Specifications for Reduced Iron as a Food Additive

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The relative biological value of eight reduced iron powders manufactured by reduction with hydrogen or carbon monoxide, or by the electrolytic or carbonyl process, was determined by a rat hemoglobin repletion assay. The particle size distribution of these powders was determined by a photographic–microscopic method. The solubility of five of the above powders in 0.2% (w/v) hydrochloric acid (pH about 1.2) at 37 °C with shaking in a 1-in. orbital stroke incubator shaker for 10 to 180 min was estimated. The relative biological value, as determined by a rat hemoglobin repletion assay, was generally found to increase with decreasing median particle size and with the solubility at different times. It was concluded from the results that a reduced iron powder to be used as a food additive should meet the following specifications in order to ensure acceptable quality. (1) The iron content of the powder should not be less than 96%. (2) At least 95% of the powder should pass through a 325 mesh sieve having a pore size of 44 μm. (3) At least 90% of the weight of the iron powder should be soluble in dilute hydrochloric acid as determined by the method described.

Enrichment of flour with thiamin, riboflavin, niacin, and iron was introduced in Canada in 1953 (Chapman and Campbell, 1957). Concerning iron, the present regulation states that "enriched white flour shall be flour to which has been added iron in a harmless carrier, in such amount that one pound of enriched flour shall contain not less than 13.0 mg and not more than 16.5 mg of iron". In enriched bread the corresponding levels of iron are 8.0 and 12.5 mg per lb (Health and Welfare Canada, 1975). The stipulated amounts of iron in enriched flour and bread were based on the levels of iron in whole wheat.

The milling industry has been using iron powder for enrichment of flour mainly because it is an economical, inert source of iron which does not affect the color or keeping quality of flour or bread. In recent years, however, the availability of iron from this source has been questioned (Cook et al., 1973; Rios et al., 1975). The biological availability of the reduced iron specified in Food Chemical Codex (National Research Council, 1972) was also not satisfactory (Shah and Belonje, 1973a). On the basis of rat assays of a number of iron powders it was concluded that at least 95% of the particles (by number) should be less than 10 μm in size so that the powder would have an acceptable bioavailability. Similarly the absorption of fine (97% particles about 5 μm) iron powder by male and female volunteers was 9% whereas the comparable value for coarse iron powder (23% particles about 25 μm and 48% larger than 30 μm) was only 3% (Höglund and Reizenstein, 1969). In view of the these results there is a need to revise the specifications for iron powder used in foods, so that an acceptable bioavailability will be ensured.

These specifications can be based on particle size distribution (Shah and Belonje, 1973a) or solubility of an iron powder in dilute hydrochloric acid (Hinton et al., 1967; Hart, 1971; Ministry of Agriculture, Fisheries and Food, U.K., 1974). Shah and Belonje (1973a) employed a photographic–microscopic method for the determination of particle size distribution whereas Motzok et al. (1975) used air elutriation to separate designated fractions. Pla et al. (1973) separated particles larger than 32 μm by sieving. Since these methods are based on varying physical principles the results are not comparable. Moreover, the equipment and the expertise required for these methods are not commonly available in food industry laboratories. It was therefore decided to investigate the parameters of a solubility test which would be met by iron powders found to have an acceptable relative biological value as determined by a rat repletion assay (Shah and Belonje, 1973b). It should be noted also that other factors, such as porosity and shape of the particles which are likely to affect the bioavailability of the iron powder, would be better reflected by its solubility than by the particle size distribution alone (Hinton et al., 1967).

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MATERIALS AND METHODS

The following food grade reduced iron powders were obtained from the manufacturers and other sources for carrying out the bioassay and the solubility tests: electrolytically reduced, 3; hydrogen reduced, 2; carbon monoxide reduced, 1; carbonyl, 2.

According to the manufacturers' specifications most of the powders contained at least 96% iron and 95% of most of them passed through a 325 mesh sieve having a pore size of 44 μm. The particle size distribution of these powders was determined as described previously (Shah and Belonje, 1973a). The relative biological value was obtained from three different rat hemoglobin repletion bioassays as described earlier (Shah and Belonje, 1973b).

For the determination of the solubility, 100 mg of a sample was added to a 500-mL glass-stoppered conical flask, which contained a single layer of 4- and 5-mm glass beads. Hydrochloric acid [250 mL of 0.2% (weight in volume)] (pH of about 1.2, simulating gastric fluid according to U.S. Pharmacopeia XIX, 1975), which had been prewarmed to 37 °C, was then added and the flask was immediately placed in a 1-in. orbital stroke incubator shaker set at 150 rpm. The flask was incubated at 37 °C with shaking for a specified time. An aliquot (2 mL) was removed and immediately shaken with a Teflon-coated magnetic stirring bar to take out any iron particles. The iron content of the aliquot was determined by atomic absorption spectroscopy (AOAC, 1975) and the amount of iron in solution was expressed as a percent of the sample weight. Five samples (two electrolytic, one hydrogen, one carbon monoxide, and one carbonyl) were used for the solubility test.

