EVALUATION OF IRON VALENCE STATE ALTERATIONS IN THERMALLY PROCESSED LIQUID MODEL SYSTEMS

by

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Human Nutrition and Foods

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September 1984

Blacksburg, Virginia

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(Abstract)

A glucose glycine liquid model system fortified with 24 ppm of iron as either ferrous sulfate, ferric orthophosphate or ferric orthophosphate + 110 ppm ascorbic acid was thermally processed as 240, 250 and 260°F for 3, 6, 9, 12 and 15 minutes. Levels of elemental, nonelemental, soluble, total ionic and ferrous iron were measured for the model systems at each processing parameter. Data from the iron profiles were evaluated for the effect of iron salt; ascorbic acid; glucose and glycine, and processing temperature and time on changes in the iron chemistry during processing.

The ferrous sulfate model system resulted in significantly higher levels of soluble and ferrous iron. Soluble iron from the ferric orthophosphate model system increased significantly with the addition of ascorbic acid. The presence of glucose and glycine prevented formation of insoluble iron hydroxides during processing of the ferrous sulfate system, promoted solubilization and ionization of iron for the ferric orthophosphate system and restricted the enhancing effect of ascorbic acid on the ferric orthophosphate profile.

The interaction of the iron salt with the model system was stimulated by the application of heat. The insignificant correlation between process lethality values and the iron profile for each iron salt

indicated that changes in the iron profile were time and temperature dependent. Kinetic parameters were calculated for all three model systems. The ferric orthophosphate iron profile was less sensitive to temperature change than either of the other two model systems. Evaluation of samples at sequential time intervals during the processing treatment allowed for a better understanding of the reaction mechanisms that occurred during processing which brought about a change in the iron profile of each model system.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following people for their encouragement and input into this research project:

My advisor, Dr. Miriam Saltmarch, and the members of my Graduate Committee, Drs. Janette Taper and Anthony Lopez;

John Chandler and especially Keith Krisa who helped me tremendously in the processing of my samples;

Carolyn Harris and Donna Soul for their assistance in the laboratory and with the computer;

Sherry Terry for her kindness and patient assistance;

Dr. Jean Phillips for her wisdom and inspiration in all aspects of my graduate career;

And, of course, to my very special friends, Sharon Cameron, Kathy Reynolds and Inger Hoelzle for helping me maintain my sanity throughout the duration of this study.

A very special thank you goes to my parents, Rita and Edwin Trayner; my brothers, Chipper and Andrew; and my fiance, David Monz, whose constant love, encouragement, understanding and support have made this all possible.

* * *

I would like to dedicate this thesis to my grandmother, Mae Perrine, in deepest appreciation for all that she has done and been to me, and as an expression of the love and admiration I have for her.

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INTRODUCTION

Iron is an essential mineral necessary for the maintenance of cellular activities vital to mammalian respiration and oxygen transport. The adult human body normally contains between 3-5 grams of iron. Most of this is recycled through the body for future use, although small amounts are lost through bleeding and normal excretory processes. Women sustain additional losses due to menstruation, pregnancy and lactation. To assure the presence of iron containing compounds the body must therefore maintain an iron balance by varying the amount of iron actually absorbed from the diet (Kuhn et al., 1968).

Intestinal absorption or biological availability of iron is influenced by several factors including bodily need, the chemical nature and amount of iron in the ingested food and a variety of other dietary conditions. It is presently assumed that a typical diet contains 6.0 mg of iron per 1000 kcalories and that an average 10% of the total iron content in a given food system is actually absorbed by the body (Morck et al., 1981). Iron deficiency anemia is defined as a hemoglobin level of less than 10 mg/100 ml. Anemia has long been recognized as a public health problem in both industrialized and developing countries. the groups most affected are mature menstruating women, particularly during pregnancy and lactation, adolescents and infants (Council on Foods & Nutrition, 1968).

The rapid growth rate during infancy necessitates that iron requirements in proportion to food intake be greater than at any other

time in life (Table 1). While there is currently insufficient data to determine the nature of the iron storage and utilization abilities of infants, the needs are estimated to be 1.0 - 1.5 mg/day of absorbed iron and concurrent requirements are set at 1.5 mg/kg body weight, up to 15 mg total (Council on Foods & Nutrition, 1968).

Human milk contains approximately 1.0 mg iron/liter of which the infant is normally able to absorb 50 percent (Weber et al., 1982). On the other hand only a fraction of iron added to a liquid formula is available to the infant for absorption. Since liquid formulas are frequently the only source of iron for infants who are not breast fed, appropriate fortification of these formulas with an easily assimilable iron compound is essential. Standards recently proposed by the Committee on Nutrition require that infants receive 1.0 mg iron/100 kcal of an infant formula diet (Anderson et al., 1982). Iron fortified formulas currently contain 12 mg iron/liter as ferrous sulfate. Therefore a liter of infant formula, which is 670 kcals, contains 1.79 mg iron per 100 kcals of formula.

Efforts to alleviate iron deficiency anemia have focused on increasing the amount of available iron in the diet through fortification of selected foods. In choosing an iron source for fortification, both functionality or product compatibility and bioavailability of the compound have to be taken into consideration. An appropriate iron compound should generally fulfill the following criteria (Zoller et al., 1980):

Table 1. RDA Values for Iron Requirements Based on a 10% Absorption Rate

Men	10 mg
Women	18 mg
Infants	.5-1.5 mg/kg body weight (up to 15 mg total)

1. The compound should not discolor the food product over long periods of storage and under extreme conditions of humidity and temperature.

2. The compound should not be present in quantities sufficient to produce off flavors in the food product.

3. The compound should be nutritionally available after processing and long periods of storage.

4. The addition of a selected compound to a given food system should be economically feasible.

In general, the compound selected for the fortification of a food system should not have any adverse affects on product quality and yet still be nutritionally safe and assimilable. However, many iron sources which exhibit the highest biological availability adversely affect the product quality. Common problems include the promotion of lipid oxidation, color changes and the development of off flavors (Lee and Clydesdale, 1979). This results in the fortification of many food products with product compatible, nonreactive iron compounds which are of poor bioavailability.

Research indicates that the biological availability of iron can be significantly altered as a result of its interaction with food components during processing (Clemens and Mercurio, 1981; Lee and Clydesdale, 1979; Thuer et al., 1971 & 1973). Changes in the physical and chemical characteristics of iron occur during storage as well (Clemens, 1982; Hodson, 1970).

This research was undertaken to gain a better understanding of the mechanisms of interaction affecting iron chemistry in a food system during processing of that system. The purpose was to relate these

mechanisms to the prediction of the value of a food system as a suitable vehicle for iron fortification with an iron compound which will be of optimal availability. Reaction kinetics and the Arrhenius equation were used to quantify the effect of thermal processing on iron chemistry.

The specific objectives of this study were to:

1. Develop a model system to be evaluated for the effect of component interaction on the iron chemistry for the model system fortified with ferrous sulfate, ferric orthophosphate and ferric orthophosphate + ascorbic acid.

2. Process the iron fortified model system at three temperatures for five times and determine the lethality values for each processing condition.

3. Evaluate the effect of component interaction including iron salt and ascorbic acid, during processing on the iron chemistry profile as determined by the chemical assay adapted from Lee and Clydesdale (1979a).

4. Utilize the processing parameters to kinetically evaluate the effect of thermal processing on the iron chemistry profile for each of the iron and iron and ascorbic acid fortified model systems.

REVIEW OF LITERATURE

Iron Chemistry

Ferric (Fe^{+3}) and ferrous (Fe^{+2}) iron are the only two oxidation states of this mineral which are stable in an aqueous environment and therefore are the only states of iron which are found occurring naturally in foods (Lee and Clydesdale, 1979). Elemental iron (Fe^{0}) may be found in food as a food additive. A basic understanding of iron chemistry is advantageous for the interpretation of the behavior of these iron compounds in food systems.

The atomic number of iron is 26, making it a transition metal with electrons falling in the 3d atomic orbital (Brown and LeMay, 1977). Ferric iron has five valence electrons while ferrous iron has six valence electrons. The electronic structures of these two oxidations states make them compatible as electron acceptor ions for ligand electron donor atoms. In general, the formation of an octahedral complex is the preferred stereochemical orientation obtained from the five or six ligand donated electrons. The strength and degree of chelation ultimately depends on the strength of the bond which forms between the metal and the ligand. Factors influencing bonding and complex formation include stereochemistry or spatial arrangement, ligand/metal concentrations and competitive interactions, and, the pH of the system being considered (May and Williams, 1980). The strongest complexes formed with ferric (Fe^{+3}) iron tend to be with oxygen donor ligands such as phenols, carbohydrates or phosphates, whereas ferrous

(Fe^{+2}) iron has a high affinity for ligands which can delocalize iron electrons into other vacant orbitals such as nitrogenous compounds or the diimine ligand orthophenanthroline (Spiro and Saltman, 1974).

In acidic aqueous solutions ferrous and ferric ions occur in hydrated forms as $Fe(H_2O)6^{+2}$ and $Fe(H_2O)6^{+3}$ respectively. As the pH is raised and the hydrogen ion concentration subsequently decreased, the water molecules give up protons to form the corresponding iron hydroxides, $FeOH_2$ and $FeOH_3$ (Lee and Clydesdale, 1979). Therefore the hydrated forms of iron are stable only under acidic conditions and the solubility of these compounds is limited, particularly for the ferric form. The maximum solubility of $Fe(OH)_3$ is 10^{-17}M at pH 7 whereas at this neutrality the solubility of $Fe(OH)_2$ is 10^{-1}M (Spiro and Saltman, 1974). As the pH is increased both iron compounds eventually form a gelatinous precipitate (May and Williams, 1980).

Iron Absorption

The body's capacity to secrete iron is limited and therefore iron balance is regulated primarily by iron absorption from the gut (McCance and Widdowson, 1937). The physiological mechanisms of iron absorption have been reviewed extensively (Forth and Rummel, 1973; Kuhn et al., 1968; and Narasinga Rao, 1981). Narasinga Rao (1981) has outlined three major phases in the absorption of iron as follows:

1. The intraluminal phase - food is digested by gastric and pancreatic enzymes and iron is released in a soluble form.

2. The mucosal phase - iron is taken up by the mucosal cells and transported across to the serosal side or retained and stored as ferritin.

3. The corporeal phase - iron is taken up by transferrin in plasma on the serosal side of the mucosal cell and carried to the liver and the haemopoietic tissues.

The critical factor in iron absorption from a particular food system is the maintenance of iron in a soluble and ionized form, particularly when subjected to the harsh pH changes throughout the gastrointestinal tract (Jacobs et al., 1966). Therefore the environment prior to mucosal absorption and transfer is of greatest importance. Iron alone or in its hydrated form is relatively insoluble and thus its absorption is low across the mucosal membrane and is largely dependent on solubility (Clydesdale, 1983). Food systems influence absorption because of the nature of the food environment and the presence of various chelating agents which interact to enhance or inhibit iron absorption in the gut (Clydesdale, 1983).

Physicochemical Properties of Food Affecting Iron Chemistry and Bioavailability

As noted earlier the solubility characteristics of iron in food systems are a function of reactivity within the food environment as well as the degree of chelation of iron compound with complexing agents also present in the food (Clydesdale, 1983). The amount of iron available from a food system is dependent on the relative concentration of soluble iron and low molecular weight iron complexes, macromolecules, and the formation of insoluble precipitates, all of which regulate

absorption (Smith, 1983). Therefore ligands which form more soluble complexes (enhancers) tend to increase bioavailability, while those which form more insoluble complexes (inhibitors) tend to have the opposite effect (Clydesdale, 1982). Examples of soluble low molecular weight iron compounds include ferrous sulfate, ferrous fumarate, ferric citrate, and iron ascorbate complexes. In contrast, insoluble or poorly available substances include iron oxide, iron hydroxide and ferric phosphate compounds (Brise and Hallberg, 1962; Pla and Fritz, 1970; and Smith, 1983). Common food components that are effective ligands include: carbohydrates, proteins and amino acids, carboxylic acids, and phosphates. These ligands can act as either enhancers or inhibitors within a food system. The nature of some of these food components as ligands which are relative to this study will be discussed here.

Carbohydrate/Sugar - Iron Complexes. The chelation of iron by sugars has been studied extensively over the last several years (Amine and Hegsted, 1974; Bates et al., 1973; Charley et al., 1963; and Stitt et al., 1962).

Charley et al. (1963) verified that fructose and other reducing sugars form stable complexes with iron over a range of pH treatments by measuring the spectral characteristics of each complex as well as the titration values and the redox potentials due to the displacement of hydrogen ions from the sugar molecule during the formation of a sugar chelate. The relative sequestering abilities of polyols and sugars for

ferric iron were found to be: fructose > sorbitol > glucose = galactose = maltose = lactose > sucrose > ribose > erythrose (Charley et al., 1963). High concentrations of the sugar are necessary for complex formation.

The effectiveness of the sugar chelate to facilitate metal ion transport is directly related to the net charge and the molecular weight of the complex (Stitt et al., 1962). Stitt et al. (1962) demonstrated the formation of a highly stable, low molecular weight ferric-fructose complex that readily enters into the mucosal cells of rats. Amine and Hegsted (1974) indicated only a slight improvement in ferric iron absorption from lactose and sucrose, a decrease in the presence of starch, and no effect due to glucose.

Bates et al. (1973) attributed the ability of fructose to act as an enhancer for ferric iron absorption as due to the reduction of ferric iron into the more soluble ferrous form. However sorbitol, a non-reducing sugar, was found to enhance iron absorption, indicating complex formation as a likely vehicle for iron absorption (Loria, et al., 1962).

Bachran and Bernhard (1980) indicated the complicated nature of iron-sugar complex formation in a study of the interaction of ferrous iron with lactose. These workers reported that the addition of NaOH to solutions containing lactose and ferrous chloride ($FeCl_2$) caused the precipitation of a lactose iron gel composed of an insoluble lactose-$Fe(OH)_2$ complex, insoluble $Fe(OH)_2$ and water. There was also the formation of a soluble lactose-Fe(OH)2 complex as well.

Protein/Amino Acid Iron Complexes. Amino acids in the diet have been shown to influence iron absorption (Kroe et al., 1963; Van Campen and Gross, 1969; and Van Campen, 1973). Kroe et al. (1963) administered histidine, asparagine, glutamic acid, glutamine, methionine, proline, phenylalanine and serine into the intestine of male rats and reported a significant increase in serum iron levels from radioisotope (Fe^{59}) labeled ferrous sulfate. Van Campen and Gross (1969) assessed the effect of specific amino acids on the intestinal absorption of radio iron (Fe^{59}) supplied in the ferric form. Of the six amino acids used, histidine and lysine increased absorption significantly whereas glutamine, glutamic acid, methionine and glycine did not. The amino acids enhanced iron absorption only when administered in the same solution with the iron, indicating that a chelation mechanism was involved. Van Campen (1973) later reported this chelation ability was subsequently lost when reactive or ionizing side groups were removed from the amino acid.

Intact polypeptide chains retain the complexing ability of their constituent amino acids within the constraints of their spatial configurations. In two related studies, Nelson and Potter (1979, 1980) investigated the ability of casein to bind iron from food grade ferrous sulfate and ferric polyphosphate and measured the distribution of iron between the soluble and insoluble protein fractions. Later these workers (Nelson and Potter, 1980) assayed the bioavailability of iron from this protein-iron complex by in vivo rat hemoglobin repletion and in vitro simulated digestion techniques. Their results indicated the

tendency of casein to bind significant amounts of iron from ferrous sulfate and ferric pyrophosphate with a greater amount of ferrous iron bound to the insoluble fraction while more ferric iron was bound to the soluble protein fraction. Relative iron binding and protein solubility were pH dependent, and the addition of ferrous sulfate further reduced protein solubility (Nelson and Potter, 1979). The casein-ferrous iron complex had an RBV (relative biological value) compared to ferrous sulfate = 100) of 82, while the casein-ferric complex, from the soluble protein fraction, had an RBV of 21 (Nelson and Potter, 1980).

