AN IN VITRO ESTIMATION OF RELATIVE IRON AVAILABILITY FROM WHEAT BRAN

By

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ABSTRACT

The effects of baking, bran particle size and other meal components on the relative availability of the endogenous iron of wheat bran were investigated using an in vitro method. The method simulated gastrointestinal digestion and measured soluble, low molecular weight iron as an estimate of available iron.

The wheat bran was incorporated into a muffin product to simulate a common domestic vehicle for fiber consumption. The muffins were blended to a slurry, adjusted to pH 2 and incubated with pepsin. Dialysis was used to adjust the pH to intestinal levels and digestion was continued with the addition of pancreatin and bile extract. Then iron from the digestion mixture which had diffused across a semipermeable membrane (6000 to 8000 molecular weight cutoff) was quantified as percent dialyzable iron.

It was found that under the conditions of the in vitro estimation, essentially no iron was available from the bran when the muffins were combined with water. When the muffins were combined with orange juice there was a very significant enhancement of iron availability.

The influence of orange juice was evaluated by comparing the relative effects of constituent organic acids. Muffins were blended to a slurry with aqueous solutions containing either ascorbic acid, citric acid or a combination of ascorbic and citric acids in amounts assumed to be present in orange juice.

Combination with ascorbic and citric acids together showed significantly greater enhancement of iron availability from wheat bran than citric acid alone which produced significantly more dialyzable iron than ascorbic acid alone. However, the increase in available iron produced by the combination of constituent organic acids was only about half of that produced by
orange juice.

It was also found that bran particle size had no significant effect on relative iron availability under the conditions of this study.

As well, it was determined that there was a significant decrease in iron availability due to baking.

Finally, the results of this study indicated that further research is necessary to examine the chemistry of iron and iron binding as related to the availability of iron from wheat bran.
# TABLE OF CONTENTS

- Abstact .............................................. ii
- List of Tables .......................................... vi
- List of Figures .......................................... vii
- Acknowledgements ...................................... viii

## I. Introduction ..................................... 1

## II. Literature Review ................................ 3

1. Iron in the Body .................................. 3
2. Iron Deficiency ................................... 3
3. Iron Balance ....................................... 4
4. Availability of Iron ............................... 6
5. Dietary Iron Sources ............................... 6
6. Chemistry of Iron ................................. 8
7. Physiology of Nonheme Iron Absorption .......... 10
8. Dietary Components Affecting Iron Absorption ... 12
9. Effects of Processing on the Availability of Nonheme Iron ................................. 14
10. Effect of Bran Particle Size on Iron Availability ........ 17
11. Effect of Orange Juice on the Availability of Iron ........ 18
14. Consumption of Whole Grain Products ............ 28
15. Availability of the Endogenous Iron of Wheat Bran ........ 32

## III. Materials and Methods .......................... 37

1. Bran ................................................. 37
2. Muffins ............................................. 38
3. Moisture Determination ............................ 38
LIST OF TABLES

I. Iron absorption from different breakfast meals ............... 13

II. Percentage of the total constituents of wheat present in the main morphological parts ........................................... 29

III. The mineral content of wheat and milled fractions ............ 30

IV. Composition of total dietary fiber of a standard wheat bran by various methods ..................................................... 33

V. Iron content of some foods calculated from tabular values ... 34

VI. Iron absorption by human subjects from rolls .................. 35

VII. Muffin recipe ......................................................... 39

VIII. Chemical composition of muffin dough based on tabular values. 40

IX. A summary of experimental conditions .......................... 52

X. Experimental data for the determination of % dialyzable iron (% DI) ................................................................. 54

XI. The effect of acid treatment on % DI from wheat bran ...... 56

XII. The effect of baking on % DI from wheat bran ............... 56

XIII. The effect of bran particle size on % DI from wheat bran .... 57
1. Schematic of the in vitro method for determination of dialyzable iron .................................................. 42
2. General plan of the experimental procedure ......................... 46
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I. INTRODUCTION

Iron is an essential element for man. It is incorporated into the structure of such body compounds as hemoglobin, myoglobin, ferritin and cytochromes. These iron-containing compounds help maintain the vital cellular activities of respiration and oxygen transport.

To assure the presence of iron-containing compounds, the body must maintain an iron balance. The iron lost daily from the body must be replenished by absorption of iron supplied by the diet.

It is well established that the absorption of dietary nonheme iron is influenced by a variety of factors. The iron status of the individual, the composition of the meal and the chemical form of iron all interact to determine the amount of nonheme iron that is absorbed by the intestinal mucosa.

It is recognized that iron deficiency is a relatively common nutritional problem worldwide. Low availability of dietary iron is considered to be one of the most significant factors responsible for the deficiency.

A balanced and varied diet provides sufficient total iron theoretically to meet the needs of the population. However, it appears that much of the dietary iron is not available for absorption. An average iron absorption from foods of only 10% is generally accepted. Therefore, it is apparent that evaluation of diets for iron adequacy requires knowledge of both the amount and availability of the iron present.

Many claims have been made for the beneficial effects of fiber in the diet. A low fiber intake has been reported to be associated with ischemic heart disease, diabetes, diverticular disease of the colon, cancer of the colon and other diseases of the gastrointestinal tract. The reported advantages of supplementation with fiber have been recognized by commercial
interests and many products now include the addition of dietary fiber. The inclusion of wheat bran in such products as bread, cereals, cookies and muffins is one method of adding fiber to the diet.

Wheat bran contains relatively high levels of iron when compared with other foods. However, the availability of the endogenous iron of this fiber source is not well understood.

The bioavailability or availability of iron describes that portion of the total iron in a food which is metabolizable. Various methods exist for the measurement of iron availability. Chemical balance techniques, animal absorption studies and human assays using radioiron have all been utilized. Several in vitro methods for the determination of iron availability have been proposed as alternatives to human and animal studies.

The purpose of this study was to estimate the relative iron availability of wheat bran using an in vitro method. The method involved simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron as an indicator of available iron. The bran was incorporated into a muffin product. The effects of baking, bran particle size and combination with known absorption-enhancing compounds on the availability of the endogenous iron of wheat bran were investigated.

It was hoped that this investigation would provide further insight into some of the factors governing the availability of the essential element, iron. It also was hoped that the results would be beneficial to an understanding of the iron nutrition of wheat bran.
II. LITERATURE REVIEW

1. IRON IN THE BODY

Iron is an essential element for human life because its presence in the heme molecule permits oxygen and electron transport (Beutler, 1980).

The total quantity of body iron varies with weight, hemoglobin concentration, sex and size of iron stores. The adult male has an average level of approximately 50 mg of iron per kg body weight and the adult female has about 35 mg per kg body weight (Beutler, 1980).

Body iron exists in two functional categories. Essential iron comprises about 70% of the total body iron and the remainder exists as nonessential storage iron found mainly in the liver, spleen and bone marrow as ferritin and hemosiderin. Approximately 85% of essential body iron is incorporated in hemoglobin, 5% in myoglobin, 10% in intracellular heme enzymes or serving as cofactors in other enzyme systems, and small amounts as transport iron bound to the plasma protein, transferrin (Beutler, 1980).

2. IRON DEFICIENCY

It is recognized that iron deficiency is a widespread nutritional problem (Narins, 1980; Lee and Clydesdale, 1979a). Cook (1978) reported that while iron deficiency is more prevalent in developing countries, the problem is global. For example, at least 10% of menstruating women in the United States are iron deficient (Cook, 1978). Low availability of dietary iron is considered to be one of the most important factors causing iron deficiency (Lee and Clydesdale, 1979a; Monsen et al., 1978).

Iron deficiency occurs when the iron supply in the blood is inadequate for normal synthesis of essential iron compounds. When iron deficiency is
well developed, insufficient iron is available for normal hemoglobin production and anemia, characterized by small, pale red blood cells, results (Beutler, 1980).

Iron deficiency is especially likely to develop in infants and children during the first two years of life, in adolescent girls and in pregnant and menstruating women (Beutler, 1980).

3. **IRON BALANCE**

To assure the presence of iron-containing compounds in the body there must be a balance between the iron absorbed and iron lost by an individual. The amount of iron that must be absorbed from food in order to maintain iron balance is determined by the amount excreted, the loss in menstrual flow or from hemorrhage, the demands of pregnancy and the needs related to growth (Beutler, 1980).

Once iron is absorbed into the body most is conserved by recycling. Iron excretion is limited. Small amounts of iron are lost in the urine, feces, skin, hair, nails and sweat (Bibeau and Clydesdale, 1976; Waddell, 1974). The main control of iron balance is achieved through regulation of iron uptake by the intestinal mucosa (Morck and Cook, 1981; Beutler, 1980).

Iron balance in men is usually not a problem. It is estimated that the adult male loses about 1 mg per day of iron. From an average dietary intake of 18 mg per day, only 6% must be absorbed to replace the normal daily losses (Subcommittee on Iron, 1979; Cook, 1978). When iron deficiency occurs in men, it almost always signifies gastrointestinal bleeding (Cook, 1978).
Iron balance in women is more difficult to maintain because the adult female has additional requirements for iron imposed by the loss of iron in menstrual blood. Blood losses due to menstruation average about 0.6 mg per day if distributed over the month. Therefore, the average total daily iron loss in the menstruating female is about 1.4 to 1.6 mg (Subcommittee on Iron, 1979). Women ingest about 10 to 12 mg of iron daily (Narins, 1980; Cook, 1978). As a result, women must absorb 12 to 15% of their daily iron intake to maintain iron balance (Subcommittee on Iron, 1979; Cook, 1978).

The daily iron requirement for women is further increased during pregnancy. When distributed over 9 months the total iron requirement for the pregnant female is 3.5 mg per day (Subcommittee on Iron, 1979). Many pregnant women cannot meet their iron requirements from the diet alone and must take iron supplements (Subcommittee on Iron, 1979; Cook, 1978).

In infancy, childhood and adolescence, there are increased iron requirements due to rapid growth and it is often difficult to maintain a positive iron balance (Subcommittee on Iron, 1979).

The iron necessary to replace daily losses must be absorbed from the diet. In developed countries, the diet provides approximately 5 to 7 mg of iron per 1000 kcal (Beutler, 1980). Theoretically, such an intake should more than adequately meet the iron needs of the population. However, the fact that iron deficiency continues to be a problem in spite of a plentiful intake indicates that not all of the iron present in the diet is capable of being absorbed (Lee and Clydesdale, 1979a). In fact, it is estimated that only 5 to 10% of dietary iron is absorbed by healthy subjects (Beutler, 1980; Lee and Clydesdale, 1979a). Therefore, it is evident that evaluation of diets for iron adequacy requires knowledge of both the amount and the availability of the iron present.
4. **AVAILABILITY OF IRON**

The availability or bioavailability of iron describes that portion of the total iron in a food which is metabolizable (Mahoney and Hendricks, 1982). The amount of iron potentially available from food depends on a number of complex and interacting factors. Iron absorption from food is affected by the chemical form of iron in the food, the amount of iron in the meal, the iron status of the person consuming the food, the presence of other food constituents in the same meal and the physiological conditions that exist in the digestive tract.

The bioavailability of iron has been discussed in several reviews (Morck and Cook, 1981; Cook, 1978; Bibeau and Clydesdale, 1976; Waddell, 1974) and textbooks (Kies, 1982; Beutler, 1980; Subcommittee on Iron, 1979). The various factors that influence the availability of dietary iron will be discussed in subsequent sections.

5. **DIETARY IRON SOURCES**

There are two chemical forms of dietary iron; heme and nonheme, and each is absorbed by a different mechanism (Morck and Cook, 1981; Monsen et al., 1978).

Heme, which contains iron in a porphyrin ring structure, is found in hemoglobin and myoglobin and accounts for approximately 40% of the iron present in animal tissues. Heme iron enters the intestinal mucosal cell as the intact porphyrin complex. Within the mucosal cell the heme is catabolized and the iron enters the same pathways as nonheme iron (Morck and Cook, 1981; Monsen et al., 1978).

Nonheme iron is present in foods such as vegetables, grains, fruits and eggs and accounts for the remaining 60% of iron in animal tissues.
It has been determined that a common nonheme iron pool is formed by foods ingested in the same meal. The implication of the pool concept is that the absorption of nonheme iron depends not only on the total amount of dietary nonheme iron but also on the effects of various intraluminal factors which either enhance or inhibit the availability of iron (Hallberg, 1980).

The absorption of heme iron from the diet is high in comparison with nonheme iron. Heme iron is prevented from interacting with other components of the diet during absorption. Therefore, the availability of heme iron is not influenced by the composition of the meal (Morck and Cook, 1981; Cook, 1978).

Heme iron provides only 5 to 10% of the total daily iron intake in developed countries. However, due to its high availability, it provides nearly one third of the iron absorbed each day from a mixed diet (Morck and Cook, 1981; Cook, 1978).

The abundance of body iron stores has only a small effect on the absorption of heme iron. An individual with no iron stores may absorb 35% of heme iron when ingested as meat while a subject with adequate iron stores may absorb 15% (Cook, 1978; Monsen et al., 1978).

The absorption of nonheme iron is sensitive to the iron status of the individual. As iron stores decrease more of the iron available in the digestive tract is absorbed and once iron stores are replete, absorption falls to a low level (Beutler, 1980). Absorption of nonheme iron by an individual with adequate iron stores will be about 2% from a meal containing components which inhibit the availability of iron. An iron deficient subject may absorb as much as 20% of nonheme iron from a meal containing
abundant absorption enhancers (Cook, 1978; Monsen et al., 1978).

The absorption of nonheme iron is more affected by the total amount of iron ingested in a meal than is heme iron. Generally, as the level of iron in the diet increases, the proportion of the iron that is absorbed decreases, but the absolute amount increases (Narins, 1980).

6. CHEMISTRY OF IRON

It is known that the chemical characteristics of iron such as valence, solubility and type of chelation influence the bioavailability of iron from foods (Nojeim and Clydesdale, 1981; Lee and Clydesdale, 1979a).

Iron has several oxidation states ranging from Fe\(^{+6}\) to Fe\(^{-2}\), depending upon the chemical environment. The ferric form, Fe\(^{+3}\), and the ferrous form, Fe\(^{+2}\), are the only states which occur naturally in foods (Lee and Clydesdale, 1979a).

