This is to certify that the

thesis entitled

Zinc Bioavailability in a Semolina/Soy Protein Mixture Was Not Affected by Extrusion Processing

presented by

Soo-Young Kang

has been accepted towards fulfillment
of the requirements for

Masters degree in Human Nutrition

Wanda L Chenoweth

Wanda L. Chenoweth

Major professor

Date December 9, 1996

ZINC BIOAVAILABILITY IN A SEMOLINA/SOY PROTEIN MIXTURE WAS NOT AFFECTED BY EXTRUSION PROCESSING

By

Soo-Young Kang

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1996

ABSTRACT

ZINC BIOAVAILABILITY IN A SEMOLINA/SOY PROTEIN MIXTURE WAS NOT AFFECTED BY EXTRUSION PROCESSING

By

Soo-Young Kang

Effects of extrusion processing on zinc bioavailability in a mixture of 85% semolina and 15% soy protein concentrate was determined using in vitro and in vivo techniques. Soluble and dialyzable zinc content of the extruded product was 15.3 and 13.0 μg/g, respectively, compared to 15.6 and 15.0 μg/g in the raw ingredients. After a zinc deficient diet period, zinc bioavailability in diets in which all of the zinc (14 ppm) was provided either by the extruded product or by the raw ingredients was determined in two groups of male rats. No differences were found in concentrations of zinc in plasma, liver and femur between rats fed the extruded and raw diets after 3 weeks. Apparent zinc digestibility was similar in the two groups. These results are consistent with the in vitro results showing no effect of extrusion processing on zinc bioavailability.

To my family

ACKNOWLEDGMENTS

I am thankful to my advisor, Dr. Wanda L. Chenoweth for her guidance, encouragement, and valuable advice throughout the entire course of the study. My appreciation is also extended to the members of the committee, Dr. Maurice R. Bennink and Dr. Dale R. Romsos.

I am also grateful to Kim Fusee for her assistance during the animal study, and Yong-Soo Chung and Ritu Ummadi for their friendship and supports.

Finally, I am particularly grateful to my mother and husband whose love and support have always been the source of my inspiration to achieve and excel.

TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES viii

INTRODUCTION i

LITERATURE REVIEW 3

1. Zinc metabolism 3
2. Assessment of zinc bioavailability 6
   a. In vitro studies 6
   b. In vivo studies 7
      1) Human studies 7
      2) Animal studies 8
3. Dietary factors affecting zinc bioavailability 10
   a. Copper-zinc interactions 10
   b. Iron-zinc interactions 11
   c. Protein-zinc interactions 13
   d. Amino acid-zinc interactions 16
4. Phytate-mineral interactions 18
   a. Chemical characteristics of phytic acid 18
   b. Interactions with minerals 19
   c. Interactions with protein and mineral 21
5. Extrusion processing 22
   a. Effect of extrusion processing on zinc bioavailability 23
   b. Effect of extrusion processing on wheat and soybean protein 24
6. Summary 29

MATERIALS AND METHODS 31

1. Preparation of extruded and raw products 31
2. Materials 32
3. Research design 32
   a. In vitro studies 32
      1) Soluble zinc analysis 33
      2) Dialyzable zinc analysis 33
   b. In vivo rat experiments 34
4. Diets 35
5. Analytical methods 37

a. Phytate 37
b. Protein determination 39
c. Mineral analyses 39
1) Wet ashing 39
2) Products and diets 40
3) Tissues 40
4) Feces 41
5) NIST standard 41
d. Statistical analyses 41

RESULTS 42

1. Analyses of products and diets 42
a. Zinc, copper, iron and phytic acid content of products 42
b. Zinc, copper, iron, phytic acid and protein content of diets 42
2. In vitro experiments 45
a. Soluble and dialyzable zinc content of products 45
3. In vivo animal experiment 45
a. Body weight, weight gain, food intake, efficiency of food utilization and total mineral intake 45
b. Organ wet weights, dry weights and % body weight of organs 48
c. Plasma concentration of zinc 51
d. Tissue concentration of zinc 51
e. Tissue concentration of copper 55
f. Tissue concentration of iron 57
g. Feces weights and concentrations of zinc, copper and iron 57
h. Apparent digestibility of protein, zinc, copper and iron 60

DISCUSSION 62

CONCLUSION 69

FUTURE RESEARCH 70

LIST OF REFERENCES 71

LIST OF TABLES

TABLE 1: Composition of diets 36

TABLE 2: Analyzed phytate, zinc, copper, and iron content of extruded and raw products 43

TABLE 3: Estimated phytate and analyzed protein, zinc, copper, iron content of diets 44

TABLE 4: Soluble, percent soluble, dialyzable and percent dialyzable zinc in extruded and raw products 46

TABLE 5: Body weights, food intake and food efficiency ratios 47

TABLE 6: Zinc, copper and iron intake 49

TABLE 7: Organ wet weights and relative organ weights 50

TABLE 8: Organ dry weights 52

TABLE 9: Plasma concentration of zinc 53

TABLE 10: Concentration and total content of zinc in tissues 54

TABLE 11: Concentration and total content of copper in tissues 56

TABLE 12: Concentration and total content of iron in tissues 58

TABLE 13: The weight of feces and concentrations and total contents of zinc, copper, iron in feces 59

TABLE 14: Apparent digestibility of protein, zinc, copper and iron 61

LIST OF FIGURE

FIGURE 1: Possible mechanism for transcellular zinc absorption in the intestinal cells....5

INTRODUCTION

Zinc is a trace mineral nutrient essential to the growth and health of all mammals, including humans. In general, meat, eggs, shellfish, legumes, and whole grain cereals have a high content of zinc. Dietary factors which influence the absorption of zinc include "extrinsic factors", that is, other chemical components of the diet that may inhibit or enhance zinc absorption, and "intrinsic factors", related to the chemical nature of the zinc itself, which may increase or decrease its biological availability (Solomons, 1982).

Fairweather-Tait (1992) defined bioavailability as the proportion of the total mineral in a food, meal or diet that is utilized for normal body function. Zinc bioavailability of various foods in a mixed meal may not be the same as that demonstrated in single food studies because of a variety of potentially complex nutrient-nutrient interactions (Wood and Zheng, 1990).

Extrusion processing, a procedure used in mass production of cereal and legume derivatives, cookies and other preshaped products, has become of major industrial importance (Harper, 1979). Interactions among food components during extrusion processing may have positive or negative effects on the bioavailability of nutrients, including bioavailability of zinc. Although processing and cooking have little effect on zinc itself in most food, zinc is a divalent ion and, as such, can form a wide variety of inorganic salts and organic zinc complexes of varying stability during processing. It is the

latter form of zinc, particularly its association with a variety of zinc proteins and possible increased risk of deficiency and toxicity, that has received the greatest share of attention in zinc biochemistry to date (Smith, 1988). Formation of stable phytate-protein-zinc complexes also has been suggested as an explanation of observed differences in zinc absorption with different methods for processing (Erdman et al, 1980).

This research will investigate the effect of extrusion processing on zinc bioavailability in a semolina product containing soy protein concentrate.

REVIEW OF LITERATURE

ZINC METABOLISM

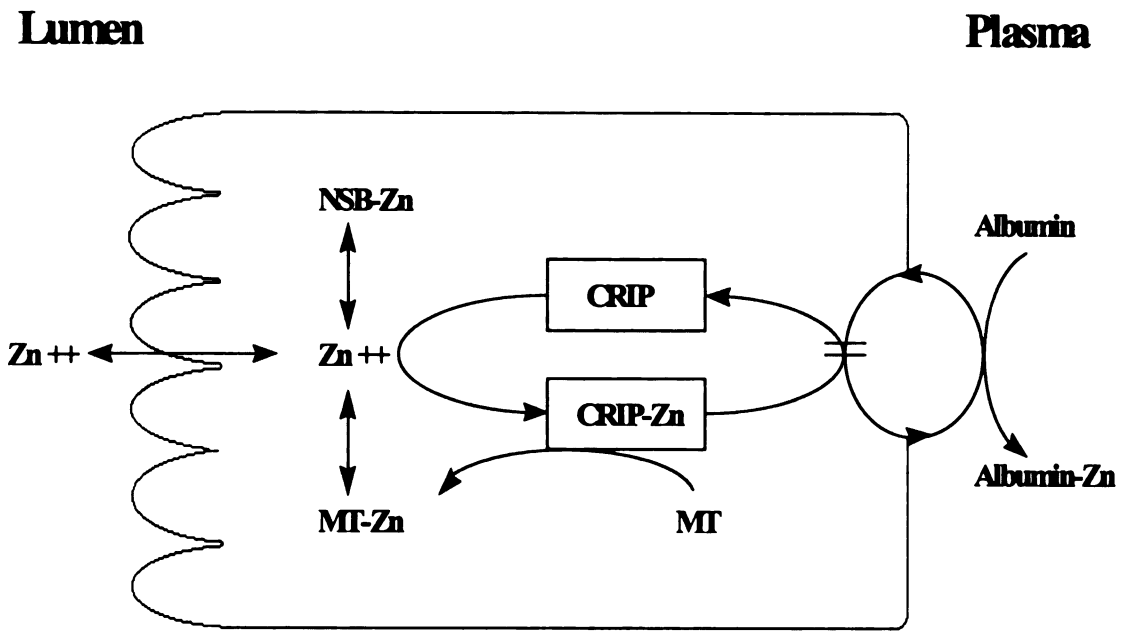
Absorption of Zn is largely a function of the presence or absence of substances in the food or meal that alter the solubility or availability of Zn compounds at the absorption site. Zn is present in all organs, tissues, fluids, and secretions of the body. Zn is primarily an intracellular ion, with well over 95% of the total-body Zn found within cells. Zn is associated with all organelles of the cell, but about 60-80% of the cellular Zn is found in the cytosol (King and Keen, 1994).