Whey protein was precipitated in the presence of ferripolyphosphate (ferric chloride and sodium polyphosphate) under acidic conditions. A ferripolyphosphate-whey protein powder was subsequently formed from the precipitate (Jones et al., 1972 and Amantea et al., 1974). The RBV of this resulting whey powder was 92 when measured by hemoglobin repletion assays with both chicks and rats. Jones and coworkers (1975) used this powder to fortify a whole milk product without the occurrence of lypolytic rancidity. The product was scored favorably when judged by taste panelists.

Iron-Ascorbate Complexes. Ascorbic acid is an effective nonheme iron absorption enhancer (Clydesdale, 1982). Several researchers, (Bjorn-Rasmussen and Hallberg, 1969; Brise and Hallberg, 1962; Callender et al., 1970; Cook and Monsen, 1977; and Monsen, 1982) have reported that increasing levels of ascorbic acid in a test meal result in a corresponding increase in iron absorption from both intrinsic and extrinsic

iron present in that meal. Callender et al. (1970) fed intrinsically labeled (Fe^{59}) hen eggs in a breakfast meal composed of bread, butter, jelly, eggs and coffee to a group of 26 subjects. When 100 mls of orange juice was added to this, mean iron absorption was increased 280% over the control meal.

In another study Bjorn-Rasmussen and Hallberg (1974) added various levels of ascorbic acid (0-200 mg) to meals of maize and 4.5 mg of iron. They found that iron absorption was increased as levels of ascorbic acid were increased in the test meal. Brise and Hallberg (1962) found this relationship to show a marked increase at a level of 200 mg of ascorbic acid (+30 mg of iron as ferrous sulfate). In a similar study, Cook and Monsen (1977) showed that the increase in iron absorption appeared to be logarithmically related to the ascorbic acid content of the ingested food. They also found that enhancement of absorption was only effective when iron and ascorbic acid were present in the stomach simultaneously. Lynch and Cook (1980) reported that ascorbic acid had minimal effect on ferrous iron and speculated that the logarithmic relationship with ferric iron compounds was due to the effect that ascorbic acid had in rendering ferric iron soluble of the alkaline pH of the duodenum.

According to Clydesdale (1982) the most important factors involved in the ability of ascorbic acid to enhance iron absorption include:

1. pH
2. complex formation
3. oxidation reduction potential.

It is the interrelationship of these three factors in a given food environment which ultimately determines the chemistry and bioavailability of iron in that food system (Smith, 1983; and Clydesdale, 1983). The interaction of these factors is delineated in the enhancement of iron absorption by ascorbic acid and will therefore be discussed here.

Oxidation reduction coupling of Fe^{+3} and Fe^{+2} is dependent upon pH (Clydesdale, 1982). The standard reduction potential for the reaction:

$$Fe^{+3}\ (aq) + e^{-} \rightarrow Fe^{+2}\ (aq) \qquad (25°C)$$

is +770 MV (Nojeim and Clydesdale, 1981b). Therefore, when ferric iron is present in a system with a redox potential less than +770 MV reduction is spontaneous. Most foods have a standard reduction potential of 400 MV or slightly less, creating a favorable environment for spontaneous reduction (Clydesdale, 1982). However, the reduction in more basic solutions:

$$Fe(OH)_3\ (solid) + e^{-} \rightarrow Fe(OH)2\ (solid) + OH^{-}$$

has a standard reduction potential of -560 MV indicating nonspontaneity in foods. Since solubility and valence are interrelated and are important in determining bioavailability, the pH and reduction potential of the food environment are therefore important as well.

Nojeim and Clydesdale (1981a) suggested that the reduction potential and dissolved oxygen within a food product may affect iron ionization thereby altering the iron profile. The same authors (Nojeim and Clydesdale, 1981b) also analyzed the effect of pH and ascorbic acid levels in both model systems and in foods. They found in all cases that the pH level of a food is a good predictor of the iron chemistry

profile. The presence of ascorbic acid, which has a reduction potential of +440 MV, favored ionization or conversion of iron to the ferrous valence state. However, as the pH was increased from 2.7 to 6.2 some of the ionized iron was oxidized and formed a soluble complex with ascorbic acid. Clydesdale (1982) proposed that ascorbic acid can act as a complexing agent and oxidize iron, or as a reducing agent, depending on the food system and the nature and concentration of the iron compound present.

Gorman and Clydesdale (1983) examined the effect of a food system on an iron abscorbate complex by measuring the stability of that complex in the food. Fe^{+2} ascorbate, and Fe^{+3} ascorbate were titrated with 0.1N NaOH and the thermodynamic stability constant was determined based on the dissociation reaction: $HA \rightarrow H^{+} + A^{-}$. The stability constants for the Fe^{+2} and Fe^{+3} ascorbate complexes were 7.69×10^{-3} - 6.95×10^{-2} and 1.90×10^{3} - 2.61×10^{4} respectively. They concluded that the Fe^{+3} ascorbate complex was more stable and was soluble in food systems.

Changes in Iron During Processing

Several studies have indicated that changes in the chemical form of iron salts and their subsequent bioavailability occur as a result of processing and storage. The stability of iron salts was first questioned by Hodson (1970) in a study involving weight control dietaries, meal substitutions popular in the 1960's and 1970's. It was found that ferric pyrophosphate salts commonly added to formulas for high quality marketing purposes (i.e. optimum flavor and color) were slowly solu-

bilized over a two to five month storage period. The ferric was gradually reduced to the ferrous form indicating a change in the valence state which influenced the solubility of the added iron source over time.

Theuer et al (1971) used the standard rat hemoglobin repletion test to study the effect of heat sterilization processing on the availability of sodium ferric pyrophosphate, ferric pyrophosphate and ferrous sulfate added to a soy based liquid infant formula. This study confirmed the effect of thermal heat processing on the bioavailabilities of ferric pyrophosphate compounds. It was found that while processing had little effect on the availability of ferrous sulfate, the bioavailabilities of ferric pyrophosphate and sodium ferric pyrophosphate were increased substantially as a result of sterilization. In a follow up study, Theuer et al. (1973) used a milk based infant formula as the fortification vehicle for the same iron salts. This time a markedly higher initial relative biological value (RBV) value was observed which was again accompanied by a substantial increase in bioavailability of iron as a result of processing. The higher initial values may be indicative of a milk enhancing factor present in milk solids or more probably a soy inhibiting factor restricting the solubilization and availability of the iron salt (Cook et al., 1981).

Shah et al. (1979) reported similarly high RBV values for iron as sodium iron pyrophosphate added to soy based infant formulas and also correlated the high values to thermal processing. By comparing RBV values measured from rat hemoglobin repletion assays of processed infant

formulas to those of breakfast and infant cereals fortified with the same iron salt, Shah and coworkers concluded that the increase in bioavailability of an iron salt was primarily due to heat treatment. In cases where no heat treatment was involved or where iron was added after the product was heated, little increase in iron bioavailability was observed.

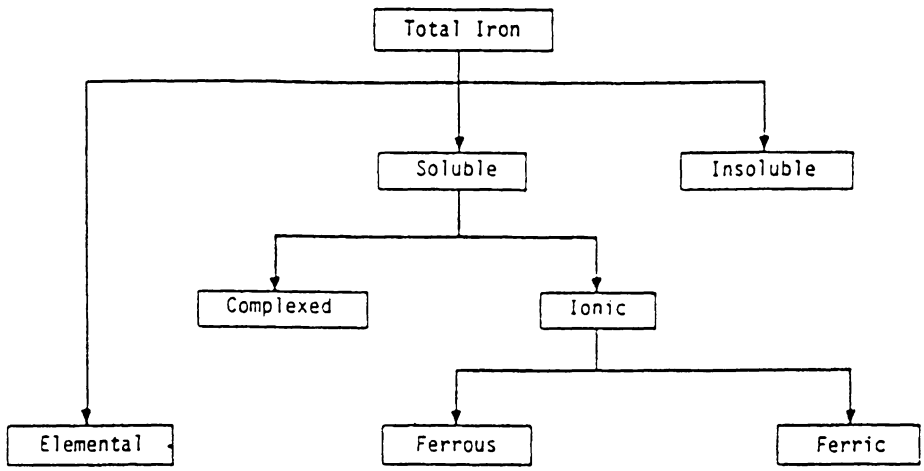
Sayers et al. (1973), intrinsically (Fe^{55}) and extrinsically (Fe^{59}) labeled maize, wheat and soy with and without various levels (50-250 mg) of ascorbic acid, then fed these foods to 66 volunteer subjects. The addition of ascorbic acid resulted in a corresponding increase in iron absorption as has been discussed. However, when ascorbic acid was added and the food subsequently processed no increase in iron absorption was found when the product was baked but a substantial increase in absorption was found when the product was boiled. These authors hypothesized that the efficacy of ascorbic acid as an enhancer was lost due to oxidative destruction at the high temperatures required for baking.

All studies previously cited assessed the bioavailability of individual iron compounds alone and when consumed as part of a meal and all reported correlation between the physical and chemical nature of an iron salt and its actual bioavailability at the time of consumption. Lee and Clydesdale (1979) recently devised a method to quantitatively and simultaneously determine the chemical forms of iron present in a food. They assumed that the chemical nature of iron in a food system is directly correlated to the usefulness of a food as a source of iron.

Their procedure identifies an iron profile for iron present in food by evaluating specific levels of elemental, ferric and ferrous iron and the nature of these valence states as either soluble, insoluble or complexed (Figure 1).

Lee and Clydesdale (1980a) used this procedure to look at the effects of spray drying and freeze drying followed by storage on an ascorbate containing fruit flavored beverage fortified with various iron compounds. They found that spray drying, but not freeze drying, had a significant effect on the iron profile of the beverage generating varying levels of elemental, ferrous, and ferric iron. The final iron profile depended on the iron salt used. Changes generally favored ferrous iron formation possibly due to the presence of ascorbic acid. The baking of a flour based biscuit product (Lee and Clydesdale, 1980b) on the other hand generated large amounts of insoluble iron regardless of the iron salt used in fortification.

Other subsequent studies (Clemens and Mercurio, 1981; Clemens, 1982) utilized the iron assay procedure to further examine the effects of specific types of processing and storage treatments on the iron profile of individual foods. the amount of soluble iron in a food just prior to ingestion was found to correlate closely with the bioavailability of iron present in that food using the rat hemoglobin repletion assay (Clemens & Mercurio, 1981; Clemens, 1982). Studies were undertaken to determine the effect of processing (Clemens and Mercurio, 1981) and storage (Clemens, 1982) on iron powders added to a liquid milk-based product. As in the previous work reported, levels of iron



[a]From Lee and Clydesdale, 1980a.

Figure 1. Classification of the Chemical Forms of Iron[a]

present were measured before and after processing. Complete iron chemistry assays (Lee and Clydesdale, 1981) were compared with relative biological values (RBV) as determined by rat hemoglobin repletion assay. Results showed that while retort processing substantially increased levels of ferrous iron, and subsequent iron bioavailability in products fortified with both carbonyl iron and elemental iron, there was no significant change in the iron profile and bioavailability of the ferric orthophosphate fortified milk-based product, even following storage. These results differ from the earlier work of Hodson (1970) and Theuer et al. (1971, 1973) in which much of the iron from the ferric based iron salts were solubilized and reduced to the ferrous form during processing and storage. This incongruence could be due to differences in the methodologies used and deserves further attention.

In general, the value of an iron salt as an available compound within a food source correlates closely with the amount of soluble iron present in that food just prior to ingestion. Researchers have indicated that the chemistry and subsequent bioavailability of iron can be significantly altered as a result of interaction of the iron with food components during processing and storage. Several mechanisms of interaction have been studied and it appears that the diverse nature of various food systems, as well as processing and storage have multiple components operating simultaneously to affect the chemical nature of an added iron compound.

Reaction Kinetics in Food Systems

According to Hill and Greiger-Block (1980), the initial objective of kinetic studies is the development of a mathematical model to define the rate of a reaction as a function of experimental variables. The rate constants determined can then be used to predict the composition of a system as a function of time (Hill and Greiger-Block, 1980), or, the extent of reaction for any time (Labuza and Riboh, 1982). A recent interest in quantitative approaches to the analysis of food quality deterioration during processing and storage has been motivated by a growing consumer awareness and by mandatory government requirements such as nutrition labeling (Saguy and Karel, 1980). Several studies have utilized Arrhenius kinetics for the prediction of nutrient losses in foods during processing and storage (Labuza, 1982).

In an effort to determine the ability of ascorbic acid to maintain a complex with iron in a soluble form, and to measure the rate of iron exchange from this sample with a mucosal/serosal acceptor, Gorman and Clydesdale (1984) utilized the thermodynamic stability constant and the kinetic stability constant respectively. The thermodynamic stability constant measures the strength of association between the metal and the ligand using the Henderson-Hasselbach equation and the relative complex concentration. The kinetic stability constant measures the half-time of iron exchange between two complexes. Bates et al. (1967) have defined the kinetic stability constant as the time required to achieve half saturation of transferrin with iron when incubated with the iron complex under study. The kinetic stability constant has been documented as a

good predictor of iron bioavailability from a particular iron complex in the gut (Forth and Rummel, 1973). However, the kinetic stability constant provides little information about the interaction of the complex in a food system (Clydesdale, 1982).

METHODS AND MATERIALS

Infant Formula Model System

A model food system was formulated with a composition (protein, carbohydrate and fat) similar to that of a commercially processed infant formula (Table 2). Reagent grade ferrous sulfate (Mallinckrodt Inc. Paris, KY, Lot No. 5572) was added to the formula at a level of 24 mg iron/liter which is equivalent to the 24 ppm level currently used to fortify infant formulas made with iron (Anderson et al., 1982). The system was made by mixing the oil, protein and sugar into a fine paste, adding the paste to the water and stirring the entire sample for 15 minutes with a magnetic stir bar. Ferrous sulfate was added after 5 minutes of stirring. The sample was refrigerated overnight. The sample pH was recorded prior to processing and measured pH 6.5. Due to problems with this system, a modified solution system was adapted which was similar to parenteral solutions. This particular system was chosen because individuals needing parenteral solutions can be considered to be another population at risk for minerals such as iron.

Modified Solution System

A simplified model solution was made containing 18.77 g/liter H_2O (0.25 molar) glycine (Abbott Laboratories, Chicago, IL, Lot No. 39-121-KA) and 252.22 g/liter H_2O (1.4 molar) glucose (Sigma Chemical Co., St. Louis, MO, Lot No. 82F-0588). Three separate batches of the solution were prepared containing 24 ppm iron as ferrous sulfate, 24 ppm

Table 2. Comparison of Model Infant Formula To A Commercially processed Infant Formula

Nutrient	Model System Percent Ingredient	Commercially Processed Infant Formula[a] Percent Ingredient
Fat	7% soybean oil	6.8% soy and coconut oil; mono and diglycerides
Carbohydrate	13% sucrose	13.6% lactose
Protein	4.5% sodium-caseinate	2.8% casein
Water	75.5%	76%
Other[b]	-	0.8% vitamins minerals carrageenan

[a]Similac, Ross Laboratories, Columbus, Ohio

[b]Both products were fortified with 24 ppm iron and 110 ppm ascorbic acid as specified. Model systems contained ferrous sulfate, ferric orthophosphate or ferric orthophasphate + ascorbic acid. Similac contained ferrous sulfate + ascorbic acid.

iron as ferric orthophosphate (Joseph Turner and Co. Chemicals, Teaneck, NJ, Lot No. 2941) and 24 ppm iron as ferric orthophosphate + 110 mg/liter ascorbic acid (Sigma Chemicals Co., St. Louis, MO, Lot No. 43F-0855; 20-200 mesh). Sample pH's measured pH 5.6, 5.6 and 4.5 respectively. All samples were refrigerated overnight prior to processing.