Ferrous and ferric ions do not occur in the free state but are hydrated as Fe\((\text{H}_2\text{O})_6^{+3}\) and Fe\((\text{H}_2\text{O})_6^{+2}\) in acidic solutions. As the pH is raised, deprotonation of the complexed water molecules occurs. Ferric ions polymerize and eventually precipitate as ferric hydroxide while most ferrous iron remains soluble in neutral and alkaline solutions (Clydesdale, 1982; Lee and Clydesdale, 1979a). At pH 7 the solubility of ferrous hydroxide is about \(10^{-1}\) M. Ferric hydroxide is much more insoluble and has a solubility of \(10^{-16}\) M at neutral pH (Lee and Clydesdale, 1979a).

Numerous studies have shown that ferrous iron is absorbed and utilized more efficiently by man than ferric iron. It has not yet been resolved whether this is due to a selective absorption mechanism or to the greater solubility of ferrous iron (Nojeim and Clydesdale, 1981; Lee and Clydesdale, 1979a).
It has also been found that the type of chelate formed with iron influences the absorption of nonheme iron. Many common food components are effective chelating agents or ligands (Lee and Clydesdale, 1979a). The absorption of chelated iron may be enhanced or inhibited depending on the nature of the specific iron complex including the solubility, molecular weight and stability of the complexes formed with iron (Clydesdale, 1982; Miller and Schricker, 1982; Monsen and Page, 1978).

Enhanced iron absorption has been observed when such compounds as ascorbic acid, sugars such as fructose, and amino acids like cysteine have been present in the meal. It has been postulated that these compounds can form low molecular weight, relatively weak chelates with ferric iron. These chelates prevent the precipitation of iron in the alkaline environment of the intestine thereby rendering otherwise insoluble iron available for absorption (Morck and Cook, 1981; Beutler, 1980; Lee and Clydesdale, 1979a; Monsen and Page, 1978).

Ascorbic acid and reducing agents in meat have also been reported to increase iron absorption from the diet by reducing ferric iron to the more soluble ferrous form (Morck and Cook, 1981; Lee and Clydesdale, 1979a).

Other dietary components such as tannins, phosphates, phytates, oxalates and carbonates have been reported to reduce the absorption of nonheme iron. It has been proposed that these iron-complexing compounds reduce absorption by the formation of insoluble precipitates or tightly bound soluble complexes with ferric iron at neutral pH (Morck and Cook, 1981; Monsen and Page, 1978; Bibeau and Clydesdale, 1976).
7. PHYSIOLOGY OF NONHEME IRON ABSORPTION

Most nonheme iron in food exists in the ferric state and is bound to food components (Miller and Schricker, 1982; Innis, 1981). As has been discussed, the iron must be soluble or ligand-bound to be available for absorption.

There are three phases in the absorption of nonheme iron. These phases are the intraluminal, mucosal and corporeal (Innis, 1981).

In the intraluminal phase, iron is released from food in a soluble form and is ionized by the acid gastric juice. The iron enters a common intraluminal nonheme iron pool (Innis, 1981; Beutler, 1980). Narins (1980) reported that less than half of the total iron in food is released by hydrochloric acid and peptic digestion and less than a third is ionized. The iron released into the stomach may be reduced to the ferrous state or chelated by ligands released during digestion (Miller and Schricker, 1982; Innis, 1981; Beutler, 1980).

When the products of gastric digestion pass from the stomach to the duodenum, bicarbonate secreted by the pancreas begins to neutralize the stomach acid and the pH increases from about 1.5 to 7.0 (Miller and Schricker, 1982; Innis, 1981). The previously described formation of sparingly soluble hydroxides with increasing pH can now be seen to have nutritional significance. At intestinal pH most of the ferric iron precipitates as ferric hydroxide unless it is prevented by a chelating agent; while most of the ferrous iron, which is not easily precipitated is still soluble (Miller and Schricker, 1982; Innis, 1981; Beutler, 1980).

In humans the mucosal cells of the duodenum have the greatest capacity for iron absorption (Miller and Schricker, 1982; Innis, 1981; Beutler, 1980). In the mucosal phase of absorption soluble iron is taken up by
brush border receptors and either immediately transported across to the serosal side or retained within the mucosal cell (Innis, 1981). The chemical form of iron that enters the mucosal cell, the nature of the receptor sites and the iron transport system within the cell are unknown (Innis, 1981; Beutler, 1980).

Recently, Miller and Schricker (1982) discussed their proposal for the form of iron which is absorbed by the intestinal mucosa. Their discussion is significant to this thesis because their proposal provided the rationale for the design of the in vitro method developed by Miller et al. (1981) which was used by this author to estimate iron availability.

Miller and Schricker (1982) assumed that absorbable iron is present in the duodenum as a soluble low molecular weight chelate. They also assumed that other forms of iron that may be present do not contribute significantly to absorbable iron. They provided several reasons why these assumptions seemed justified. Miller and Schricker (1982) stated that:

1. Iron may be absorbed as the intact chelate or the chelate may transfer its iron to an acceptor on the mucosal cell surface. Absorption and exchange would be much more rapid with soluble forms of iron since insoluble forms would have limited contact with the mucosal cell surface.

2. Iron bound to large molecular weight ligands may be available but absorption would be limited to an iron transfer mechanism since large molecules are generally not absorbed intact. Large molecular weight soluble chelates of available iron would most likely involve proteins as the ligand and digestion would quickly transform them into low molecular weight chelates.

3. Polymerized iron, even when soluble, is probably not readily available ...

In an earlier study, Peters et al. (1971) concluded that iron is absorbed only as the free ferrous ion or as low molecular weight complexes capable of passing through a membrane by simple diffusion.
The final phase of nonheme iron absorption is the corporeal phase where iron is taken up by plasma transferrin on the serosal side of the mucosal cell. The exact transfer mechanism is not known but it is believed to involve a membrane receptor (Innis, 1981).

8. **DIETARY COMPONENTS AFFECTING IRON AVAILABILITY**

It is well established that the availability of nonheme iron can be influenced by components of foods ingested concomitantly that either enhance or inhibit the absorption of iron from a meal (Morck and Cook, 1981; Rossander et al., 1979; Monsen et al., 1978). The effect of dietary components on nonheme iron availability is related to the chemistry and physiology of iron absorption which has been discussed previously.

Numerous studies have been carried out on the effect of various dietary factors on iron absorption and these are reviewed by Morck and Cook (1981).

One study which examined the interaction of various foods on iron absorption will be discussed. Rossander et al. (1979) measured the absorption of nonheme iron from 9 Western type breakfasts in 129 subjects using extrinsic labelling with radioiron. In the study, the iron absorption from a basal breakfast consisting of coffee (150 ml), two wheat rolls with margarine (12 g), one with orange marmalade (10 g) and the other with cheese (15 g), was standardized against the absorption of a reference dose of ferrous ascorbate. The basal breakfast was then used as the standard against which iron absorption from other breakfast meals was compared.

As shown in Table I, there was a wide variation in the bioavailability of iron in the different breakfast meals. The greatest effects were seen with tea which reduced the absorption to less than half and with
Table I. Iron absorption from different breakfast meals

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Composition</th>
<th>Corrected Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal breakfast (coffee)</td>
<td>0.16</td>
</tr>
<tr>
<td>2</td>
<td>with orange juice</td>
<td>0.40</td>
</tr>
<tr>
<td>3</td>
<td>with boiled egg</td>
<td>0.19</td>
</tr>
<tr>
<td>4</td>
<td>with scrambled egg and bacon</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>with cornflakes and milk</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>Basal breakfast (tea)</td>
<td>0.07</td>
</tr>
<tr>
<td>7</td>
<td>with orange juice</td>
<td>0.21</td>
</tr>
<tr>
<td>8</td>
<td>with scrambled egg and bacon</td>
<td>0.12</td>
</tr>
<tr>
<td>9</td>
<td>Basal breakfast (chocolate milk)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*a* Adapted from Rossander et al. (1979).

*b* Corrected to correspond to an absorption of 0.16 mg from the basal breakfast with coffee.
orange juice which increased the absorption two and a half times.

9. EFFECTS OF PROCESSING ON THE AVAILABILITY OF NONHEME IRON

Almost all foods are processed in some form before they are consumed. It seems possible that some of these processes may influence the availability of iron. Recently, Lee (1982) reviewed the effect of processing on the bioavailability and chemistry of iron in food.

Heat treatment of foods is a major form of processing and it appears to have a varying effect on iron availability.

Theuer et al. (1971, 1973) investigated the effects of sterilization on the availability of supplemental iron added to infant formulas. In their first study, Theuer et al. (1971) prepared experimental batches of liquid soy isolate infant formula products without added iron and with various iron salts by standard commercial techniques. The effect of processing was assessed directly with three iron salts that were also added without processing to lyophilized product made without added iron. Iron availability was evaluated by measuring hemoglobin response in anemic rats fed measured amounts of the lyophilized products.

It was found that heat sterilization had little effect on the availability of ferrous sulfate. However processing did increase the relative availability of ferric pyrophosphate from 39 to 93% and of sodium iron pyrophosphate from 15 to 66%.

In a similar experiment, Theuer et al. (1973) determined the relative availability of various iron salts when incorporated into a liquid milk-based infant formula. It was found that sterilization increased the relative availability of ferric pyrophosphate from 75 to 125% and of sodium iron pyrophosphate from 40 to 60%.
Wood et al. (1978) conducted experiments to determine the effects of heat and pressure processing (1,055 g/cm² and 121°C for 15 min) on the bioavailability of various iron phosphate salts using the chick hemoglobin repletion technique. Heat and pressure processing resulted in a significant enhancement of the relative biological value (RBV) of sodium ferric pyrophosphate from 14 to 66% and of ferric pyrophosphate from 7 to 90%. Processing did not significantly alter the RBV of ferrous sulfate or ferric orthophosphate.

Clemens and Mercurio (1981) determined the availability of carbonyl iron, electrolytic iron and ferric orthophosphate in a canned cocoa-containing milk-based product. The relative biological values were determined by hemoglobin repletion assay in rats before and after retort processing. It was found that there was a three-fold increase in the RBV of both carbonyl iron (from 65 to 202%) and electrolytic iron (from 123 to 297%) following retort processing. The RBV of ferric orthophosphate remained unchanged.

Disler et al. (1975) showed that when sugar fortified with ascorbic acid and ferric orthophosphate was added to corn meal porridge before cooking there was a several-fold enhancement in the availability of iron from ferric orthophosphate than if it was added after heating. However, the effect of heating ferric orthophosphate alone, without ascorbate, was not determined.

Fritz and Pla (1972) used the hemoglobin repletion assay in both rats and chicks to compare the bioavailability of various iron sources when the iron was added directly to the test diet and when the iron was a component of a food which was then added to the test diet. Foods were processed in several ways. For example, farina enriched with ferric
orthophosphate was cooked and ferrous sulfate was added to a biscuit mix and baked into biscuits. It was concluded that processing had little or no effect on the bioavailability of iron.

Pla et al. (1973) used a hemoglobin repletion assay with chicks and rats to determine the RBV of iron from bread. Bread was baked from flour enriched with ferrous sulfate and reduced iron. It was found that there was no evidence for any effect of baking on the bioavailability of the iron sources.

Coccodrilli et al. (1976) determined the RBV of elemental iron powders which were commercially processed into a bran flake breakfast cereal. It was observed that commercial cereal processing procedures did not significantly alter the expected RBV of the iron sources to rats.

Verma et al. (1977) baked bread with flour containing different forms of elemental iron. It was found that the iron sources had similar availability to rats whether they were added directly to the diet or baked into bread and then added to the diet.

In a recent study, Schricker and Miller (1982) used an in vitro method to estimate the relative availability of various iron fortification sources. The effect of baking on fortification iron availability in bread was evaluated. The different forms of iron were added to the bread either before baking or during homogenization of the baked bread. The bread was analyzed alone or as part of a complex meal. A reduction in available iron was observed as a result of baking with the complex meal but not with the bread meal.

The literature indicates that processing generally increased the bioavailability of added iron when the process involved heating a predominately aqueous food (Clemens and Mercurio, 1981; Wood et al.,
1978; Disler et al., 1975; Theurer et al., 1971, 1973). Dry heat processing was found to have little effect on the bioavailability of added iron (Verma et al., 1977; Coccodrilli et al., 1976; Pla et al., 1973; Fritz and Pla, 1972).

There is little information in the literature on the effect of heat processing on the bioavailability of iron naturally present in foods.

Sayers et al. (1973) measured the absorption of intrinsic and added iron in three different vegetable foods using a double radioiron method with iron deficient human subjects. Maize was prepared as porridge, soya beans as biscuits and wheat as whole wheat bread. All of the foods were combined with ascorbic acid so that differences in bioavailability due to baking per se could not be determined. It was found that iron in cooked maize porridge increased in bioavailability when cooked in combination with ascorbic acid. However, iron in baked soya biscuits or baked bread did not change in bioavailability when cooked with ascorbic acid.

10. **EFFECT OF BRAN PARTICLE SIZE ON IRON AVAILABILITY**

A review of the literature indicates that the effect of bran particle size on the availability of iron from bran has not been considered.

It is hypothesized by this author that a decrease in the particle size of bran may increase the amount of iron available for absorption by increasing the surface area of the fiber exposed to the digestive process.
11. **EFFECT OF ORANGE JUICE ON THE AVAILABILITY OF IRON**

The ability of orange juice to enhance iron availability has been well documented (Monsen, 1982; Schricker and Miller, 1982; Rossander et al., 1979).

Orange juice contains a variety of organic acids including citric, ascorbic, malic and oxalic. Citric acid is the most predominant organic acid in orange juice. The average content is 0.60 to 0.75 g of citric acid per 100 ml (Nagy, 1978; Clements, 1964). The content of ascorbic acid in fresh orange juice is about 50 mg per 100 ml of juice (Health and Welfare Canada, 1979).

The ability of ascorbic acid to enhance nonheme iron absorption from foods has been extensively studied. Monsen (1982) recently reviewed the roles of orange juice and ascorbic acid in nonheme iron absorption. It is interesting that most studies which have demonstrated the ability of orange juice to enhance nonheme iron availability have attributed the absorption promoting property of the orange juice to the presence of ascorbic acid and have not considered the possible influence of citric acid (Monsen, 1982; Rossander et al., 1979).

