron from flour to dough to bread. Steinkamp *et al.* (1) refer to unpublished data indicating that during baking ferrous sulfate is converted partially to the ferric state.

A more complete knowledge of the chemical state of iron in bread would be helpful in elucidating its dietary value as a source of iron in human nutrition.

This paper presents the results of the available and total iron content in three different types of commercial bread, in both crumb and crust, by the chemical (2,2-dipyridyl) procedure (10,11) before and after ashing; changes in the available iron content during the baking process in both enriched and unenriched flour; and the actual chemical state of iron in bread.

---

[1] The data presented are taken from a thesis presented in partial fulfillment of the requirement for the degree of Master of Science by Joseph Leichter, January 1966.

MATERIALS AND METHODS

Bread and Ingredients

The total and available iron content in both crust and crumb were determined on the following three commercial breads: white enriched, sour French enriched, and rye. The breads were obtained locally in a fresh state. Wheat gluten and pentosans were obtained from D. K. Mecham of the U.S. Dept. Agr., Albany, California. Wheat starch (purified powder) was obtained from Matheson Coleman & Bell, Norwood, Ohio.

To investigate the changes in available iron content during the baking process, bread was made from enriched (Gold Medal) and unenriched (Oroweat) white flours.

Baking

The baking test procedure used was essentially that reported in *Cereal Laboratory Methods* (12). The bread was made from a dough of the following composition: flour 100 g., compressed Fleischmann yeast 3 g., salt 1 g., sugar 2.5 g., and distilled water 60 ml. The doughs after mixing were fermented for 2 hr. and 35 min. and punched; 25 min. later they were panned and proofed for 55 min. at 30°C., and then baked for 20 min. at 230°C. Samples of dough were taken immediately after mixing and made into a thin layer on a tin plate to facilitate air-drying. The air-dried dough was ground in a Waring Blendor for 5 min. Another sample was taken after 2 hr. and 55 min. of fermentation and treated in the same way. The baked bread was sliced, air-dried, and ground, as was the dough. The crust was separated from the crumb.

Iron Analysis

Total iron was determined by the AOAC method (13). Available iron was determined by the 2,2-dipyridyl procedure described by Theriault and Fellers (11) with the following modifications: 1) acetate buffer, pH 4.4, used instead of pH 5.5; 2) colorimetry of pigment formed in a ½-in. cell in a B&L Spectronic 20 colorimeter at 520 mμ; 3) reduction of the time of iron extraction from bread to 1 hr. through use of a rotary agitator.

Separation of Crust and Crumb

The crust was separated from the crumb by cutting the surface layers of bread slices with a sharp knife. The demarcation between the zones of brown crust and white crumb was sharp enough to permit separation with a fair degree of accuracy. Our results on proportion of crust to crumb solids were similar to those reported previously (14).

Iron-Binding by Bread

The previously described dipyridyl procedure was employed for the study of the iron-binding capacity of bread and for the determination of the actual state of iron present in bread. Ferrous and ferric iron solutions were used. To prevent oxidation of the ferrous iron solution the tests were carried out in atmosphere of carbon dioxide after previous evacuation of air. Air-dried ground bread (1 g.) was weighed into a 70-ml. culture tube (screw cap with rubber liner); 10 ml. of pH 4.4 buffer acetate and freshly boiled water previously cooled in a stream of CO_2 were added to make a total volume of 50 ml. when the required aliquot of iron (ferrous) was added. Air was evacuated by suction with a water aspirator and the vacuum was released with CO_2 gas which was bubbled through the solution for 10 min. After 10 min. with CO_2 still continuing to bubble, a known amount of the ferrous iron solution was added. The gassing tube was then removed, the screw cap replaced, and the tube agitated on the rotary agitator, for 1 hr. After removal from the rotary agitator, the tube was allowed to stand for 10 min., and the contents were filtered. Sodium hydrosulfite was added to the filtrate and available iron determined as described above.

The difference between the iron added and the iron determined in the filtrate was taken as the iron absorbed by the bread.

The method used for ferric iron-binding capacity by bread and bread constituents was essentially the same as that described above, except that the evacuation and gassing with CO_2 was eliminated, and iron in the ferric state was added.

RESULTS

Total and Available Iron Content of Commercial Bread

The results of the total and available iron determination in the three types of bread, shown in Table I, indicate that the different breads do not vary significantly in percentage of available iron, either in crust or in crumb. The available iron in each case is identical with the total iron within the experimental error. The total iron data were determined after ashing. These results correspond to those reported by Shackleton and McCance (15) for iron in brown bread. They reported the ionizable iron content, as percent of total iron, to range from 72–83% in whole-meal bread, 87–90% in white bread, to 95–100% in brown bread. They reported white flour to contain 93% ionizable iron. In contrast, Elvehjem *et al.* (4) reported wheat to contain 47% available iron when tested by Hill's procedure (10). Apparently the higher phytic acid content of wheat bound more of the iron present.