Control Systems

Model solution samples with ascorbic acid levels of 0 mg and 110 mg/liter containing no added iron were processed and assayed as blanks. Water solutions containing 24 ppm iron as ferrous sulfate, 24 ppm iron as ferric orthophosphate and 24 ppm iron as ferric orthophosphate + 110 mg/liter ascorbic acid were processed and assayed as controls.

Thermal Processing Treatments

Aliquots of 7 ml of sample solution were pipetted into 8 ml Wheaton 200 glass vials (Fisher Scientific, Fairlawn, NJ) leaving minimal head space within the container. Glass vials were used in place of metal cans to minimize the effect of iron interaction between the food system and the canning container. Ten vials containing sample were processed for each treatment variation. All samples were subjected to thermal sterilization in the mini retort processor located at the VPI&SU Department of Food Science and Technology pilot processing laboratory.

The internal temperature of the canned sample was measured during processing with a type T (copper-constantan) plastic tube and rod

thermocouple (O.F. Ecklund, Cape Coral, FL) placed .50 cm from the bottom of the vial. Positioning of the thermocouple was decided from a series of cold point determination tests conducted prior to processing (Appendix A) to locate the area of slowest heating (Lopez, 1981). The vials were positioned upright into compartments of a wire mesh basket (8.9 cm x 15.2 cm) sized to fit the internal area of the processor barrel (10.2 cm x 22.2 cm). The temperature within the processor barrel was recorded by manually joined thermocouple wires attached to the upper and lower end of the container basket. Temperatures were recorded at 16 second intervals by the Omega Data Logger, Model 271 (Omega Engineering Inc., Stamford, CT).

All samples were processed at temperatures of 240, 250 and 260°F for 3,6, 9, 12 and 15 min. at each temperature. The processor barrel was heated to the designated temperature prior to loading. The time lapse for manual loading did not exceed 5 seconds. Process timing began when the retort temperature reached the processing temperature ($t \doteq 48$ sec). Samples were cooled in an ice bath immediately following processing. The significance of this set up, including vial size and the use of the mini retort processor, was to minimize the come up time and the cool down time during the processing procedure and allow for a more accurate determination of the lethality value (Fo) during steam processing time only.

Process Lethality Determination

The General or Graphical Method (Lopez, 1981 and Patashnik, 1953) was used to determine the lethality of F values obtained from the time temperature data for the sample vials. Temperatures recorded at equal (16 sec) time intervals were converted to equivalent lethal values using the lethal rates chart from Lopez (1981) and multiplying the value given for 1 minute by 16/60 fraction of time. The resulting lethal values were then summed together and the total process lethality or F value was recorded (Appendix B).

Chemical Analysis of Processed Samples

Processed samples were assayed in duplicate according to the procedure outlined by Lee and Clydesdale (1979a) for the levels of elemental, nonelemental, soluble, ionic and ferrous iron as follows:

Elemental Iron.

1. Stir together five vials or a total of 35 ml of sample for 15 minutes in a 100 ml glass beaker with a 3 cm magnetic teflon stir bar to magnetically extract elemental iron.
2. Remove stir bar from sample with non-metallic forceps and rinse with distilled deionized water.
3. Place stir bar in 30 ml concentrated HCl (American Scientific Products, McGow Park, IL) and allow to sit overnight.
4. Dilute solution to 100 ml and read on the atomic absorption spectrophotometer.

Nonelemental Iron.

1. Pour 10 ml of the sample into a 400 ml beaker and add 30 ml concentrated HCl.

2. Wet ash the mixture, heating over ceramic heating plate until completely charred.

3. Resolubilize ash with 100 ml of 30% HCl and allow to sit overnight.

4. Filter solution through Whatman #541 filter paper, collect filtrate, and read for nonelemental iron atomic absorption spectrophotometer.

Centrifuge (Sorvall RC-2) remaining sample (≐ 25 ml) in 50 ml centrifuge tubes at 11,000 rpm = 14,900 g for 10 minutes under nitrogen. Immediately collect supernatant for analysis.

Soluble Iron. Total soluble iron is measured as the iron remaining in solution after centrifugation.

1. Pipet a 10 ml aliquot of supernatant into a 100 ml volumetric flask.

2. Add 30 ml of concentrated HCl and bring to volume with distilled deionized water.

3. Hold sample overnight and read on the atomic absorption spectrophotometer.

Actual concentrations of individual elemental, nonelemental and soluble iron were measured in parts per million on a Perkin Elmer Atomic Absorption Spectrophotometer (Model 502) against Fisher certified reference standards in a 30% HCl solution.

Ferrous and Ionic Iron. Bathophenanthroline reagent (G. Frederick Smith Chemical Co., Columbus, OH, Lot No. G-3) was used to measure ionic iron because of its known ability to react exclusively with ferrous iron to form a deep-red colored complex (Lee and Clydesdale, 1979a; Lee and

Stum, 1960). Ferrous iron was measured directly. Total ionic iron (ferrous + ferric) was measured after reacting the sample with a standard reducing agent, hydroxylamine hydrochloride (Sigma Chemical Co., St. Louis, MO, Lot No. H-9876). Ferric iron was measured by difference (Ionic - Fe^{+2} = Fe^{+3}).

The bathophenanthroline procedure for the determination of ionic and ferrous iron was as follows:

1. Add 1 ml of the reducing agent hydroxylamine hydrochloride (10 gm to 100 ml volume with 50% EtOH) to one of two 60 ml separatory funnels.

2. Pipet 1 ml aliquot of sample supernatant to both separatory funnels.

3. Pipet 1 ml sodium acetate buffer, pH 4, to each separatory funnel. 41 ml of 0.2 M glacial acetic acid (Fisher Scientific Co., Fairlawn, NJ, Lot No. A-82) + 9 ml of 0.2 M sodium acetate (Fisher Scientific Co., Lot No. 794930) diluted to 100 ml volume with distilled deionized water.

4. Bring samples to 15 ml total volume with distilled deionized water.

5. Add 15 ml of bathophenanthroline (0.120 gm/100 ml of 95% ethanol) to each flask, shake, and allow to react for 30 sec.

6. Add 10 ml chloroform (G. Frederick Smith Co., Columbus, OH), shake and allow separation to occur.

7. Drain batho-chloroform complex into 25 ml volumetric flask and bring to volume with 95% ethanol.

Levels of ferrous and ionic iron were measured, in parts per million by relative depth of color on the spectrophotometer (Bausch and Lomb Spectronic 2000) at 433 nm. Standards were made from a stock iron solution consisting of Baker Analyzed Reagent Grade iron wire (J. T.Baker Chemical Co., Phillipsburg, NJ, Lot No. 120375).

Extraction of the bathophenanthroline iron complex with chloroform was necessary to prevent color formation due to the presence of small amounts of iron in the hydroxylamine hydrochloride solution (Lee and Clydesdale, 1979). The addition of sodium acetate buffer to the mixture was necessary to create a pH optimum for the bathrophenanthroline reaction to occur. A 30 sec reaction time was critical to allow minimum but uniform exposure of the sample to an altered pH (Platt and Clydesdale, 1984).

Procedure Modification for the Analysis and Infant Formula

A modification of the procedure outlined by Lee and Clydesdale (1979) was necessary for the determination of the chemical forms of iron in a protein containing infant formula because of the difficulty of complete protein precipitation by centrifugation alone. For the determination of soluble, complexed and ionic iron a precipitation procedure was added as follows (Clemens, 1984): An aliquot containing 30 ml of the infant formula was pipetted into a 150 ml beaker and 5 ml of concentrated HCl, 10 ml of 95% ethanol and 30 ml of deionized water were subsequently added with constant stirring. The ethanol was added to prevent reduction of the ionic iron. The solution was filtered through Whatman #541 filter paper. The supernatant was assayed for total ionic, ferrous, and soluble iron as above. Complexed iron was determined by dry ashing the precipitate and diluting with 100 ml of 30% HCl. This solution was read on the atomic absorption spectrophotometer for iron concentration in ppm.

Determination of Iron Profile

The instrumental values were converted into actual concentration values in parts per million. These values were subsequently recorded as percentages of the total iron present in that system. Values for elemental, nonelemental, soluble, ionic and ferrous iron were determined directly. Ferric, insoluble and complexed iron values were measured by difference (Table 3).

Kinetic Models for Iron Concentration

The kinetic parameters of soluble, ferrous and ionic iron concentrations were determined for the three sample formulas, ferrous sulfate, ferric orthophosphate and ferric orthophosphate + ascorbic acid, processed at different temperature conditions. A general kinetic scheme was used to define and predict the processing temperature on the iron chemistry profile in the model liquid system (Labuza, 1982).

Reaction order was determined by a graphical representation of the data as iron concentration vs. time (t) at a given temperature (T). The slope was calculated for the best fit line and the reaction order determined as zero order or first order depending on the relationship being linear or logarithmic respectively. The slope of this line is the rate constant (k) for the reaction at the given temperature.

A first order relationship was determined for the change in iron concentration with a positive rate change value indicating an increase in product concentration. The rate of change in iron concentration is represented by the following equation:

Table 3. Forms of Iron Measured[a] by the Iron Chemistry Assay

By Atomic Absorption	By Batho	By Difference
EL Elemental	Ionic	I-F Ferric
NE Nonelemental	F Ferrous	SO-I Complexed
SO Soluble		NE-S Insoluble

[a]From Lee and Clydesdale, 1980a.

$$\ln \left[\frac{A}{A_o} \right] = - k_I \quad t \qquad (1)$$

where: A = iron concentration

A_o = original iron concentration

k_I = rate constant

t = time

Change in iron concentration is a temperature dependent reaction. Therefore the Arrhenius relationship should hold where

$$k = k_o \, e^{-Ea/Rt} \qquad (2)$$

where: Ea = activation energy in cal/mole

R = gas constant 1.986 cal/mole °K

k_o = preexponential factor

T = absolute temperature in °K.

A semilog of ln k vs. 1/T °K yields a straight line with a slope of - Ea/R. From this the activation energy was determined. The Q_{10} values of the reactions were then calculated using the following relationship:

$$\log_{10} Q_{10} = \frac{(2.189)(Ea \text{ cal/mole})}{(T_{°K})\,(T_{°K} + 10)} \qquad (3)$$

(Labuza, 1982).

Statistical Analysis

Pairwise means and standard deviations were determined for individual variation values. Analysis of variance was performed on the

data and differences in iron profiles were determined using Duncan's multiple range test and linear regression analysis (Ott, 1977).

RESULTS AND DISCUSSION

Model Infant Formula System

Preliminary testing of the procedure for iron chemistry determination outlined by Lee and Clydesdale (1979) was done using the model infant formula system described above. This system was originally chosen as the vehicle for iron fortification because of its importance as an iron source to a large percentage of the population (Anderson et al., 1982; Roos et al., 1975; and the Council on Foods and Nutrition, 1968) and because of its convenience to the iron chemistry procedure as a liquid system necessarily subjected to thermal sterilization.

The model infant formula sample could not be assayed using Lee and Clydesdale's original procedure (1979). This was due to the formation of a proteinaceous floc after centrifugation of the sample which could not be separated by either further centrifugation or filtration. The nature of this floc prevented accurate determination of soluble iron using the atomic absorption spectrophotometer. It also caused the formation of a pink colored emulsion when treated by the bathophenanthroline procedure, preventing the determination of total ionic and soluble iron.

A modification of the chemical procedure from Lee and Clydesdale (1979) was used (Clemens, 1984) to facilitate protein separation and provide for accurate determination of the iron chemistry in the model infant formula system. The results obtained using this procedure are shown in Figure 5. The absence of ferrous iron coupled with the high

level of insoluble iron in both model systems indicates that the iron may be complexing with the protein which is subsequently acid precipitated. The large quantity of precipitate formed as a result of treatment with the hydrochloric acid indicated that much of the protein was remaining soluble even with centrifugation. This protein-iron complex may also explain the formation of a milky pink emulsion in the centrifuged supernatant during the bathophenanthroline assay for ionic iron.

The high ionic values obtained from the chemical assay of the water sample (Table 4) show that the absence of ionic iron in the model formula samples was not strictly due to the complete reduction of ionic iron in the presence of hydrochloric acid and was therefore not the result of the harsh nature of this procedure modification. The treatment may have some effect however because only 64% of the ferrous sulfate iron originally added was recovered in the ferrous (Fe^{+2}) form. In fact, only 73% of the ferrous sulfate added remained soluble indicating possible reduction of the ionic iron to the insoluble elemental form in the presence of hydrochloric acid (Spiro and Saltman, 1974).

The formation of a protein-iron complex has been previously discussed (Nelson and Potter, 1979, 1980 and Jones et al., 1972, 1975). Nelson and Potter (1979) found large amounts of both ferrous and ferric iron bound to the insoluble fraction of protein in a sodium casein - water solution. Protein solubility is highly pH dependent. Protein tends to denature and precipitate at a reduced pH. Sodium caseinate demonstrated maximum iron binding capacities for both ferrous and ferric iron at pH 5-6 (Nelson and Potter, 1979).

Table 4. Iron Profile[a] as Determined by the Modified Chemical Procedure[b] for Processed[c] Model Infant Formula Systems and for a Processed[d] Water Blank, All Containing 24 ppm of Iron

	Iron Content in ppm			
Sample	Soluble	Insoluble[e]	Total Ionic	Ferrous(Fe^{+2})
Ferrous Sulfate	3.70±0.47	22.10±0.82	3.06±0.85	0
Ferric Ortho-phosphate	2.35±0.47	23.18±1.42	0.56±0.09	0
Water[f]	17.45±0.33	0	15.72±0.18	15.28±0.46

[a]See figure 2.

[b]Clemens and Mercurio (1984).

[c&d]Processed at 250°F for 3 minutes.

[e]Insoluble or complexed iron was measured directly by dry ashing the precipitate.

[f]Water contained 24 ppm of iron from ferrous sulfate.

The pH of the model infant formula sample was 6.5. At this pH the ferrous and ferric iron salts added to the sample were bound to the sodium caseinate. The reduction of pH by the sodium acetate buffer (pH 4) may explain the apparent emulsion formation during the bathophenanthroline procedure for ionic iron determination. For the same reason, the hydrochloric acid treatment described by Clemens (1984) also causes the precipitation of the sodium caseinate-iron complex.

Iron complexation does not necessarily imply poor bioavailability. Nelson and Potter (1980) found that iron bound to various protein sources (wheat gluten, soy isolate and casein) was released upon digestion by acid and proteolytic enzymes in a readily assimilable form and was therefore highly bioavailable. Jones et al. (1975) found similar results from a ferripolyphosphate-whey protein complex. The procedure of Lee and Clydesdale (1979), however, cannot distinguish between a highly bioavailable iron complex and one of poor bioavailability. In fact, this procedure determines complexed iron by difference. The modified assay described by Clemens (1984) allows for direct determination of complexed iron, but again cannot differentiate bioavailability.

Process Lethality

The General Method was the method used to calculate process lethality. The F value is defined as the equivalent time in minutes at a given temperature, of all heat delivered to a container with respect to its capacity to destroy spores or vegetative cells of a particular

microorganism. The effect upon bacteria of a thermal process with a certain F value depends on the Z value of the bacteria or its spores. The Z value denotes the temperature (T) in degrees farenheit required to bring about a tenfold change in the thermal death time (TDT) or time for sterilization of a sample contaminated with a specific organism. The thermal death time and, ultimately, the z value depend on the resistance of the organism to thermal destruction (Lopez, 1981 and Bonwart, 1981). A z value of 18 was assumed for the purposes of this study because it is the value most commonly calculated for Clostridium botulinum and is generally used in thermal sterilization process determinations.