Day-to-day variation in the results of the solubility test was checked with two electrolytic, one hydrogen reduced, and one carbonyl powder.

RESULTS AND DISCUSSION

The particle size distribution (by number percent) and the relative biological value (RBV, $FeSO_4$ = 100%) of eight iron powders are given in Table I. On the basis of median particle size, the iron powders can be divided into four categories. In the first category are the two carbonyl

Table I. Particle Size Distribution by Number (Percent) and Relative Biological Value ($FeSO_4$ = 100%) of Iron Powders

Iron powders	RBV,[a] %	μm						
		Median size	<5	6–10	11–20	21–30	31–40	>40
Electrolytic I	32	8.2	24.0	39.0	27.7	6.6	1.8	0.9
Hydrogen reduced I	18	21	4.5	11.3	34.4	29.0	13.0	8.0
Hydrogen reduced II	24	20	16.0	9.0	25.0	23.0	15.0	12.0
Electrolytic II	37	7.6	24.0	42.0	28.0	4.4	1.0	0.6
Carbon monoxide reduced	12	28		6.5	22.0	29.5	23.5	18.5
Carbonyl iron I	61	3.8	69.0	29.8	1.2			
Carbonyl iron II	58	2.9	86.0	13.6	0.4			
Electrolytic III	16	9.7	21.0	30.0	32.0	9.5	4.8	2.7

[a] The 95% fiducial limits were generally 20% below and 25% above the estimated RBV.

Table II. Solubility of Iron Powders (Percent Dissolved)

Time, min	Hydrogen reduced II	Electrolytic II	Carbon monoxide reduced	Carbonyl II	Electrolytic III
10	15.6	54.4	9.8	70.8	19.1
20	24.4	68.9	14.9	91.1	32.3
30	32.9	79.8	20.1	96.9	41.1
60	52.1	92.9	35.1	95.5	64.9
90	63.6	99.4	48.5	96.9	79.4
120	72.8	99.9	57.3	96.6	83.8
150	78.7	94.9	64.5	95.6	90.9
180	83.2	94.9	70.2	95.6	90.9
Median particle size, μm	20	7.6	28	2.9	9.7

powders having median particle sizes of 2.9 and 3.8 μm. The size of almost all the particles in these powders is 10 μm or less. The second consists of electrolytically reduced iron powders, which have a median particle size of 7.6–9.7 μm. Approximately 50 to 65% of their particles are not more than 10 μm in size. The third category comprises the hydrogen reduced powders, having a median particle size of 20 and 21 μm and the corresponding percentages for particles of size 10 μm or less being 25 and 15.8. In the fourth category there is the carbon monoxide reduced powder, which has the maximum median particle size of 28 μm and all but 6.5 of its particles are larger than 10 μm in size. The relative biological value (RBV) of these iron powders decreases from 61 to 12% as the median particle size increases from less than 4 to 28 μm although the correlation is not linear. A similar effect of particle size on biological availability has been reported by Fritz et al. (1975) and Pennell et al. (1975). Moreover, the latter group has reported that in human subjects also, hydrogen reduced and electrolytic powders having 7–10-μm particles were much better absorbed than the corresponding fractions having 20–26-μm particles. Using a radiolabeled iron powder separated into fine and coarse fractions and incorporated into bread, Höglund and Reizenstein (1969) observed a similar effect of particle size on iron absorption by the volunteers who consumed the bread.

The RBV of electrolytic iron obtained by us was less than the 45–50% reported by Pla et al. (1973) and Pennell et al. (1975). Similarly Pla et al. (1973) found the RBV of carbon monoxide reduced iron powder to be 19% whereas our corresponding result is only 12%. The variation in the RBV could be due to many factors including differences in the composition of the basal diet and those in the powders from different manufactures or due to the manner of handling of the samples. One of the major differences between the basal diets used by these two groups and by us was that we added 25% corn starch in our diet whereas they replaced it with degermed yellow corn meal. Another difference was that in many assays they used dried skim milk powder as the source of protein instead of casein. Skim milk powder has been reported to cause mild diarrhea in rats (Shah and Belonje, 1973b) but its effect on the availability of iron is not known. Amine and Hegsted (1971) have reported that starch reduces iron absorption significantly. It should be noted, however, that the RBV of carbonyl iron reported by Fritz et al. (1975) agreed fairly well with our result.