Zn is absorbed all along the small intestine; only small amounts are absorbed in the stomach and large intestine. Most of Zn is probably absorbed in the jejunum. Sandstrom and Lonnerdal (1989) reported that free Zn is able to form coordination complexes with various exogenous and endogenous ligands, such as amino acids, phosphates, and other organic acids

The mechanism by which Zn enters mucosal cells is unknown. Zn uptake across the brush border surface occurs by both a carrier-mediated mechanism and diffusion. Research to elucidate the mechanism of Zn absorption (Cousins, 1989) has consistently suggested the involvement of one or more carrier molecules in the absorption process, but viable candidates have not been identified. Hempe and Cousins (1992) showed that cysteine-rich intestinal protein (CRIP) binds Zn during transmucosal Zn transport, and that CRIP functions as an intracellular Zn carrier. CRIP is a 77-amino acid, 8.6-kDa

protein with seven cysteine residues (Birkenmeir and Gordon, 1986). Hempe and Cousins (1992) also suggested that CRIP is a saturable, intracellular Zn transport protein. Metallothionein (MT), a cysteine-rich, low-molecular-weight protein, has high affinity for Zn, Cu and other heavy metals. MT is also suggested to inhibit Zn absorption in competition with CRIP. They present the hypothetical model of Zn absorption shown in Figure 1 to describe the envisioned roles of CRIP and MT in transcellular Zn transport. Following a meal, Zn moving from the lumen into the enterocyte may associate with a variety of intracellular constituents, including CRIP, MT and various nonspecific Zn-binding constituents (NSB). Zn bound to CRIP would thus increase the intracellular concentration of Zn that can diffuse across the cell for exchange and export at the basolateral membrane, thereby enhancing the rate of Zn absorption. The possible involvement of a basolateral membrane-bound acceptor/carrier molecule is indicated (=). MT inhibits Zn absorption by limiting Zn binding to CRIP. When the luminal Zn concentration is high, Zn bound to MT would be free to associate with NSB. NSB could thus serve as a labile pool of Zn in the intestinal cell. The model indicates that transcellular Zn absorption could be regulated by dietary or physiological factors that alter either CRIP or MT gene expression. The number of Zn-binding sites in CRIP is not known.

Zn trapped within the intestinal cell is eventually lost in the feces in the normal course of mucosal cell turnover. Transport of Zn across the serosal membrane is carrier mediated and occurs by an adenosine triphosphate (ATP)-driven mechanism. Zn is released by the intestinal cells at the basolateral-serosal surface into the mesenteric capillaries and is carried by the portal blood to the liver. About 0.05 mmol (3 mg) of Zn



CRIP: cysteine-rich intestinal protein

MT: metallothionein

NSB: nonspecific zinc-binding constituents

== : possible involvement of a basolateral membrane-bound acceptor/carrier molecule

Figure 1: Possible mechanism for transcellular zinc absorption in the intestinal cells.

(Adapted from Hempe and Cousins, J. Nutr. 1992)

is normally circulating in the plasma at any given moment. Harris and Keen (1989) reported that this Zn is portioned among albumin (57%), α-2-macroglobulin (40%), and amino acids (3%). These loosely bound albumin and amino acid fractions of circulating Zn provide the transport and delivery of Zn to tissues.

There is no specific Zn "store." Nonetheless, some sources of endogenous Zn are retained preferentially in certain tissues such as bone in response to a decrease in dietary Zn. The major route for endogenous Zn excretion is into the gastrointestinal tract with ultimate loss in the feces. Fecal losses are a combination of unabsorbed dietary Zn and endogenous Zn secretions. Pancreatic secretions are the major source of endogenous Zn losses. Other sources include biliary and gastroduodenal secretions, transepithelial flux of Zn from the mucosal cells, and sloughing of old mucosal cells into the gut. Surface losses through desquamation of skin, outgrowth of hair, and sweat contribute up to 15 umol (1 mg) of Zn daily. Other sources of loss include the semen and menstrual secretions (Hambidge et al, 1986).

ASSESSMENT OF ZINC BIOAVAILABILITY

In Vitro Studies

In the past few years in vitro methods to assess the bioavailability of minerals and trace elements have gained popularity because of their accuracy, speed of analysis and relatively low costs. In vitro methods offer an appealing alternative to human and animal studies. Most of the in vitro methods simulate gastrointestinal conditions. In the small intestine several components of our food form soluble or insoluble complexes with

minerals and trace elements. The solubility of minerals and trace elements after simulation of the conditions in the stomach (pH 1-2, 37 °C) and small intestine (pH 6.5-8, 37 °C) as a measure of in vivo bioavailability has been studied by Narasinga Rao and Prabhavathi (1978), Wien and Schwartz (1983), Hunt et al (1987), Sandberg et al (1989), Schwartz and Nevins (1989) and Turnland et al (1990). The authors reported contradictory results with respect to the correlation between in vitro and in vivo bioavailability.

Miller et al (1981) used the dialyzability of Fe under simulated conditions of the stomach and the small intestine as an index for its bioavailability. This method has been modified by some other researchers for estimation of the bioavailability of Fe and Zn. Promising correlations between in vitro dialyzability and in vivo bioavailability were reported (Schricker et al, 1981; Hazell and Johnson, 1987; Hurrell et al, 1988; Sandstrom and Almgren, 1989). However, it is concluded that the prediction of bioavailability with in vitro methods is relative rather than absolute because not all important physiological factors can be simulated in vitro. Thus, in vitro methods may be useful primarily for ranking purposes.

In Vivo Studies

Human studies. In vivo experiments with experimental subjects are the best way to study the bioavailability of minerals and trace elements to man. Estimation of apparent absorption using the balance study technique requires long periods on a constant diet (Schwartz et al, 1986) to achieve equilibrium and is not a sensitive method for the identification of individual factors that influence Zn absorption. For example, Turnlund et

al (1982) showed that fecal Zn samples collected from 1 day's Zn intake can also contain non-absorbed fractions of Zn from the previous days' meals as well as fractions of endogenous Zn excreted over the same period of time. Using a radionuclide, Zn absorption can be determined from measurements of the whole-body retention of the isotope. The meals are labeled with radioisotope extrinsically or intrinsically. In recent years there has been interest in the use of stable isotopes such as ^{67}Zn as a safe alternative to study Zn metabolism, particularly in infants and pregnant women. The increase in plasma Zn after a Zn load also has been used as a method to identify dietary and physiological factors affecting Zn absorption. One of major limitations of this method is that the dose of Zn needed to produce detectable increases of plasma Zn concentration is much higher than the normal dietary level of Zn. Human studies, however, are time-consuming and very expensive, and often quite variable results are obtained which are difficult to interpret. As an alternative, the rat is often used as a model for man.

Animal studies. In many studies in experimental animals, stock diets are fed for long periods prior to the test period. These diets are usually formulated to provide at least a minimum supply of most nutrients. The use of Zn-deficient diets in such experimental models could also induce systemic changes in the intestine that may influence the outcome of Zn absorption studies. Sandstrom and Lonnerdal (1989) summarized intestinal uptake models for assessing Zn bioavailability: 1) Animals in which a segment of the small intestine is tied off or a loop of intestine is externalized. The intestine is then perfused with a solution of Zn and by measurements of changes in Zn concentration, transfer rates and kinetics of Zn transport can be determined. 2) Another approach is to

use everted gut sacs; intestinal segments are surgically removed, everted. 3) Brush border membrane vesicles (BBMV) prepared by physically removing the mucosa and selectively precipitating the BBMVs. These methods have potential drawbacks including effects of anesthesia on metabolism and absence of intact innervation and blood supply. The advantage of the above approaches is, of course, that many conditions can be closely controlled and, thus, effects of pH, ionic strength, concentrations of ions, ligands, competitors and stimulatory factors can be determined. Radioisotope studies in animals have been used in a way not possible in human subjects. Information can be gained about the metabolic fate of the absorbed Zn; tissue can be homogenized and the molecular localization of the element can be determined. Fox et al (1994) reported that the isotope such as ^{65}Zn was more likely to behave in the same manner as the intrinsic Zn than the stable isotope ^{67}Zn.

Total femur Zn and body weight of weaning rats were shown to be suitable parameters for a Zn bioassay (Momcilovic et al, 1975). Femur Zn was compared to body weight as a suitable parameter for the determination of Zn bioavailability. They concluded that a slope-ratio bioassay was appropriate for the determination of the bioavailability of Zn, when the logarithm of total femur Zn was plotted against the level of dietary Zn. Forbes and Parker (1977) were able to successfully measure Zn bioavailability from soy flour utilizing male weaning rats in a slope ratio assay procedure patterned after Momcilovic et al (1975). Hettiarachchy and Erdman (1984) also determined the bioavailability of Zn in mature winged bean seed flour in a slope ratio assay by measuring weight gain and total tibia Zn.

DIETARY FACTORS AFFECTING ZINC BIOAVAILABILITY

A theoretical framework for interactions between minerals and trace elements was set by the studies by Hill and Metrone (1970). These investigators postulated that elements with similar physicochemical properties may compete for common pathways and act antagonistically to each other biologically. In biologic systems Zn is virtually always in the divalent state. Zn typically forms complexes with a coordination number of 4 with a tetrahedral disposition of ligands around the metal (King and Keen, 1994). These parameters are identical to those for Cu^{2+} and Cd^{2+}, and therefore these elements would be expected to interact with Zn. Another interaction, less readily applicable in the basis of common structural features of the ions involved, is that between Zn and Fe (Solomons, 1986). In this case, the mutual affinity of Zn and Fe for a biological carrier such as transferrin, capable of binding many different elements, explains the interaction. Zn also readily complexes to amino acids, peptides, proteins, and nucleotides. Zn has an affinity for thiol and hydroxy groups and for ligands containing electron-rich nitrogen as a donor.

