Changes in Iron Forms During Extrusion Processing

R. S. KADAN and G. M. ZIEGLER, JR.[1]

Cereal Chem. 64(4):256–259

ABSTRACT

Cereal mixes consisting of corn meal, rice flour, and soy protein isolate were fortified with three iron sources ($FeSO_4$, Fe_2O_3, and electrolytic iron [EI]), ascorbic acid, citric acid, and an antioxidant, butylated hydroxyanisole, in various combinations. Each mix was extruded to produce a simulated breakfast/snack food. Analysis of the extruded food for metallic, soluble, and complexed iron in aqueous slurry and under simulated stomach digestion conditions was performed. Metallic iron was subjected to electron spectroscopy for chemical analysis (ESCA). Results showed that for $FeSO_4$ and EI an extruded food contained more soluble iron under simulated stomach conditions than an unextruded mix. In aqueous slurries, nearly all iron from $FeSO_4$ and Fe_2O_3 and about three-fifths of the EI had complexed with the mix during extrusion. The addition of organic acids and antioxidant to the mix containing $FeSO_4$ and EI increased soluble iron under simulated stomach conditions. The addition of Fe_2O_3 to the mix with or without additives did not appreciably increase soluble iron under simulated stomach conditions. As expected, most of the soluble iron was in the ferric form in aqueous slurries and in the ferrous form in simulated stomach conditions. ESCA studies confirmed that when atmospheric oxygen was present during processing, the exterior of electrolytic iron particles was susceptible to oxidation to ferric form. The ESCA data further indicated that the outer layer of starting EI, as well as the metallic iron recovered from extruded foods and iron rust, had similar oxidation states, as shown by binding energies for iron and oxygen.

Iron-deficiency anemia in individuals of all age groups is a continuing public health concern in both developing and developed countries, although this problem is more prevalent among pregnant women, the elderly, and young children (WHO 1975). Low iron bioavailability is one of the most important factors responsible for iron deficiency. Nutritional iron deficiency reaches its greatest prevalence and severity in populations subsisting predominantly on cereals and legumes (Bothwell et al 1979, Hallberg 1981), because, generally, iron is poorly absorbed from them. Cereal products and legumes make up the bulk of the staple diets in large parts of the world. Meat consumption is much greater in Western countries, but there has recently been a trend to reduce the intake of meat and to increase that of unrefined carbohydrates. These dietary patterns have important implications for iron nutrition.

Dietary iron can be divided into two distinct forms, heme and nonheme, because of their separate absorption pathways. The largest part is nonheme iron. Supplementing diets with additional, easily absorbable iron may improve the iron nutrition. This improvement may be accomplished by fortifying commonly used plant foods with inorganic iron salts or improving iron availability in habitual diets that have adequate iron levels but are poorly absorbed. Processing of foods containing nonheme iron affects the chemical form of the iron and probably its bioavailability (Hodson 1970; Theuer et al 1971, 1973; Lee and Clydesdale 1978; Hallberg 1981). Many factors affect iron absorption in man, such as an individual's need, composition of the diet, valency, solubility, ease of ionization, and the degree of chelation or complex formation of the iron and food components (Forth and Rummel 1973, Jacob and Greenman 1969, Monsen and Cook 1979, Morck and Cook 1981, Saltman 1965). Some food components such as soy protein products have an inhibitory effect on nonheme iron absorption (Cook et al 1981), whereas others such as ascorbic acid promote iron absorption (Bothwell et al 1979).

The problem of determining the amount of iron that is absorbed from the diet (i.e., biologically available iron) is well recognized. In vivo animal or human feeding studies are ideal; however, they are time-consuming and expensive for screening large numbers of food samples. Several in vitro techniques, wherein the chemical environment of stomach and upper (small) intestine is simulated artificially, have been reported with varying degrees of success (Narasinga Rao and Prabhavathi 1978, Lock and Bender 1980, Miller et al 1981, Kadan and Ziegler 1986). Nevertheless, the chemical state of iron in processed food and its conversion under simulated gastric digestion conditions can provide valuable information about its potential bioavailability (Smith 1983).