Values used for the calculation of process lethality (F) were abstracted from the Lethal Rates Table for z=18 (Lopez, 1981). Individual lethal rate values depicted in this table are described as Fo/t and are derived from the thermal death time (TDT) curve (time in minutes for 90% destruction of spores vs. temperature °F) illustrated in Figure 2 as follows

$$\frac{\log t - \log Fo}{\log 10} = \frac{250-T}{z}$$

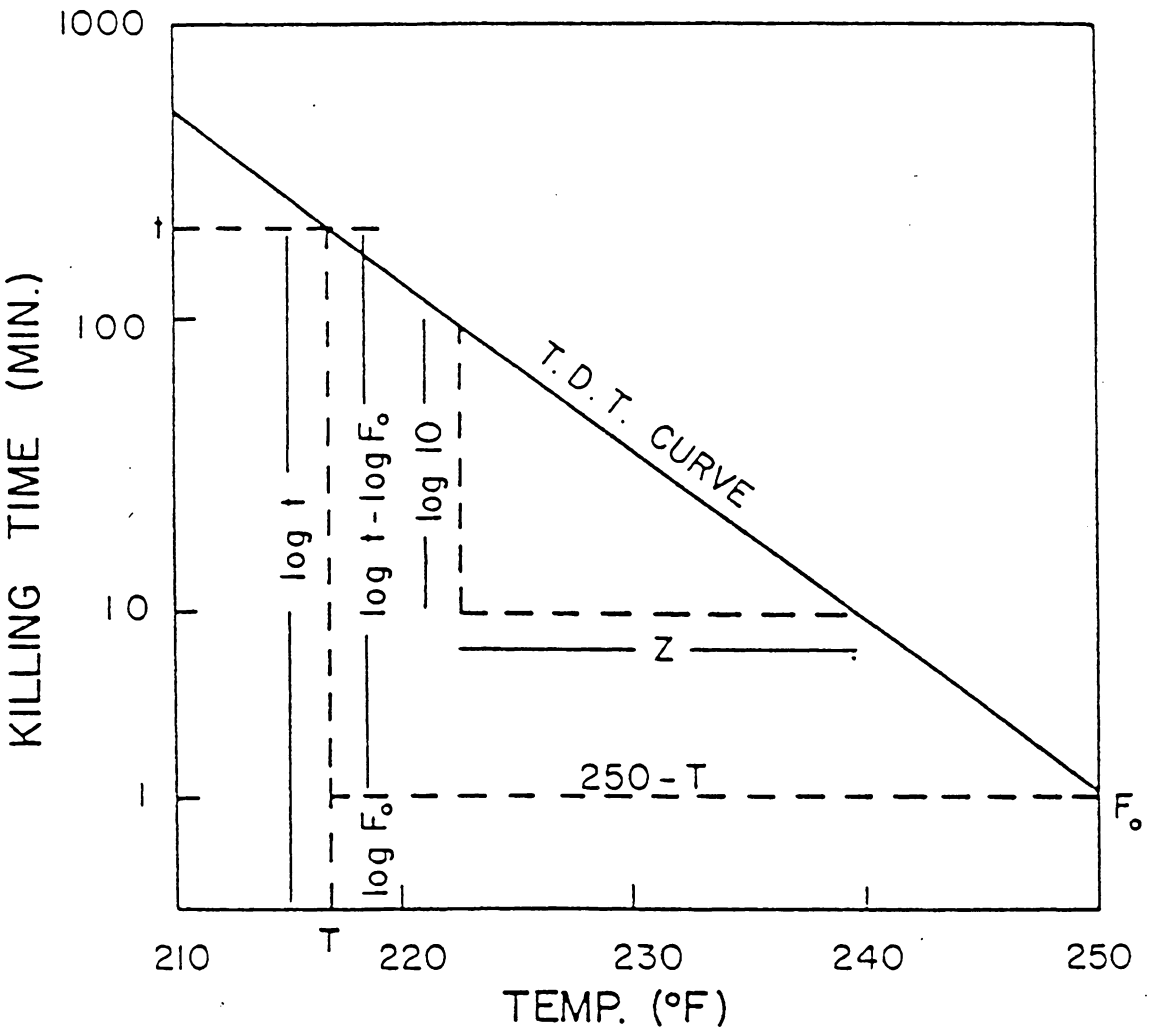
where T = temperature (°F) at which lethality is being calculated.

t = time required to destroy organism at desired T (°F)

F_o = number of minutes required to destroy a microorganism for a given z value and/or the desired temperature.

Log 10 = 1, therefore:

$$\log \frac{t}{Fo} = \frac{250 - T}{z}$$



(Adapted from Desrosier, 1970)

Figure 2. Theory for the General Method of Process Evaluation Derived from the Thermal Death Time (T.D.T.) Curve.

or

$$\frac{t}{Fo} = \text{antilog}\ \frac{250-T}{z} .$$

Since process lethality is expressed per minute the lethal rate:

$$\frac{Fo}{t} = \frac{1}{\text{antilog}\ \frac{250-T}{z}} .$$

(Lopez, 1984).

This value represents the lethal rate at any temperature (T) and any z value.

The calculated lethality values for the different processing conditions are summarized in Table 5. Process lethality was calculated as described above for each of the glucose/glycine samples containing either ferrous sulfate, ferric orthophosphate or ferric orthophsophate + ascorbic acid, which were processed. Not surprisingly, there was no significant difference ($p > .01$) in process lethality for each of the samples processed regardless of the iron compound they contained.

Analysis of Model Systems

A model system consisting of glucose, glycine and water was finally chosen as the vehicle for iron fortification because of the difficulties initially encountered using the infant formula model system (see above). The iron profile for the simplified system was easily determined using the method described by Lee and Clydesdale (1979a).

Results obtained for the iron profile of samples containing either ferrous sulfate, ferric orthophosphate or ferric orthophosphate + ascorbic acid and processed at different times and temperatures with

Table 5. Mean Lethality Values (F) for Samples[a] Processed at Different Times and Temperatures.[b]

Time Minutes	Process Lethality[c] 240°F	250°F	260°F
3	0.539±0.023	2.287±0.076	6.256±0.041
6	1.247±0.031	4.547±0.244	17.822±0.065
9	1.978±0.031	7.718±0.025	25.090±0.066
12	2.793±0.030	10.208±0.048	34.975±0.056
15	3.472±0.034	12.704±0.039	45.516±0.246

[a]Values are averages for each glucose glycine solution containing either ferrous sulfate, ferric orthophosphate or ferric orthophosphate + ascorbic acid.

[b]See Appendix B for example of raw data.

[c]Lethality values are cummulative for process time.

different lethality values are given as percentages of 24 ppm total iron in Table 6 through 8. An iron profile normally consists of elemental, soluble, insoluble, ionic, complexed, ferric and ferrous iron present in a single system. Values for elemental iron were not given here because no elemental iron was detected in the samples from the iron salts indicated. Insoluble iron was not listed but can be determined as the difference between total iron (100%) and soluble iron (%). The iron profile as given is relevant to the discussion of the relationship between the iron chemistry and the nature of the model system.

As depicted in Table 6 for ferrous sulfate the only significant change (p 0.01) in iron chemistry is an increase in the percentage of ferrous iron and simultaneous decrease in ferric iron over time. This occurs as the ferric iron is reduced to its ferrous form within the parameters of the model system, and occurs to a greater extent as temperature increases. There is, however, an initial decrease in ferrous iron at 240°F and 250°F followed by an increase as processing time increases. This effect is most noticeable at lower temperatures. At 240°F the percentage of ferrous iron decreases through 9 minutes, at 250°F it decreases through 6 minutes and at 260°F there is no decrease, but a consistent increase in ferrous iron over processing time.

The iron profile for ferric orthophosphate is illustrated in Table 7. There is a significant increase ($p < 0.05$) in soluble, total ionic, ferrous and ferric iron over processing time and temperature. The increase in soluble iron appears to be somewhat erratic and is again prefaced by a decrease which is more noticeable at lower temperatures

Table 6. Iron Profile[a] for a Glucose/Glycine Solution Fortified with Ferrous Sulfate and Processed at Different Times and Temperatures

Ferrous Sulfate Process Temp. °F	Process Time min.	Lethal Value (F)	Percent of Total Iron[b] Soluble	Complexed	Ionic Iron Total	Fe^{+2}	Fe^{+3}
-c	0	0	94.21±2.80	7.5±2.32	87.71±1.85	61.25±0.94	26.46±1.40
240	3	0.54	92.19±1.85	0	92.73±0.38	56.33±0.35	36.40±0.36
	6	1.25	98.79±0	5.59±0.47	93.12±0.94	56.02±0.74	37.10±0.84
	9	1.98	94.83±1.88	7.93±1.30	86.90±0.73	53.02±0.20	33.88±0.46
	12	2.79	92.21±5.60	0	94.29±0.35	57.79±1.30	36.50±0.82
	15	3.47	94.83±1.88	0	95.46±0.47	76.12±0.94	19.35±0.70
250	3	2.28	105.38±4.18	15.03±2.10	90.35±0.03	63.38±2.42	26.97±1.22
	6	4.54	102.29±1.76	17.27±1.63	85.02±1.50	54.58±0.18	30.44±0.84
	9	7.72	102.29±1.76	13.41±1.18	88.88±0.59	62.44±3.74	26.44±2.16
	12	10.21	94.85±1.74	8.04±1.38	86.81±1.03	69.62±1.12	17.19±1.08
	15	12.70	105.12±1.74	14.12±0.96	91.00±0.18	79.58±2.06	11.42±1.12
260	3	6.26	96.14±3.74	5.18±2.10	90.96±0.47	71.50±0	19.46±0.24
	6	17.82	92.19-1.85	0.89±1.63	91.33±1.41	75.79±0.47	15.54±0.94
	9	25.09	102.73±1.85	16.50±1.85	86.23±1.85	78.12±0.77	8.11±1.31
	12	34.98	96.14±3.74	8.00±3.33	88.14±2.92	80.04±0.47	8.10±1.70
	15	45.52	97.48±1.85	7.79±1.02	89.69±0.20	82.46±0.47	7.23±0.34

[a]See Figure 1.

[b]Solution originally contained 24 ppm of iron as ferrous sulfate.

[c]Indicates preprocessed solution fortified with 24 ppm iron.

Table 7. Iron Profile[a] for a Glucose/Glycine Solution Fortified with Ferric Orthophosphate and Processed at Different Times and Temperatures

Process Temp. °F	FOP[b] Process Time min.	Lethal Value (F)	Percent of Total Iron[c]				
			Soluble	Complexed	Ionic Iron		
					Total	Fe^{+2}	Fe^{+3}
-d	0	0	19.67±7.48	19.67±3.74	0	0	0
240	3	0.54	5.14±1.85	1.81±1.40	3.33±0.94	1.54±0.65	1.79±0.80
	6	1.25	17.04±0	9.02±.04	8.02±0.09	6.04±0.73	1.98±0.82
	9	1.98	20.99±1.85	8.18±1.06	12.81±0.27	7.69±0.38	5.12±0.32
	12	2.79	31.52±1.85	16.77±0.92	14.75±0.00	10.50±0.35	4.25±0.18
	15	3.47	31.52±1.85	10.67±1.20	20.85±0.56	15.48±0.68	5.37±0.62
250	3	2.28	7.94±1.80	0.98±1.22	6.96±0.65	5.04±0.35	1.92±0.50
	6	4.54	39.00±0	24.12±1.75	14.88±0.35	8.35±0.56	6.53±0.46
	9	7.72	37.77±1.74	19.56±0.96	18.21±0.18	13.42±0.18	4.79±0.18
	12	10.21	35.29±1.77	11.89±1.08	23.40±0.38	18.46±0.18	4.94±0.28
	15	12.70	35.27±5.27	10.0213.015	25.25±0.76	19.94±0.38	5.31±0.57
260	3	6.26	65.81±9.34	57.83±4.86	7.98±0.38	7.98±0.38	0
	6	17.82	39.44±1.85	21.23±1.02	18.21±0.18	14.33±0	3.88±0.09
	9	25.09	46.04±0	24.79±0	21.25±0	15.81±0.56	5.44±0.28
	12	34.98	43.42±0	18.86±0.28	24.56±0.56	20.46±0.77	4.10±0.66
	15	45.52	90.88±3.71	57.13±2.03	33.75±0.35	28.29±0.18	5.46±0.26

[a]See Figure 1.

[b]Ferric Orthophosphate.

[c]Solution originally contained 24 ppm of iron as ferric orthophosphate.

[d]Indicates preprocessed solution fortified with 24 ppm of iron as ferric orthophosphate.

Table 8. Iron Profile[a] for a Glucose/Glycine Solution Fortified with Ferric Orthophosphate + Ascorbic Acid Processed at Different Times and Temperatures

Process Temp. °F	FOP + AA[b] Process Time min.	Lethal Value (F)	Percent of Total Iron[c]				
			Soluble	Complexed	Ionic Iron		
					Total	Fe^{+2}	Fe^{+3}
-d	0	0	53.94±3.74	30.15±1.96	23.79±0.18	22.33±1.88	1.46±1.03
240	3	0.54	54.81±3.50	18.41±1.94	36.40±0.38	35.20±0.18	1.20±0.28
	6	1.25	78.44±8.81	42.17±5.06	36.27±1.32	34.67±2.24	1.60±1.78
	9	1.98	56.06±5.28	15.54±2.92	40.52±0.56	37.46±0	3.06±0.28
	12	2.79	64.77±3.50	13.87±2.03	50.90±0.56	47.69±0.20	3.21±0.38
	15	3.47	72.83±0.88	20.62±0.91	52.21±0.94	49.69±1.15	2.52±1.04
250	3	2.28	66.00±1.77	31.06±0.98	34.94±0.20	35.08±0.77	0
	6	4.54	61.04±1.77	21.04±0.98	40.00±0.18	38.00±0	2.00±.04
	9	7.72	62.29±0	14.19±0.06	48.10±0.12	44.67±0.41	3.43±0.26
	12	10.21	75.94±1.74	21.09±1.71	54.85±1.68	51.96±0.18	2.89±0.93
	15	12.70	69.73±3.50	9.42±2.12	60.31±0.74	55.14±6.57	5.17±3.66
260	3	6.26	61.04±1.77	21.98±1.26	39.06±0.74	36.33±0.30	2.73±0.52
	6	17.82	69.73±0.04	23.90±0.67	45.83±1.30	44.77±0.20	1.06±0.75
	9	25.09	73.46±1.77	25.54±0.94	47.92±0.12	46.77±0.38	1.15±0.25
	12	34.98	72.21±3.53	17.36±2.04	54.85±0.56	51.94±0.56	2.91±0.56
	15	45.52	66.00±1.77	7.00±1.45	59.00±1.12	55.14±1.68	3.86±1.40

[a]See Figure 1.

[b]Ferric Orthophosphate + Ascorbic Acid.

[c]Solution originally contained 24 ppm of iron as ferric orthophosphate.

[d]Indicates preprocessed solution fortified with 24 ppm of iron as ferric orthophosphate.

and nonexistent at 260°F. The increase in total ionic iron is coupled with a simultaneous increase in both ferrous and ferric iron, although the percentage of ferrous iron becomes proportionately greater than that of ferric iron over time. The percentage of ferrous iron and total ionic iron increase with increasing temperature, whereas the percentage of ferric iron does not.