Copper-Zinc Interactions

Cu-Zn interactions have been of particular interest. Large quantities of ingested Zn can interfere with Cu absorption. Because of the necessity of Cu for Fe metabolism, anemia may result (Patterson et al, 1985). The apparent Cu retention and fecal excretion are clearly modulated by the concentration and duration of dietary Zn ingestion. Festa et al (1985) showed that an intake of 150 mg Zn per day for 2 weeks reduced apparent Cu

retention in a group of young men. There is some evidence that even relatively low levels of dietary Zn may interfere with Cu absorption. In adult males, it was shown that an intake of Zn only 3.5mg/day above the RDA reduced apparent Cu retention when Cu was ingested at the level of 2.6mg/day (Festa et al, 1985). Studies in rats using isolated, vascularly perfused rat intestines and dietary concentrations of 5, 30 and 180 mg Zn/kg and 1, 6, 36 mg Cu/kg have not, however, shown mutually interactive effects on their absorption (Oestreicher and Cousins, 1985). A high intake of Cu does not inhibit Zn absorption. Valberg et al (1984) found no effect of 5 mg Cu on the absorption of 0.5 mg Zn in water in humans. In human diets the relation between Zn and Cu is almost always a higher Zn than Cu content, and Cu is practically not a serious inhibitor of Zn absorption.

It is understood that the key to the interaction between Zn and Cu lies in the induction of intracellular MT. MT is a Cu- and Zn- binding protein that is induced by high levels of Zn and Cu in the diet (Bremmer and Beattie, 1990). MT concentration in intestine responds to an increased absorption of Zn, Cu and Cd but seems to be more influenced by dietary Zn than by Cu (Oestreicher and Cousins, 1985). A high intake of Zn induces the synthesis of MT in the mucosal cell. This protein sequesters Cu, making it unavailable for serosal transfer and thus decreases Cu absorption. This property has been put into therapeutic use as a means to limit the absorption of Cu in Wilson's disease. The long-term Zn therapy, at a dose of 100 to 200 mg/day of elemental Zn, suffices to prevent the continued accumulation of Cu in the liver or the reaccumulation after a period of penicillamine therapy (Brewer et al, 1987; Sandstead, 1987).

Iron-Zinc Interactions

The interaction between Fe and Zn is still controversial. Pharmacological doses of Fe are often given to groups of subjects with high Zn requirements such as pregnant women and teenagers. Furthermore, diets like infant formulas are often enriched with Fe. A negative effect of Fe on Zn absorption could therefore have serious implications for supplying Zn to vulnerable groups. High levels of Fe in the diet have been shown to inhibit Zn absorption by competition with endogenous low molecular weight ligands (Evans and Johnson, 1981; Gordon, 1983). Ferrous iron, but not heme iron, has been known to inhibit Zn absorption when the molar proportion of Fe is two or three times that of Zn. The effects of ferric ion on Zn absorption are less marked than those of the divalent form (Solomons et al, 1983). The interaction of Fe and Zn and competitive inhibition of Zn uptake by excess Fe in the ratio of 2:1 or greater appear to have a measurable effect on human Zn nutriture. Pregnant women and artificially fed infants consuming Fe supplements or Fe-fortified formulae may develop Zn deficiency, suggesting a negative impact of excessively high Fe:Zn ratios in diet (Solomons and Cousins, 1984). Using body retention of ^{65}Zn as an index of absorption, Valberg et al (1984) found that both inorganic Fe and heme Fe inhibited Zn absorption in humans from 6-mg doses of Zn as Zn chloride. Inorganic Fe had no effect, however, on ^{65}Zn absorption from an extrinsically labeled turkey test meal. Similar differences in the effects of Fe on Zn absorption depending upon whether the Zn is supplied in aqueous solution or from a meal have been observed by Sandstrom et al (1985). The absorption of Zn from infant formulas by adults was not influenced by differences in the Fe content of the two formulas (Lonnerdal et al, 1984). Solomons (1986) has reviewed studies of the interaction

between Fe and Zn and suggested that the total amount of ionic species determines the effect on absorption of Zn. He claimed that when the total amount of ions given as a single oral dose in solution is greater than 25 mg, a measurable effect on human Zn nutriture can be expected. The different effect of Fe on Zn absorption from a solution and from a food exemplifies the complex interactions between promoters and antagonists of absorption. The practical implication of these results could be that higher amount of Fe than that of Zn can reduce the efficacy of a Zn supplement while normal levels of Fe used in enrichment of food do not seem to significantly impair Zn supply. However, most studies of Zn absorption have been performed in healthy non-pregnant adults, and the effect of Fe could be different in groups with higher Zn or Fe needs. Most, but not all, of the direct Zn absorption studies in human subjects, in which the total acute oral dose has exceeded 25 mg of Zn, have shown an inhibition of Zn uptake by pharmacological doses of ferrous iron (Solomons and Jacobs, 1981; Solomons et al, 1983; Meadows et al, 1983). However, Castillo-Duran and Solomons (1991) measured Zn absorption in healthy volunteers following ingestion of 400 g of cooked beef containing 16 mg of Zn. Fe/Zn ratios of 1:1, 4:1-but not 7:1-achieved by addition of 16, 64, and 112mg of ferrous iron in the form of ferrous sulfate produced a significant reduction in the plasma Zn response.

Protein-Zinc Interactions

The effect of dietary protein on Zn absorption in humans is also controversial. Both the type and the amount of protein appear to affect the degree of Zn absorption. Significantly lower fecal Zn losses were observed in adult males fed a high protein diet (150g/d), achieved by intake of a bread enriched with purified protein sources, as

compared with an intake of 50 g protein/d and similar Zn intakes (Greger and Snedeker, 1980). Urinary Zn excretion, however, was increased on the higher protein intake and the apparent retention of Zn was not statistically different. Much research has been done on the absorption of the Zn present in milk itself. Zn in cow's milk is less available than in human milk. Many observations suggest that the protein content and composition of milk diets to a large extent influence the intestinal handling of Zn. In cow's milk, Zn is found predominantly bound to the high-molecular-weight protein, casein (Cousins and Smith 1980; Harzer and Kauer, 1982; Blakeborough et al, 1983), and it was suggested early on that Zn bound to casein may not be utilized by the young infant (Lonnerdal et al, 1980). From in vitro dialysis experiments a Zn-binding capacity of 8.4 μg Zn/mg bovine casein has been observed at slightly alkaline pH (Harzer and Kauer, 1982). This study also indicated that Zn is complexed to the negatively charged phosphate groups of casein. Human milk contains a lower concentration of casein which is also less phosphorylated than bovine casein. Casein separated from human milk by ultracentrifugation was found to contain 14% of the total Zn content; albumin bound 28% and the remaining Zn was equally distributed between fat and low-molecular-weight ligands (Lonnerdal et al, 1982). In human adults, dietary proteins are digested to 80-90% in the small intestine where the protein-bound Zn is probably rendered available for absorption. Incomplete hydrolysis of the casein in cow's milk may lead to decreased absorption of Zn in infants (Lonnerdal et al, 1985). Cow's milk and lactose-free cow's milk significantly reduced Zn absorption in lactose-tolerant and lactose-intolerant postmenopausal women when fractional Zn absorption was studied with a dual-isotope technique using ^{65}Zn and ^{67}Zn (Wood and Hanssen, 1988). Greger et al (1989) reported that rats fed supplemental lactose or

unhydrolyzed cow's milk retained more Zn and Mg in bone. Wood and Zheng (1990) reported no significant effect of milk or calcium phosphate supplementation on either Zn absorption or retention in postmenopausal women. They concluded that increased milk consumption with meals has no detrimental consequences on Zn nutriture in elderly women.

In view of the high affinity of Zn for undigested protein, even a minor impairment in protein digestion could affect Zn absorption (Wapnir, 1990). Istfan et al (1983) measured Zn absorption, with the aid of the stable isotope ^{70}Zn, from a formula diet with either egg protein or a soy protein concentrate as the sole source of protein and with 8.5 or 12.8 mg of added Zn as Zn chloride. No significant difference in Zn absorption between the diets was observed.

The most questioned protein source, in terms of its effects on mineral absorption, has been soy isolate. Forbes and Parker (1977) showed that Zn in a soy protein product was less available than that from a highly available source, $ZnSO_4$, though the presence of soy protein did not affect availability of Zn from other sources in the diet. Lo et al (1981) suggested that this inhibitory action in isolated soy protein may relate to 1) the residual amount of phytate remaining after the isolation and purification procedure, which may produce an elevated phytate to Zn ratio, 2) the structure and nature of soy protein itself. A much lower absorption of Zn from a soy-based infant formula compared with human milk or cow's milk formula has been observed in adults (Sandstrom et al, 1983), and growth studies of children recovering from protein-energy malnutrition also imply a poor availability of Zn from soy-based formulas (Golden and Golden, 1981). Zn in soy formula is associated with the large-molecular-weight proteins and the solubility of Zn

bound to this soy protein fraction at physiological pH is low (Sandstrom et al, 1983). Although a lower small intestinal protein digestion of a 25% soy protein diet compared with a meat diet has been observed in ileostomates (Sandstrom et al, 1986), this had no significant effect on apparent Zn absorption. When all meat protein was replaced by soy protein, a lower fractional absorption than expected from the dietary Zn content was observed in studies using the extrinsic labeling radionuclide technique to measure Zn absorption from a composite meal (Sandstrom et al, 1987).

Amino Acids-Zinc Interactions

Competition between Zn and other elements for absorption binding sites can influence absorbability. Meat, liver, eggs, and seafood are considered to be good sources of Zn because of the relative absence of chemical constituents that inhibit Zn absorption and because of certain amino acids that improve Zn solubility. Cysteine and histidine enhance Zn absorbability by forming stable complexes with Zn. Greger and Snedeker (1980) also observed that supplementing a low protein diet with sulphur-containing amino acids increased urinary Zn excretion in adult males. While this could indicate a higher absorption of Zn, it could also be due to other changes in Zn metabolism induced by the changed amino acid intake (Snedeker and Greger, 1981). Snedeker and Greger (1983) studied the effects on Zn absorption of a variety of dietary protein levels with and without supplements of histidine and cysteine. Apparent absorption of Zn was higher when rats were fed a 45% lactalbumin diet or a 15% lactalbumin diet supplemented with histidine and cysteine than when fed the unsupplemented 15% protein lactalbumin diet. An improvement in Zn absorption was also observed in rats when cysteine and

methionine were added to a soy protein diet; absorption was similar to that from a lactalbumin-containing diet (Greger and Mulvaney, 1985). Magee and Lugeye (1985) showed that chicks that received an amino acid supplement, without additional mineral reinforcement, had an improvement in their Zn status, indicating that amino acids sufficed to improve the mineral balance of birds without extra doses of the element. The greater Zn absorption achieved by amino acids in the small intestine appeared to be due to both mediated and nonmediated transport mechanisms for amino acids. Greger (1988) suggested that amino acids may improve absorption by forming soluble complexes with Zn.