Extrusion processing is an economical and popular method for manufacturing ready-to-eat cereals, expanded snacks, textured vegetable proteins, and other food items (Horn 1979). It is especially suited for processing cereal-based foods. Foods can be processed at relatively low moisture contents (about 20–30%) and high cooking temperatures (about 150–180° C) using this technique. Other commonly used food processes have been examined for their role in affecting the chemical changes in iron forms during processing (Lee and Clydesdale 1978, 1979, 1980; Kadan and Ziegler 1984, 1985, 1986), but there have been none on the effects of the extrusion process. This paper reports the results of changes in iron forms in a typical extruded cereal-based food and under simulated stomach digestion conditions.

MATERIALS AND METHODS

A cereal mix consisting of 50% corn meal (Lauhoff Grain Co.), 25% rice flour (RL-100, Riviana Food Inc.), and 25% soy-protein isolate (Supro 710, Ralston Purina Co.) was moistened with deionized water to 25% moisture, conditioned overnight in a sealed plastic bag, and extruded the next day. Appropriate amounts of ferrous sulfate ($FeSO_4 \cdot 7H_2O$ [A.R.]), electrolytic iron (EI; A-131, Glidden-Durkee), and ferric oxide (Fe_2O_3 [Baker A. R.]) were added in various combinations to attain a level of 400 μg of iron per gram of the mix on dry basis. Electrolytic iron A-131 is a fine powder (325 mesh), having an irregular, dendritic (fern like) form, of high purity elemental iron obtained by electrolytic deposition. Nearly 90% of the powder was less than 30 μm in size. Certain additives, 100 ppm of ascorbic acid (AA), and 500 ppm of antioxident (AO) Tenox R (Eastman Kodak Co., Kingsport, TN), consisting of 20% butylated hydroxyanisole (BHA), 20% citric acid, and 60% propylene glycol, were also evaluated with the three iron sources. The water-soluble additives (ferrous sulfate [$FeSO_4$], ascorbic acid, and Tenox R) were dissolved in deionized water and blended with the cereal mix in a Hobart mixer. The water-insoluble additives (ferric oxide [Fe_2O_3] and EI) were mixed with a small portion of rice flour in a mortar and pestle and blended with cereal mix before adding the required amount of water. The overnight conditioned mix was extruded using a Brabender extruder (model #125-20, G. 8 R.) at 30 rpm. The extruder temperatures were 140° C in zones 1, 2, and 3, and 120° C in zone 4. The collet (extrudate) had cooking, expansion, and texture characteristics similar to ready-to-eat cereals and snacks. The collet was allowed to cool and dry

[1]Southern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 19687, New Orleans, LA 70179.

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overnight at room temperature. The material was milled using a Rietz mill and a 0.081-cm screen. The milled material containing 7–9% moisture was stored at 0° C under a blanket of nitrogen and analyzed within eight weeks.

Iron forms in the milled material were determined in an aqueous slurry (pH 7) by using a modified approach of Lee and Clydesdale (1979) and by incubating in a pepsin-dilute HCl mixture (Kadan and Ziegler 1984), as shown in Figure 1. A 2-g sample was suspended in 50 ml of nitrogen-purged double-distilled water in a 250-ml Erlenmeyer flask with a clean 5-cm Teflon-coated magnetic stirring bar. The flask head space was blanketed with nitrogen, stoppered, and metallic iron (MI) was extracted by stirring for 20 min. The magnetic stirring bar was removed with nonmetallic forceps, rinsed, and the MI transferred into an Erlenmeyer flask containing 25 ml of 3*N* HCl. The extraction was repeated until there was no visible MI held on the surface of the magnet. The extracted MI was dissolved over a mildly heated hot plate for about 15 min. The MI thus extracted was analyzed for total iron content by atomic absorption spectroscopy (AAS) and by the bathophenanthroline reaction, which earlier work (Kadan and Ziegler 1984) had shown can characterize the MI iron into its Fe^{+2} and Fe^{+3} ion contents. For measuring soluble and insoluble iron (bound or complexed), an aliquot of metallic iron-extracted slurry was centrifuged at 3,000 × *g* for 15 min. The supernatant liquid was treated with 30% trichloroacetic acid (TCA) solution (10 ml supernatant liquid plus 2 ml of TCA solution) and again centrifuged at 3,000 × *g* for 15 min. The final supernatant was analyzed for Fe^{+2} and Fe^{+3} ion contents by the bathophenanthroline reaction. The two precipitates were combined and wet ashed with concentrated HNO_3. Then the residue was dissolved in dilute HCl, filtered, and analyzed by AAS for its iron content. For measuring iron forms under simulated stomach conditions, a 3-g milled sample was suspended in 20 ml of nitrogen-purged double-distilled water. Then 16 ml of 0.1*N* HCl solution containing 0.16 g of pepsin (500 units per milligram of protein) (Sigma Chemical Co., St. Louis, MO) was added in a 250-ml Erlenmeyer flask. The flask head space was purged with nitrogen, stoppered, and incubated at 37° C for 2 hr. The material was then handled as described above for insoluble and soluble iron.