However, a recent study by Kojima et al. (1981) examined the effects of ascorbic acid, citric acid and orange juice on the solubilization of iron from cooked pinto beans using an in vitro method. In one experiment, a dual incubation procedure was carried out at pH 2 and then pH 6. It was found that a 10 mM solution of ascorbic acid solubilized 45% of the iron from a cooked pinto bean suspension. Virtually all of the soluble iron was present in the ferrous state. A 10 mM solution of citric acid solubilized about 50% of the iron and 25% of the soluble iron was in the ferrous form. It was found that the effects of ascorbic acid and
citric acid were additive and 10 mM solutions of each combined solubilized approximately 70% of the iron from a cooked pinto bean suspension. Almost all of the soluble iron was in the ferrous state.

The effect of orange juice was also investigated using a dual incubation procedure. The solubilization of iron from a cooked pinto bean suspension in the presence of fresh orange juice was as great as a combination of 10 mM citric acid and 10 mM ascorbic acid. Kojima et al. (1981) concluded that while other factors may play a role, the solubilization of iron from beans by orange juice is probably due to the effects of citric and ascorbic acids.

A survey of the literature indicates that few studies have looked at the effect of orange juice and its constituent organic acids on the availability of iron from wheat or wheat bran.

Kuhn et al. (1968) examined the effect of chelates on food iron absorption using a double radioiron tag method on humans. Wheat which had been intrinsically labelled was cooked and fed as a small cake. Two moles of ascorbic acid per mole of iron were added immediately before oral administration. Comparison of the absorption of iron from wheat in the same individual with and without ascorbic acid showed that ascorbic acid increased absorption 2.5 times.

Sayers et al. (1973) determined the absorption of iron from maize, wheat and soya bean using a double radioiron method in iron deficient human subjects. The addition of 50 mg ascorbic acid to unsupplemented maize porridge increased the average iron absorption from about 7% to 24%. The addition of ascorbic acid to soya biscuits or whole wheat bread prior to baking did not affect the absorption of intrinsic or extrinsic iron. It was suggested that the lack of effect was probably
due to the fact that ascorbic acid was destroyed during baking.

12. METHODS FOR THE DETERMINATION OF IRON AVAILABILITY

Many methods exist for determining the quantity of iron available for absorption from foods and these have been reviewed in several textbooks (Beutler, 1980; Hallberg, 1980; Narins, 1980).

The oldest method for measuring food iron absorption is the chemical balance technique. By this method absorption equals the difference between oral intake and fecal loss. Generally, the technique is tedious, insensitive and inaccurate. The very small amount of iron absorbed relative to the iron content of the diet makes the difference difficult to measure with precision. As well, differentiation between excreted and unabsorbed iron is not possible (Morck and Cook, 1981; Beutler, 1980; Narins, 1980).

The most frequently used animal bioassay is the rat hemoglobin repletion test. The method is described by Fritz et al. (1978, 1974) and it has been adopted as the standard method of analysis for the bioavailability of iron by the Association of Official Analytical Chemists (AOAC, 1980, sections 43.217 - 43.219).

In this test, rats are made anemic on a low iron basal diet. The anemic animals are then fed a diet containing graded levels of iron provided by either a reference standard of highly available ferrous sulfate or the test source. After two weeks the hemoglobin response to the test source of iron is compared to the response observed in a comparable group of animals fed identical levels of iron from the reference standard. The results are expressed as the relative biological value (RBV) of the test iron source relative to ferrous sulfate which is given a value of 100.
There are advantages and disadvantages to the use of animal assays such as the hemoglobin repletion test. The principal advantage is the use of an intact biological system (Miller and Schricker, 1982). However, there are several disadvantages which include the problems associated with extrapolating results from rats to humans. Fritz and Pla (1972) reported that the absorption enhancing effect of ascorbic acid has not been demonstrated in rats. They postulated that species such as rats, which do not need a dietary source of ascorbic acid, produce enough ascorbic acid in their bodies to minimize any effect from dietary sources. It has also been reported that while humans preferentially absorb ferrous iron; ferric and ferrous iron are equally well absorbed by rats (Narins, 1980).

As well, the hemoglobin repletion test probably overestimates the RBV of iron sources because anemic animals use iron more efficiently. However, it is necessary to use depleted animals in order to measure a response to dietary iron (Miller and Schricker, 1982). The requirement for graded levels of iron in the diets means that when whole foods are used the composition of diets is usually not constant between groups (Miller and Schricker, 1982). Finally, animal absorption studies are expensive.

Radioisotope techniques allow the most precise measurements of dietary iron absorption in humans (Narins, 1980). Isotopic techniques are also used with animals but the disadvantages of animal studies have already been discussed.

Radioisotopes were first used to measure the absorption of iron from single foods which had been intrinsically labelled with radioactive iron prior to harvest or slaughter. Studies with intrinsically labelled
foods demonstrated that iron absorption from different foods varied widely and that absorption was greater from foods of animal origin (Hallberg, 1980; Narins, 1980). However, intrinsic studies provided little information about iron absorption from a complete meal.

A major advance in the study of the bioavailability of iron was made when the extrinsic tag method was demonstrated to be a valid technique (Hallberg, 1980). It has been determined that all of the nonheme iron of foods ingested in the same meal becomes part of a common nonheme iron pool in the gut. Absorption of nonheme iron can be measured by adding a radioactive inorganic iron tracer to a food or composite meal. Isotopic exchange between the tracer and the native nonheme iron results in complete and uniform labelling of the nonheme iron (Hallberg, 1980).

Whole body retention of radioiron is the most reliable, sensitive and quantitative method for measuring iron absorption in humans. The general procedure is to count the natural background radioactivity in the fasting subject. The subject then ingests food containing a known amount of radioiron. The radioactivity of the whole body is counted after 4 hours and after 14 to 20 days when the unabsorbed iron has been eliminated from the body (Narins, 1980).

Radiolabelled heme iron exchanges completely with the pool of heme iron in the gut. Therefore, it is possible by using the two pool extrinsic radioiron tag method to measure the absorption of heme and nonheme iron in the same meal (Beutler, 1980; Hallberg, 1980).

Human studies using radioiron have proven to be very successful. However, such studies are expensive, complicated and time consuming. As well, the administration of radioisotopes to humans is not always possible or desirable (Miller and Schricker, 1982).
13. IN VITRO METHODS FOR THE DETERMINATION OF IRON AVAILABILITY

In vitro methods offer an appealing alternative to human and animal studies and they have been used to estimate iron availability for at least 50 years (Miller and Schricker, 1982). In vitro methods can be simple, rapid and inexpensive (Miller et al., 1981). While human and animal studies are necessary to measure actual absorption and utilization of food iron, in vitro techniques can be used to measure the amount of dietary iron that is potentially available for absorption (Narasinga - Rao and Prabhavathi, 1978).

The determination of ionizable iron has been used as an estimate of available iron. Ionizable iron has been determined as that fraction of the total iron in a food that will react with a complexing agent such as $\alpha,\alpha'$-dipyridyl, tripyridyl triazine or bathophenanthroline to form a chromagen which can be quantified spectrophotometrically (Miller and Schricker, 1982; Narins, 1980).

Schackleton and McCance (1936) estimated the ionizable iron of a variety of foodstuffs by extraction with a sodium acetate-acetic acid buffer at pH 5.5 in the presence of sodium hydrosulfite, followed by measurement of the coloured iron complex formed with $\alpha,\alpha'$-dipyridyl. Leichter and Joslyn (1967) used a similar procedure to estimate available iron in 3 different types of bread.

However, the determination of ionizable iron in foods is probably of little value since the conditions in the gastrointestinal tract which influence iron availability are not considered (Narasinga-Rao and Prabhavathi, 1978).
Other in vitro methods of predicting the availability of dietary iron have concentrated on duplicating the chemical environment of the stomach with subsequent measurement of the soluble iron released by simulated gastric digestion.

Jacobs and Greenman (1969) estimated the amount of iron released from a number of foods by peptic digestion. Various foods were incubated with pepsin and hydrochloric acid. The digest was centrifuged and the supernatant analyzed for ionizable iron by a tripyridyl triazine method. Total soluble iron, which measured both ionizable and complexed iron, was also determined. It was considered that the quantities found represented the amount of iron expected to be present in soluble form in the stomach and therefore available for absorption.

Hart (1971) used an analysis similar to that employed by Jacobs and Greenman (1969) to compare the availability of iron in enriched white bread with that of iron naturally present in wholemeal bread.

In another in vitro method, Lock and Bender (1980) measured the proportion of iron liberated from 20 foods after incubation with human gastric juice. The digest was centrifuged and the supernatant analyzed for soluble iron by atomic absorption spectroscopy. It was suggested that the iron solubilized by human gastric juice was "chemically available".

However, the use of simulated gastric digestion probably does not determine the true availability of iron since most dietary iron is absorbed from the small intestine and not from the stomach (Narasinga-Rao and Prabhavathi, 1978).

Narasinga-Rao and Prabhavathi (1978) developed an in vitro method for the determination of available nonheme iron in foods. Their method attempted to simulate the chemical environment of the stomach and small
intestine. Food was subjected to treatment with pepsin and hydrochloric acid at pH 1.35 at 37°C for 1.5 h. The solution was subsequently adjusted to pH 7.5 with sodium hydroxide and filtered. Ionizable iron was determined in the filtrate by reaction with \( \alpha,\alpha' \)-dipyridyl.

Narasinga-Rao and Prabhavathi (1978) used their method to estimate the availability of iron from various foods and diets. It was reported that the percent ionizable iron at pH 7.5 in a number of diets correlated highly with percent iron absorption from the same diets observed in adult males. It was also observed that the ionizable iron at pH 7.5 increased in the presence of ascorbic acid and meat extract while it decreased in the presence of phytate and tannins; similar to the effects of these factors on iron absorption in human subjects.

Hazell et al. (1978) used an in vitro digestion procedure, which simulated the physiological conditions of the stomach and the small intestine, to investigate the availability of iron from meat.

Another in vitro method to estimate food iron availability was developed by Miller et al. (1981). They reported that their method differs from previously published in vitro methods such as that of Narasinga-Rao and Frabhavathi (1978) in two important ways. The pH adjustment from gastric to intestinal levels is gradual and reproducible and only low molecular weight soluble iron is used in the estimation of available iron. Recognition of these important differences in methodology prompted the selection of the in vitro method of Miller et al. (1981) for use in this study. Details of the method have been described elsewhere (Miller and Schricker, 1982; Miller et al., 1981; Schricker et al., 1981) but for the purpose of discussion the method, as adapted for this study, will be outlined briefly.
The method involved digesting aliquots of homogenized meals, adjusted to pH 2 and mixed with pepsin in a 37°C shaking water bath for 2 h. After the pepsin digestion, aliquots were removed for measurement of titratable acidity. The pH of the pepsin digests was then raised to 7 using sodium bicarbonate. An amount of sodium bicarbonate equivalent to the titratable acidity (the amount of potassium hydroxide required to raise the pH of pepsin digest to 7.5) was placed in a segment of dialysis tubing (mol. wt. cutoff 6000 to 8000) which was placed in the digest. A second 2.5 h incubation at 37°C in a shaking water bath was conducted in the presence of a pancreatin-bile extract mixture. At the end of the incubation, the dialysis bags were removed, rinsed and the dialysate weighed and analyzed. Dialysable iron was determined by a modified bathophenanthroline method (Clark, 1962; Lee and Stumm, 1960; Smith et al., 1952). Total iron was determined in the muffin samples by atomic absorption spectroscopy according to the method of Maurer (1977).

In the original method, dialyzable iron was also determined by counting $^{59}\text{Fe}$ activity. Miller et al. (1981) reported that the two methods for the determination of dialyzable iron were highly correlated. In the original method, food samples were analyzed for both heme and nonheme iron. However, Miller (1982) noted that in meals which do not contain meat, the amount of heme iron is insignificant and nonheme and total iron are therefore identical.

Miller et al. (1981) discussed their rationale for choosing various experimental procedures. They explained that the use of a bicarbonate solution in dialysis tubing allows for a slow increase in pH before and during pancreatin digestion. As well, this method of pH adjustment permits dialysis of iron to occur during the neutralization process and thus
more closely parallels the in vivo situation. The use of dialysis tubing of a specific pore size permits discrimination between high and low molecular weight soluble iron complexes and is based on the assumption that absorbable iron is present in the duodenum as a low molecular weight soluble chelate.

Schricker et al. (1981) compared the in vitro method of Miller et al. (1981) with rat and human extrinsic tag methods for estimating food iron availability. Complex meals were used in the comparison. When the criterion for comparison was the ability to show statistically significant differences between iron availability in the various meals, there was substantial agreement between the in vitro and human in vivo methods.

An in vitro method, such as that developed by Miller et al. (1981), has certain limitations. These include uncertainties over the use of an artificial system, the inexact duplication of in vivo conditions and the inability to account for the effects of such factors as active transport and brush border binding proteins (Miller and Schricker, 1982). As a result of these limitations, Miller et al. (1981) noted that in vitro measurement of iron availability must be a relative rather than an absolute indication of availability.

However, an in vitro method for the estimation of relative iron availability is rapid and inexpensive. Other advantages of a method such as that developed by Miller et al. (1981) include reduced variability compared to in vivo methods and the ability to precisely control conditions during the determinations (Miller and Schricker, 1982).
14. **CONSUMPTION OF WHOLE GRAIN PRODUCTS**

The consumption of foods made with unrefined cereals and their byproducts, such as bran, is increasing (Turnlund, 1982). This may be illustrated by the number of "new" whole grain breakfast cereals that have recently been introduced into supermarkets. The Health Protection Branch of Health and Welfare Canada has recommended that more whole grain foods be included in the Canadian diet (Health and Welfare Canada, 1977).

Recommendations for the consumption of whole grain products have been made for several reasons. Whole grain products contain not only calories and other nutrients commonly associated with cereals, but also significant amounts of essential minerals which may not be present in refined cereal products (Turnland, 1982; O'Dell et al., 1972).

Kent (1975) reported the percentage of the total constituents of wheat present in the main morphological parts (Table II). It was found that 67, 23 and 10% of the total mineral content was present in the bran, endosperm and germ respectively.

O'Dell et al. (1972) analyzed the distribution of elements among the morphological components of the wheat kernel and determined that approximately 15.1, 27.6, 78.6 and 6.4% of the total iron was present in the germ, endosperm, aleurone layer and hull respectively.