The amino acid with the highest affinity toward Zn, histidine, may be involved in Zn homeostasis. There have been attempts to titrate the optimum concentration of histidine in rodent diets in order to maintain normal Zn level in serum. Increases in this amino acid from 0 to 0.40% correlated with an increase in serum Zn and Cu (Koo et al, 1986). However, diets with 8% histidine in the diet produced low plasma Zn and severe tissue Zn deficiency in rats (Wensink and Van den Hamper, 1988). Moderate amounts of histidine have given clear results in human. Ingestion of Zn complexed with histidine at a ratio of 1:2 or 1:12 increased serum Zn concentration 25% more than ingestion of Zn sulfate. Zinc histidine complexes are better absorbed than zinc sulfate in humans (Scholmerich et al, 1987). Wapnir et al (1986) suggested that proline and tryptophan have affinities toward Zn almost as great as that of histidine in humans. It has been postulated that the increased availability of Zn from animal (meat) sources is caused by a " meat factor". Sulfhydryl compounds such as cysteine and glutathione that are high in meat may bind Zn and increase absorption efficacy (Hortin et al, 1991).

PHYTIC ACID-MINERAL INTERACTIONS

Chemical Characteristics of Phytic Acid

Phytic acid, myoinositol hexaphosphate (1,2,3,4,5,6 hexakis dihydrogen phosphate), is a naturally occurring compound formed during the maturation of seeds and cereal grains. In the seeds of legumes, it accounts for about 70% of the phosphate content and is structurally integrated with the protein bodies as phytin, a mixed potassium, magnesium, and calcium salt of inositol (Erdman, 1979). Phytic acid is the most abundant form of phosphorous in plants. Sandberg et al (1987) showed that during food processing and digestion, inositol hexaphosphate can be partially dephosphorylated to produce degradation products, such as penta-, tetra-, and triphosphate, by the action of endogenous phytases, which are found in most phytic acid-containing seeds.

Phytic acid has been considered as an antinutrient due to its inhibitory effect on mineral bioavailability. The conformation of this highly polar compound explains its high affinity toward di- and trivalent cations (Wapnir, 1990). The most striking chemical impact of phytic acid is its strong chelating ability with multivalent cations, especially di- and trivalent cations, to form cation-phytic acid complexes. Zhou and Erdman (1995) reviewed that the complexes are usually soluble at acidic pH, but they have limited solubility at neutral pH, a pH near to that in the small intestine. The insolubility of the complexes is regarded as the major reason for the reduced bioavailability of phytic acid-mineral complexes. Minerals of concern would include Zn, Fe, Ca, Cu as well as phosphorous itself from phytic acid. In particular, phytic acid-induced Zn deficiency has been extensively reported.

Interactions with Minerals

The phytate to Zn molar ratio has been suggested as a parameter to predict Zn bioavailability. Oberleas and Harland (1981) proposed that in humans adequate dietary Zn can be provided by a daily diet having a phytate to Zn molar ratio of 10 or less. In rats, low-phytate wheat brans with phytate to Zn molar ratios of 8 or less were equivalent to Zn sulfate as dietary sources of Zn for growth (Morris and Ellis, 1980b). Ellis and Morris (1981) also reported that reduction of the soybean phytate:Zn molar ratio from 33 to 13 improved the bioavailability of soybean Zn to rats. Thus they found that femur Zn values of rats fed soybean at a phytate:Zn molar ratio of 13 were significantly greater than values of rats fed untreated soybean as the Zn source. Lo et al (1981) found that phytate has a marked effect on Zn absorption when the Zn status of the animal is low but has little effect when the phytate-Zn molar ratio is nine or less and the physiological Zn status is adequate. It has been suggested that this difference in absorption relates to the fact that phytate is hydrolyzed in the intestinal lumen by the Zn-dependent enzyme, phosphatase or phytase, in Zn-adequate but not in Zn-deficient animals. On the basis of animal experiments to date, a daily phytate: Zn molar ratio of 10 or less is thought to be acceptable in providing adequate dietary Zn, and daily ratios consistently above 20 may jeopardize Zn status (Zhou and Erdman, 1995).

Phytate also forms complexes with more than one mineral in a complex. The Ca content of the diet is of vital importance to the negative impact of phytic acid on Zn bioavailability. Ca exaggerates the inhibitory effect of phytic acid on Zn bioavailability by forming insoluble Ca-phytic acid-Zn complexes. The interaction is so strong that a [phytic acid] x [Ca] /[Zn] molar ratio has been found in some studies to be a better

predictor of Zn bioavailability than [phytic acid]/[Zn] molar ratio alone (Bindra et al, 1986; Davies et al, 1985). In an in vitro study, Gifford-Steffen and Clydesdale (1993) observed that at pH 5.5, there was a significant increase in soluble Zn and soluble phytate at the level of 0.25 mmol Ca compared to the higher levels (0.49-4.94 mmol) due to the possible formation of a soluble Ca-phytate-Zn complex. Various combinations of Ca (4.94 mmol), Zn (0.0071 mmol) and phytate (0.284 mmol) were added either to soy concentrate, casein or torula yeast. The presence of phytate in the protein+Zn+phytate treatment of soy concentrate resulted in a highly significant reduction in solubility of both protein and Zn. $CaCO_3$ significantly reduced Zn solubility in all proteins, both with and without phytate. Ca solubility also was significantly reduced but only when phytate was present. Phytate solubility decreased at pH 5.5 when Ca was present. This work supported the hypothesis that increasing amounts of Ca in a diet containing Zn and phytate might reduce the bioavailability of Zn. However, in vitro solubility does not necessarily correlate with bioavailability.

Dietary levels of Ca used in animal experiments are frequently much higher than those in human diets. Addition of milk and cheese to a phytic acid-containing bread improved the absorption of Zn (Sandstrom et al,1980). However, the content of many other nutrients also was increased by the addition of milk, and it was concluded that the increase in protein content of the meal was counterbalancing the negative effect of phytic acid. When calcium chloride was added to a phytic acid-containing soy isolate formula, an improvement of Zn absorption was also observed (Lonnerdal et al, 1984). These results indicate that at the levels of Ca and phytic acid found in most human diets,

the Ca-phytate-Zn interaction observed in animal studies has limited relevance with regard to Zn absorption in humans.

Interactions with Protein and Mineral

According to Cheryan (1980), phytic acid has an electrostatic interaction with proteins. At a low pH, below the isoelectric point of proteins, terminal lysyl, histidyl, or arginyl groups could be positively charged. Any of these groups can directly form a complex with a negatively charged phytate anion. At the same time, the protein molecule could bind with additive phytate anions, depending on the number of positively charged groups and conformational conditions. At intermediate pH values, only the lysyl and arginyl groups are still positively charged, so that a slight possibility of electrostatic interactions exists. If the pH is very high, the interaction between phytic acid and protein is diminished (Lásztity and Lásztity, 1990).

The presence of other components in the food system gives additional possibilities for protein-phytic acid interactions. If polyvalent cations, such as Zn or Ca are present, the cations may form a bridge between the phytate anion and a negatively charged group of protein, thus allowing the binding of phytic acid by proteins at neutral and high pH. Formation of stable protein-phytic acid-Zn complexes has been suggested as an explanation of observed differences in Zn absorption with different methods for processing of soya protein (Erdman et al,1980). Forbes and Erdman (1983) suggested that these complexes could be thermodynamically stable and resistant to proteolytic digestion in the gastrointestinal tract. Phytate has been suggested to influence protein digestibility. Sandstrom et al (1986) observed in ileostomy patients that complete recovery of phytic

acid from a soy protein-based diet in small intestinal digesta was associated with a concomitant reduction in protein digestibility. Ritter et al (1987) reported that in vitro digestibility (determined by equilibrium dialysis method) of soy protein with lower phytate content (0.07%) was greater than soy protein with a higher content (1.41%). Honig and Wolf (1991) investigated the associations of essential minerals with specific proteins or phytic acid by gel filtration techniques, and they found associations of protein with phytic acid and minerals in defatted soybean flakes. There was a significant negative correlation between phytic acid and in vitro protein digestibility of soybean and other legumes (Chitra et al, 1995). Singh et al (1991) claimed that phytic acid reduced protein digestibility in groundnut.

EXTRUSION PROCESSING

Harper (1989) defined extrusion cooking as a high-temperature short-time (HTST) technique for production of a range of foods and food ingredients-especially plant proteins, starches, and cereal products. Extrusion cooking has some unique features compared to other heat processes in that the material is subjected to intense mechanical shear. It is capable of breaking covalent bonds in bipolymers.

Food extruders are generally divided into two classifications, single-screw and twin-screw. Because of its improved pumping action, the corotating twin-screw extruder is finding increased use to produce products which require precise process control (Huber, 1991). The operation of an extruder has been summarized by Harper (1989). Ingredients are released from the feed hopper into the preconditioner at a controlled rate.