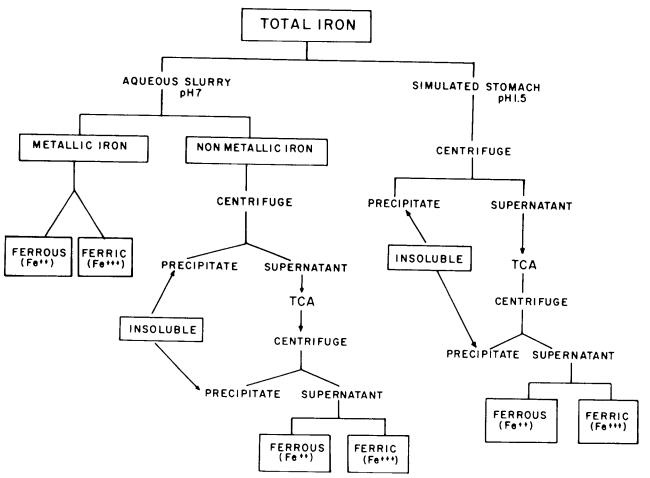

Fig. 1. Classification of the iron forms in extruded food.

The magnetically extracted MI was also subjected to electron spectroscopy for chemical analysis (ESCA) for iron and oxygen spectra (Siegbahn et al 1967). The data was compared with the starting EI and an iron rust sample obtained from food processing pilot plant equipment.

Each experiment was repeated and every sample analyzed in duplicate; thus, the values given are the averages of four individual determinations. Also, sufficient time was devoted in analytical technique development to get a repeatability of 90% between the samples.

RESULTS AND DISCUSSION

The mean values for distribution of the iron forms in aqueous slurries and simulated stomach conditions are presented in Table I. Data indicated that, for all three iron sources, there were distinct

TABLE I
The Effect of Organic Acids and Antioxidant on Iron Distribution (μg/g) in Extruded Foods and in Unextruded and Extruded Cereal Mixes

Sample Description	Aqueous (pH 7) Slurry														Simulated Stomach (pH 1.5)							
	Metallic								Soluble								Soluble					
	Fe^{+2}		Fe^{+3}		Total		Insoluble		Fe^{+2}		Fe^{+3}		Total		Insoluble		Fe^{+2}		Fe^{+3}		Total	
	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.	Ext.	Unext.
Control mix	12	···	14	···	26	···	12	···	4	···	8	···	12	···	13	···	16	···	···	24	40	···
Control mix + EI[a]	88	59	43	280	131	339	228	45	7	7	12	19	19	26	281	372	69	0	17	38	97	38
+ EI + AA[b]	77	···	144	···	220	···	190	···	8	···	15	···	23	···	202	···	178	···	32	···	210	···
+ EI + AO[c]	52	···	170	···	222	···	150	···	6	···	3	···	9	···	198	···	170	···	10	···	180	···
+ EI + AA[b] + AO[c]	27	···	182	···	208	···	195	···	3	···	7	···	10	···	183	···	203	···	18	···	221	···
+ $FeSO_4$[d]	0	0	2	2	2	2	344	343	9	12	10	62	19	74	297	373	63	4	19	33	82	31
+ $FeSO_4$[d] + AA[b]	3	···	6	···	9	···	399	···	2	···	10	···	12	···	273	···	56	···	31	···	87	···
+ $FeSO_4$[d] + AO[c]	27	···	16	···	42	···	374	···	15	···	16	···	31	···	298	···	130	···	10	···	139	···
+ $FeSO_4$[d] + AA[b] + AO[c]	14	···	19	···	33	···	396	···	6	···	30	···	36	···	321	···	100	···	9	···	109	···
+ Fe_2O_3[e]	0	8	0	10	0	18	349	346	2	9	9	23	11	32	342	375	22	16	7	19	29	35
+ Fe_2O_3[e] +AA[b]	19	···	13	···	32	···	317	···	11	···	3	···	14	···	331	···	39	···	6	···	45	···
+ Fe_2O_3[e] + AO[c]	16	···	10	···	26	···	381	···	3	···	6	···	9	···	374	···	16	···	14	···	30	···
+ Fe_2O_3[e] + AA[b] + AO[c]	0	···	36	···	36	···	384	···	4	···	9	···	13	···	370	···	30	···	4	···	34	···