The outer layers of wheat consisting of the bran (composed of the hull and aleurone layer) and the germ with their high content of minerals are removed during milling (Turnlund, 1982). The content of several essential trace elements in wheat, wheat fractions and wheat flour is compared in Table III. Only some of the trace nutrients are added back during fortification. In Canada, wheat flour is fortified with thiamine,
Table II. Percentage of the total constituents of wheat present in the main morphological parts

<table>
<thead>
<tr>
<th>Part</th>
<th>Weight (g/100 g grain)</th>
<th>Starch</th>
<th>Protein</th>
<th>Fiber</th>
<th>Fat</th>
<th>Minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bran</td>
<td>15</td>
<td>0</td>
<td>20</td>
<td>93</td>
<td>30</td>
<td>67</td>
</tr>
<tr>
<td>Endosperm</td>
<td>82</td>
<td>100</td>
<td>72</td>
<td>4</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td>Germ</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

*aAdapted from Kent (1975).*
Table III. The mineral content of wheat and milled fractions\(^a\)

<table>
<thead>
<tr>
<th>ppm</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>18-31</td>
<td>21-63</td>
<td>24-37</td>
<td>1.8-6.2</td>
</tr>
<tr>
<td>Bran</td>
<td>74-103</td>
<td>56-141</td>
<td>72-144</td>
<td>8.4-16.2</td>
</tr>
<tr>
<td>Germ</td>
<td>41-58</td>
<td>100-144</td>
<td>101-129</td>
<td>7.2-11.8</td>
</tr>
<tr>
<td>Flour</td>
<td>3.5-9.1</td>
<td>3.4-10.5</td>
<td>2.1-3.5</td>
<td>0.62-0.63</td>
</tr>
</tbody>
</table>

\(^a\)Adapted from Turnlund (1982)
Interest in unrefined cereals and their byproducts has also increased because numerous hypotheses have linked increased consumption of whole grains and dietary fiber to beneficial health effects. Several authors have reviewed the effects of dietary fiber intake on man (Kelsay, 1978; Burkitt, 1977; Spiller and Amen, 1974).

A low fiber intake has been reported to be associated with ischemic heart disease, diabetes, constipation, appendicitis, hiatus hernia, hemorrhoids, diverticular disease of the colon and cancer of the colon (Kelsay, 1978; Burkitt, 1977; Spiller and Amen, 1974). The claims of health benefits have resulted in widespread recommendations to include more fiber in the diet (Turnlund, 1982). The addition of wheat bran to such products as bread, cakes, muffins and cookies is one method of increasing the dietary fiber content of the diet (Polizzoto et al., 1983).

Dietary fiber is a loosely defined group of polymers present in varying degrees in all plants and natural plant products (i.e., grains, vegetables, and fruits). The most important of these polymers are cellulose, hemicellulose, lignin and pectin. Cellulose is a high molecular weight polymer of repeating units of β-linked glucose. Hemicelluloses are made up of long chains of such monosaccharides as xylose, galactose, glucose, mannose and arabinose. Pectic substances are mostly polymers of galacturonic acid. Lignin is made up of repeating phenyl-propane units and is not a carbohydrate (Spiller and Amen, 1974). Various methods exist for the determination of dietary fiber in foods including the determination of crude fiber, acid detergent fiber (ADF) and neutral detergent fiber (NDF). These and other methods have been discussed
by Kelsay (1978).

Anderson and Clydesdale (1980a) analyzed the total dietary fiber content of a standard wheat bran using various methods. The results of their analyses are shown in Table IV. Anderson and Clydesdale (1980a) also reported that the dietary fiber of a standard wheat bran was composed of 28.3, 8.7, 3.2 and 3.0% hemicellulose, cellulose, lignin and pectin respectively.

Although wheat bran is being consumed in the diet, very little is known about the availability of the endogenous iron of this fiber source.

15. AVAILABILITY OF THE ENDOGENOUS IRON OF WHEAT BRAN

Wheat bran contains relatively high levels of iron when compared with other foods as shown in Table V. However, a survey of the literature reveals that little work has been done on the availability of iron from bran.

Some studies have compared the absorption of iron from whole grain and refined wheat products (Ranhotra et al., 1979; Bjorn-Rasmussen, 1974). Since the iron in whole grain flour is provided by both bran and endosperm, the availability of iron from bran itself cannot be ascertained. For example, Bjorn-Rasmussen (1974) measured the percentage iron absorption from rolls with and without added bran. Turnlund (1982) calculated the results to reflect the amount of iron absorbed in mg (Table VI). These results showed that the total amount of iron absorbed remained essentially the same while the percentage bran added to the rolls increased. However, because bran contains a significant amount of iron, the percentage absorption of iron decreased.
Table IV. Composition of total dietary fiber of a standard wheat bran by various methods\textsuperscript{a}

<table>
<thead>
<tr>
<th>Method</th>
<th>Dietary fiber content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractionation</td>
<td>44.12</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>46.0</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>40.2</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>11.9</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>8.91</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Adapted from Anderson and Clydesdale (1980a).
Table V. Iron content of some foods calculated from tabular values

<table>
<thead>
<tr>
<th>Food</th>
<th>mg iron/100 g edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, whole fluid</td>
<td>trace</td>
</tr>
<tr>
<td>Brown rice, cooked</td>
<td>0.5</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>0.7</td>
</tr>
<tr>
<td>Tuna, canned</td>
<td>1.9</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>1.9</td>
</tr>
<tr>
<td>Whole egg</td>
<td>2.2</td>
</tr>
<tr>
<td>Pork, roasted</td>
<td>2.9</td>
</tr>
<tr>
<td>Ground beef, cooked</td>
<td>3.2</td>
</tr>
<tr>
<td>Raisins</td>
<td>3.6</td>
</tr>
<tr>
<td>Liver, calf, cooked</td>
<td>14.4</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>15.0</td>
</tr>
<tr>
<td>Molasses, blackstrap</td>
<td>16.0</td>
</tr>
</tbody>
</table>

*aHealth and Welfare Canada, 1979.*
Table VI. Iron absorption by human subjects from rolls<sup>a</sup>

<table>
<thead>
<tr>
<th>Percent bran added to rolls</th>
<th>Iron content of flour and bran (mg/100g)</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg</td>
</tr>
<tr>
<td>0.0</td>
<td>0.48</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.085</td>
</tr>
<tr>
<td>1.7</td>
<td>0.62</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.088</td>
</tr>
<tr>
<td>3.3</td>
<td>0.75</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.107</td>
</tr>
<tr>
<td>6.7</td>
<td>0.93</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.059</td>
</tr>
<tr>
<td>10.0</td>
<td>1.20</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.102</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adapted from Turnlund (1982) based on data reported by Bjorn-Rasmussen (1974).
Many other studies have looked at the effect of adding wheat bran to a meal on the absorption of dietary nonheme iron (Reinhold, 1982; Reinhold et al., 1981; Simpson et al., 1981). Studies such as these have provided little information about the availability of iron from bran per se. However, it is generally agreed that the availability of iron from whole grains and wheat bran is poor (Turnlund, 1982; Erdman, 1981).

Hussain et al. (1965) examined the availability of iron from intrinsically labelled wheat. Whole wheat flour was cooked into pancakes and fed to human subjects. The average absorption of wheat iron was 4.5% in 21 healthy subjects and 7.8% in 21 iron deficient subjects.

Elwood et al. (1968) determined the absorption from bread by humans of the iron naturally present in bran. Bread was made from white flour with added intrinsically labelled bran. It was found that iron from wheat bran was less well absorbed than iron from ferric ammonium citrate added to bread made from white flour.

In another study, Layrisse and Martinez-Torres (1971) examined the absorption of iron from various intrinsically labelled foods and found that the absorption from wheat was 5%.

Finally, Erdman (1981) reported that various components of bran including phytate, phosphate and dietary fiber have the ability to chelate minerals. It was suggested that the availability of iron from bran may depend on the digestability of the various iron complexes formed with the bran components.
III. MATERIALS AND METHODS

1. BRAN

Hard red wheat bran was obtained from the American Association of Cereal Chemists (AACC, St. Paul, MN). Upon receipt of the bran some was sifted to obtain two different particle sizes. Five hundred g of bran was sifted on a series of Nalgene sieves fitted with a pan and cover, for 15 min using an automatic shaker (Eberbach Corporation, Ann Arbor, MI). The bran which passed through mesh No. 10 (2.00 mm diameter) but which was retained on mesh No. 20 (1.00 mm diameter) was collected and subsequently referred to as #10 bran. After sufficient #10 bran was obtained some was ground in a porcelain ball mill to further decrease the particle size. After grinding, the bran was sifted and that portion which passed through mesh No. 40 (0.0425 mm diameter) was collected and subsequently referred to as #40 bran. The bran was very difficult to grind and it took 72 to 96 h to obtain enough #40 bran for one muffin batch. This limited the number of experiments that could be done with #40 bran. It was assumed that the porcelain ball mill did not contribute iron to the bran.

Samples of bran were analyzed for moisture content and total iron. The bran was stored in plastic bags at approximately 4°C.

The particle size distribution of each bran fraction (#10 and #40 bran) was determined by a method similar to that recently described by Polizotto et al. (1983).
2. MUFFINS

All of the muffins used for this study were prepared by combining the ingredients listed in Table VII. The muffins were made using either one of the two particle sizes of bran. The muffin product was formulated so as to eliminate, as much as possible, all sources of dietary iron except that contributed by the wheat bran. The chemical composition of the muffin dough is shown in Table VIII. The moisture content and total iron of the unenriched flour were verified experimentally.

For each experiment, two separate batches of muffin dough were prepared. For each muffin batch, half of the dough was baked and half was unbaked. Paper-lined muffin tins were filled with about 65 g portions of dough. The muffins were baked in a preheated oven (Despatch Oven Company, Minneapolis, MN) at approximately 200 °C for 20 min. After cooling at room temperature for 45 min the muffins were frozen in plastic bags at about -25 °C. The unbaked dough was divided into approximately 65 g portions and immediately frozen in plastic bags. Samples of muffin dough and cooled, baked muffin were taken for moisture determination prior to freezing.

3. MOISTURE DETERMINATION

The moisture content of bran and flour was estimated by drying a known weight of the sample in a vacuum oven at 100 °C for approximately 5 hours as described by AOAC (AOAC, 1980, sections 14.002 - 14.003). The analyses were in triplicate and mean values were calculated.

The moisture content of baked and unbaked muffins was estimated by mixing a known weight of sample with quartz sand and water and heating on a hot plate until almost dry as described by AACC (AACC, 1978, section
Table VII. Muffin recipe

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>235 g fluid skim milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>230 g unenriched, untreated wheat flour&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 g AACC Certified hard red wheat bran</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 g corn oil&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 g granulated white sugar&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 g whole egg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 g baking powder&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 g salt&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Purity brand donated by Maple Leaf Mills, Toronto, Ont.
<sup>b</sup>Townhouse brand, Empress Foods Ltd., Vancouver, B.C.
<sup>c</sup>BC Sugar, Vancouver, B.C.
<sup>d</sup>Magic brand, Standard Brands Canada Ltd.
<sup>e</sup>Windsor brand, Canadian Salt Co., Ltd., Montreal, Que.
Table VIII. Chemical composition of muffin dough based on tabular values\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight (g)</th>
<th>Moisture (%)</th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Total CHO (g)</th>
<th>Iron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fluid skim milk</td>
<td>235</td>
<td>90</td>
<td>82</td>
<td>8.2</td>
<td>tr</td>
<td>11.8</td>
<td>0.091</td>
</tr>
<tr>
<td>unenriched flour\textsuperscript{c}</td>
<td>230</td>
<td>12</td>
<td>837</td>
<td>24.2</td>
<td>2.3</td>
<td>175.0</td>
<td>1.8</td>
</tr>
<tr>
<td>wheat bran\textsuperscript{d}</td>
<td>150</td>
<td>9</td>
<td>254</td>
<td>24.9</td>
<td>2.0</td>
<td>99.0</td>
<td>24.75</td>
</tr>
<tr>
<td>corn oil</td>
<td>75</td>
<td>0</td>
<td>663</td>
<td>0.0</td>
<td>75.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>white sugar</td>
<td>60</td>
<td>tr</td>
<td>231</td>
<td>0.0</td>
<td>0.0</td>
<td>59.7</td>
<td>0.057</td>
</tr>
<tr>
<td>whole egg</td>
<td>50</td>
<td>75</td>
<td>79</td>
<td>6.0</td>
<td>6.0</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>baking powder\textsuperscript{c,e}</td>
<td>16</td>
<td>tr</td>
<td>21</td>
<td>tr</td>
<td>tr</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>salt</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

| Total                       | 818        | 186          | 2167          | 63.3        | 85.3    | 351.5         | 27.80     |

\textsuperscript{a} Health and Welfare Canada (1979).

\textsuperscript{b} 0 = none, tr = trace, (-) = no suitable value found but measurable amount may be present.

\textsuperscript{c} USDA (1975).

\textsuperscript{d} AACC analysis.

\textsuperscript{e} Contains starch, monocalcium phosphate and sodium bicarbonate.
The samples were then dried in a vacuum oven at 100°C for approximately 5 h. Duplicate determinations for both baked and unbaked muffin samples were made for each muffin batch.

4. IN VITRO ESTIMATION OF RELATIVE IRON AVAILABILITY

Since adaptations were made to the method of Miller et al. (1981) to accommodate the conditions and facilities existing in the Department of Food Science at the University of British Columbia and because the in vitro determination is new and interesting, the protocol followed by this author will be described in detail. A flow diagram of the method is shown in Figure 1.

4.1 Reagents and Materials

Water - The water used throughout the analyses was distilled, deionized (d.d.) water.

Glassware - All glassware was washed, rinsed with d.d. water, soaked overnight in 1N HCL and rinsed again with d.d. water.

Orange juice - Bel-Air brand frozen orange juice concentrate (Empress Foods Ltd., Vancouver, B.C.). The orange juice was reconstituted with three volumes of d.d. water.

Ascorbic acid - (Matheson, Coleman and Bell Manufacturing Chemists, Norwood, OH).

Citric acid - (Matheson, Coleman and Bell Manufacturing Chemists, Norwood, OH).
Figure 1. Schematic of the in vitro method for determination of dialyzable iron.