Raw granular food ingredients are uniformly moistened or heated by contact with water or steam in the preconditioner before entering the extruder. When the food enters the extruder, the extrusion screw sequentially conveys and heats food ingredients and works them into a continuous plasticized mass while rotating in a tightly fitting barrel. As the flights on the extruder screw convey the food materials down the barrel, the mechanical energy used to turn the screw is dissipated causing a rapid rise in the temperature of the food ingredients. The resulting plasticized feed ingredients are then forced through a die. The pressure drop across the die and rapidly converts the high-temperature water in the product to steam and causes puffing to occur. The advantages of extrusion processing over conventional cooking procedures are its relatively low cost, high productivity, energy efficiency, ability to produce high quality products, ability to process dry, viscous materials, and ease of production of new foods and product shapes (Harper, 1989)

Effect of Extrusion Processing on Zinc Bioavailability

Because the bioavailability of minerals is influenced by phytate, the effect of processing on the phytate content of extruded products is therefore of interest. Anderson et al (1981) reported a 13-35% reduction in phytate content in an extrusion-cooked, bran-enriched product. Because Zhou et al (1992) showed phytate reduction in soybean protein increased Zn bioavailability in rats, one might postulate that extrusion processing would increase Zn bioavailability. Kivisto et al (1986), however, suggested that Zn bioavailability was lower in extruded than in unprocessed bran due to a processing induced deactivation of the phytase naturally present in cereals and legumes.

In contrast, mineral bioavailability was not affected by extrusion processing in some studies. There was no alteration in the absorption of Fe from potato and maize containing low levels of phytate as a result of extrusion cooking at 120-140 °C in rats (Fairweather-Tait et al., 1987). Other studies conducted with normal adults and using the stable isotopes, ^{58}Fe and ^{67}Zn, revealed that extrusion cooking of a bran flour mixture at a maximum temperature of 140 °C produced no effect on the retention of these two elements (Fairweather-Tait et al 1989). The test meals were comprised of 100 ml pasteurized full-fat milk plus 40 g of a 1:1 mixture of wheat bran and wheat flour. Because Platt et al (1987) reported that milk exerted a positive effect on the phytate induced precipitation of Zn and Fe and thus their potential bioavailability, any changes in Zn availability caused by extrusion processing may have been counteracted by the milk. Moreover, there were differences in the composition of diets and variations in extrusion conditions. Therefore the conflicting results on the effects of extrusion on Zn availability reported by various researchers may be partially explained by the differences in temperature, pressure, and moisture conditions used during extrusion processing.

Effects of Extrusion Processing on Wheat and Soy Protein

The traditional classes of wheat endosperm proteins as separated by fractional extraction using the procedure of Osborne (1907) include: 1) the gluten proteins (gliadins, LMW glutenins, and residue proteins or HMW glutenins) and 2) the nongluten or soluble proteins (albumins and globulins). Wheat flour and gluten are important feedstocks. The importance of chemical and physical reactions on mass

transport and heat transfer during extrusion has been recognized, and the importance of chemical reactions in both starch and protein fractions of commonly extruded food materials has been recognized. Wheat flour has a unique composition compared to other feedstocks. Though protein comprises 10-14% (wt) of the total flour, it is responsible for the viscosity and elasticity of dough to a great extent. Gluten is comprised off glutenin and gliadin. Glutenin, a linear, asymmetric protein with molecular weights > a million Daltons, is responsible for the viscoelastic properties of gluten (Strecker et al, 1995). Edwart (1988) determined the influence of disulfide bonds on gluten viscoelastic properties and structure. Glutenin was hypothesized as a trifunctional monomer capable of polymerizing via disulfide bond formation. Schofield et al (1983) analyzed the effects of heat on sulfhydryl-disulfide interchange reactions and concluded they were primary bonds responsible for network formation upon thermosetting. The reaction mechanism in gluten during extrusion processing was dominated by polymerization providing a buffer to shear forces (Strecker et al, 1995).

Soybeans are high in protein but also contain a number of minor constituents traditionally considered to be antinutritional factors. These include phytic acid, trypsin inhibitors, saponins and isoflavones. Soybean processing changes the content of these minor constituents in various ways. During the course of soybean processing to change physicochemical properties, the chemical composition may change as well. Most texturized soy proteins are produced from soy flours or soy protein concentrates by extrusion. Extruders compress and work soy ingredients into a flowing, hot, plastic mass (Rockey et al. 1993).

Soy protein products are mainly used as ingredients in formulated foods and seldom are seen by the public. Most soy proteins are derived from " white flakes," made by dehulling, flaking and defatting soybeans by hexane extraction. These may then be milled into defatted flours or grits containing ~50-54% protein; extracted with ethanol or acidic waters to remove flavor compounds and flatulence sugars, producing soy protein concentrates containing 65-70% protein (Lucas and Riaz, 1995). The main objective in producing soy protein concentrates is to remove strong-flavor components and the flatulence sugars (stachyose and raffinose), but other soluble compounds and some minerals also are extracted. In turn, both the protein and dietary fiber contents are increased. Approximately 20% of total nitrogen is lost as albumins when preparing isoelectric-leached soy protein concentrates. Most soy proteins are globulins, whose solubility and dissociation are greatly affected by pH and salt content of the solution. In native soybean protein molecules, most amino acid residues responsible for the chemical reactions during processing of soybean protein foods-such as cysteine(-SH), cystine (S-S), and hydrophobic amino acid residues- are buried in the inside region of the molecule, inaccessible to water. These residues become reactable with each other through the exposure from the inside by heat denaturation during extrusion processing. The unique texture of texturized soybean products produced by extrusion is the result of both the intermolecular interchange reaction between the exposed -SH and S-S groups and the intermolecular hydrophobic reaction among the exposed hydrophobic amino acid residues (Fukushima, 1991).

Gujska and Khan (1991) showed that a high degree of protein insolubility was found in pinto and navy beans after extrusion at 110-150°C. The results suggested

formation of ternary phytate-protein-mineral complexes during extrusion processing and these complexes decrease the solubility of protein. Prudencio-Ferreira and Areas (1993) showed that disulfide linkages, and hydrophobic and electrostatic interactions were the main stabilizing mechanisms for the 3-dimensional structure and moisture contents of soy protein. Infrared spectra showed the presence of β-sheet anti-parallel structures. Peptide bonds were of negligible importance in extrusion texturization of soy protein. It is well established that extrusion processing decreases the solubility of semolina proteins. Dexter and Matsuo (1977) previously reported that extrusion processing decreased the solubility of semolina proteins. Various studies have also reported that high-temperature drying of pasta resulted in decreased protein solubility (De-Stefanis and Sgrulletta, 1990; Aktan and Khan, 1992). Ummadi et al (1995) showed that extrusion processing at 50 and 96 °C caused a marked decrease in the percentage of total protein present as albumin, globulin, gliadin, and glutenin fractions with a corresponding increase in the insoluble residue fraction. Extrusion at a higher temperature (96 °C) caused a greater increase in insoluble residue than did the lower temperature (50 °C). The results also suggested that extrusion may have promoted the formation of additional disulfide bonds, although Dexter and Matsuo (1977) reported that extrusion of semolina at 50 °C did not produce any significant changes in disulfide bond levels. The role of disulfide bonds in thermal extrusion effects on protein solubility has been studied previously. Hager (1984) showed that disulfide bonds contribute to the new, extended protein networks produced by extrusion of soy concentrate. Rhee et al (1981) reported that extrusion at 138 °C reduced protein solubility; an increase in protein solubility was achieved by the use of 2-mercaptoethanol and SDS, suggesting disulfide linkages may be responsible for the

decrease in protein solubility after extrusion. Li and Lee (1994) showed that the solubility of protein in wheat flour extrudate increased at an extrusion temperature of 60 and 90 °C but not at 120 and 160 °C. They reported that disulfide bond and hydrophobic interactions occurring during the extrusion processing caused changes in solubility of protein in extruded wheat flour and egg white. Extrusion processing imparts thermal and mechanical energy to dough and causes unfolding and reassociation of the protein molecules, forming new linkages. Wheat flour protein was more highly crosslinked by disulfide bonds with extrusion cooking, and some covalent bond cross-linking was formed during extrusion (Koh and Schaich, 1994).

Extrusion may also influence protein digestibility of products. Dahlin and Lorenz (1993) reported that in vitro protein digestibility of extruded wheat was improved by extrusion. Products extruded at 100-150 °C showed the highest protein digestibility. However, Rudolph et al (1983) and Knabe et al (1989), in studies with growing and finishing pigs, reported lower apparent ileal digestibility values of crude protein and amino acids in extruded soybean than in soybean meal. In a study by Fan et al (1995), pigs were fed on maize starch-based diets formulated to contain 160 g/kg of crude protein from soybean meal, soybean meal plus extruded soybean. The apparent ileal digestibility values of crude protein and most of the amino acids were higher in soybean meal than in extruded soybean. They found that high residual trypsin inhibitor activity in extruded soybean was 2.5-4.0 times higher. In contrast, Godinez et al (1992) reported that digestibility of bean protein is related to the presence of trypsin inhibitors. Inhibition of trypsin, an enzyme actively involved in proteolysis, could possibly impair protein

digestibility, and inhibitors are inactivated by thermal treatment such as extrusion processing.

SUMMARY

The purpose of this research is to determine the effect of extrusion processing on Zn bioavailability. High temperature short time extrusion processing has become increasingly used for texturization of plant proteins (Harper,1986). To date there have been limited studies to determine bioavailability of Zn in extruded products. According to Sandberg and her colleagues (1986), extrusion processing of a high-fiber cereal causes a loss of endogenous phytase activity, the phytate hydrolytic enzyme present in whole grains, but does not alter the availability of the protein, fat or starch. The apparent absorption of certain minerals in a high fiber cereal product was altered, as determined in studies with seven ileostomy patients (Kivistö et al, 1986). Although absorption of Fe was unaltered, that of Zn decreased by 41%. In addition, significant reductions in absorption of Mg (-31%) and P (-11%) were observed. Anderson at al (1981) showed a 13 to 35% reduction in phytate content in an extrusion-cooked, bran-enriched product. Other studies reported that reduction in phytate content in soybean products improved Zn bioavailability in rats (Zhou et al, 1992). Thus, one might expect improvement of Zn bioavailability in extruded products containing phytate. However, the studies by Fairweather-Tait et al (1989) revealed that extrusion cooking of a bran flour mixture produced no effect on the retention of Fe and Zn in humans.