[a] Electrolytic iron at the rate of 400 μg/g.
[b] Ascorbic acid at the rate of 100 ppm.
[c] Tenox R at the rate of 500 ppm.
[d] $FeSO_4 \cdot 7H_2O$ at the rate of 1,990 μg/g.
[e] Fe_2O_3 at the rate of 572 μg/g.

differences in iron form distribution between unextruded and extruded samples. Nearly all of the added EI was recovered as MI from unextruded mix; during extrusion processing about two-thirds of it reacted with the mix components. The increased recovery of MI from extruded samples containing EI and AA indicated that AA protected EI from interacting with the cereal mix components. The addition of either AO or the mixture of AO and AA did not increase the protective effect over AA alone. However, these additives did not protect the MI from oxidation, as the majority of it was found in the Fe^{+3} form by further chemical analysis. The magnet does not attract Fe^{+2} or Fe^{+3} forms of iron, therefore significant amounts of elemental iron (Fe°) must have been present in MI, extracted from both extruded and unextruded mixes. Analysis of MI by bathophenanthroline reaction indicated there were nearly five times more Fe^{+3} than Fe^{+2} ions in the unextruded mix. The extrusion process appeared to have reduced Fe^{+3} ions to Fe^{+2} ions, because the MI from extruded material had about twice the amount of Fe^{+2}. It should be pointed out that, when analyzed by the bathophenanthroline reaction, starting EI yields nearly 100% Fe^{+2} ions. Therefore, the extent of conversion of EI (Fe°) into Fe^{+2} ions during extrusion processing cannot be determined by this technique, because the bathophenanthroline reaction cannot distinguish between Fe° and Fe^{+2} ions. Unexpectedly, the addition of both $FeSO_4$ and Fe_2O_3 decreased appreciably the amount of MI in both unextruded and extruded samples as compared with control, suggesting that these iron sources promote the interaction of MI and cereal mix components.

An attempt was made to study the EI and MI by characterizing iron and oxygen contents by ESCA. This is a technique for studying the energy distribution of electrons ejected from a material that has been irradiated with a source of ionization radiation, such as X-rays. It is considered a useful technique to provide quantitative information about binding energies (eV), changes in valence states that involve the atom as a function of its chemical environment (Yin and Adler 1978). Unfortunately, only the exterior 20 or so angstroms (Å), i.e., almost vanishingly small amounts of the test sample, can be studied at a time (Siegbahn et al 1967). The comparison of ESCA data on binding energies for iron and oxygen elements from EI, MI, and rust showed (Table II) that the top layers of all three iron samples had been oxidized more or less to the same extent, indicating, as expected, the susceptibility of EI to oxidation to Fe^{+3} form during handling and processing.

ESCA has been refined to remove successive surface layers (about 20–50 Å at a time) by using ion-sputtering to determine the in-depth composition (Yin and Adler 1978). However, the attempts to follow this approach did not succeed because of various technical difficulties. Ideally, it should have been possible to quantify the extent of oxidation of EI due to processing and other conditions.

The distribution of nonmetallic iron in aqueous slurries was generally not affected by added iron, except in unextruded mixes or when certain additives were in extruded samples (Table I). For example, the addition of all three iron sources increased the soluble iron in unextruded mixes as compared with control. The effects of additives were selective in extruded samples. The addition of AA increased soluble iron relative to the control only in the case of added EI. The addition of AO with and without AA increased soluble iron in presence of additional $FeSO_4$. Nearly all the added $FeSO_4$ and Fe_2O_3 resulted in precipitated or insoluble iron. In the case of EI, even in unextruded mix, there was considerable interaction with the cereal mix, as indicated by the increase in both insoluble and soluble iron over the control. ESCA data (Table II) indicated that the outer layer of EI had been oxidized to Fe^{+3} form, even before it was added to the mix. The Fe^{+3} form, which has extremely low ($\sim 10^{-18}$ *M*) water solubility at neutral pH (Forth and Rummel 1973, Spiro and Saltman 1974), can nevertheless react with food. Little information is available about the nature of precipitated or insoluble iron. Iron is capable of reacting or complexing with various food components (Nelson and Potter 1979, Rosanoff and Kennedy 1982, Platt and Clydesdale 1984) both under neutral conditions and under simulated gastrointestinal pH conditions. However, the exact significance and the role of precipitated or insoluble iron in human nutrition is still not well understood. The majority of soluble iron in aqueous slurry (pH 7.0) was found in the Fe^{+3} form, as suggested by Milazzo and Caroli (1978).