TABLE I

TOTAL AND AVAILABLE IRON RECOVERED FROM BREAD[a]

(Average of duplicate runs)

TYPE OF BREAD	TOTAL IRON[b]		AVAILABLE IRON		AVAILABLE IRON	
	Crumb	Crust	Crumb	Crust	Crumb	Crust
	γ/g. bread		*γ/g. bread*		*% of total*	
White enriched	33.3	32.3	33.1	33.2	99.3	102.8
Rye	20.2	21.4	21.1	21.9	104.5	102.3
Sour French enriched	33.8	36.8	32.6	32.6	96.4	88.6
			Average			99.0
			Average deviation			± 4.3

[a] Moisture-free basis.

[b] On ashed sample.

Changes in Available Iron Content during Mixing, Fermentation, Proofing, and Baking

Along with determination of available iron, total iron content of the flour, the unfermented and fermented dough, and the bread (separately for crust and

TABLE II

CHANGES IN AVAILABLE IRON DURING MIXING, FERMENTATION, AND BAKING[a]

	TOTAL IRON		AVAILABLE IRON		
	Average[b]	Difference between Duplicates	Average[b]	Difference between Duplicates	Percent of Total
	γ/g.	%	γ/g.	%	%
Unenriched flour	18.4	0	13.0	21	71
Unenriched dough					
Unfermented	19.3	12	18.4	16	95
Fermented	19.8	5	19.2	6	97
Unenriched bread					
Crust	20.5	6	20.0	0	96
Crumb	20.0	5	20.3	5	100
Enriched flour	37.3	15	30.1	9	81
Enriched dough					
Unfermented	40.1	0	33.1	18	83
Fermented	38.7	9	33.8	12	88
Enriched bread					
Crust	40.5	8	37.3	9	92
Crumb	39.8	0	38.9	0	98
Average difference between duplicates (%)		5.6		9.6	

[a] Moisture-free basis.
[b] Average of duplicate determinations.

crumb) was estimated from the same samples. This was done for enriched and unenriched flour. The results (Table II) show that with both enriched and unenriched flour there was a slight increase in availability of iron on fermentation. The differences, however, are small and could be due to turbidity in the initial stages.

The percentages of total available iron for the enriched and unenriched items are similar. It thus appears that the percentage of total available iron is not influenced by whether the iron comes from the enrichment mix or is present naturally in the unenriched flour. The data in Table II show that the available iron in flour is about 80% of the total iron, which is about 20% lower than in bread. This increase of available iron in bread may be due to the fact that some sites of iron-binding in protein were denatured during the baking process. Fermentation also seems to increase the solubility of iron, as shown in Table II. These results are in agreement with studies of the effect of heat on the availability of the iron in beef muscle by Oldham (16). She reported that heat renders the iron of beef muscle, of which at least 50% is in the organic form, as available for hemoglobin synthesis as the iron in an inorganic iron salt.

The available iron in the two types of bread made from the enriched and unenriched flour is identical with the total iron content, which is in agreement with the results obtained for the three commercial breads reported in Table I.

Actual State of Iron in Bread, and Iron-Binding Capacity by Bread and Bread Ingredients

When bread was suspended in a pH 4.4 acetate buffer solution or in an aqueous solution, no iron was liberated from the bread into the solution. On addition of a reducing agent (sodium hydrosulfite), the bread iron was released into the solution. The iron determined in the solution after filtration by the 2,2-dipyridyl procedure agreed with the iron content determined after ashing of the bread sample. Since the reducing agent (sodium hydrosulfite) reduces ferric iron to the ferrous state and also liberates ferric iron from its complexes, it appears that ferrous iron does not form such complexes.

To obtain data on relative binding of ferrous and ferric iron by bread, the following experiment was carried out. To a series of aqueous suspensions of bread a known volume of standard ferric chloride solutions was added, and the resulting mixture was shaken well and filtered. The iron present in the filtrate was estimated. The same experiment was carried out with a ferrous ammonium sulfate solution. As shown by the data in Table III, there was little difference between the percentage of free ferrous and ferric iron present when the experiment was carried out in presence of air. This was suspected to be due to oxidation of added ferrous salt. When the tests with ferrous salt were carried out in absence of air, less ferrous iron was bound, as shown in Table IV. This was accomplished by carrying out the tests in an inert CO_2 atmosphere after previous evacuation of the air.

TABLE III

IRON (FERRIC AND FERROUS) BINDING BY BREAD CRUMB (WHITE) IN PRESENCE OF AIR
(γ iron/1 g. bread, moisture-free basis; av. of triplicate runs)

IRON ADDED	FERRIC IRON		FERROUS IRON	
	Free	Adsorption[a]	Free	Adsorption[a]
γ	γ	%	γ	%
100	8	92	8	92
200	22	89	20	90
400	40	90	49	88
600	60	90	76	88
800	110	86	140	83

[a] Percent added iron adsorbed by bread solids.