The heating of proteins during processing has been shown to modify their ternary and quaternary structures. Extrusion processing has the potential to alter protein structure, solubility, and digestibility by heat, pressure, and shear (Phillips, 1989). Denaturation could, under some circumstances, facilitate digestion (Wapnir, 1990). However, in extrusion cooking, some unique features are present because the product is subjected to high pressure in combination with severe shear. The degree of mixing and homogenization during extrusion cooking might decrease diffusion barriers or break chemical bonds and thus, increase reactivity in a food system (Björk and Asp, 1983). Interactions among food components such as phytate, protein and Zn during extrusion cooking may have positive, negative or no effects on the bioavailability of Zn. Since extrusion cooking is used increasingly for production of weaning food and breakfast cereals, thorough knowledge of the effects on nutritional value is essential

MATERIALS AND METHOD

PREPARATION OF EXTRUDED AND RAW PRODUCTS

Semolina is wheat flour milled from durum wheat. Semolina (30 mesh) with approximately 65% extraction and enriched with niacin, iron, thiamin and riboflavin, was obtained from the North Dakota Mill & Elevator, Grand Folks, ND. Enriched semolina is known to have added amounts of 3.13 mg iron, 0.53 mg thiamin, 0.49 mg riboflavin and 2.86 mg niacin per 100 g (USDA,1989). Semolina (85%) and soy protein concentrate (15%) (Procon 2000, Central Soya , Fort Wayne, IN) were used to make a product in the shape of small "O's". A corotating twin-screw extruder (Creusot-Loire, Model 45) was used to extrude the semolina /soy product at the temperature of 92 to 96°C. Water was injected into the feed at the rate of 0.12 L/min. The screw was operated at 900 rpm, and the feeder was set to deliver 2.06 kg of the semolina/soy mixture per a minute. The final product was dried in a vat dryer/ blower to a moisture content of 8 to 9%. The raw ingredients and the extruded product were put in plastic bags and stored in a cubicle (18°C). The extruded product was ground using a blender prior to analyses.

MATERIALS

The following chemicals were purchased from J. T. Baker Chemical (Phillipsburg, NJ): potassium hydroxide, sodium hydroxide, sodium sulfite, sodium bisulfite, EDTA (disodium salt), 30% hydrogen peroxide, concentrated sulfuric acid, zinc, copper, and iron standard solutions (1000 ppm). Bovine liver (No 1557a) and wheat flour (No 1567a) were purchased from National Institute of Standards and Technology (NIST) (Gaithersburg, MD). Methoxyflurane was from Pitman-Moore (Mundelein, IL); hydrochloric acid and ether were from Mallinckrodt (Paris, Kentucky); ultrapure concentrated nitric acid and ammonium molybdate were from Fisher Scientific (Pittsburgh, PA). Glycerol, pepsin (from porcine stomach mucosa), pancreatin (from porcine pancreas) and bile extract (porcine) were from Sigma Chemical Co. (St. Louis, MO).

RESEARCH DESIGN

In Vitro Studies

Quantitative determination of soluble zinc was done by the assay described by Gifford-Steffen and Clydesdale (1993) with modifications. Dialyzable zinc was estimated using a modification of an in vitro method developed by Miller et al (1981). Mineral content (zinc, copper and iron) of the extruded and raw ingredients was determined using atomic absorption spectrophometry (AAS) (Perkin-Elmer Model 2380, Norwalk, CT).

Soluble Zinc Analysis. Triplicate samples (2 g) of ground extruded or raw product were added to 100 ml of 0.01 N HCl. The mixture was placed on a magnetic stirrer at medium speed for 30 min. The pH was adjusted to 2.0 ± 0.05 with HCl and stirred an additional 30 min. The pH was then adjusted to 5.5 ± 0.05 with NaOH followed by a 30 minute period of mixing. Two 30-ml aliquots of each sample were centrifuged at 3335 x g for 20 min (Sorvall RC-5B, Dupont). A 10-ml aliquot of the supernatant of each sample was pipetted into a 25-ml Erlenmyer flask and wet ashed using HNO_3 and H_2O_2.

Dialyzable Zinc Analysis The method involves simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight zinc. It differs from soluble zinc analysis method in two important ways: 1) pH adjustment from gastric to intestinal levels is gradual and 2) only low molecular weight, soluble zinc is used in the estimation of dialyzable zinc. The method involves a two-stage digestion: stage 1 (pepsin digestion) and stage 2 (pancreatin digestion). The amount of zinc which passes into a dialysis sac (dialyzable zinc) during stage 2 is used as an indicator of zinc availability. Specifics of the method follow:

Triplicate samples (5 g) of extruded or raw product were placed in a 125-ml Erlenmyer flask. Fifty ml of 0.1 N HCl was added to the product to form a slurry, and the pH was adjusted to 2.0 ± 0.05 by dropwise addition of 6 N NaOH with rapid stirring. A blank containing no sample but otherwise prepared as above was also run to determine the contribution of zinc in the enzymes to dialyzable zinc. A 5 ml aliquot of pepsin solution (10g pepsin/100ml 0.1N HCl) was added to each flask. The flasks were inverted several times to mix and incubated for 2 h in a 37 °C shaking water bath. The contents of

each flask were mixed with 5 ml of the pancreatin-bile suspension (0.4g pancreatin, 2.5g bile extract/100ml 0.1M $NaHCO_3$) and titrated to pH 7.5 with 0.5 N KOH. The aliquots were then incubated in a 37°C shaking water bath for 30 min. A dialysis bag with a molecular weight cutoff of 6000-8000 Da was prepared and put into each sample. The bag contained a volume of 0.5 N $NaHCO_3$ equal to the volume of 0.5 N KOH required to titrate the pancreatin-bile-sample mixture to pH 7.5, plus double deionized water (DDW) to bring the total volume to 20 ml. Incubation was continued for 2h. A 2 ml aliquot of the dialysate was taken and wet ashed prior to zinc analysis by AAS.

In Vivo Animal Experiment

The in vivo bioavailability of zinc in the extruded product and the raw ingredients was determined in weaning Sprague-Dawley male rats (Harland , Indianapolis, IN). Rats were housed individually in suspended stainless steel cages in a temperature- and humidity- regulated room with alternating 12-hour light/dark cycle with lights on at 7:00 am. Temperature was maintained at 20-22 °C, humidity at 68-70%. Food consumption and body weights were measured at least twice weekly. Rats were housed in metabolic cages during the last week of the experimental diet period for daily collections of feces. Food intake was measured daily during the fecal collection period. Distilled water was supplied ad libitum throughout the experiment using glass water bottles with plastic caps and stainless steel drinking tubes. Care and treatment of rats were approved by the All University Committee on Animal Use and Care at Michigan State University.

During 8 days of adaptation the rats were fed rat chow containing adequate zinc. A zinc deficient basal diet was then fed for 10 days to maximize the efficiency of zinc

absorption. At the end of the zinc deficient basal diet period, six rats were killed to get baseline data. Rats were then assigned to the following three experimental diet groups: extruded diet (n=13); raw diet (n=6); control diet (n=13). At the end of wk 1 of the experimental diet period, six rats each in extruded and control groups were killed. During week 3, daily fecal samples were collected for seven days. The fecal samples were wiped to remove attached hair and food. The fecal samples were combined and dried in a drying oven to a constant weight. At the end of three weeks, rats were anesthetized with methoxyflurane and killed by drawing blood from the heart using 3ml syringes rinsed with 1000 units/ml sodium heparin solution. Blood samples were centrifuged (International Centrifuge, Model UV) at 1500 x g for 20 minutes; plasma was removed and frozen. Livers, kidneys, and femurs were removed, rinsed in saline, cleaned of adhering matter, weighed and frozen.

DIETS

The diets were formulated in accordance with the general guidelines prepared by the American Institute of Nutrition (Reeves et al, 1993) with modifications. Composition of the basal zinc deficient diet and three test diets is listed in Table1. The protein source in the basal diet was spray dried egg white. An AIN-93 mineral mix without zinc, copper and iron was purchased from Dyets (Bethlehem, PA). Cupric carbonate and ferric citrate were premixed with Dyetrose and then incorporated into the basal diet to provide recommended levels of copper and iron. Because of the relatively low phosphorous content of egg white, potassium phosphate was added in order to provide an adequate

TABLE 1

Composition of diets [1]

Component	Basal	Extruded	Raw	Control
		g/kg		
Extruded product	--	516	--	--
Raw product	--	--	543	--
Egg white[2]	220	107	95	234
Cornstarch	380.5	--	--	366.5
Dyetrose[3]	132	109.5	94.5	132
AIN-93G mineral mix[4]	35	35	35	35
(Zn, Cu and Fe free)				
		mg/kg		
Zinc carbonate[5]	--	--	--	27.0
Cupric carbonate[6]	9.6	--	--	9.2
Ferric citrate[7]	269.5	--	--	208.7
Potassium phosphate[8]	5461	--	--	5403

[1]The following ingredients (g/kg) were added to all diets: sucrose, 100; soybean oil (0.02% TBHQ, Dyets, Inc., Bethlehem, PA), 70; fiber (Cellulose, FCC, Dyets, Inc., Bethlehem, PA), 50; AIN-93 vitamin mix (Dyets, Inc., Bethlehem, PA), 10; and choline bitartrate, 2.5.

[2]Egg white, spray dried (Dyets, Inc., Bethlehem, PA)

[3]Dyets, Inc., Bethlehem, PA

[4]Contained (g/kg mineral mix): Sucrose, 221; $CaCO_3$, 357; KH_2PO_4, 196; $K_3C_6H_5O_7 \cdot H_2O$, 70.8; NaCl, 74; K_2SO_4, 46.6; MgO, 24; $MnCO_3$, 0.63; KIO_3, 0.01; $Na_2SeO_3 \cdot 0.01$; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.008; $Na_2O_3Si \cdot 9H_2O$, 1.45; $CrK(SO_4) \cdot 12H_2O$, 0.275; LiCl, 0.02; H_3BO_3, 0.08; NaF, 0.06; $NiCO_3$, 0.03; NH_4VO_3, 0.007.