Sample blended to slurry

\[ \downarrow \]

Homogenate adjusted to pH 2 with 6N HCL

\[ \downarrow \]

Incubation with pepsin 2h, 37°C shaking water bath

\[ \downarrow \]

Titratable Acidity

Incubation with pancreatin-bile 30 min, 37°C shaking water bath

\[ \downarrow \]

Addition of dialysis bag

Incubation with pancreatin-bile 2 h, 37°C shaking water bath

\[ \downarrow \]

Collection of dialysate pH 7-7.5

\[ \downarrow \]

Analysis of dialysate

\[ \downarrow \]

% Dialyzable iron

Pepsin - The solution was prepared just before use. Sixteen g pepsin (from porcine stomach mucosa, sigma P700, Sigma Chemical Co., St. Louis, MO) were suspended in 0.1N HCL and brought to 100 ml with 0.1N HCL.

Pancreatin-Bile - The solution was prepared just before use. Four hundred mg pancreatin (from porcine pancreas, Sigma P1750, Sigma Chemical Co., St. Louis, MO) and 2.5 g bile extract (porcine, Sigma B8631, Sigma Chemical Co., St. Louis, MO) were dispersed in 0.1 M sodium bicarbonate (Fisher Scientific Co., Fair Lawn, NJ) and brought to 100 ml with 0.1 M sodium bicarbonate.

Bathophenanthroline solution - A 0.001 M solution was prepared by dissolving 0.0334 g of bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) (Sigma B1250, Sigma Chemical Co., St. Louis, MO) in 50 ml of ethyl alcohol. The solution was heated in a water bath to dissolve the reagent, cooled and brought to 100 ml with d.d water.

Hydroxylamine Hydrochloride - Reagent grade hydroxylamine hydrochloride contains appreciable amounts of iron. An adaptation of the method described by Smith et al. (1952) was used to purify the hydroxylamine hydrochloride. One hundred ml of a 20% aqueous solution of hydroxylamine hydrochloride (Sigma H9876, Sigma Chemical Co., St. Louis, MO) were prepared and placed in a 250 ml conical separatory funnel. Three to 4 ml of 0.001 M bathophenanthroline were then added. Next, 10 to 20 ml of chloroform (BDH Chemicals, Toronto) were added to the separatory funnel, the contents of the funnel were shaken and the layers allowed to separate for 5 min. Then the colored bottom layer containing the
chloroform and extracted red iron impurity was drawn off and discarded. The separation was repeated until a colorless solution of hydroxylamine hydrochloride was obtained. The iron-free hydroxylamine hydrochloride solution was stored in a glass bottle at 4°C.

Protein precipitant solution - The solution was prepared just before use. Ten g trichloroacetic acid (Fisher A322, Fisher Scientific Co., Fair Lawn, NJ), 50 ml 20% purified hydroxylamine hydrochloride and 10 ml concentrated HCL were combined and brought to 100 ml with d.d. water.

Chromagen solution - The solution was prepared just before use. Twenty-five mg bathophenanthroline sulfonate (4,7-diphenyl-1,10-phenanthroline disulfonate) (Sigma 1375, Sigma Chemical Co., St. Louis, MO) were dissolved in 4M sodium acetate in a 100 ml volumetric flask. The solution was heated to approximately 80°C in a water bath to dissolve the reagent, cooled and brought to 100 ml with 4M sodium acetate.

Dialysis tubing - Spectrapor brand dialysis tubing with molecular weight cutoff of 6000-8000 (Fisher 08-670C, Fisher Scientific Co., Fair Lawn, NJ) was cut into 15 cm lengths and soaked in d.d. water until required.

Sample bottles - Wheaton brand glass, 120 ml bottles with plastic snap caps (Fisher 03-335 10D, Fisher Scientific Co., Fair Lawn, NJ) were used for the pancreatin-bile digestion.

Atomic absorption spectrophotometer - A Perkin Elmer model 4000 (Perkin Elmer, Norwalk, CT) double beam atomic absorption spectrophotometer with air-acetylene flame was used to measure iron at 248.3 nm.
4.2 Procedure

The general plan of the experimental procedure is shown in Figure 2. For each muffin batch the in vitro analysis was carried out separately at the same time on baked and unbaked samples. One slurry was prepared using baked muffins and a separate slurry was made with unbaked muffin dough.

4.2.1 Slurry preparation

Frozen muffin samples were thawed at room temperature prior to blending. The slurry components were homogenized in a Waring blender (Waring Products Division, Dynamics Corp. of America, New Hartford, CT) to a creamy consistency. The slurry was adjusted to pH 2 with 6N HCL while still in the glass blender jar. With the blender running at a low speed, acid was added to the slurry dropwise by pipette. At intervals, the blender was stopped and the pH of the slurry determined using an Accumet brand pH meter (Fisher Scientific Co., Fair Lawn, NJ).

4.2.2 Pepsin digestion

From each slurry, 2x 100g aliquots of muffin mixture were transferred to 250 ml erlenmeyer flasks. Ten ml of the pepsin suspension were added to each flask (Since Sigma P700 pepsin is about 32% pepsin, this is equivalent to about 0.5 g pepsin). The flasks were sealed with parafilm and aluminum foil and the slurries were incubated for 2 h in a 37°C shaking water bath (Magni-Whirl, Blue M Electric Co., Blue Island, IL).

After incubation, samples were removed from each of the flasks of pepsin digest as follows: 1) Twenty g aliquots of the pepsin digest were transferred to each of 3x120 ml snap cap bottles. These aliquots
Figure 2. General plan of the experimental procedure

Experiment 1

Muffin Batch A

Baked Muffins

continues as for unbaked muffins

Unbaked Muffins

Muffin Batch B

continues as for Muffin Batch A

Pepsin Digest 1

Dialysate 1

Dialysate 2

Pepsin Digest 2

Dialysate 3
were frozen until required for the pancreatin-bile digestion. 2) A fourth 20 g aliquot was transferred to a 30 ml beaker. Each beaker was sealed with parafilm and aluminum foil and the samples were frozen until required for the determination of titratable acidity.

4.2.3 Titratable acidity

The frozen aliquots were thawed in a 37°C water bath. Five ml of the pancreatin-bile suspension were added to each beaker of thawed digest. Using a pH meter, the titratable acidity was determined by titrating the combined pepsin digest pancreatin-bile extract mixture with 0.5N potassium hydroxide (Fisher Scientific Co., Fair Lawn, NJ) to pH 7.5 and recording the volume of potassium hydroxide required.

4.2.4 Preparation of dialysis bags

Each prepared dialysis bag contained a total volume of 20 ml. The volume of each dialysis bag was made up of an amount of 0.5N sodium bicarbonate (Fisher Scientific Co., Fair Lawn, NJ) equivalent to the amount of potassium hydroxide used in the titratable acidity determination and d.d. water. The dialysis bags were stored in a sodium bicarbonate solution of equivalent concentration until just before use. String was used to close the bags.

4.2.5 Pancreatin-bile digestion

The frozen aliquots were thawed in a 37°C shaking water bath. Then one prepared dialysis bag was placed in each bottle of thawed digest and the bottles were sealed with plastic caps. Care was taken that each bag was completely covered with pepsin digest. The samples were incubated
in a 37 °C shaking water bath for 30 min. Then, 5 ml of the pancreatin-bile solution were added to each bottle of digest and the incubation was continued for an additional 2 h.

4.2.6 Collection of dialysate

At the end of the incubation period, the dialysis bags were removed from the digest and rinsed by dipping in d.d. water. One end was cut off each bag and the contents were transferred into a preweighed test tube. The test tube was then reweighed and the weight of the dialysate determined by difference.

4.2.7 Analysis of dialysate

Bathophenanthroline reactive iron was measured in the dialysates immediately after the pancreatin-bile incubation. A 10 ml aliquot of each dialysate was transferred to a clean test tube. Five ml of the protein precipitant solution were added to each tube of dialysate. Then the tubes were covered with parafilm and aluminum foil and were left to sit overnight at approximately 4 °C.

The next morning the tubes of dialysate were removed from refrigeration. The addition of the protein precipitant solution had caused the samples to turn cloudy. To obtain a clear solution for analysis, the tubes were heated in a boiling water bath for 10 min. After cooling to room temperature, each dialysate was transferred to a 50 ml centrifuge tube and centrifuged (RC-2B Automatic Superspeed Refrigerated Centrifuge, Ivan Sorvall Inc., Norwalk, CT) at 8000 rpm for 15 min.
Each dialysate was analyzed in triplicate and a mean value was calculated. For each dialysate, 3 ml aliquots of clear supernatant were transferred to each of 3 test tubes. Then 1.5 ml of chromagen solution were added. Each tube was mixed using a vortex mixer (Thermolyne Corp., Dubuque, IA) and allowed to react 10 min prior to reading the absorbance of the solution at 533 nm. A Cary 210 spectrophotometer (Varian Associates Inc., Palo Alto, CA) was used to determine absorbance and d.d. water was used to zero the instrument. For each run, three reagent blanks and two iron standards (0.5 ppm and 1.0 ppm iron in 0.1N HCL) were treated and analyzed by the same procedures as the dialysates.

A sample blank was prepared for each dialysate in order to account for any absorbance due to the color of the dialysate itself. For each dialysate, one 3 ml aliquot of clear supernatant was transferred to a test tube and 1.5 ml of d.d. water were added. After mixing, the absorbance of the sample blanks was read at 533 nm and the value subtracted from the absorbance for the bathophenanthroline reactive iron determined for each dialysate.

A standard curve was prepared using iron standards made up in 0.1N HCL (see Appendix A). The equation of the line was determined by linear regression on a Monroe calculator (Litton Business Systems Inc.). The concentration of iron in each dialysate was determined using the standard curve and was calculated as ug Fe/ml dialysate.
4.2.8 Determination of nonheme iron

As mentioned, the total iron content and nonheme iron content of a muffin product are essentially identical (Miller, 1982). Duplicate samples of baked and unbaked muffin from each muffin batch were analyzed for total iron by atomic absorption spectroscopy.

Frozen muffin samples were thawed at room temperature. Aliquots of thawed muffin were wet ashed by boiling with a mixture of hydrochloric and nitric acids according to the method of Maurer (1977). The digested samples were cooled, made up to volume with d.d. water and filtered (Whatman 541). The clarified solution was analyzed for iron by atomic absorption spectroscopy. Reagent blanks were carried through the entire ashing procedure. The concentration of iron in muffins was calculated on a dry weight basis.

The total iron content of bran and unenriched flour was determined in triplicate by the same method.

4.2.9 Calculation of percent dialyzable iron

The results were calculated as percent dialyzable iron (%DI) according to the following equation:

\[
\% \text{DI} = \frac{A \times B \times 100}{C \times D}
\]

where:
- \(A\) = ug Fe/ml dialysate
- \(B\) = ml dialysate
- \(C\) = ug nonheme Fe/g muffin (dry weight basis)
- \(D\) = g muffin in 20 g aliquot pepsin digest (dry weight basis)
5. **RESEARCH DESIGN AND ANALYSIS OF DATA**

Two preliminary studies were carried out to determine if the results obtained by Miller et al. (1981) using their in vitro method could be reproduced. Two test meals were formulated and prepared to duplicate those used by Miller et al. (1981). The experimental results obtained were similar to those presented by Miller et al. (1981) and indicated that the methodology could be used to estimate relative iron availability. The results of the preliminary studies are presented in Appendix B.

Seven experiments were conducted to estimate the relative availability of the intrinsic iron of wheat bran when incorporated into a muffin product. Baked and unbaked muffins from each batch were blended to a slurry separately with either water, orange juice or aqueous solutions of ascorbic acid, citric acid or ascorbic and citric acids in amounts assumed to be provided by 250 ml of orange juice. A summary of the conditions varied in each experiment is presented in Table IX.

The statistical analysis was performed using an Amdahl 470/8 computer and a program adapted by Greig and Osterlin (1978). The data were analyzed by two factor analysis of variance and mean values of % dialyzable iron were compared by Duncan's multiple range test (Zar, 1974).
Table IX. A summary of experimental conditions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Bran Particle Size</th>
<th>Slurry Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td># 10</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 ml d.d. water</td>
</tr>
<tr>
<td>2</td>
<td># 40</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 ml d.d. water</td>
</tr>
<tr>
<td>3</td>
<td># 10</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 ml d.d. water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130 mg ascorbic acid</td>
</tr>
<tr>
<td>4</td>
<td># 10</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ml orange juice</td>
</tr>
<tr>
<td>5</td>
<td># 40</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ml orange juice</td>
</tr>
<tr>
<td>6</td>
<td># 10</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 ml d.d. water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 g citric acid</td>
</tr>
<tr>
<td>7</td>
<td># 10</td>
<td>130 g muffin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 ml d.d. water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130 mg ascorbic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 g citric acid</td>
</tr>
</tbody>
</table>
IV. RESULTS

The data obtained from each of the experiments are summarized in Table X. Results of the analysis of variance and Duncan's multiple range test for the effects of acid treatment, baking and bran particle size on the availability of iron from wheat bran are presented in Tables XI, XII, and XIII respectively. The effect of bran particle size was not determined directly but was based on observations of significant differences in % DI between acid treatments which varied only in bran particle size. Superscripts are used to designate where or if significant differences exist among mean values. Details of the analysis of variance and Duncan's multiple range test are discussed in Appendix C.

Other experimental results which are not directly related to the discussion of iron availability from wheat bran are shown in Appendix D.
Table X. Experimental data for the determination of % dialyzable iron (% DI).