The iron profile for the model solution containing ferric orthophosphate + ascorbic is illustrated in Table 8. From this data it is evident that there is a simultaneous increase ($p < 0.01$) in both ferrous and total ionic iron and a decrease in the level of complexed iron over time. The amount of soluble iron is increased, however there is no consistent trend in these values over time or temperature. The same is true for the ferric iron. The increase in total ionic iron and ferrous iron is greater at 250°F and 260°F than at 240°F. The decrease in complexed iron is greater at 250°F and 260°F than at 240°F.

The values for each iron profile in Tables 6 through 8 were listed as a function of process lethality as well as temperature and time. As the time increases at a given temperature, the lethality (F) value for that process also increases. However, the lethal rate (Fo/t) is temperature dependent with higher temperatures yielding greater lethality per minute than lower temperatures.

Discussion of Model Systems

According to Lee (1982) changes in the chemical and physical forms of exogenous iron in a food system during processing may be explained in part by:

1. the food matrix or product composition
2. processing conditions
3. The iron salt used for fortification
4. the interaction of these components

The impact of these factors on the iron profile obtained from a processed model system containing glucose and glycine fortified with iron is discussed below as follows.

The unfortified model system used in this study consisted of 0.25 molar glycine, 1.4 molar glucose and distilled deionized water. An unfortified sample processed as a blank yielded no detectable levels of iron, therefore, all results for the iron profile were recorded in parts per million directly from the processed sample (Appendix C).

The nature of this simplified model system limits the effective change in iron chemistry to the following variables:

1. the iron salt used for fortification
2. the presence of glucose
3. the presence of glycine
4. the presence of ascorbic acid
5. processing temperature
6. processing time
7. the interaction of these variables.

In order to quantify these variables, rate constants were calculated for the change in the amount of ferrous iron based on a first order or concentration dependent reaction. These values are summarized in Table 9.

Table 9. Rate Constants (k)[a] for the Increase in Ferrous (Fe^{+2}) Iron During Processing of the Model System and Water Controls

	Rate Constant		
	Processing Temperature °F		
Sample	240	250	260
Ferrous Sulfate	0.0601[b]	0.0413[c]	0.0176
Ferric Orthophosphate	0.1218	0.1161	0.1190
Ferric Orthophosphate + Ascorbic Acid	0.0475	0.0556	0.0536
Water + FS[d]	-	-0.0235	-
Water + FOP[e]	-	0	-
Water + FOP + AA[f]	-	0.0556	-

[a]Rate constants were derived from the slope of the best fit line for $\ln \frac{A}{Ao}$ vs. time.

[b]There was an initial decrease in slope; for 0-9 min. k = -0.0145.

[c]There was an initial decrease in slope; for 0-6 min. k = -0.0192.

[d]FS = Ferrous Sulfate

[e]FOP - Ferric Orthophosphate

[f]FOP + AA = Ferric Orthophosphate + Ascorbic Acid

The Iron Salt Used for Fortification. The type of iron salt used for fortification strongly affected the iron profile for the sample recorded throughout processing. The higher rate constant for ferric orthophosphate (Table 9) is indicative of a faster rate of increase in the amount of ferrous iron than for the ferrous sulfate sample. However, in spite of its slower rate constant, ferrous sulfate provides and maintains higher overall levels of soluble, total ionic and ferrous iron (Table 6) than the ferric orthophosphate samples (Table 7). A comparison of these two samples at 240°F is illustrated graphically in Figure 3.

Ferric orthophosphate is known to be poorly available to man (Cook and Monsen, 1981 and Waddell, 1973). This is probably due to the low solubility of ferric iron, particularly at a high pH. Coccodrilli et al. (1976) found that the RBV (relative biological value) of ferric orthophosphate correlated closely with its solubility. Lee and Clydesdale (1980b) found a decrease in insoluble iron coupled with an increase in ferrous iron in a ferric orthophosphate fortified ascorbate containing beverage stored for three days at ambient temperature.

The ferric orthophosphate iron profile illustrated in Table 7 follows a similar increase in ferrous iron and decrease in insoluble (increase in soluble) iron indicating an increase in solubility and a probable increase in bioavailability as a result of processing. Ferric orthophosphate is widely used in industry because of its light color and chemical inertness within a food system (Lee and Clydesdale, 1980). Therefore the processing of a sample containing ferric orthophosphate is

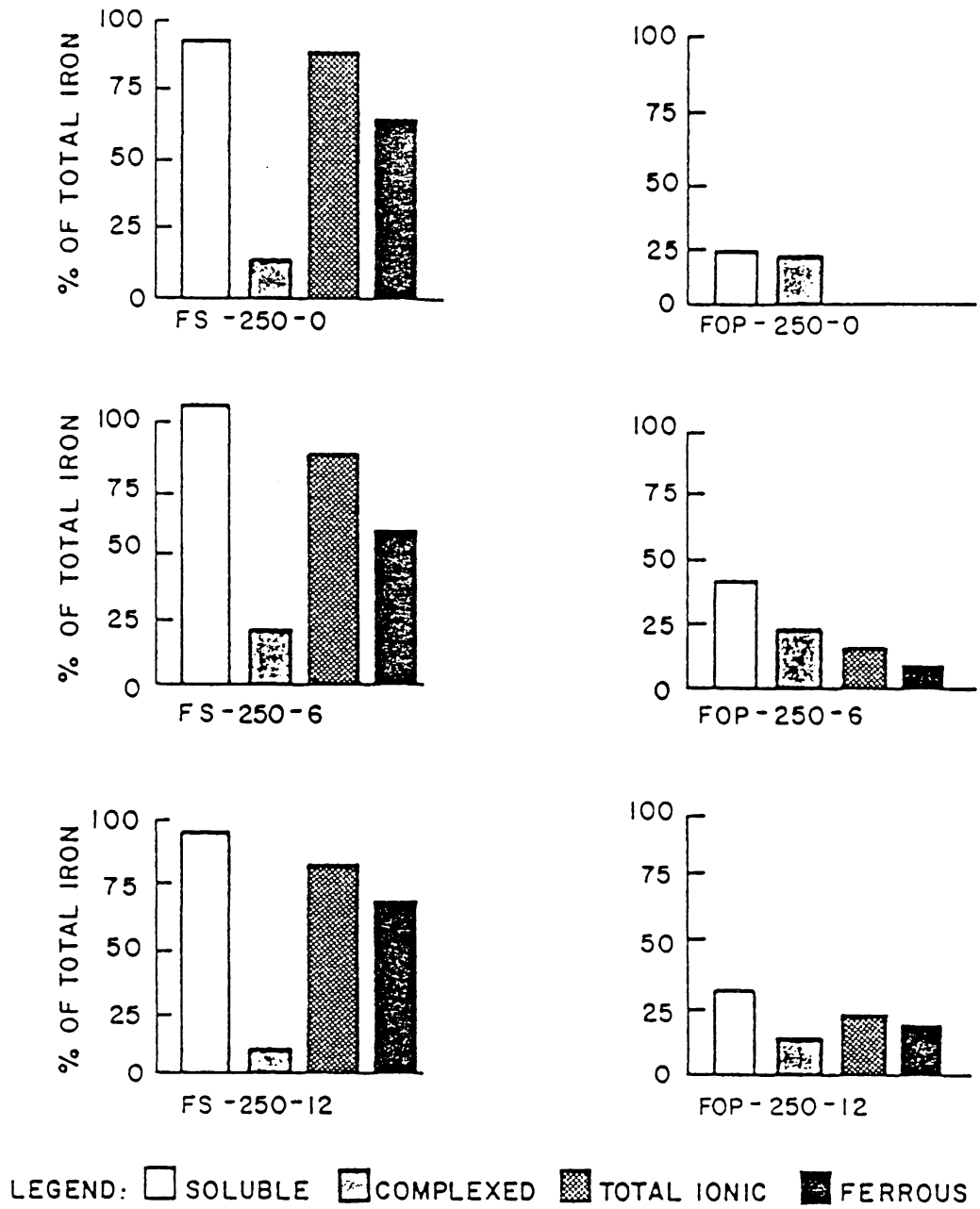


Figure 3. Bar Graph Depicting Differences in Ferrous Sulfate and Ferric Orthophosphate Iron Profiles.

beneficial to the use of this iron salt as a utilizable source of iron for fortification.

In contrast, ferrous sulfate is known for its high bioavailability (Cook and Monsen, 1981). This is probably due to its high solubility constant making it very soluble in food systems. It is a very reactive compound as well, as is known to promote rancidity and the formation of oxidized flavors and produce discoloration in the appropriate foods (Edmondson, et al., 1971; Lee and Clydesdale, 1979b; and Zoller, et al., 1980). The ferrous sulfate iron profile illustrated in Figure 9 is indicative of the highly soluble nature of ferrous sulfate as an iron compound in a food system. The formation of a brown-orange color was detected in the water control processed with ferrous sulfate. Discoloration in the glucose glycine sample could not be distinguished due to non enzymatic browning products in the processed system.

Glucose and Glycine. The iron profile for a food product is highly dependent on the chemical matrix of that food product. The chemical matrix of the model system consisted of glucose, glycine and water. The effect of these components can be seen in Tables 6-9 and in Table 10 by comparing the rate constants and iron profiles from the iron fortified model systems with the iron fortified water controls.

The presence of glucose and glycine significantly enhanced ($p < 0.01$) the levels of soluble and ionic iron in the model system containing ferrous sulfate (Figure 4). In fact, thermal processing of the water control + ferrous sulfate sample resulted in the formation of

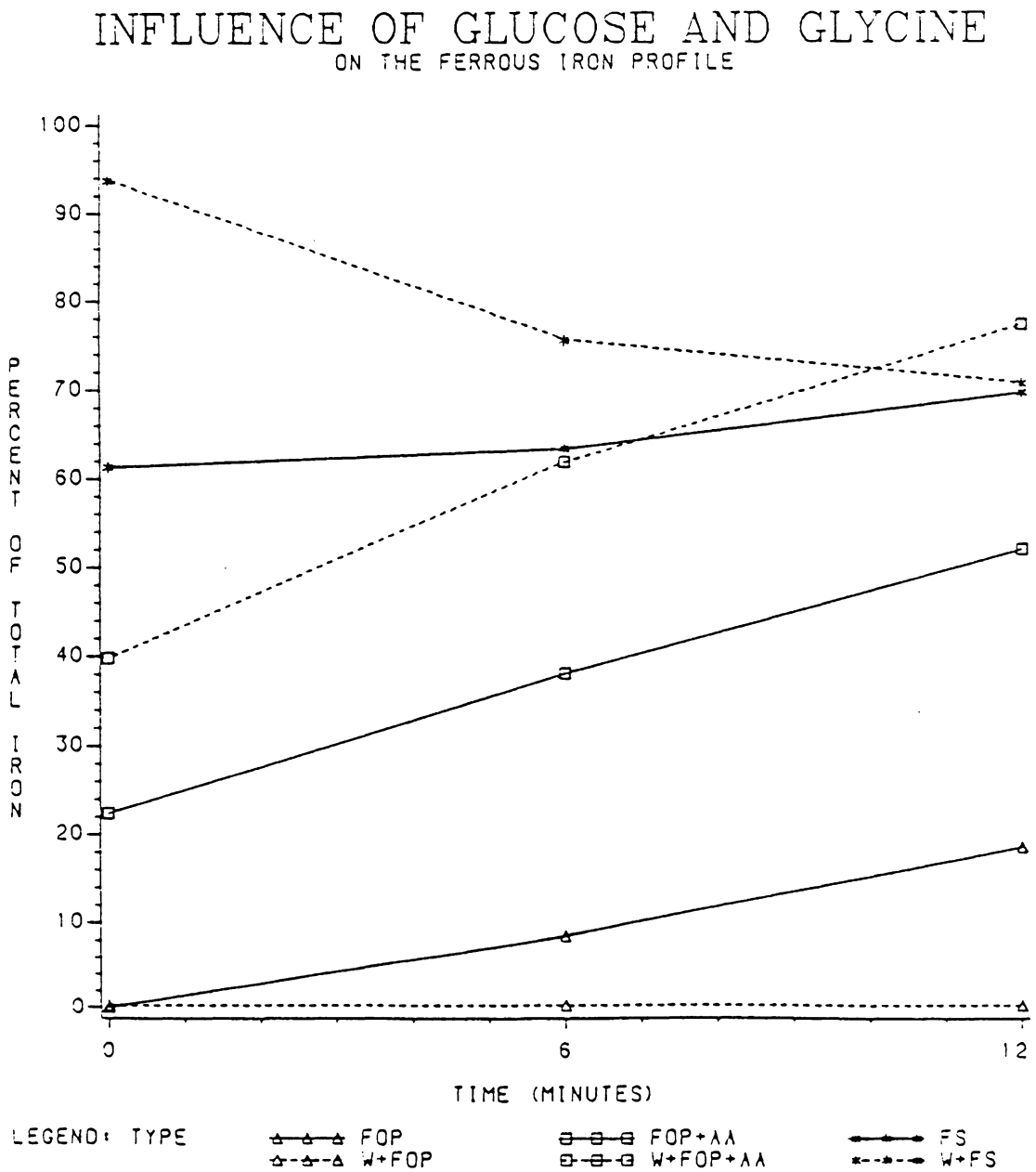


Figure 4. Comparison of the Effect of Glucose and Glycine on the Ferrous Iron Profile for the Model Systems Containing Ferrous Sulfate, Ferric Orthophosphate, and Ferric Orthophosphate + Ascorbic Acid with their Respective Water Blanks Processed at 250°F.

insoluble iron hydroxides and a decrease in the levels of soluble and ionic iron. This was accompanied by a detectable browning in the water sample during processing. The rate constants for ferrous iron in the model system + ferrous sulfate sample and the water + ferrous sulfate control at 250°F were 0.0413 and -0.0235 respectively. However, the rate constant from 0-6 minutes for ferrous iron in the model system at 250°F was -0.0192 indicating an initial decrease in the amount of ferrous iron. Therefore heating appears to be required for the observed enhancing effect of glucose and glycine on ferrous sulfate.

Glucose and glycine actually promote the solubilization and ionization of ferric orthophosphate in the model system (Figure 4). In contrast, however, the enhancing effect of ascorbic acid appears limited by these components (Figure 4).

Pollack et al. (1964) reported that glucose, despite its ability as a reducing sugar to form a weak iron complex (Charley, et al., 1963), has no significant effect on iron absorption.

Amino acids are known to chelate iron and thereby enhance its absorption from the gut (Kroe et al., 1963 and Van Campen, 1973). However, Van Campen and Gross (1969) noted that glycine is not as effective as other amino acids in its ability to chelate iron and enhance availability. These author also reported that when histidine was added to an Fe^{59} solution which contained ascorbic acid, it increased absorption above that observed from ascorbic acid alone.

The data from the iron chemistry profiles obtained in this study indicate an increase in availability based on iron chemistry and a

decreased enhancement effect of ascorbic acid in the presence of glucose and glycine. These results appear contradictory to the work of Van Campen and Gross (1969). This could be due to methodological differences (biological vs. chemical), or to the effect of thermal processing treatment on the component interactions within the model system.

Ascorbic Acid. The effect of ascorbic acid on the iron profile for ferric orthophosphate solutions processed with and without 110 ppm of ascorbic acid is illustrated in Figure 5. A significance increase in soluble and ionic iron ($p < 0.01$) was found due to the presence of ascorbic acid. Again, a higher rate constant is noted for the change in ferrous iron in the ferric orthophosphate sample (Figure 3), while a higher overall level ($p < 0.01$) of soluble, total ionic and ferrous iron is noted for the ferric orthophosphate sample with ascorbic acid (Table 9). These values correlate with data supporting the enhancing effect of ascorbic acid on iron bioavailability (Callender et al., 1970 and Monsen, 1982).