The results of the solubility test on five iron powders are given in Table II. From these results it is evident that the smaller the median particle size, the greater was the solubility. Moreover, the rate of dissolution of the powders also varied inversely as the median particle size. As mentioned above, the relative biological value also had a similar inverse relationship with the median particle size. It should be pointed out that although the median particle size of the electrolytic powder III was similar to the other two electrolytic powders (Table I) its rate of dissolution and the solubility at 90 min were less than that of the electrolytic powder II. This was in agreement with its low RBV, indicating that the in vitro solubility is a better predictor of RBV. It is pertinent to note that the three powders were obtained from different sources and it is likely that the process of manufacture of the powder III had an adverse effect on the particles in terms of solubility and availability.

Since the mean half residence time of food in the stomach is about 90 min in man (Oser, 1965) and also in the rat (Farris and Griffith, 1949) this would be a suitable time for a solubility test. Attempts by Pla et al. (1976) to correlate solubility in dilute hydrochloric acid with biological availability were not encouraging when they suspended the samples in dilute acid for 3 h with mechanical shaking or for 72 h with occasional manual

Table III. Day-to-Day Variation in Solubility of Iron Powder in 90 min (Percent Dissolved)

Day	Electrolytic I	Hydrogen reduced I	Electrolytic II	Carbonyl I
1	90.9 ± 2.8 (3.0)[a]	53.9 ± 2.5 (4.6)	93.5 ± 0.2 (0.2)	96.3 ± 0.8 (0.9)
2	92.9 ± 1.6 (1.8)	57.7 ± 2.0 (3.4)	96.4 ± 1.6 (1.7)	100.1 ± 2.2 (2.2)
3	90.4 ± 1.0 (1.1)	59.9 ± 7.2 (12.0)	94.5 ± 2.3 (2.4)	96.5 ± 3.8 (4.0)
1, 2, 3	91.4 ± 1.9 (2.0)	57.2 ± 4.5 (7.8)	94.8 ± 1.8 (1.9)	97.6 ± 2.8 (2.8)
Median particle size, μm	8.2	21	7.6	3.8

[a] Average ± standard deviation (coefficient of variation as percent).

shaking. They observed an excellent correlation for hydrogen reduced and electrolytic iron samples, however, when the solubility was determined for 1 min. Their results for three carbonyl iron samples having particles less than 10 μm in size were not consistent with those for the above two types of powders. This was attributed to the unique chemical and physical properties of the carbonyl powders. From our results (Table II), however, it is evident that at shorter times the differences between solubilities of different powders are maximized whereas they are reduced at longer times. It is, therefore, essential to employ an optimal time for the solubility test. Ninety minutes is suggested above on a physiological basis.

The variation in the solubility of four iron powders determined in triplicate on 3 days is indicated by the results given in Table III. The hydrogen reduced iron powder which had the largest median particle size and the lowest solubility showed the maximum coefficient of variation of 12.0% on one day and also the highest overall coefficient of variation (7.8%). The corresponding values for the other three powders of acceptable quality did not exceed 3%.

From these results, and from data reported previously by others, it is concluded that iron powder used as an iron source should meet the following specifications to ensure acceptable bioavailability. (1) The iron content of the powder should not be less than 96%. (2) At least 95% of the powder should pass through a 325 mesh sieve having a pore size of 44 μm. (3) At least 90% of the weight of the powder should be soluble iron, as determined in triplicate by the method described.

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Nuclear Magnetic Resonance Analysis of Bulk Food Additive Chemicals. 1. Food Chemicals Codex Chemicals, Group 1

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A number of representative food additive chemicals that are listed in the Food Chemicals Codex and do not have analytically useful ultraviolet (UV) chromophores have been studied by nuclear magnetic resonance (NMR). The value of the latter technique has been investigated both in qualitative and quantitative analyses. Conditions for the analyses have been established in that appropriate solvents and internal standards have been selected. Results indicate that the qualitative identification is specific and that the quantitative measurements are reproducible to within 1.5% relative standard deviation.

Prior to 1958, it was incumbent upon food processors to provide detailed procurement specifications when ordering bulk food chemicals from primary manufacturers or distributors. In 1958, the Industry Liaison Panel and other sources requested from the Food Protection Committee of the National Academy of Sciences that a Food Chemicals Codex (FCC) be produced, comparable in many respects to the drug compendia, the United States Pharmacopeia (USP) (1974), and the National Formulary (NF) (1974). As a result, the first bound copy of the FCC (1976) was published in 1966.

The FCC was given quasi-legal recognition, a state which

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