<table>
<thead>
<tr>
<th>Experiment&lt;sup&gt;a&lt;/sup&gt; (Acid Treatment)</th>
<th>Baking Effect</th>
<th>Muffin Batch (Duplicate)</th>
<th>ug Fe&lt;sup&gt;b&lt;/sup&gt; g muffin (dry weight)</th>
<th>% DI&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baked</td>
<td>1</td>
<td>67.36 ± 0.21</td>
<td>0.007 ± 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>69.67 ± 1.65</td>
<td>0.005 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>70.13 ± 2.86</td>
<td>0.005 ± 0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>68.24 ± 1.95</td>
<td>0.002 ± 0.004</td>
</tr>
<tr>
<td>2</td>
<td>Baked</td>
<td>1</td>
<td>63.45 ± 1.79</td>
<td>0.003 ± 0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>63.24 ± 3.99</td>
<td>0.002 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>67.25 ± 2.02</td>
<td>0.002 ± 0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65.78 ± 2.65</td>
<td>0.007 ± 0.005</td>
</tr>
<tr>
<td>3</td>
<td>Baked</td>
<td>1</td>
<td>64.65 ± 0.35</td>
<td>0.078 ± 0.084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65.56 ± 2.83</td>
<td>0.042 ± 0.043</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>68.72 ± 0.74</td>
<td>0.017 ± 0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>68.87 ± 2.55</td>
<td>0.067 ± 0.053</td>
</tr>
<tr>
<td>4</td>
<td>Baked</td>
<td>1</td>
<td>66.53 ± 0.73</td>
<td>4.213 ± 0.217</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>64.23 ± 0.80</td>
<td>4.420 ± 0.189</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>66.00 ± 2.22</td>
<td>4.993 ± 0.144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>67.82 ± 0.06</td>
<td>4.748 ± 0.331</td>
</tr>
<tr>
<td>5</td>
<td>Baked</td>
<td>1</td>
<td>67.50 ± 0.04</td>
<td>3.920 ± 0.135</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65.41 ± 0.12</td>
<td>4.197 ± 0.245</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>67.36 ± 0.09</td>
<td>4.445 ± 0.172</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>64.88 ± 1.71</td>
<td>4.927 ± 0.285</td>
</tr>
</tbody>
</table>
Table X. (continued)

<table>
<thead>
<tr>
<th>6</th>
<th>Baked</th>
<th>1</th>
<th>68.13 ± 2.68</th>
<th>1.792 ± 0.099</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65.82 ± 2.27</td>
<td>1.872 ± 0.117</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>69.47 ± 0.61</td>
<td>2.367 ± 0.097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>66.69 ± 0.15</td>
<td>2.200 ± 0.174</td>
</tr>
<tr>
<td>7</td>
<td>Baked</td>
<td>1</td>
<td>63.44 ± 0.91</td>
<td>2.518 ± 0.086</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65.27 ± 1.47</td>
<td>2.255 ± 0.085</td>
</tr>
<tr>
<td></td>
<td>Unbaked</td>
<td>1</td>
<td>67.35 ± 0.85</td>
<td>2.602 ± 0.117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>66.22 ± 0.71</td>
<td>2.672 ± 0.067</td>
</tr>
</tbody>
</table>

^a Refer to Table IX for complete details of experimental conditions.

^b Values are expressed as means ± standard deviation, n=2.

^c Values are expressed as means ± standard deviation. The mean values were calculated from 6 values for % DI, 3 from each of 2 digests. Refer to Figure 2 for clarification of the experimental plan.
Table XI. The effect of acid treatment on % DI from wheat bran.

<table>
<thead>
<tr>
<th>Acid Treatment (^a) (Experiment #1)</th>
<th>&quot;Acid&quot; Tested</th>
<th>% DI(^b, c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 water</td>
<td>0.005 ± 0.006 (^h)</td>
<td></td>
</tr>
<tr>
<td>2 water</td>
<td>0.003 ± 0.005 (^h)</td>
<td></td>
</tr>
<tr>
<td>3 ascorbic acid</td>
<td>0.051 ± 0.058 (^h)</td>
<td></td>
</tr>
<tr>
<td>4 orange juice</td>
<td>4.594 ± 0.374 (^p)</td>
<td></td>
</tr>
<tr>
<td>5 orange juice</td>
<td>4.372 ± 0.429 (^p)</td>
<td></td>
</tr>
<tr>
<td>6 citric acid</td>
<td>2.057 ± 0.267 (^j)</td>
<td></td>
</tr>
<tr>
<td>7 citric &amp; ascorbic acids</td>
<td>2.512 ± 0.182 (^k)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Refer to Table IX for complete details of experimental conditions.

\(^b\) Values are expressed as means ± standard deviation. (n = 24)

\(^c\) Means not sharing the same superscript are significantly different \((p<0.05)\)

Table XII. The effect of baking on % DI from wheat bran.

<table>
<thead>
<tr>
<th>Baking</th>
<th>% DI(^a, b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked muffins</td>
<td>1.809 ± 1.759 (^h)</td>
</tr>
<tr>
<td>Unbaked muffins</td>
<td>2.075 ± 2.009 (^j)</td>
</tr>
</tbody>
</table>

\(^a\) Values are expressed as means ± standard deviation. (n = 84)

\(^b\) Means not sharing the same superscript are significantly different \((p<0.05)\)
Table XIII. The effect of bran particle size on % DI from wheat bran.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Bran Particle Size</th>
<th>% Dialyzable Iron(^{a,b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td># 10</td>
<td>0.005 ± 0.006(^{h})</td>
</tr>
<tr>
<td>2</td>
<td># 40</td>
<td>0.003 ± 0.005(^{h})</td>
</tr>
<tr>
<td>4</td>
<td># 10</td>
<td>4.594 ± 0.374(^{p})</td>
</tr>
<tr>
<td>5</td>
<td># 40</td>
<td>4.372 ± 0.429(^{p})</td>
</tr>
</tbody>
</table>

\(^{a}\)Values are expressed as means ± standard deviation. (n = 24)

\(^{b}\)Means not sharing the same superscript are significantly different (p < 0.05).
IV. DISCUSSION

1. INTERPRETATION OF RESULTS

It was the purpose of this study to examine if acid treatment, baking and/or bran particle size had any effect on the availability of iron from wheat bran. As in any scientific analysis, the results of this study were influenced by experimental error. However, the statistical model (see Appendix C) was designed to take the variability due to experimental error into consideration in the determination of any significant treatment effects. As a result, the treatment effects themselves were found to significantly influence the availability of iron from wheat bran in spite of the fact that significant variation could be attributed to experimental error in the determination of iron availability.

It should be noted that the determination of statistically significant treatment effects does not necessarily indicate that they are of any practical significance. However, the results found in this study did seem reasonable and attempts have been made to discuss the experimental findings based on information provided in the literature.

2. AVAILABILITY OF IRON FROM WHEAT BRAN

Results of Experiments 1 and 2, as shown in Table XI illustrate that essentially none of the iron from wheat bran was available for absorption when bran muffins were combined with water. The form of iron present in wheat bran is not known. Work carried out by Camire and Clydesdale (1982, 1981) suggested that the endogenous iron of bran exists in a water insoluble form and may be complexed with protein. Morris and
Ellis (1982) recently determined that over 60% of the endogenous iron in wheat bran is present as monoferric phytate (MFP) which is probably bound to protein and other cellular components.

Morris and Ellis (1982) investigated the availability of iron fed as MFP using the hemoglobin repletion test in rats. It was found that the iron from purified MFP prepared from extracts of wheat bran and 2 synthetic preparations was highly available.

Simpson et al. (1981) measured the absorption of iron fed as free MFP using the extrinsic tag method in humans. The MFP was added to meals of both high and low availability. It was determined that iron fed as MFP was no less well absorbed by human subjects than the dietary nonheme iron in the meals.

However, in contrast to the reported availability of iron fed as free MFP, it is generally recognized that the availability of endogenous iron from wheat bran is poor (Turnlund, 1982; Erdman, 1981). Numerous studies have shown that when wheat bran was added to a meal the absorption of dietary iron was decreased (Morris and Ellis, 1982; Reinhold, 1982; Reinhold et al. 1981; Simpson et al., 1981). Although the nature of the inhibition is unknown it has been attributed to the formation of complexes of iron with such compounds as phytate and dietary fiber (Simpson et al., 1981). It seems reasonable to assume that the same binding mechanisms may be responsible for the complexation and poor availability of the endogenous iron of wheat bran. Therefore, the findings of some recent studies of the interaction of iron with wheat bran will be discussed.

Thompson and Weber (1979) investigated the binding of endogenous copper, zinc and iron in 6 fiber sources, including wheat bran, using an in vitro method. A dual incubation procedure at pH 0.65 and then at
pH 6.8 was used to simulate the changes in pH that occur during the
in vivo digestion process. It was found that most minerals were released
into solution after incubation at pH 0.65 but were bound after incubation
at pH 6.8. These results suggest that while the endogenous minerals
of bran may not be bound at the very acidic pH of the stomach they may
be rebound as the pH is raised and thereby become unavailable for absorption
in the duodenum.

Simpson et al. (1981) investigated the effect of whole wheat bran
and its components on the absorption of nonheme dietary iron using a
double isotope technique in humans. In their first experiment, test meals
consisted of 2 muffins and a milkshake. The muffins were either plain or
contained 6 g of wheat bran. It was found that bran muffins reduced
absorption of dietary nonheme iron by 74% compared to absorption from a
meal containing plain muffins.

In their next experiment, Simpson et al. (1981) examined the effect
of bran phytate on iron absorption. They found that dephytinized bran
produced the same degree of inhibition of dietary nonheme iron absorption
as did whole bran and they suggested that the inhibitory effect of bran
should not be attributed to its phytate content.

As reported by Maga (1982), the binding of iron by phytate has been
extensively studied but many of the data are contradictory. Some investigators
have shown phytate to be inhibitory to iron absorption whereas others have
shown it to have no effect.

In a third experiment, Simpson et al. (1981) attempted to identify
the fraction of wheat bran that inhibits iron absorption. Dephytinized
bran was separated into a soluble, phosphate-rich fraction and an insoluble,
high-fiber fraction. The total phosphorus content was 13 mg in insoluble
bran and 166 mg in soluble bran; neutral detergent fiber was 4.6 g in insoluble bran and was undetectable in the soluble fraction. It was found that inhibition was greater with soluble than with insoluble bran, suggesting that phosphate was more inhibitory than fiber. However, these workers concluded that they could not clearly identify the bran fraction responsible for the inhibition of iron absorption.

In another study, Reinhold et al. (1981) investigated the binding of iron by wheat bran and the dietary fiber of wheat bran using an in vitro method. Neutral detergent fiber (NDF), which contains cellulose, lignin, hemicellulose and pectin; and acid detergent fiber (ADF), which is comprised of cellulose and lignin, were prepared from AACC wheat bran for use in the investigation. Native wheat bran, dephytinized wheat bran and cellulose were also examined in the study.

The quantity of iron bound by wheat NDF was about 0.38 µg of iron per g of NDF. Binding of iron was minimal, although appreciable, below pH 4.0, but increased rapidly above pH 5.0 to a maximum near pH 7.0.

Reinhold et al. (1981) attempted to explain the binding of iron by wheat NDF. They observed that the uptake of iron with increasing pH produced a sinusoidal curve with its midpoint at about pH 5.8 which suggested the presence of a proton-dissociating group. It had been reported that titration of wheat bran yielded a curve with an inflection near pH 6.0 and that iron binding by wheat gluten increased between pH 4.0 and 7.0. It was postulated that fiber-bound protein could be similarly active. However, removal of nearly all of the fiber-bound protein by treatment of NDF with peptidases failed to alter the pH dependence of iron binding. It was then suggested that the binding of iron by NDF could be due to the unsubstituted uronic acid groups of hemicellulose.
Acid detergent fiber of wheat was found to bind approximately 0.25 μg of iron per mg of ADF. The binding of iron by ADF was attributed to its cellulose component. Native bran behaved like NDF with respect to iron uptake and response to pH but had a lower affinity for iron. Finally, dephytinized wheat bran was found to bind iron as effectively as native bran. It was concluded by Reinhold et al. (1981) that the dietary fiber of wheat bran appears to bind dietary nonheme iron. It seems possible that dietary fiber may also have the ability to bind the endogenous iron of bran.

Camire and Clydesdale (1981) investigated the metal binding capacity of wheat bran and fractionated wheat bran from which water soluble polysaccharides, protein and starch had been removed. The binding of added ferrous sulfate was determined using an in vitro method. It was found that the fractionation treatment resulted in significantly more iron bound in comparison to the unfractionated wheat bran. It was suggested that this could be due to the fact that the lignin, cellulose and hemicellulose concentrations were increased as a result of the fractionation treatment. Results from the same study had indicated that lignin had a high metal binding capacity.

As mentioned, the iron from wheat bran was found to be unavailable for absorption under the conditions of in vitro method used in the present study. It is postulated that at least some of the endogenous iron of wheat bran may exist in a form such as monoferric phytate which is complexed with protein components of the bran. During peptic digestion at acidic pH the iron may be released from bran in a soluble form. However, at neutral and alkaline pH the iron may be rebound to various components of the dietary fiber of wheat bran and made unavailable for
absorption. The iron in wheat bran may also exist in a form which is not solubilized during the digestion procedure and is therefore not potentially available for absorption.

It is also possible that other ingredients of the muffin product such as egg or milk may bind any iron released from wheat bran. However, the effect of dietary components on the absorption of dietary nonheme iron is a topic in itself. Since all of the experiments utilized the same muffin formulation, it has been assumed that any possible effects of muffin ingredients were constant and therefore not of any significance in the present study of relative iron availability.

Clydesdale (1982) recently reviewed several proposed mechanisms for the binding of cations by the dietary fiber component, pectin, and suggested that the proposed mechanisms could have significance in iron availability. A simple model postulated that acidic polysaccharides such as pectin could be converted into anion exchangers by complexing with trivalent cations, which have the ability to bind a variety of anions.

Another model, described in detail by Clydesdale (1982), suggested that two carboxylic acid groups, which belong to neighboring galacturonic acid residues in the linear chain of pectin, could participate in binding with one trivalent cation. A third carboxylic acid group could be furnished by a neighboring linear chain and result in complete neutralization of the trivalent cation; thereby inhibiting its cationic nature. It was suggested that insoluble pectin fibers which have been neutralized with a trivalent cation would have a greater affinity for various anions than a fiber which has been neutralized by a divalent cation, due to a greater density of positive charge.
Clydesdale (1982) suggested that such reactions could partially explain the greater availability of ferrous iron compared to ferric iron. It was postulated that perhaps there is a tendency for trivalent ferric iron to form cationic complexes while divalent ferrous iron may tend to form neutral complexes. As a result, the reactivity of the ferric complex would increase, thereby increasing its potential for further binding and decrease its potential availability. Conversely, the ferrous complex would be soluble and more chemically inert so that it would be more available for absorption.
3. EFFECT OF ORANGE JUICE ON IRON AVAILABILITY

The effects of various acid treatments on the relative availability of iron from wheat bran, as estimated by % dialyzable iron (% DI), are compared in Table XI. Among the acid treatments examined in the present study, orange juice showed the most significant (p < 0.05) enhancement of iron availability; resulting in % DI values of approximately 4.59% and 4.37% from "meals" containing muffins made from # 10 and # 40 bran respectively.