There is no significant difference ($p < 0.01$) between the rate constants for ferrous iron at 250°F in both the water control + ascorbic acid and the ferric orthophosphate + ascorbic acid samples (Table 10), although again the overall level of ferrous iron is higher in the water control profile. Therefore glucose and glycine appear to limit the enhancing effect of ascorbic acid on the ferric orthophosphate iron

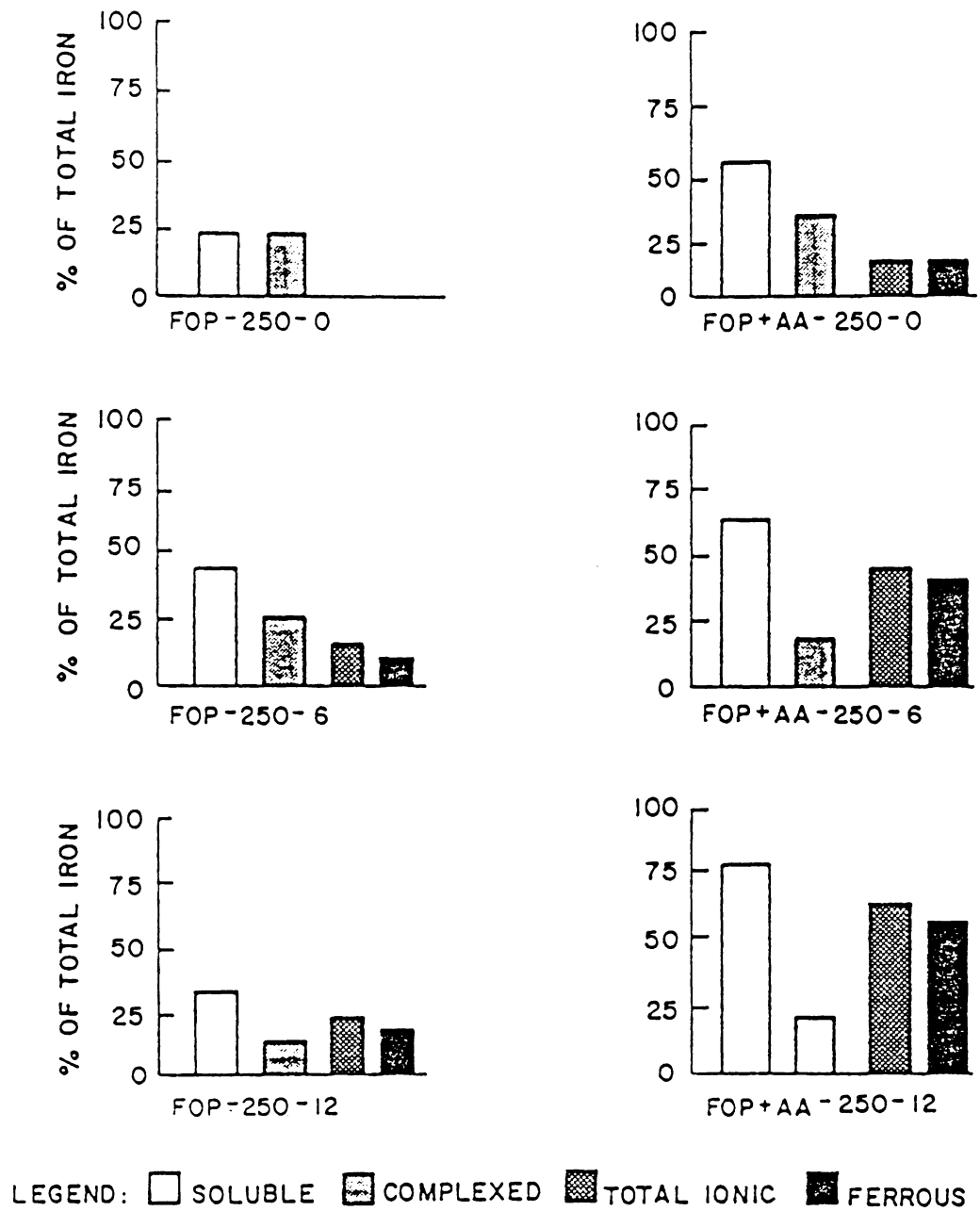


Figure 5. Bar Graph Depicting the Enhancing Effect of Ascorbic Acid on the Solubilization and Ionization of Ferric Orthophosphate.

Table 10. Iron Profile[a] of Water Fortified with Either Ferrous Sulfate, Ferric Orthophosphate or Ferric Orthophosphate + Ascorbic Acid and Processed at 250°F for Different Times

Iron Source	Process Time min.	Percent of Total Iron[b]				
		Soluble	Complexed	Ionic Iron		
				Total	Fe^{+2}	Fe^{+3}
FS[c]	0	90.88±1.77	0	101.12±2.06	93.67±0.94	7.45±1.50
	6	78.42±1.77	0	82.46±0	75.60±1.68	6.86±0.84
	12	77.19±7.04	0.88±4.18	76.31±1.32	70.69±2.27	5.62±1.82
FOP[d]	0	0	0	0	0	0
	6	2.50±3.53	2.50±1.76	0	0	0
	12	0.69±0.97	0.69±0.48	0	0	0
FOP+AA[e]	0	46.10±5.28	5.83±2.92	40.27±0.56	39.73±0.20	0.54±0.38
	6	71.52±2.33	5.62±1.58	65.90±0.38	61.90±0.74	4.00±0.56
	12	87.14±3.50	5.31±1.75	81.83±0.00	77.38±0.06	4.45±0.03

[a]See Figure 2.

[b]All solutions were originally fortified with 24 ppm of the designated iron compound.

[c]Ferrous Sulfate.

[d]Ferric Orthophosphate.

[e]Ferric Orthophosphate + Ascorbic Acid.

profile, however, the rate of change for ferrous iron due to ascorbic acid is unaffected by these two factors.

Table 11 summarizes the rate constants calculated for the increasing levels of total ionic iron over time in the ferric orthophosphate and ferric orthophosphate + ascorbic acid. From the iron profile data illustrated in Tables 8 and 9 it appears that iron added as ferric orthophosphate is solubilized, ionized and reduced to the bivalent form during processing in the presence of ascorbic acid.

Nojiem and Clydesdale (1981) reported that ascorbic acid promoted the reduction of iron at low pH (pH 2.7) and the oxidation of iron at high pH values (pH 6.2). The absence of the expected iron precipitates at higher pH values (Nojiem and Clydesdale, 1981) further indicates the formation of an iron ascorbate complex.

The data from this study seems to fit the mechanism recently proposed by Clydesdale (1982) for the apparent contradiction behavior of iron in the presence of ascorbic acid. This mechanism (Clydesdale 1982; and Gorman and Clydesdale, 1983) is based on the interrelationship between solubility, pH, reduction potential and chelation as already discussed (see above). It is possible that the interaction of these components could be stimulated by the high temperatures inflicted on the product during processing. Variation in the amount of complexed iron and total ionic iron over time indicates the formation and subsequent destabilization of an iron ascorbate complex. At pH values of the ascorbic acid containing model system (pH 4.5) the decomplexed ascorbate promotes the reduction of the freed ferric iron to ferrous iron. This

Table 11. Rate Constants (R) for the increase in Total Ionic Iron During Processing of the Ferric Orthophosphate and Ferric Orthophosphate + Ascorbic Acid Model Systems

	Rate Constants		
	Processing Temperature °F		
Sample	240	250	260
Ferric Orthophosphate	0.1425	0.1010	0.1061
Ferric Orthophosphate + Ascorbic Acid	0.0481	0.0602	0.0534

ongoing interaction explains the simultaneous increase in the amount of ionic and ferrous iron and the erratic increase in ferric iron.

Temperature and Time Effect. The nature of the processing treatment imparted on a food system also affects the degree of change in the iron profile of that food system (Lee, 1982). The application of heat to a system for a known amount of time, or to achieve a certain lethality value, implies thermal processing of that system. Thermal processing involves the addition of energy to a food system. In this experiment, heat, in the form of steam, was applied to the model system. The temperature and time parameters used for processing (240, 250 and 260°F for 3-15 min) were selected based upon the values used by Clemens and Mercurio (1981) of 123°C for 6 minutes for the processing of a liquid milk based product.

The temperature dependence of the change in iron chemistry during processing was evaluated by the Arrhenius equation. Figure 6 illustrates a typical semilog plot of the rate constants for the changes in ferrous iron in the ferric orthophosphate + ascorbic acid containing model system vs. the reciprocal of the temperature in degrees Kelvin. The activation energy (Ea) values, in Kcals/mole, were determined from the slope of the best fit line derived from Figure 6. The Ea values determined for ferrous iron in all three model systems are listed in Table 12. The Q_{10} values listed in Table 12 were derived from the activation energy values as another means of evaluating temperature sensitivity of the change in the ferrous iron profile. The Ea and Q_{10}

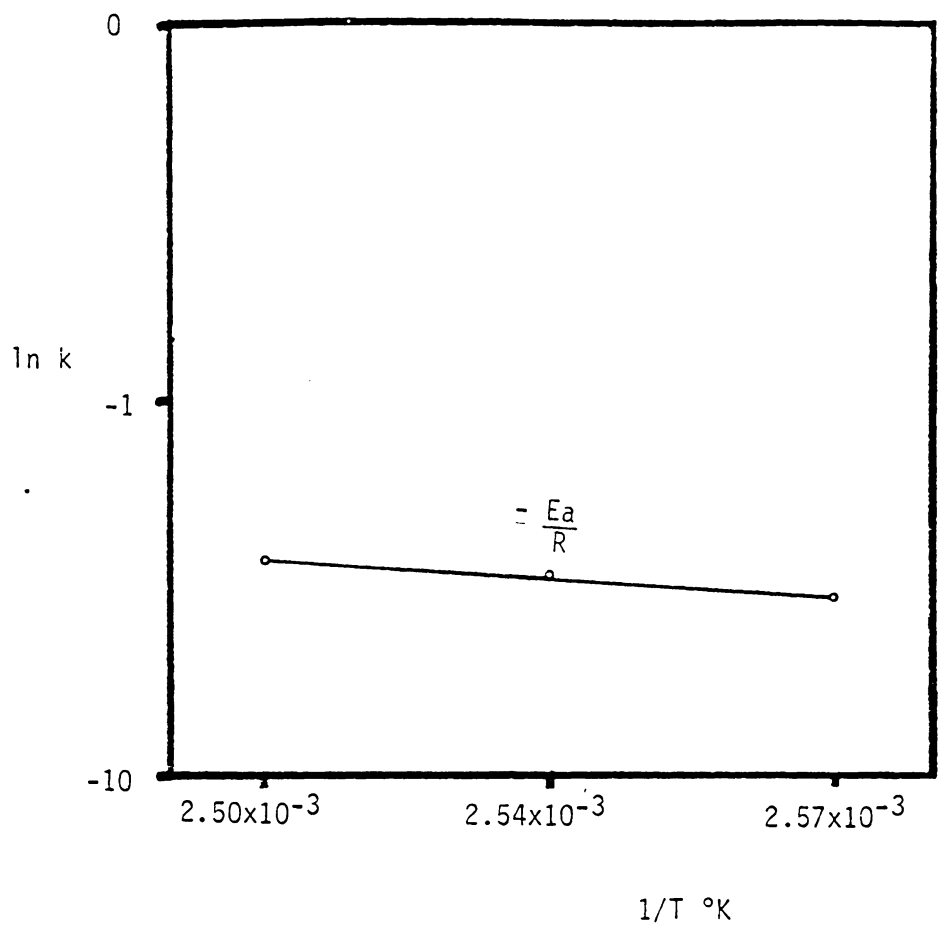


Figure 6. Semilog Plot for the Change in Rate Constant for Ferrous Iron in Ferric Orthophosphate + Ascorbic Acid Model System.

Table 12. Activation Energy and Q_{10} Values for Changes in Ferrous Iron During Processing as a Function of Iron Salt

Iron Salt	Ea(kcal/mole)	Q_{10}[a]
Ferrous Sulfate	7.24	1.24
Ferric Orthophosphate	0	0
Ferric Orthophosphate + Ascorbic Acid	3.38	1.10

[a] Q_{10} calculated for T_1 = 115°C/239°F and T_2 = 125°C/257°F.

values determined for the changes in total ionic iron from the ferric orthophosphate and ferric orthophosphate + ascorbic acid model systems are listed Table 13.

The Ea values for ferrous iron changes in the ferrous sulfate, ferric orthophosphate, and ferric orthophosphate + ascorbic acid fortified model systems were 7.24, 0, and 3.38 kcal/mole respectively. The corresponding Q_{10} values were 1.24, 0, and 1.10 respectively. Thus, the ferrous sulfate containing sample appears to be the most temperature sensitive for ferrous iron increase while the ferric orthophosphate samples did not appear to indicate any particular temperature sensitivity in this temperature range. The addition of ascorbic acid to the ferric orthophsophate model system increases the temperature sensitivity of that system. Ascorbic acid similarly increases the temperature sensitivity of the total ionic iron in the ferric orthophosphate model system.

The increased temperature sensitivity could be due to the degree of solubilized ionic iron already present in the system as a result of the environmental factors previously discussed. It appears that the lower the amount of solubilized ionic iron originally present in the system, the less temperature sensitive the system appears to be.

The results reported in this study substantiate the positive effect of thermal processing on the ionization of iron, particularly to the ferrous form. These results appear to support the effect of processing an increased iron bioavailability (Hodson, 1970; Lee and Clydesdale, 1980; Lee, 1982; and Theuer et al., 1971, 1973). However, the results

Table 13. Activation Energy and Q_{10} Values for Changes in Total Ionic Iron During Processing of the Ferric Orthophosphate and Ferric Orthophosphate + Ascorbic Acid Model Systems

Iron Salt	Ea(kcal/mole)	Q_{10}[a]
Ferric Orthophosphate	0	0
Ferric Orthophosphate + Ascorbic Acid	1.483	1.04

[a] Q_{10} calculated for T_1 = 115°C/239°F and T_2 = 125°C/257°F.

reported for ferric orthophosphate are contradictory to those reported by Clemens and Mercurio (1981) for a liquid milk based product. These authors reported that the ferric orthophosphate remained insoluble even after processing. This could be the protein complex effect as discussed previously. Therefore the effect of processing as reported by the chemical assay described by Lee and Clydesdale (1979a) depends on the food system being considered.

A Critical Evaluation of the Methodology

Clemens and Mercurio (1981) and Clemens (1981) reported a high correlation between iron bioavailability as measured by the rat hemoglobin repletion assay and the iron chemistry profile developed by Lee and Clydesdale (1979a). Changes in iron bioavailability were synchronous with changes in ionic, ferrous and soluble iron (Lee, 1982). However, in spite of its seemingly high correlation there are several weak points in the methodology developed by Lee and Clydesdale which deserve to be mentioned.

The limitations of this methodology in certain food systems, specifically inadequate separation in protein containing liquid beverages, have already been discussed. This problem was remedied with modifications to the procedures as described by Clemens (1984) and mentioned previously. In this study the iron profile determined using Clemens' modifications classified all the iron as complexed because it was precipitated and assayed with the protein. According to Nelson and Potter (1980) iron from this protein complex is available for

absorption. However, Lee and Clydesdale's method (1979a) cannot distinguish complexed iron of this nature for bioavailability.

As mentioned previously, complexed iron is determined as the difference between soluble and total ionic iron. However, soluble iron is measured directly by the atomic absorption spectrophotometer while total ionic iron is measured colorimetrically using the bathophenanthroline assay and the UV spectrophotometer. Consequently, the source of error for complexed iron determination is notably increased. This could explain the indiscriminate variation in the levels of complexed iron reported in this study (Tables 6-9).