TABLE II
Electron Binding Energies of Iron Samples

Sample Description	Binding Energies (eV)	
	Iron	Oxygen
Electrolytic iron	712.0	532.0
Metallic iron	712.3	531.3
Rust (stainless steel)	712.6	532.7
Iron (Fe°)[a]	710.0	...
Oxygen (O)	...	532.0

[a]From Siegbahn et al 1967.

A comparison of iron distribution from three iron sources, under simulated stomach conditions, showed that extruded material in all cases had more soluble iron than did unextruded mix. As indicated by the oxidation-reduction potentials (Milazzo and Caroli 1978), the majority of soluble iron (pH 1.5) was in Fe^{+2} form in the extruded material, whereas Fe^{+3} ions predominated in the unextruded mix. No suitable explanation is available for this remarkable difference between the two sets of samples. Perhaps the interactions between various ingredients during extrusion cooking reduced some of the Fe^{+3} form into Fe^{+2}. Similar observations were made in our laboratory between unbaked and baked bread products (Kadan and Ziegler 1986).

AA is known to be a major enhancer of iron absorption in cereal foods (Sayers et al 1973, Bothwell et al 1979, Hallberg 1981). Nojeim and Clydesdale (1981) showed that AA favored iron ionization at pH values from 2.7 to 6.2 and favored Fe^{+2} valence state at pH 2.7. Other organic acids, such as citric acid, and antioxidants, such as BHA, can also affect iron absorption, even though their relative roles remain to be defined.

Under simulated stomach conditions, the iron distribution data showed that adding either organic acids or antioxidant increased soluble iron when added with EI and $FeSO_4$ but not with Fe_2O_3. The effect tended to be additive (i.e., the addition of Tenox R with AA had more soluble iron than AA alone). It is not clear why the addition of Fe_2O_3 alone or even in combination with organic acids and antioxidant did not significantly affect the soluble iron. Poor solubility of Fe_2O_3 in aqueous solution is well known, but in the presence of organic acids and low pH of simulated stomach conditions one would expect a significant increase in its soluble form (Spiro and Saltman 1974). Therefore, it is hypothesized that added Fe_2O_3 irreversibly complexed with certain cereal mix components during extrusion cooking, but the nature of such complexes is a matter of conjecture. As indicated by Milazzo and Caroli (1978), most of the soluble iron was in Fe^{+2} form under low pH of simulated stomach conditions. Indirectly, it can be inferred that any increase in soluble iron or the Fe^{+2} ion under simulated stomach conditions due to any additive or processing condition could result in increased iron bioavailability from the food system.

In conclusion, the results of this study showed that the addition of both EI and $FeSO_4$ into an extruded cereal food resulted in increase in soluble iron under simulated stomach conditions. For these iron sources, extruded food always had more soluble iron than the unextruded but conditioned mix. Fe^{+2} ions predominated under acidic stomach conditions but Fe^{+3} predominated under neutral pH conditions. The addition of organic acids and an antioxidant tended to increase both soluble iron and its Fe^{+2} form under simulated stomach conditions. The addition of Fe_2O_3, with or without additives, did not seem to affect soluble iron.

EI was more sensitive to the presence of various additives. The addition of organic acids and antioxidant promoted interactions with the cereal mix, i.e., more metallic iron was recovered in the presence of additives. ESCA studies indicated that the outer surface of EI is essentially oxidized to Fe^{+3} form. It remains so in extruded food, with or without additives. The recovery of MI and its iron forms also showed that the interior of iron particles essentially stays in Fe° form. The extent of oxidation of the outer

surface could not be quantified because of various technical difficulties.

ACKNOWLEDGMENTS

We thank Ruth R. Benerito and Oscar Hinojosa for ESCA analyses and interpretations.

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[Received March 20, 1986. Accepted April 6, 1987.]