TABLE IV

IRON (FERRIC AND FERROUS[a]) BINDING BY BREAD CRUMB (WHITE)
(γ iron/1 g. bread, moisture-free basis; av. of triplicate runs)

IRON ADDED	FERRIC IRON		FERROUS IRON	
	Free	Adsorption[b]	Free	Adsorption[b]
γ	γ	%	γ	%
100	8	92	6.5	93.5
200	22	89	22	89
400	40	90	122	70
600	60	90	190	68
800	110	86	350	56

[a] Air excluded in ferrous test.
[b] Percent added iron adsorbed by bread solids.

The results obtained for the ferric and ferrous iron-binding capacity by bread are presented in Table IV. As can be seen, the adsorption of ferric iron by bread is significantly higher than for the ferrous iron. Because of the agreement between values for total iron determined after ashing and those for available iron, it was not expected that ferrous iron would be adsorbed by bread. The reason that there was partial adsorption of the ferrous iron by bread may be that oxidation of the ferrous iron was not eliminated completely during the experiment.

As for the ferric iron-binding by bread, if other conditions are equal, the amount of iron compound formed is a function of the increasing concentrations of iron added, as shown in Tables III and IV.

TABLE V

IRON (FERRIC) BINDING CAPACITY BY WHITE BREAD CRUST AND CRUMB

(γ iron/1 g. bread, moisture-free basis; av. of triplicate runs)

IRON ADDED	FREE IRON	
	Crumb	Crust
γ	γ	γ
100	8	10
200	22	26
400	40	40
600	60	70
800	110	120

The above tests were done separately for bread crumb and for bread crust (Table V), and no significant differences were found. From the observations made, it appears that ferric iron forms complexes with bread.

The comparative iron-binding capacity of gluten, starch, and pentosans, measured similarly by adding various amounts of ferric iron per g. of suspended solid, indicated that of these most of the iron was bound by gluten and least by pentosans. The iron-binding capacity of gluten did not change appreciably in the range of 1,000 to 8,000 mg./g. (average 76% bound); the iron-binding capacity of starch increased from 14% at 100 γ/g. to 35% at 600; that for pentosans remained at 6% in the range of 300 to 1,300 γ/g.

Acknowledgment

The authors wish to express their appreciation to S. Eisenberg, Anresco Laboratories, San Francisco, California, for use of laboratory facilities in carrying out the tests; and also to D. K. Mecham, U. S. Dept. Agr., Albany, California, for providing samples of gluten and pentosans.

Literature Cited

1. STEINKAMP, R., DUBACH, R., and MOORE, C. V. Absorption of radioiron from iron-enriched bread. Arch. Internal Med. **95**: 181–193 (1955).
2. MOORE, C. V., DUBACH, R., MINNICH, V., and ROBERTS, H. K. Absorption of ferrous and ferric radioactive iron by human subjects and by dog. J. Clin. Invest. **23**: 755–762 (1944).
3. HAHN, P. F., JONES, E., LOWE, R. C., MCNEELY, G. R., and PEACOCK, W. The relative absorption and utilization of ferrous and ferric iron in anemia as determined with the radioactive isotope. Am. J. Physiol. **143**: 191–196 (1945).

4. Elvehjem, C. A., Hart, E. B., and Sherman, W. C. The availability of iron from different sources for hemoglobin formation. J. Biol. Chem. **103:** 61–70 (1933).
5. Harris, R., Mosher, M., and Bunker, J. The nutritional availability of iron in molasses. Am. J. Digest. Diseases **6:** 459–462 (1939).
6. Vaughan, O. W., and Knauff, R. E. Chemical state and nutritional availability of iron in a prepared infant formula. J. Dairy Sci. **44:** 1650–1654 (1961).
7. Bothwell, T. H., and Finch, C. A. Iron metabolism. Little, Brown: Boston (1962).
8. Granick, S. Iron metabolism in animals and plants. *In* Trace elements, C. A. Lamb, O. Y. Bentley, and J. M. Beattie (eds.), p. 365–382. Academic Press: New York (1958).
9. Blumberg, H., and Arnold, A. Comparative biological availabilities of various forms of iron in enriched bread. Cereal Chem. **24:** 303–314 (1947).
10. Hill, R. A method for the estimation of iron in biological material. Proc. Roy. Soc. London **B107:** 205–214 (1930).
11. Theriault, F. R., and Fellers, C. R. Effect of freezing and of canning in glass and in tin on available iron content of foods. Food Res. **7:** 503–508 (1942).
12. American Association of Cereal Chemists. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minnesota (1962).
13. Association of Official Agricultural Chemists. Official and tentative methods of analysis (9th ed.), p. 159. The Association: Washington, D.C. (1960).
14. Kennedy, B. M., and Joslyn, M. A. Changes in iron, thiamine and riboflavin content of flour during dough formation and baking. Baker's Dig. **40:** 60–62 (1966).
15. Shackleton, L., and McCance, R. A. The ionizable iron in foods. Biochem. J. **30:** 582–591 (1936).
16. Oldham, Helen G. The effect of heat on the availability of the iron of beef muscle. J. Nutrition **22:** 197–203 (1941).

[Received August 1, 1966. Accepted December 12, 1966]