Total nonelemental iron is determined using a wet ashing, acid dilution and filtration pretreatment of the sample which is then measured by atomic absorption spectrophotometric analysis. Price and Roos (1969) obtained a 96% recovery of iron from fruit juice using the dry ashing procedure. However, Bake and Smith (1974) found that dry ashing of plant tissues resulted in a decrease in the apparent levels of iron and other minerals due to the presence of high levels of extraneous ions.

Despite conflicting reports, Lee and Clydesdale (1979a) recommend the ashing procedure described by Price and Roos (1969) particularly for samples with little organic matter and few extraneous inorganic ions. The model infant formula system initially used in this study contained high levels of organic matter due to the presence of protein and fat. Dry ashing of this sample yielded variable results for total nonelemental iron (Appendix C).

Finally, Gorman and Clydesdale (1983, 1984) reported the formation of a thermodynamically favorable bathophenanthroline-iron complex in the presence of other ligands, particularly ascorbic acid. These authors observed that the bathophenanthroline assay yielded inaccurately high results for total ionic and ferrous iron and did not correctly account for the stable iron-ascorbate complex normally present in food systems. Bathophenanthroline promoted the destabilization of the ferric-ascorbate complex, the reduction of ferric to ferrous iron in the presence of bathophenanthroline and the subsequent formation of a highly stable batho-ferrous iron complex (Figure 7). This pink colored complex was then measured for ferrous iron content on the spectrophotometer. Gorman and Clydesdale (1983) suggest that care should be taken when using bathophenanthroline as an ion reagent in the presence of other ligands. Therefore the results obtained from the ferric orthophosphate + ascorbic acid samples in this study should be interpreted as higher values than they actually are.

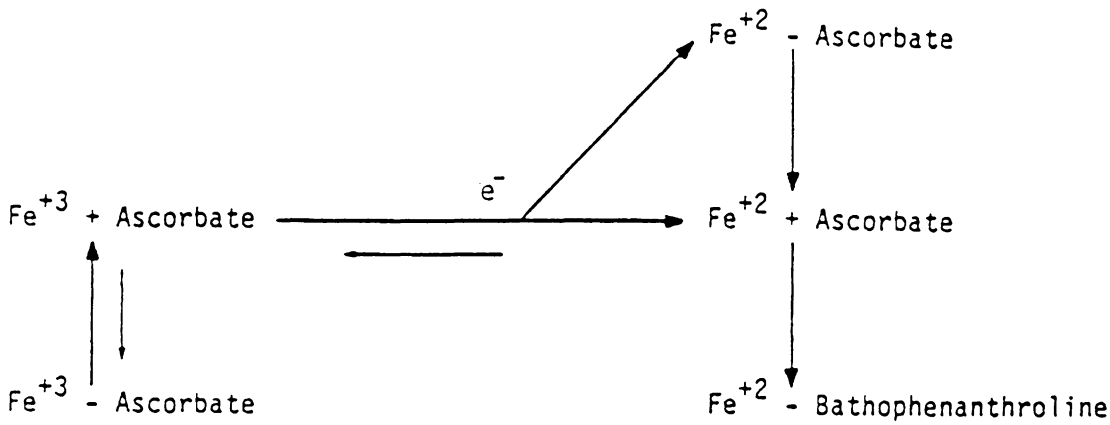


Figure 7. A Diagramatic Explanation of the Behavior of Iron in the Presence of Ascorbic Acid and Bathophenanthroline (from Gorman and Clydesdale, 1983).

SUMMARY AND CONCLUSIONS

A model system containing glucose and glycine was developed and fortified with 24 ppm of iron as either ferrous sulfate, ferric orthophosphate, or ferric orthophosphate + 110 ppm ascorbic acid. These samples were then thermally processed at 240, 250 and 260°F for 3, 6, 9, 12, and 15 minutes. Lethality values were determined for each processing treatment administered to the model systems. Each processed sample was then assayed for the quantitative determination of elemental, total ionic, ferrous, ferric, soluble and complexed iron using the chemical method developed by Lee and Clydesdale (1979a). These data were used to calculate the chemical profiles of iron which evaluated the level of each form of iron as a percentage of the total iron used for fortification. Kinetic parameters, i.e. rate constants, activation energies and Q_{10} values, were calculated for the ferrous iron profile of all three model systems and for the total ionic iron profile of the ferric orthophosphate and the ferric orthophosphate + ascorbic acid fortified model systems.

The iron profiles were evaluated and compared for effects due to the iron salt used for fortification, the presence of glucose and glycine, and for the interaction of these effects as a result of varying conditions of processing time and temperature. Ascorbic acid was also studied for its effect on the ferric orthophosphate model system during processing.

The iron salt used for fortification significantly effected the level of soluble, ionic and ferrous iron originally present in the model system. The preprocessed ferrous sulfate sample contained 95.2% of total iron in the soluble form, while the ferric orthophosphate sample contained only 19.6% soluble iron. Processing increased soluble iron in ferric orthophosphate sample but had no effect on soluble iron in the ferrous sulfate sample. The ferrous iron profile increased for both samples as a result of processing. Ascorbic acid increased the original percentage of soluble iron in the ferric orthophosphate sample. This value and the ferrous iron profile also increased as a result of processing.

The effect of the glucose-glycine model system was evaluated by comparing the iron profile from the model system fortified with each iron salt to that of the iron profile for the respective iron salt in water. Comparisons were made for the samples processed at 250°F. Glucose and glycine prevented the formation of insoluble iron hydroxides in the ferrous sulfate model system. This effect occurred during thermal processing of the ferrous sulfate model system. Solubilization and ionization of the iron salt was actually promoted by the presence of glucose and glycine. However, the enhancing ability of ascorbic acid on ferric orthophosphate was slightly inhibited by the glucose-glycine food matrix.

The interaction of the iron salt with the environmental matrix was stimulated by the application of heat to the model system. Lethality values calculated for each processing condition correlated with the

increase in the ferrous iron profile for three model systems over time at a given processing temperature. However, there was no correlation between iron profiles for a sample and the corresponding lethality values over all conditions of time and temperature.

Kinetic parameters for ferrous iron and total ionic iron were calculated to further elucidate the effect of processing temperature and time on the ionization of ion from each of the model systems. The ferric orthophosphate fortified model system had higher rate constants and lower Ea and Q_{10} values for ferrous iron than did the ferrous sulfate fortified model system. The addition of ascorbic acid to the ferric orthophosphate sample significantly reduced the rate constant and simultaneously increased the Ea and Q_{10} values for the ferrous iron. Similar results were reported for the increase in total ionic iron from ferric orthophosphate due to the presence of ascorbic acid. There was no increase in total ionic iron for the ferrous sulfate model system therefore kinetic parameters for total ionic iron were not determined. Thus it appears that the lower the amount of solubilized ionic iron originally present in the system, the less temperature dependent the reaction is to an increase in the solubilized ionic iron in that system. The extent of the change in the iron chemistry appears to depend on the environmental conditions surrounding the iron salt.

Thermal processing resulted in a significant increase in the percentage concentration of ferrous iron regardless of the iron salt used for fortification of the model system. The extraction of samples at sequential time intervals during an entire processing treatment and

evaluation for changes in the iron chemistry at each interval allowed for a better understanding of the individual reaction mechanisms that occurred during processing. The use of controlled model systems further simplified determination of these mechanisms.

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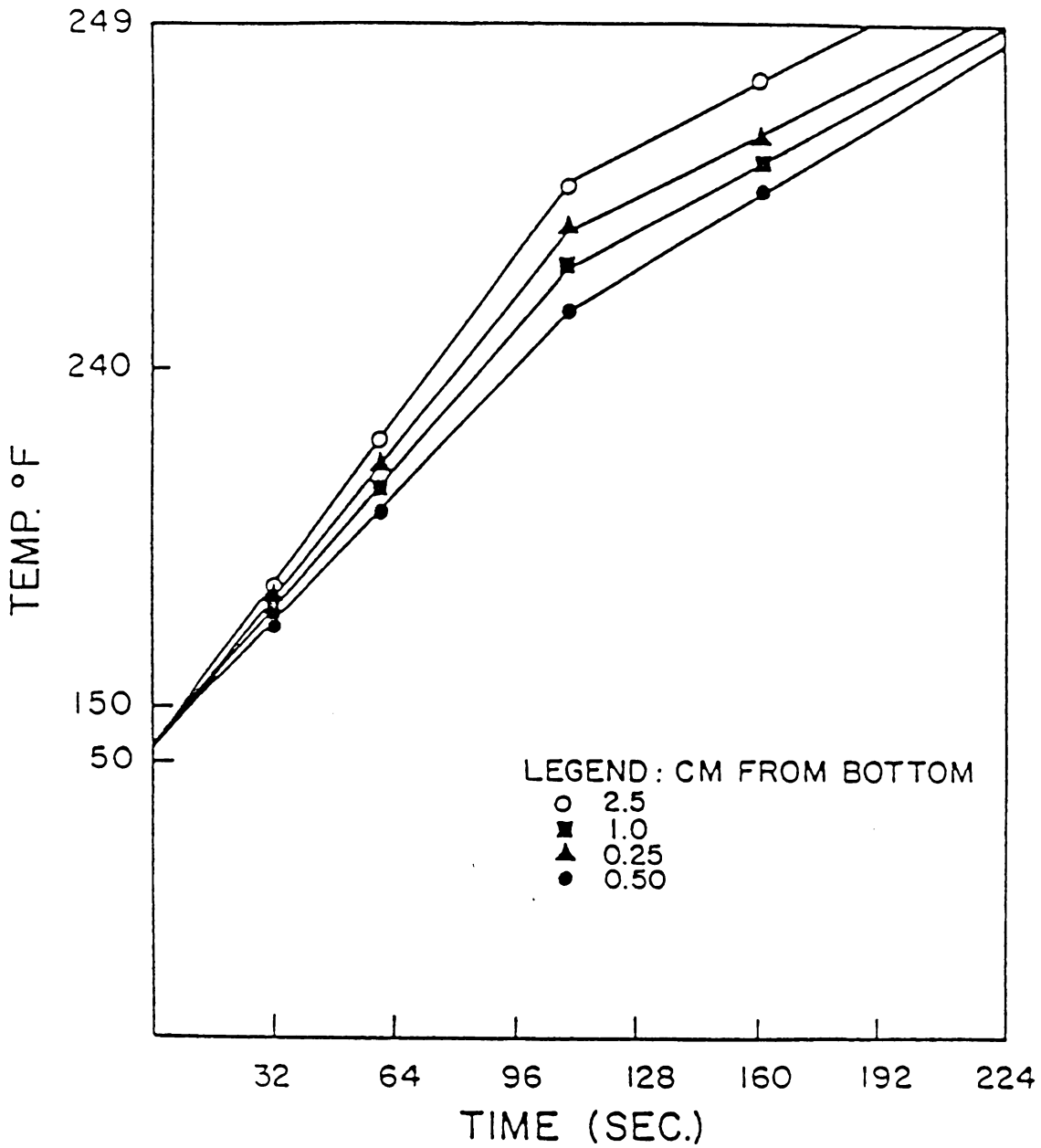
APPENDIX A

COLD POINT DETERMINATION FOR GLUCOSE/GLYCINE MODEL SYSTEM



APPENDIX B

Sample Lethality Data (F) For Unfortified Model System Processed at 240°F for 3-15 minutes.

Blank-240-3

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
0	75.9	0	0
16	84.7	0	0
32	139.6	0	0
48	184.1	0	0
64	209.0	.005	.0014
80	222.0	.028	.0076
96	229.1	.069	.0186
112	233.1	.115	.0310
128	235.4	.154	.0416
144	236.7	.182	.0491
160	237.7	.207	.0559
176	238.4	.227	.0613
192	238.6	.233	.0629
208	239.1	.248	.0670
224	239.0	.245	.0662
*240	239.1	.248	.0670
*256	196.7	.001	.0003
272	124.8	0	0
			Fo - 0.5299

*Cooling Time

APPENDIX B

Sample Lethality Data (F) For Unfortified Model System Processed at 240°F for 3-15 minutes.

Blank-240-6

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
0	74	0	
16	97.1	0	
32	159.3	0	
48	196.0	.001	.0003
64	216.0	.013	.0035
80	224.3	.037	.0100
96	229.7	.075	.0202
112	232.8	.111	.0300
128	234.5	.138	.0373
144	236.2	.171	.0462
160	236.5	.178	.0481
176	237.1	.192	.0518
192	237.4	.200	.0540
208	238.5	.230	.0621
224	238.8	.239	.0645
240	239.0	.245	.0662
256	239.1	.248	.0670
272	238.9	.242	.0653
288	239.4	.258	.0700
304	239.1	.248	.0670
320	239.0	.245	.0662
336	239.2	.252	.0680
352	239.1	.248	.0670
368	239.0	.245	.0662
384	239.3	.254	.0686
400	239.0	.245	.0662
*416	239.0	.245	.0662
*432	181.7	0	0
*448	121.2	0	0
			Fo = 1.2320

*Cooling Time

Blank-240-9

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
0	75.3	0	0
16	100.7	0	0
32	163.0	0	0
48	198.3	.001	.0003
64	216.0	.013	.0035
80	225.2	.042	.0113
96	230.3	.080	.0216
112	233.1	.115	.0310
128	234.5	.138	.0373
144	235.4	.154	.0416
160	236.1	.169	.0456
176	236.4	.176	.0475
192	236.9	.187	.0505
208	237.0	.190	.0513
224	237.4	.200	.0540
240	237.8	.210	.0567
256	238.0	.215	.0580
272	238.3	.224	.0605
288	238.8	.239	.0645
304	239.1	.248	.0670
320	239.0	.245	.0662
336	239.1	.248	.0670
352	239.0	.245	.0662
368	239.1	.248	.0670
384	238.8	.239	.0645
400	239.0	.245	.0662
416	239.1	.248	.0670
432	239.1	.248	.0670
448	239.2	.252	.0680

Blank-240-9 (Continued)

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
464	239.1	.248	.0670
480	239.0	.245	.0662
496	239.0	.245	.0662
512	239.1	.248	.0670
528	239.0	.245	.0662
544	239.0	.245	.0662
560	239.1	.248	.0670
576	239.0	.245	.0662
592	239.1	.248	.0670
*608	239.0	.245	.0662
*624	184.3	0	0
640	132.0	0	0

Fo = 1.9665

Blank-240-12

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
0	75.5	0	0
16	121.2	0	0
32	164.7	0	0
48	203.3	.002	.0005
64	219.0	.019	.0051
80	226.0	.046	.0124
96	230.2	.079	.0213
112	232.9	.112	.0302
128	234.2	.133	.0359
144	235.4	.154	.0416
160	236.3	.173	.0467
176	237.0	.190	.0513
192	237.4	.200	.0540
208	237.9	.213	.0575
224	238.5	.230	.0621
240	238.8	.239	.0645
256	239.1	.248	.0670
272	239.0	.245	.0662
288	239.3	.254	.0686
304	239.3	.254	.0686
320	239.4	.258	.0694
336	239.5	.261	.0705
352	239.6	.264	.0713
368	239.3	.254	.0686
384	238.9	.242	.0653
400	238.9	.242	.0653
416	239.1	.248	.0670
432	239.4	.258	.0697
448	239.0	.245	.0662

Blank-240-12 (Continued)

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
164	239.1	.248	.0670
180	239.2	.252	.0680
196	239.1	.248	.0670
512	239.0	.245	.0662
528	239.1	.248	.0670
544	239.3	.254	.0686
560	239.3	.254	.0686
576	239.3	.254	.0686
592	239.2	.252	.0680
608	239.4	.258	.0697
624	239.1	.248	.0670
640	239.1	.248	.0670
656	239.0	.245	.0662
672	239.3	.254	.0686
688	239.4	.258	.0697
704	239.4	.258	.0697
720	239.2	.252	.0680
736	239.1	.248	.0670
752	239.4	.258	.0697
768	239.4	.258	.0697
*784	239.3	.254	.0686
*800	179.9	0	0
816	120.3	0	0