[5]Fisher Scientific, Fair Lawn, NJ

[6]J.T. Baker Chemical Co. Phillipsburg, NJ

[7]$FeC_6H_5O_7 \cdot H_2O$, Matheson Coleman & Bell, Norwood, OH

[8]Monobasic, Mallinckrodt, Paris, KE

amount of phosphorous. The control diet was similar to the basal diet except for an increase in the amount of zinc and minor adjustments in the protein and iron to be similar to amounts in the extruded and raw diets. The ground extruded product and the raw product ingredients were used to provide all of the zinc (14 ppm) in the extruded and raw experimental diets, respectively. This level of zinc is adequate but low compared to the recommended level in the AIN-93 diet. Approximately 50% of the total protein was supplied by egg white, and the remainder by the extruded product or raw product ingredients. Adjustments in the amounts of cornstarch and Dyetrose were made to compensate for carbohydrate in the semolina.

ANALYTICAL METHODS

Phytate

The phytate content of the extruded and raw products was determined using the anion-exchange method of Harland and Oberleas (1986). The procedures were as follows: Triplicate samples (2.000 g) of ground extruded and raw products were accurately weighed and placed in 125ml Erlenmyer flasks. Forty ml of 2.4% HCl was added and the flasks were covered with parafilm and shaken vigorously for 3 hr at room temperature. The mixture was centrifuged and then filtered through Whatman No. 1 filter paper. Plastic columns (4.0 ml with 2 frits) were washed with 3ml of DDW and packed with 0.5 g of chloride exchange resin (AG 1-X8, 200-400 mesh) in a water slurry. Before use, the packed columns were washed with 10 ml of 0.7 M NaCl to assure chloride saturation of the resin and then with 15 ml of DDW. A blank for one batch was prepared by mixing

1 ml 2.4% HCl with 1 ml EDTA-NaOH reagent (0.11 M EDTA and 0.75M NaOH), diluted to 25 ml with DDW, and then poured onto the column. One milliliter of the sample extract was mixed with 1 ml of the EDTA-NaOH reagent, diluted to 25 ml with DDW and transferred onto the column to elute most of the inorganic phosphate. The remaining inorganic phosphate was eluted with 15 ml of 0.1 M NaCl. The phytate was eluted from the column with 15 ml of 0.7 M NaCl and collected in a 25 ml Erlenmyer flask. Concentrated H_2SO_4 (0.5 ml) and concentrated HNO_3 (3.0 ml) were added, and then the mixture was digested on low heat until all acid was evaporated. DDW (10 ml) was added to the flask and heated on low temperature for 10 minutes and then allowed to cool. The content of the flask was quantitatively transferred to a 50-ml volumetric flask. Two ml of molybdate solution (2.5% ammonium molybdate in 1 N H_2SO_4) was added, and the contents mixed; 1 ml of sulfonic acid reagent (0.16% 1-amino-2-naphthol-4-sulfonic acid, 1.92% Na_2SO_3, and 9.60% $NaHSO_3$ in DDW) was added, and the flask was made to volume with DDW. After standing for 15 minutes, the absorbance was read at 640 nm. The phosphorous values were calculated from a standard curve and converted to phytate using a conversion factor of 3.55.

The standard curve was prepared by pipetting 1, 3 or 5 ml aliqouts of phosphorous standard solution (80 μg P/ml) into 50 ml volumetric flasks and then adding 20 ml of DDW. After the flask was mixed thoroughly, 2 ml of molybdate solution was added, and the content mixed. One ml of sulfonic acid reagent was then added, and the contents mixed well. The flask was made to volume with DDW and mixed well. The absorbance was read at 640 nm after 15 min (Spectronic 21, Baush & Lomb).

Protein Analysis

The protein content of the diets and dried feces was analyzed by micro kjeldahl nitrogen analysis using a modification of the AOAC (1984) method. Concentrated sulfuric acid (5 ml) and one catalyst tablet (potassium sulfate and selenium; Tecator Co., England) were added to each of the digestion tubes containing preweighed samples. Triplicate samples were digested using gradually increased temperatures until digestion was complete (Tecator 1016 Digestor, Tecator Co., England). The samples were distilled using a Buchi 323 distillation unit (Switzerland) and then titrated (716 DMS Titrino, Switzerland). The protein content was calculated on a dry weight basis using a nitrogen conversion factor of 5.70.

Mineral Analyses

Wet ashing. Triplicate 0.5 g samples of ground samples were weighed and placed in 25 ml Erlenmyer flasks with 10 ml concentrated HNO_3. The flask was placed on a hot plate at low heat for 1 day and then allowed to go to dryness. Five ml of 30% H_2O_2 was added. If a white ash was not obtained, more H_2O_2 (5 to 10 ml) was added. When digestion was complete, samples were diluted with acid and DDW prior to analyzing minerals by AAS.

Products and diets. The extruded product, raw product ingredients and rat diets were wet ashed. For copper analysis, the ashed samples were diluted with 1ml 0.1N HCl and 9ml DDW for products and 1ml 0.1N HCl and 4ml DDW for diets. Diluted product samples were further diluted 1:10 with DDW for zinc and iron analyses. Diluted diet samples were diluted 1:10 or 1:5 with DDW for zinc and iron analyses.

Tissues. Livers and kidneys were freeze-dried (Unitrap II, Virtis Co., Gardiner, NY) to a constant weight. Liver samples were crushed manually. Duplicate 0.5 g liver samples and one kidney (approximate 0.16g) were soaked overnight in 10 ml concentrated HNO_3 to allow tissues to dissolve in the acid before wet ashing. If a white ash was not obtained or particles were present in the flask when diluted with 0.1 N HCl, repeat ashing was necessary. Ashed samples were diluted with 5 ml 0.1N HCl for copper analysis. Diluted ashed liver and kidney samples were further diluted (1: 26 and 1:16, respectively) for analyses of zinc and iron.

Femur samples (one from each rat) were soaked in ether for 24 hours to extract fat and allowed to dry briefly at room temperature. The femur samples were placed in acid-washed ceramic crucibles and dried in a drying oven (Blue M, Blue Island, IL) to a constant weight and then dry ashed in a muffle furnace (Temco, Model No 293C, Barber-Coleman, Rockford, IL) at 600 °C for 40 hours. When the femur samples were cool, 2 ml of 3 N HCl was added. Diluted samples were further diluted (1: 1) for copper analysis. For femur zinc and iron, 0.1 ml of samples was diluted with 5.9 ml of DDW.

Feces. The dried feces were ground in a blender, weighed and wet-ashed. Ashed samples were diluted with 10 ml 1 N HCl. Further dilution (1:30) was made for fecal zinc and iron.

NIST Standard. Standard reference materials of bovine liver and wheat flour were digested and analyzed using the same procedures as for tissues samples to check the validity of the methodology.

Plasma. Plasma samples were diluted 1: 10 with deionized water for analysis of zinc by AAS. Standard solutions were made by diluting stock standards with a 5%

glycerol solution. The glycerol solution was used to compensate for the viscosity of the serum.

Statistical Analyses

The Student's t-test was used to compare data obtained at the end of week 1 of the experimental period. Data at the end of experiment (week 3) were subjected to a one-way analysis of variance at the 95% confidence level to determine the significance of differences between means. Significant differences among the treatments were determined by Fischer's least significant differences (LSD) test when appropriate. All percentage data were log transformed prior to statistical analyses.

RESULTS

Analyses of products and diets

Zinc, copper, iron and phytic acid content of products. The analyzed amounts of zinc, copper and iron were similar in the extruded and raw products (Table 2). The accuracy of analytical procedures were verified by analysis of NIST wheat flour. The analyzed zinc, copper and iron concentrations in wheat flour (13.3±0.5, 2.1±0.4 and 12.5±0.9 μg/g, respectively) were within the range of certified values. Literature values for the phytate content of wheat flour and soy protein concentrate are 0.28% and 1.25-2.7%, respectively (Oberleas and Harland, 1981; Anderson and Wolf, 1995). We estimated that the mixture of 85% semolina/15% soy protein concentrate had 0.43-0.64% of phytate in it. The analyzed values for the products used in this experiment were low (0.34% and 0.37% for the extruded and the raw semolina/soy protein product) compared to literature values and also similar to each other.

Zinc, copper, iron, phytic acid and protein content of diets. The analyzed amounts of zinc, copper and iron were similar in the extruded and raw diets (Table 3). However concentrations of zinc and iron in the control diet were 14% and 27% higher than in the extruded diet and 17% and 15% higher than in the raw diet.

TABLE 2

Analyzed phytate, zinc, copper, and iron content of extruded and raw products[1]

	Phytate(mg/g)	Analyzed mineral Content (μg/g) Zinc	Copper	Iron
Extruded product	3.42±0.79	27.2±0.8	7.08±0.20	57.5±1.5
Raw product	3.73±0.39	25.8±0.4	6.25±0.07	54.0±1.8

[1]Each value represents mean ± SEM of six determinations.

TABLE 3

Estimated phytate and analyzed protein, zinc, copper, iron content of diets[1]

Diets	Phytate (mg/g diet)[2]	Protein (%)	Analyzed Mineral Content (mg/kg diet) Zinc	Copper	Iron
Basal	--	16.9±0.2	3.8±0.3	5.07±0.2	60.1±1.3
Extruded	1.76	18.5±0.1	14.8±0.5	5.46±0.2	40.7±2.6
Raw	2.02	17.8±0.3	14.5±0.8	5.67±0.4	44.7±2.2
Control	--	17.6±0.1	16.9±0.8	5.48±0.3	51.6±2.5

[1]Each value represents mean ± SEM of six determinations.

[2] Phytate values are obtained by calculation.

Concentrations of phytate in the diets containing the extruded and raw product was calculated to be 0.18% and 0.20%, respectively based on the analyzed amount of phytate in the extruded product or raw ingredients. It was assumed that basal and control diets contained no phytate.