In order to evaluate the effect of orange juice on the availability of iron from wheat bran; ascorbic acid, citric acid and a combination of ascorbic and citric acids, in amounts assumed to be present in 250 ml of orange juice, were added to muffin "meals". The substitution of orange juice with a solution of ascorbic and citric acids resulted in a % DI value of 2.51%; while citric acid alone and ascorbic acid alone resulted in % DI values of about 2.06% and 0.051% respectively. The combined effect of citric and ascorbic acids was significantly (p < 0.05) less than the effect of orange juice, but was significantly (p < 0.05) greater than the effect of citric acid alone. However, the ability of citric acid to enhance iron availability was significantly (p < 0.05) greater than that of ascorbic acid alone.

It is known that the availability of iron is related to the chemical state of iron. In a recent study, Kojima et al. (1981) examined the effect of ascorbic acid, citric acid and orange juice on the solubilization of iron from cooked pinto beans using an in vitro method. Bean suspensions were incubated with the test solutions at 37°C. After centrifugation of the mixtures, the supernatants were analyzed for iron. Although the study does not examine the availability of iron from wheat bran, it does
provide insight into the effect of orange juice and its constituent organic acids on the solubilization of iron from a vegetable food.

In a single incubation procedure a 10 mM solution of ascorbic acid was incubated with a cooked pinto bean suspension for 30 min at pH 2. Approximately 50% of the iron from the bean suspension was solubilized and virtually all of the soluble iron was in the ferrous state. In another procedure, 10 mM solutions of ascorbic acid were incubated with the bean suspension at variable pH. It was found that ascorbic acid was maximally effective in solubilizing iron in the pH range from 1.5 to 5. Throughout the pH range almost all of the supernatant iron was found in the ferrous form.

Kojima et al. (1981) postulated that the ability of ascorbic acid to enhance the solubilization of iron from a cooked pinto bean suspension may occur via a reductive iron release mechanism. The ascorbic acid may be able to reduce tightly bound ferric iron to the ferrous form with subsequent release of the iron from the ligands associated with the insoluble phase.

The effect of citric acid on iron solubilization was also investigated. It was found that the ability of a 10 mM solution of citric acid to solubilize iron from a cooked pinto bean suspension was maximal near pH 6 and had little if any effect in the acidic range. A 10 mM solution of citric acid solubilized approximately 46% of the iron from a cooked pinto bean suspension and 25% of the soluble iron was in the ferrous state.

A dual incubation procedure was used to examine the effect of combining citric acid and ascorbic acid. The bean suspension was first incubated at pH 2 for 30 min in the presence of a 10 mM solution of ascorbic acid. After the pH was raised to 6.0, a 10 mM solution of citric acid was
added and the mixture reincubated for an additional 30 min. It was found that when ascorbic and citric acids were combined their effects were additive and virtually all of the complexed iron was in the reduced form.

Kojima et al (1981) suggested that the solubilization of iron from a cooked pinto bean suspension by citric acid may be by chelation of iron in the ferric form.

The effect of various beverages on iron solubilization was examined using a dual incubation procedure. It was found that three orange juice preparations; Tang, frozen reconstituted, and fresh orange juice, were active in solubilizing iron from a cooked pinto bean suspension. Fresh orange juice solubilized approximately the same amount of iron as did a combination of 10 mM citric acid and 10 mM ascorbic acid in a dual incubation experiment. It was proposed that the ability of orange juice to solubilize iron was most likely due to its content of citric and ascorbic acids.

In another recent study, Reinhold et al. (1981) investigated the binding of iron by the fiber of wheat bran using an in vitro method at pH 6.45. It was found that ascorbic acid and citric acid, among other compounds such as cysteine, EDTA and phytate, strongly decreased the binding of iron by wheat bran and by the neutral detergent fiber of wheat bran. Generally, inhibition of iron binding occurred at less than 1 mM/L and was proportional to the log of the concentration of the inhibitor. However, the behaviour of ascorbic acid was unique in that inhibition was small and constant at concentrations below 0.3 mM/L. However, when this concentration was exceeded, binding became proportional to the log of the concentration of ascorbic acid. It was concluded that among the inhibitors of iron binding examined, ascorbic acid was the least active
at low concentrations because of the plateau of inhibiting activity shown by it.

Reinhold et al. (1981) suggested that the ability of ascorbic and citric acids to inhibit the binding of added iron by wheat bran indicates that these compounds may promote iron absorption by their ability to release iron from its combination with dietary fiber.

The exact mechanism by which ascorbic acid enhances the absorption of nonheme iron is not completely understood. Brise and Hallberg (1962) and Monsen and Page (1978) suggested that the effect of ascorbic acid was mainly due to its reducing action within the intestinal lumen. Ascorbic acid may increase the absorption of nonheme iron from foods by converting ferric iron to the more available ferrous form. Other workers suggested that ascorbic acid forms a soluble chelate with ferric iron and thereby prevents the formation of unabsorbable polymers and precipitates at neutral and alkaline pH (Conrad and Schade, 1968; Crosby, 1968).

In a recent review, Clydesdale (1982) proposed a mechanism for the effect of ascorbic acid on increasing the availability of nonheme iron. It was explained that at acidic pH, ferrous and ferric ions are soluble and exist as hydrates, with the standard reduction potential of
\[ \text{Fe}^{3+} (aq) + e^- \rightarrow \text{Fe}^{2+} (aq) \] being +770 mv. In the presence of ascorbic acid, which has a standard reduction potential of +440 mv, the formation of ferrous iron will take place spontaneously. At acidic pH, both ferrous-ascorbate and ferric-ascorbate complexes may also form. However, the overall effect of ascorbic acid at acidic pH is to act as a reducing agent. This implies that in an acidic solution containing ferric iron and ascorbic acid that the ferrous form will predominate and ferri-
ascorbate complexes will tend to destabilize. It was further explained that as the pH is raised, the hydrates of iron begin to lose protons and form their respective hydroxides which are increasingly insoluble with increasing pH. In basic solutions the standard reduction potential of \( \text{Fe(OH)}_3(s) + e^- \rightarrow \text{Fe(OH)}_2(s) + \text{(OH)}^- \) is -560 mv. Since most foods have a standard reduction potential of +400 mv, the formation of ferric hydroxide is favored with and without ascorbic acid. Therefore, the effect of ascorbic acid as the pH is raised appears to be to maintain the ferric form of iron in solution by forming a ferric-ascorbate complex.

A review of the literature reveals that there has been little reference to the ability of citric acid to enhance iron availability. However, it is recognized that citric acid is an effective chelating agent (Lindsay, 1976). Any molecule or ion with an unshared electron pair can coordinate or complex with metal ions. Foods contain a variety of compounds (called chelating agents or ligands) which have the ability to chelate metal ions (Furia, 1964). The steric location and number of groups on a ligand for combination with metal ions determines the characteristics of the metal complexes. For example, EDTA has the ability to form 6 coordinating bonds with iron resulting in a stable chelate which may not liberate iron for absorption while, citric acid, which has 3 carboxylate groups to bind metal, forms an unstable complex that holds iron in solution at neutral and weakly alkaline pH (Benjamin, 1967).

The formation of a chelate involves an equilibrium reaction, \( M + Y \leftrightarrow MY \), where \( M \) is the metal ion, \( Y \) is the coordinating agent and \( MY \) the metal complex. The rate at which a metal complex is formed is determined by the stability or equilibrium constant of the metal complex which is given by the following equation:
As the stability constant increases, more of the metal is complexed and the metal in the complex is more tightly bound (Lindsay, 1976; Furia, 1964).

The formation of a metal chelate is also influenced by pH. Kojima et al. (1981) found that citric acid was most effective in solubilizing iron from a cooked pinto bean suspension at pH 6.0 and had little effect at pH 2. They suggested that the carboxylic acid groups of citric acid are protonated at acidic pH which interferes with their ability to bind iron. As reported by Lindsay (1976), a gradual increase in pH allows dissociation of the carboxyl groups and enhances chelating efficiency.

In foods, stability constants alone are of limited value in predicting the effectiveness of a chelate because of interference by a variety of competing substances such as other metal ions, precipitants and other chelating agents (Furia, 1964). Hydroxyl ions will compete with the metal ion for the chelating agent resulting in the formation of an insoluble hydroxide. As described by Furia (1964), this involves the competition of a complexing agent (Y) and a precipitating agent (X) in the reaction, \( MX + Y \rightleftharpoons MY + X \). If the reaction is forced to the right and if the metal complex (MY) is soluble, then the precipitate (MX) will dissolve. This will occur when the stability constant of the metal complex (MY) is greater than the reciprocal of the solubility product of the precipitate (MX), when the components M, X and Y are present in equimolar concentrations.

There is very little information on the stability constants and solubility products of various iron complexes as they exist in foods.
Knowledge of these values would aid in the understanding of the binding mechanisms and availability of food iron.

The pH dependence for the binding of iron by dietary fiber has been described by Thompson and Weber (1979). As well, the effect of pH on the action of ascorbic and citric acids has been discussed (Kojima et al., 1981; Lindsay, 1976). It should be noted that in the present study, each of the muffin slurries was adjusted to pH 2.0 prior to the pepsin digestion and each slurry was subsequently adjusted to approximately pH 7.0 during the dialysis of iron. Therefore, differences in pH among the acid treatments was not a factor in the determination of relative iron availability.

The experimental results obtained in the present study were not all as expected. The most surprising observation was the lack of any significant \( p < 0.05 \) effect of ascorbic acid on the relative availability of iron from wheat bran as determined by the in vitro method. When bran muffins were combined with an amount of ascorbic acid assumed to be present in orange juice (Experiment 3, Table XI), the resulting \% DI value was not significantly \( p < 0.05 \) different than from muffins combined with only water (Experiments 1 and 2, Table XI). This appears to indicate that the observed significant \( p < 0.05 \) absorption enhancing effect of orange juice (Experiments 4 and 5, Table XI) was probably not due, in any great extent, to its ascorbic acid content. It is possible that the concentration of ascorbic acid was insufficient to enhance the availability of iron from wheat bran. The apparent dependency of the efficacy of ascorbic acid on concentration has been observed (Clydesdale, 1982; Reinhold et al., 1981).
The ability of ascorbic acid to enhance the availability of nonheme iron is well established (Monsen, 1982). It is hypothesized by this author that ascorbic acid, present in adequate concentration, may enhance the availability of iron from bran by its ability to act as a reducing agent at acidic pH. During peptic digestion, protein-bound iron may be released from bran in the ferric form. Then ascorbic acid present in the digest may reduce the iron to the more available ferrous form. It is also possible that iron may remain tightly bound as ferric iron to components of wheat bran. Ascorbic acid may reduce the ferric form to the ferrous form and thereby release iron from its association with bran.

The results from Experiment 6 (Table XI) indicate that much of the observed effect of orange juice may be attributed to the action of its citric acid content. Orange juice contains approximately 10 times more citric acid than ascorbic acid (Nagy, 1978; Clements, 1964). Based on information presented in the literature, it is suggested by this author that citric acid may enhance the availability of iron from wheat bran, as measured by the in vitro method, by its ability to chelate ferric iron at alkaline pH. For example, the stability constant of the ferric-citrate complex may be greater than the reciprocal of the solubility products of various iron-fiber complexes. As a result, chelation by citric acid could liberate ferric iron from bran in a soluble, available form.

Results from Experiment 7 (Table XI) show that ascorbic and citric acids combined appeared to have an effect greater than the sum of the individual acids. Kojima et al. (1981) demonstrated a similar effect on iron solubilization but they combined equal concentrations of ascorbic and citric acids, which is not representative of the actual acid
content of orange juice. In the present study, the addition of ascorbic and citric acids to muffin "meals" in amounts assumed to be present in orange juice, enhanced iron availability from bran, as measured by the in vitro method, to only half the extent of combining muffins with orange juice. It is possible that either the other organic acids present in orange juice, such as oxalic and malic acids, and/or some other component of the juice may also be responsible for enhancing the availability of iron from wheat bran.

4. EFFECT OF BAKING ON IRON AVAILABILITY

The heat treatment received during baking was found to significantly (p < 0.05) decrease the relative availability of iron from wheat bran, as determined by the in vitro method, when compared to the availability of endogenous iron from unbaked muffin products. The effect of baking on % dialyzable iron (% DI) is shown in Table XII. As outlined in Appendix C, the baking effect was determined to be essentially the same for each acid treatment.

As has been discussed, previous studies have found baking to have little effect on the availability of dietary nonheme iron. However, other recent studies have shown that processing changes the chemical form of iron present in some foods (Lee and Clydesdale, 1981; Lee and Clydesdale, 1980a; Lee and Clydesdale, 1980b; Lee and Clydesdale, 1979b). Changes in the chemistry of iron may be important since the chemical form of iron is known to influence iron availability.

Lee and Clydesdale (1979b) developed a method for the simultaneous analysis of the chemical forms of iron, including elemental, soluble, complexed and ionic, added to or endogenous to foods. In one study,
Lee and Clydesdale (1980a) investigated the effects of baking on the chemical forms of iron in biscuits. Almost all of the endogenous iron of unenriched wheat flour was initially found to be either in the insoluble or ferric form. After the unenriched flour was baked in biscuits, the distribution of iron forms was measured. It was found that there was an increase in insoluble iron with a corresponding decrease in ferric iron.

The effect of baking on the chemical form of various iron enrichment sources was also investigated. Each of the iron sources was added to unenriched flour, baked as biscuits and the resulting "iron profile" measured. It was found that very large changes in the iron profile resulted from the baking process. The major effect of baking was the production of insoluble forms of iron; even when a highly soluble form of iron, such as ferrous sulfate, was used for enrichment.

Camire and Clydesdale (1981) investigated the effect of wet and dry processing treatments on the ability of AACC wheat bran and major fractions of dietary fiber to bind metals, including iron, in an in vitro system at pH 5.0 to 7.0. It was found that toasting wheat bran at 177° C for 1 h caused a highly significant increase in the amount of added ferrous iron bound by bran.

In an earlier study, Anderson and Clydesdale (1980b) had examined the effect of processing on the dietary fiber content of AACC wheat bran. Their results showed that toasting wheat bran significantly increased the apparent amount of "lignin" in the bran. The content of "lignin" increased from 2.87% at time 0 to 4.28% after 30 min and 12.02% after 60 min of toasting at 177° C. An overall increase in dietary fiber found after toasting indicated to these workers that the increase in "lignin" content was due to fractions of the wheat bran other than the dietary
fiber constituents. It had been previously reported that heating above 50°C was found to cause the formation of Maillard browning products which were insoluble in 72% sulphuric acid and therefore isolated along with the true lignin fraction.