Fo = 2.7970

APPENDIX B (Continued)

Blank-240-15			
Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
0	75.5	0	0
16	99.4	0	0
32	138.9	0	0
48	197.1	.001	.0003
64	210.9	.007	.0019
80	223.1	.032	.0086
96	229.4	.072	.0194
112	233.0	.114	.0308
128	234.7	.141	.0381
144	235.8	.163	.0440
160	236.5	.178	.0481
176	237.1	.192	.0518
192	237.4	.200	.0540
208	238.6	.233	.0629
224	238.9	.242	.0653
240	239.1	.248	.0670
256	239.0	.245	.0662
272	239.1	.248	.0670
288	239.2	.252	.0680
304	239.0	.245	.0662
320	239.0	.245	.0662
336	239.0	.245	.0662
352	239.0	.245	.0662
368	239.0	.245	.0662
384	239.1	.248	.0670
400	239.3	.254	.0686
416	239.2	.252	.0680
432	239.1	.248	.0670
448	239.1	.248	.0670
464	239.2	.252	.0680

Blank-240-15 (Continued)			
Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
480	238.9	.242	.0653
496	238.9	.242	.0653
512	239.1	.248	.0670
528	239.0	.245	.0662
544	239.0	.245	.0662
560	239.0	.245	.0662
576	239.1	.248	.0670
592	238.9	.242	.0653
608	239.1	.248	.0670
624	238.8	.239	.0645
640	239.0	.245	.0662
656	239.0	.245	.0662
672	239.1	.248	.0670
688	239.1	.248	.0670
704	239.0	.245	.0662
720	239.1	.248	.0670
736	239.0	.245	.0662
752	239.0	.245	.0662
768	239.2	.252	.0680
784	239.0	.245	.0662
800	239.0	.245	.0662
816	239.1	.248	.0670
832	239.0	.245	.0662
848	239.1	.248	.0670
864	239.0	.245	.0662
880	239.0	.245	.0662
896	239.1	.248	.0670
912	239.1	.248	.0670
928	239.0	.245	.0662
944	239.0	.245	.0662

Blank-240-15

Heating time (sec)	Temp °F	Lethal Rate	Fo/Sec
*960	239.0	.245	.0662
*976	180.3	0	0
992	122.7	0	0
			Fo = 3.4876

Appendix C

Iron Profile in Parts Per Million

Sample	Temp °F	Time min.	Ferrous	Total Ionic	Nonelemental	Soluble
FS	-	0	14.70±0.23	21.04±0.44	23.86±0.23	22.28±0.67
	240	3	13.52±0.08	22.26±0.09	22.92±0.56	22.12±0.44
		6	13.45±0.18	22.35±0.23	23.55±0.66	23.71±0.00
		9	12.72±0.05	20.86±0.18	21.49±1.34	22.76±0.45
		12	13.87±0.31	22.63±0.08	22.92±1.56	22.13±1.34
		15	18.27±0.23	22.91±0.11	21.81±0.00	22.76±0.45
	250	3	15.21±0.58	21.86±0.01	23.96±0.42	25.29±0.90
		6	13.10±0.04	20.40±0.36	22.76±0.42	24.55±0.44
		9	14.98±0.90	21.33±0.14	22.76±1.27	24.55±0.44
		12	16.71±0.27	20.83±0.25	25.14±0.42	22.76±0.90
		15	19.10±0.50	21.84±0.04	24.55±0.42	25.14±0.44
	260	3	17.16±0.00	21.83±0.11	25.60±0.90	23.08±1.80
		6	18.19±0.11	21.92±0.34	25.92±1.34	22.12±0.44
		9	18.75±0.18	20.70±0.44	23.71±0.00	24.66±0.44
		12	19.21±0.11	21.16±0.70	21.80±1.79	23.08±0.90
		15	19.79±0.11	21.52±0.05	24.34±0.00	23.40±0.44
FOP		0	0.00	0.00	22.76±1.34	4.72±1.82
	240	3	0.37±0.16	0.80±0.23	20.54±0.90	1.24±0.44
		6	1.14±0.18	1.92±0.21	20.54±0.90	4.09±0.00
		9	1.84±0.09	3.08±0.06	22.44±0.90	5.04±0.44
		12	2.52±0.08	3.54±0.00	23.08±0.90	7.56±0.48
		15	3.72±0.16	5.00±0.13	21.80±0.90	7.56±0.42
	250	3	1.21±0.08	1.67±0.16	20.98±0.42	1.90±0.43
		6	2.00±0.13	3.57±0.08	21.87±0.85	9.36±0.00
		9	3.22±0.04	4.37±0.04	21.87±1.68	9.06±0.41
		12	4.43±0.04	5.62±0.09	20.36±0.42	8.47±0.47
		15	4.78±0.09	6.06±0.18	18.60±0.41	8.46±1.27

Appendix C (Continued)

Sample	Temp °F	Time min.	Ferrous	Total Ionic	Nonelemental	Soluble
	260	36	1.92±0.09	1.92±0.09	21.46±0.49	15.80±2.42
		6	3.44±0.00	4.37±0.04	22.12±1.34	9.46±0.44
		9	3.80±0.13	5.10±0.00	22.75±0.44	11.05±0.00
		12	4.91±0.18	5.90±0.13	22.44±0.89	10.42±0.00
		15	6.79±0.04	8.10±0.08	24.18±0.23	21.81±0.89
FOP+AA	-	0	5.36±0.45	5.71±0.04	23.24±0.22	12.94±0.90
	240	3	8.45±0.04	8.74±0.09	24.19±0.42	13.16±0.84
		6	8.32±0.54	8.70±0.32	21.50±0.84	18.82±2.11
		9	8.99±0.00	9.72±0.13	23.89±0.00	13.46±1.27
		12	11.44±0.05	12.22±0.13	23.45±0.21	15.94±0.84
		15	11.92±0.28	12.53±0.23	23.59±0.42	17.48±0.21
	250	3	8.48±0.18	8.38±0.05	20.62±1.27	15.84±0.42
		6	9.12±0.00	9.60±0.04	20.91±0.00	14.65±0.42
		9	10.72±0.10	11.54±0.18	23.00±0.42	14.95±0
		12	12.47±0.04	13.74±0.40	22.40±0.42	18.22±0.42
		15	13.24±1.57	14.48±0.18	22.10±1.69	16.74±0.84
	260	3	8.72±0.07	9.38±0.18	24.19±2.11	14.65±0.42
		6	10.74±0.05	11.00±0.31	20.92±0.84	16.74±0.85
		9	11.22±0.09	11.50±0.03	22.40±1.27	17.63±0.51
		12	12.46±0.13	13.16±0.13	21.80±1.27	17.33±0.84
		15	13.23±0.40	14.16±0.27	22.40±1.27	15.84±0.42
Water Blanks						
FS	250	0	22.48±0.23	24.27±0.50	22.95±0.42	21.81±0.42
		6	18.14±0.40	19.79±0.00	23.60±0.00	18.82±0.42
		12	16.96±0.54	18.32±0.32	22.55±0.21	18.52±1.69
FOP	250	0	0.00	0.00	23.45±0.21	0.00
		6	0.00	0.00	24.04±0.20	0.60±0.84
		12	0.00	0.00	23.30±0.00	0.16±0.23

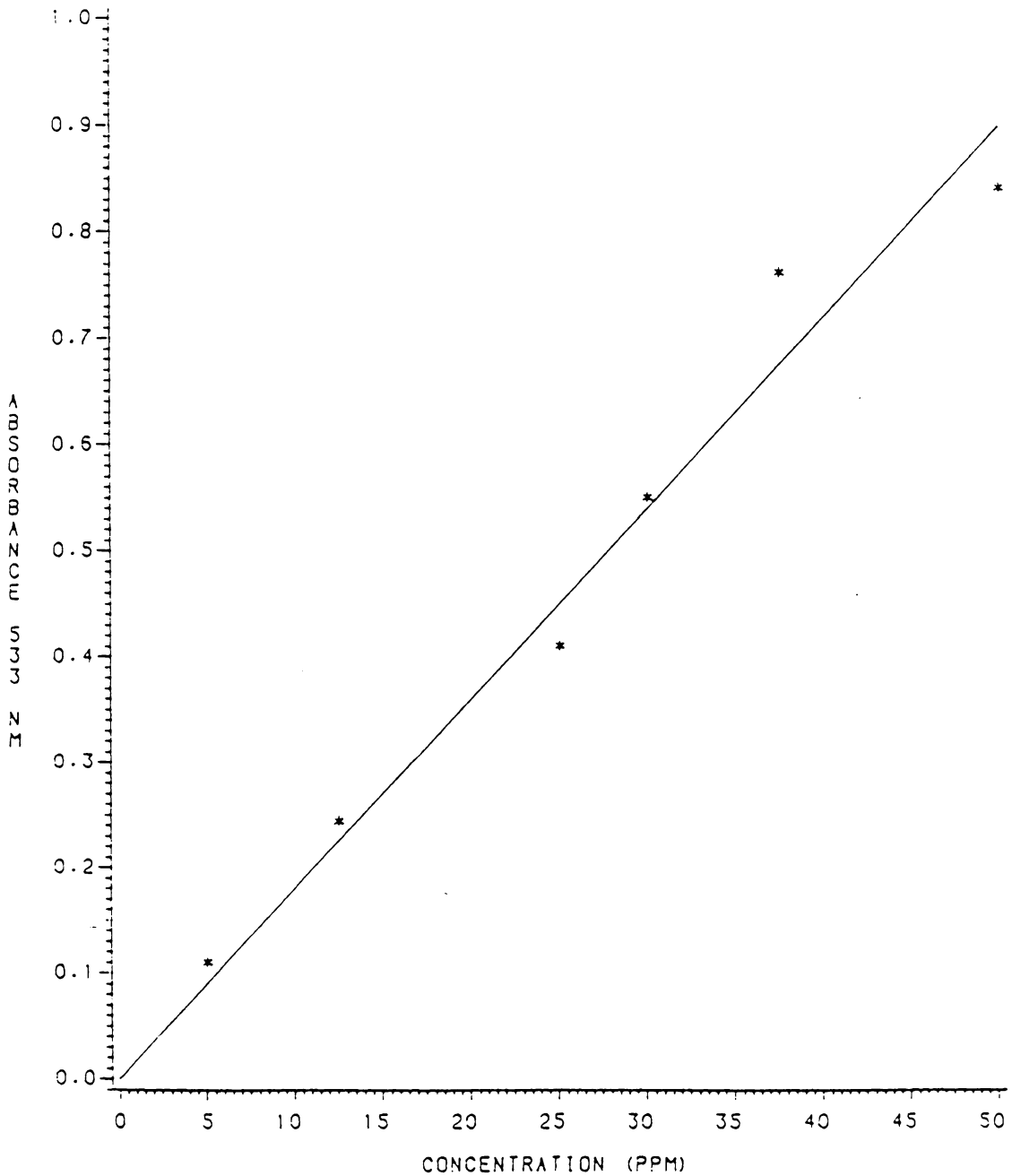
Appendix C (Continued)

Sample	Temp °F	Time min.	Ferrous	Total Ionic	Nonelemental	Soluble
FOP+AA	250	0	9.54±0.05	9.66±0.13	23.74±0.20	11.06±1.27
		6	14.86±0.18	15.82±0.09	22.25±0.21	17.16±0.56
		12	18.57±0.01	19.64±0.00	23.51±0.30	20.91±0.84

APPENDIX D

STANDARD CURVE

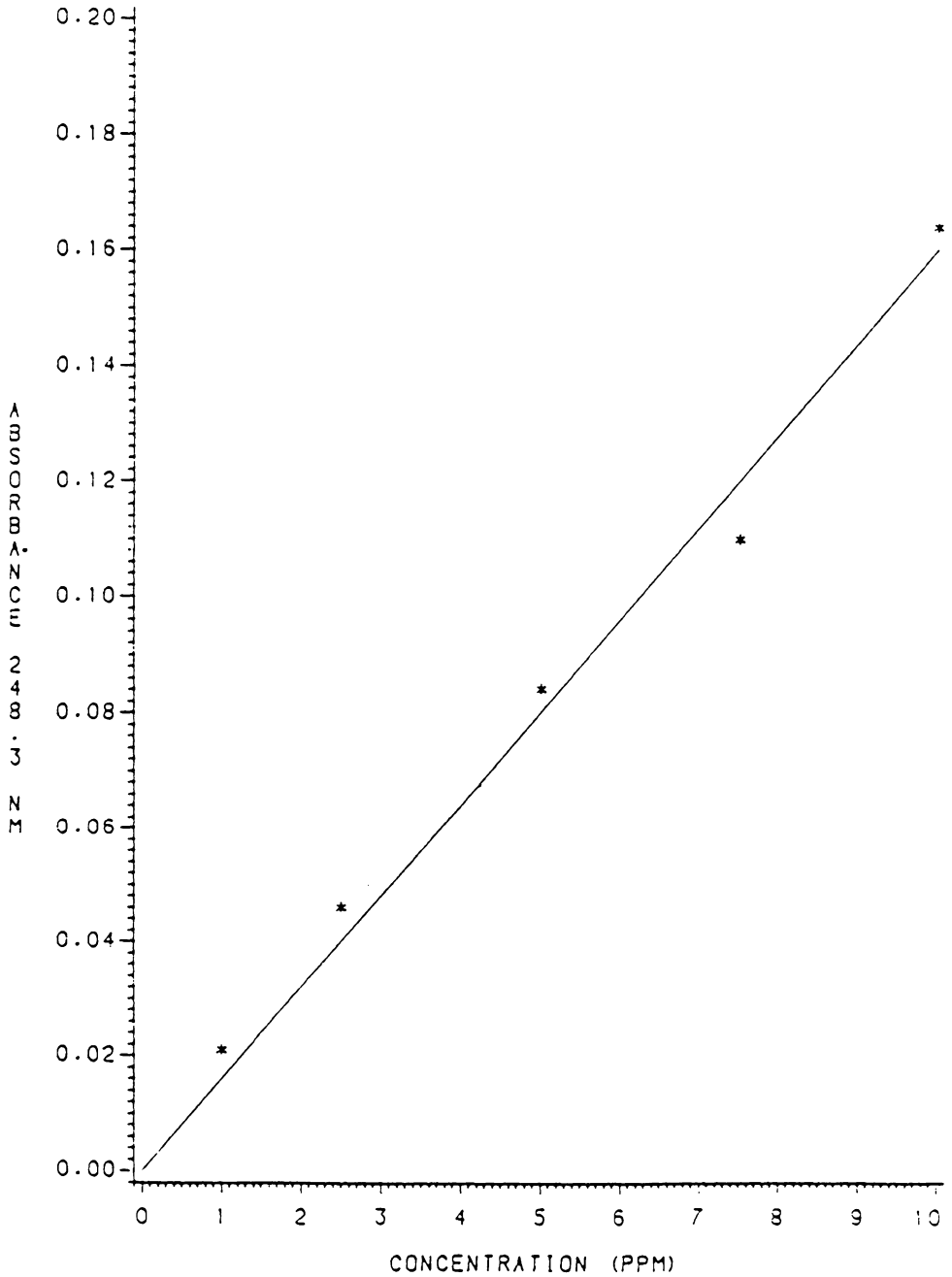
FOR FERROUS AND IONIC DETERMINATION



APPENDIX E

STANDARD CURVE

ELEMENTAL NONELEMENTAL AND SOLUBLE IRON



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