Based on the work by Anderson and Clydesdale (1980), Camire and Clydesdale (1981) concluded that the increased binding of some metals by wheat bran as a result of toasting could be due to the small increase in the amount of "lignin" formed during the heating process. Lignin and other components of dietary fiber have been shown to have high metal binding capacities (Clydesdale, 1982; Camire and Clydesdale, 1981).

Based on the information reported by other workers, it is postulated by this author that the baking of muffins may have resulted in a significant (p<0.05) decrease in the relative availability of iron from bran, as measured by % DI, due to the formation of substances during the baking process which were inhibitory to iron absorption. These substances may be similar to the dietary fiber component, lignin, and have the ability to complex iron in an insoluble form.

However, it is recognized that numerous factors interact to affect the availability of dietary nonheme iron. Therefore, it is probable that the observed effect of baking on the relative availability of iron from bran is of little practical importance compared to the influence of other dietary constituents, such as orange juice, consumed at the same time as the baked product.
5. **EFFECT OF BRAN PARTICLE SIZE ON IRON AVAILABILITY**

As shown in Table XIII, a decrease in the particle size of bran had no significant \((p<0.05)\) effect on the availability of iron from wheat bran, as determined by the in vitro method. There was no significant \((p<0.05)\) difference in dialyzable iron between pairs of experiments in which the only factor varied was bran particle size (Table IX).

It was hypothesized by this author that a decrease in bran particle size would increase the exposure of wheat bran to the digestive process and increase the liberation of endogenous iron from bran. However, even if more iron was liberated from finely ground bran (#40) during digestion, the iron may not have been available for absorption. As has been discussed, chemical and dietary factors in the gut influence the availability of dietary nonheme iron. It appears that under the conditions of this study, bran particle size is not a factor in the availability of iron from wheat bran.
6. LIMITATIONS OF THE STUDY

In the present study, the availability of iron from wheat bran was estimated using an in vitro method. The method simulated gastrointestinal digestion but did not account for such factors as active transport or brush border binding proteins which may play a role in the absorption of food iron in vivo. Only soluble, low molecular weight iron, capable of passing through a membrane by simple diffusion, was measured as an estimate of available iron. Therefore, because of the uncertainties involved with the determination of iron availability using an artificial system, the results from the present study represent the relative availability rather than the absolute availability of iron from wheat bran. No conclusions regarding the absorption of iron from bran in vivo can be made. The actual absorption and utilization of food iron can only be measured using a biological system.

However, comparison of results utilizing the in vitro method of Miller et al. (1981) and human in vivo methods for the determination of iron availability has shown good agreement between the methods (Schricker et al., 1981). For this reason, the observed effects of such factors as orange juice and baking on the availability of iron from bran in vitro probably approximates their effects in the human body and provide a basis for further investigation of iron availability from wheat bran.
VI. CONCLUSIONS

There has been little information published on the availability of the endogenous iron of wheat bran. In this study, the in vitro method developed by Miller et al. (1981) was found to provide a relatively rapid and inexpensive estimation of the amount of iron available for absorption from wheat bran. The method was based on the assumption that iron is absorbed as soluble, low molecular weight complexes capable of passing through a membrane by simple diffusion.

The endogenous iron from wheat bran was found to be virtually unavailable for absorption when the muffins were combined with water. On the basis of results obtained, it is postulated that iron released from wheat bran during peptic digestion at acidic pH is rebound to components of dietary fiber at neutral and alkaline pH.

Orange juice was found to significantly increase the availability of iron from wheat bran. The citric acid present in orange juice was determined to be at least partly responsible for the ability of orange juice to promote iron absorption. It is postulated that citric acid enhances the availability of iron by chelating with ferric iron at neutral and alkaline pH and releasing it from wheat bran in a soluble form.

Although ascorbic acid has been shown to increase the availability of nonheme iron, it appears that the effect of ascorbic acid is dependent on concentration. The concentration of ascorbic acid used in this study appeared to be inadequate for the promotion of iron availability from wheat bran.

However, when ascorbic and citric acids were combined in amounts assumed to be present in orange juice they appeared to have an effect
greater than the sum of the individual acids. Ascorbic acid may increase the availability of iron from wheat bran in conjunction with citric acid by its ability to reduce ferric iron to the more soluble ferrous state and/or its ability to chelate ferric iron and form soluble complexes at neutral and alkaline pH.

Baking was found to significantly decrease the availability of endogenous iron from wheat bran. It is postulated that the heat treatment provided during baking results in the formation of insoluble forms of iron which are unavailable for absorption.

Finally, bran particle size was found not to be a factor in the availability of iron from wheat bran.

In conclusion, although the results of this study have provided some insight into the availability of iron from wheat bran, further research is necessary to determine the chemistry of iron and iron binding. A clearer understanding of the chemical nature of iron in wheat bran and the influence of individual and interacting factors on the chemical behavior of iron in the gut may eventually lead to improved availability of the essential element iron from wheat bran.
REFERENCES


Innis, S. 1981. Lecture material, HUNU 517. School of Home Economics, University of British Columbia, Vancouver, B.C.


Lee, K. and Clydesdale, F.M. 1979a. Iron sources used in food fortification and their changes due to food processing. CRC Critical Reviews in Food Science and Nutrition. 11: 117.


Maurer, J. 1977. Extraction method for the simultaneous determination of Na, K, Ca, Mg, Fe, Cu, Zn and Mn in organic material using AAS. Lebensmittel-Untersuchung und-Forschung. 165: 1.


APPENDIX A  STANDARD CURVE FOR DETERMINATION OF DIALYZABLE IRON

A standard curve was prepared using 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 1.6, 2.0 and 3.0 ppm iron standards in 0.1N HCL. Determinations of absorbance were made in replicates of 6. The data for the standard curve were analyzed by linear regression and yielded the following information:

\[
\begin{align*}
\bar{X} & = 0.810 \\
\bar{Y} & = 0.146 \\
\beta & = 0.181 \\
\alpha & = 0.000 \\
Sy\cdot x & = 0.005 \\
r & = 0.999
\end{align*}
\]

where:
- \( \bar{X} \) = mean of \( X \) values
- \( \bar{Y} \) = mean of \( Y \) values
- \( \beta \) = slope of the best fit regression line
- \( \alpha \) = \( Y \) intercept
- \( Sy\cdot x \) = standard error of estimate
- \( r \) = correlation coefficient

Given the general equation for a straight line as:

\[
Y = \alpha + \beta X
\]

the equation of the line for the standard curve was calculated as:

\[
Y = 0.000 + 0.181 X
\]
Table I. Composition of test meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Component</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ground beef, cooked</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Bread, white enriched</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Green beans, frozen</td>
<td>23.25</td>
</tr>
<tr>
<td></td>
<td>Fluid whole milk</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>87.25</td>
</tr>
<tr>
<td>2</td>
<td>Ground beef, cooked</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Bread, white enriched</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Green beans, frozen</td>
<td>23.25</td>
</tr>
<tr>
<td></td>
<td>Orange juice, frozen, reconstituted</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>87.25</td>
</tr>
</tbody>
</table>

Table II. Total iron and % dialyzable iron (%DI) determined in the test meals.

<table>
<thead>
<tr>
<th>Meal</th>
<th>Nonheme Iron mg/100 g meal</th>
<th>% DI&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Published&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are expressed as means ± standard deviation, n=6.
<sup>b</sup>Calculated from tabular values (Health and Welfare Canada, 1979).
<sup>c</sup>As reported by Miller et al. (1981).
APPENDIX C  STATISTICAL ANALYSIS

The data were analyzed by two factor analysis of variance (ANOVA) with subsampling. Such an analysis is also called a nested or hierarchical ANOVA. This design is used when each group of data consists of subgroups and results in a complex partitioning of variability.

Percent dialyzable iron ($\%$DI) was measured in 7 different experiments. Within each experiment there were 2 muffin batches. Analysis of 2 muffin batches duplicated the experiment. Within each muffin batch, $\%$ DI was determined in both baked and unbaked muffins. In the determination of $\%$ DI, the digestion procedure was duplicated within each baking effect. From each digest, 3 dialysates were collected and analyzed for dialyzable iron.

The following statistical model was developed:

$$Fe = A + B + AB + D(A) + BD(A) + C(ABD) + E$$

where: $A$ = the effect of acid treatment. Acid treatment refers to either water, orange juice, ascorbic acid, citric acid or a combination of ascorbic and citric acids. There are 7 acid treatments as defined by the 7 experiments.

$B$ = the effect of baking. There are 2 baking treatments (baked and unbaked) within each muffin batch.

$AB$ = the interaction effect of acid treatment and baking.

$C$ = pepsin digest. There are 2 aliquots taken for pepsin digestion within each baking treatment.

$D$ = duplicate. Each of the 2 muffins batches within each experiment is referred to as a duplicate.

$D(A)$ = duplicate error term. This term considers the error due to variability between duplicates within each acid treatment.
BD(A) = consistency error term. This term measures the variability among duplicates within the baking treatments.

C(ABD) = digest error term. This term considers the error due to differences between digests.

E = residual error.

The expected mean squares (EMS) table and ANOV are shown in Tables I and II respectively. The purpose of the ANOV was to determine if the acid treatment and/or baking treatment had a significant effect on the availability of iron from wheat bran. The ANOV also determined if there was an interaction between the 2 treatments.

Sources of variation other than the effects of acid treatment or baking could influence the experimental results. However, the statistical model was designed to take the error due to variability between duplicate determinations and/or the variability between digests into consideration in the determination of any significant treatment effects.

The effect of acid treatment on % DI was tested against the duplicate error term. The ANOV (Table II) indicated that there was a very significant difference between duplicate determinations. However, this source of variation was taken into consideration and a significant difference in % DI due to acid treatment was still found.

The effect of various acid treatments was analyzed by comparing mean % DI of baked and unbaked samples combined for each acid treatment by Duncan's multiple range test (Zar, 1974).

The effect of baking on % DI was tested against the consistency error term. Although the ANOV (Table II) showed that significant error could be attributed to variability among duplicates within the baking treatments, the effect of baking was still found to be significant.
Table I. Expected mean squares table.

<table>
<thead>
<tr>
<th>Source #</th>
<th>Source</th>
<th>EMS</th>
<th>Tested Against</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>$s^2 + 1,4,6$</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>$s^2 + 2,5,6$</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>AB</td>
<td>$s^2 + 3,5,6$</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>D(A)</td>
<td>$s^2 + 4,6$</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>BD(A)</td>
<td>$s^2 + 5,6$</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>C(ABD)</td>
<td>$s^2 + 6$</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>$s^2$</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Analysis of variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>F Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>97.45</td>
<td>731.59</td>
<td>0.0000</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>2.98</td>
<td>32.67</td>
<td>0.0009</td>
</tr>
<tr>
<td>AB</td>
<td>6</td>
<td>0.47</td>
<td>5.17</td>
<td>0.0251</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>0.13</td>
<td>8.92</td>
<td>0.0000</td>
</tr>
<tr>
<td>BD</td>
<td>7</td>
<td>0.09</td>
<td>6.10</td>
<td>0.0002</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>0.01</td>
<td>0.78</td>
<td>0.7697</td>
</tr>
<tr>
<td>ERROR</td>
<td>112</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>167</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The effect of the baking treatments was examined by comparing mean % DI of all baked samples in all acid treatments to mean % DI of all unbaked samples in all treatments by Duncan's multiple range test (Zar, 1974).

The variability due to differences among digests did not directly influence the determination of significant treatment effects but was taken into consideration indirectly.

As shown by ANOV (Table II), the interaction effect of acid treatment and baking was found to be significant. As illustrated in Figure 1, the significant interaction occurs only at the extremely low levels of % DI. It appears that the baking effect is constant for each acid at more measurable levels of dialyzable iron.
Figure 1. A plot of the interaction of acid treatment and baking effect.

- o baked
- x unbaked
APPENDIX D  ANALYSIS OF SAMPLES

Table I. Particle size distribution of two bran fractions showing percentage of sample retained.

<table>
<thead>
<tr>
<th>Sieve number</th>
<th>Sieve mesh</th>
<th>% Retained # 10 Bran</th>
<th>% Retained # 40 Bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1.00 mm</td>
<td>71.63</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>420 u</td>
<td>28.37</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>246 u</td>
<td>-</td>
<td>58.88</td>
</tr>
<tr>
<td>80</td>
<td>177 u</td>
<td>-</td>
<td>32.89</td>
</tr>
<tr>
<td>120</td>
<td>125 u</td>
<td>-</td>
<td>6.70</td>
</tr>
<tr>
<td>20</td>
<td>74 u</td>
<td>-</td>
<td>1.52</td>
</tr>
</tbody>
</table>

aTyler test sieves.

Table II. Average moisture content of flour, bran and muffins.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicates</th>
<th>% Moisturea</th>
</tr>
</thead>
<tbody>
<tr>
<td>flourb</td>
<td>3</td>
<td>11.90 ± 0.07</td>
</tr>
<tr>
<td>AACC bran</td>
<td>3</td>
<td>9.70 ± 0.06</td>
</tr>
<tr>
<td># 10 bran</td>
<td>3</td>
<td>9.78 ± 0.07</td>
</tr>
<tr>
<td># 40 bran</td>
<td>3</td>
<td>9.51 ± 0.14</td>
</tr>
<tr>
<td>Baked muffins</td>
<td>30</td>
<td>30.98 ± 1.10</td>
</tr>
<tr>
<td>Unbaked muffins</td>
<td>30</td>
<td>35.82 ± 0.95</td>
</tr>
</tbody>
</table>

aValues are expressed as means ± standard deviation.

bPurity unenriched wheat flour, Maple Leaf Mills, Toronto, Ontario.
Table III. Total iron content of flour and bran.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total iron (ppm)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>flour</td>
<td>10.52 ± 0.27</td>
</tr>
<tr>
<td>AACC bran</td>
<td>171.71 ± 5.80</td>
</tr>
<tr>
<td># 10 bran</td>
<td>178.88 ± 7.12</td>
</tr>
<tr>
<td># 40 bran</td>
<td>190.00 ± 10.39</td>
</tr>
</tbody>
</table>

¹Values are expressed as mean ± standard deviation, n=3.

²Purity unenriched flour, Maple Leaf Mills, Toronto, Ont.