In Vitro Experiments

Soluble and dialyzable zinc content of products. The analyzed amount of soluble zinc and percent soluble zinc were similar in extruded and raw products (Table 4). The soluble zinc content of the extruded product was 15.3 μg zinc/g compared to 15.6 μg/g in the raw ingredients, respectively. These amounts represent approximately 56% of the total zinc. The extruded product contained 13.0 μg dialyzable zinc/g compared to 15.0 μg/g in the raw ingredients or 47.8 and 58.1 % of total zinc, respectively. These results indicate that extrusion processing had little effect on the bioavailability of zinc as evaluated by these in vitro techniques.

In Vivo Animal Experiment

Body weights, weight gain, food intake, efficiency of food utilization and total mineral intake. Rats fed the basal zinc deficient diet consumed an average of 8.0 g food/day and gained 1.4 g/day (Table 5). During the first week of the experimental diet period, rats fed the extruded diet consumed more food than rats fed the control egg white diet, although total zinc intakes were similar. Rats fed the extruded diet also consumed

TABLE 4

Soluble, percent soluble, dialyzable and percent dialyzable zinc in extruded and raw products[1]

Product	Soluble Zn (μg/g)	Soluble Zn (%)	Dialyzable Zn (μg/g)	Dialyzable Zn (%)
Extruded	15.3±3.0	56.5±10.9	13.0±0.5	47.8±1.7
Raw	15.6±1.7	56.6± 6.5	15.0±0.2	58.1±0.8

[1]Each value represents mean ± SEM of six determinations.

TABLE 5

Body weights, food intake and food efficiency ratios[1,2]

Diets	Initial Weight (g)	Final Weight (g)	Weight Gain (g/d)	Food Intake (g/d)	Food Efficiency Ratios (%)[3]
			Zn deficient		
Basal	108±3	125±4	1.4±0.2	8.0±0.2	17.4±2.7
			Week 1[4]		
Extruded	125±4	172±3	6.6±0.5	11.6±0.3[a]	57.1±4.5
Control	124±5	163±6	5.5±0.4	10.1±0.3[b]	54.6±3.8
			Week 3[5]		
Extruded	122±6	236±7[b]	5.4±0.1[b]	14.2±0.4[b]	38.5±1.0[b]
Raw	126±5	248±3[b]	5.8±0.3[b]	15.8±0.3[c]	37.0±0.9[ab]
Control	123±5	211±9[a]	4.2±0.3[a]	12.4±0.5[a]	34.0±1.3[a]

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Food Efficiency Ratio = (weight gain, g/ total food intake, g) x 100 %
[3]Data were log-transformed prior to statistical analysis.
[4]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[5]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

more copper but less iron than those fed the control diet. At the end of week 3 of the experimental diet period, the mean body weight (211±9 g) of rats in the control group was significantly lower than that of the extruded (236±7 g) and raw (248±3 g) groups. There was no significant difference in the body weight of the extruded and raw groups. The lower total weight gain of rats in the control group, which was 89% of that in the extruded group and 85% of that in the raw group, can be explained by their significantly lower intake of food. Food efficiency ratio was lower in rats fed the control diet compared to the extruded or raw diet.

The total dietary intakes of zinc, copper and iron are shown in Table 6 . Because of differences in food intake and slight differences in mineral content of the diets, the total copper intake during the experimental diet period was lowest in rats fed the control diet and highest in rats fed the raw diet. However, the total zinc intake was not statistically different in all three groups. The total iron intake in the control group was higher than that in the extruded group because of the higher content of iron in the diet.

Organ wet weights, dry weights and % body weight of organs. After consuming the experimental diets for one week, rats fed the control diet had higher femur relative weight compared to rats fed the extruded diet (Table 7). At the end of the basal diet period, rats had similar wet and relative weights to those in rats that were killed at wk 1 except for liver wet weight and relative femur weight. At the end of wk 3 of the experimental diet period, rats fed the raw diet had significantly higher liver weight than rats fed the control diet. Rats fed the semolina/soy protein diets had significantly higher mean kidney weight than rats fed the control diet. However, there was no significant

TABLE 6

Zinc, copper and iron intake[1]

Diets	Zinc	Copper (μg/ daily)	Iron	Total Mineral Intake (mg) Zinc	Copper	Iron
			Zn deficient			
Basal	30.7±0.9	40.7±1.2	483±14	0.37±0.01	0.49±0.01	5.79±0.17
			Week 1[1]			
Extruded	175±4	63.6±1.6[b]	474±12[a]	1.22±0.03	0.44±0.01[b]	3.32±0.08[a]
Control	171±4	55.6±1.4[a]	523±13[b]	1.20±0.03	0.39±0.01[a]	3.66±0.09[b]
			Week 3[3]			
Extruded	213±6	77.4±2.2[b]	577±16[a]	4.46±0.13	1.62±0.05[b]	12.11±0.34[a]
Raw	228±5	89.6±1.8[c]	706±14[c]	4.79±0.10	1.88±0.04[c]	14.83±0.30[c]
Control	211±8	68.0±2.6[a]	640±25[b]	4.40±0.17	1.43±0.06[a]	13.43±0.52[b]

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

TABLE 7

Organ wet weights and relative organ weights[1]

Diets	Wet weights (g)			Relative Weights (g wet wt/100g body wt)		
	Liver	Kidneys	Femur	Liver	Kidney	Femur
			Zn deficient			
Basal	5.40±0.42	1.07±0.03	0.51±0.03	4.29±0.23	0.85±0.02	0.41±0.02
			Week 1[2]			
Extruded	7.34±0.40	1.43±0.04	0.56±0.01	4.27±0.20	0.83±0.03	0.33±0.00[a]
Control	7.10±0.48	1.38±0.06	0.57±0.02	4.36±0.24	0.85±0.03	0.35±0.01[b]
			Week 3[3]			
Extruded	9.32±0.32[ab]	1.74±0.03[b]	0.71±0.02	3.94±0.05	0.74±0.01	0.30±0.00[ab]
Raw	10.01±0.21[b]	1.82±0.05[b]	0.73±0.01	4.03±0.06	0.73±0.02	0.29±0.00[a]
Control	8.26±0.54[a]	1.52±0.07[a]	0.66±0.03	3.89±0.12	0.72±0.02	0.31±0.01[b]

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

difference between the mean wet weight of kidneys in rats fed the extruded and raw diets. Mean femur wet weight was not statistically different in rats fed the three diets whereas relative weight of femur was significantly different only between the raw and control groups. The same trend was observed in the mean liver, kidney and femur dry weight values for rats fed the three diets (Table 8). These results could be related to differences in body weights. Body weights of rats in the extruded and raw diet were also significantly higher than those fed the control diet.

Plasma concentration of zinc. Plasma zinc concentration in rats fed the control was higher compared to value at the end of basal zinc deficient diet period, and was similar to that in rats fed extruded diet at the end of wk 1 (Table 9). Plasma concentration of zinc was significantly higher in rats fed the control diet than in rats fed extruded and raw diets at the end of wk 3. There was no significant difference in plasma zinc concentrations between rats fed the extruded and raw diets suggesting no effect of extrusion processing on zinc bioavailability.

Tissue concentration of zinc. At the end of the zinc deficient basal diet period, zinc concentrations in liver and kidney were 73.2 and 82.7 μg/g and similar to those in rats fed the extruded or the control diet after wk 1 (Table 10). Concentration of zinc in the femur and total zinc in liver, kidney and femur were lower compared to rats fed the two experimental diets for 1 week. After 1 week, the concentrations of zinc and total content of zinc in the femur of rats in the control group were significantly higher than those in rats fed the extruded diet. Rats fed the control diet, however, had significantly higher total

TABLE 8

Organ dry weights[1]

Diets	Liver (g)	Kidneys (g)	Femur (g defatted dry wt)
		Zn deficient	
Basal	1.67±0.14	0.28±0.01	0.24±0.01
		Week 1[2]	
Extruded	2.10±0.13	0.34±0.02	0.29±0.00
Control	2.36±0.13	0.33±0.01	0.30±0.01
		Week 3[3]	
Extruded	2.88±0.12^{ab}	0.43±0.01^{b}	0.40±0.01
Raw	3.07±0.06^{b}	0.46±0.01^{b}	0.41±0.01
Control	2.61±0.17^{a}	0.38±0.01^{a}	0.38±0.01

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

TABLE 9

Plasma concentration of zinc[1]

Diets	Plasma Zn (μmol/L)
	Zn deficient
Basal	10.3±1.40
	Week 1[2]
Extruded	21.7±1.25
Control	22.5±1.84
	Week 3[3]
Extruded	23.7±0.93[a]
Raw	25.1±1.01[a]
Control	27.6±1.04[b]

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

TABLE 10

Concentration and total content of zinc in tissues[1]

Diets	Liver	Kidney	Femur	Tissue Total Zinc (μg Zn)		
	(μg Zn/g dry wt)		(μg Zn/g defatted dry wt)	Liver	Kidneys	Femur
			Zn deficient			
Basal	73.2±4.6	82.7±3.9	109±5	120± 8	23.3±1.5	25.9±0.8
			Week 1[2]			
Extruded	80.3±3.8	89.6±0.7	128±3[a]	168±10	30.0±1.2	37.4±0.6[a]
Control	75.3±1.8	94.1±2.6	154±6[b]	178± 9	31.0±1.4	45.4±2.6[b]
			Week 3[3]			
Extruded	87.8±2.1	81.9±1.2[a]	157±2[a]	252± 8[b]	35.1±0.8[a]	62.9±1.5[a]
Raw	89.9±2.1	85.4±1.7[a]	163±3[a]	276± 8[b]	39.0±0.7[b]	67.4±1.3[a]
Control	85.8±2.2	94.0±1.2[b]	216±6[b]	223±10[a]	35.9±1.3[ab]	82.8±2.9[b]

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

femur zinc than rats fed the extruded and the raw diet possibly due to absence of phytate in the diet and different source of protein. After week 3 of the experimental diet period, the concentration of zinc in kidneys of rats fed the extruded or raw diets was significantly lower than in rats fed the control diets. Total liver zinc was significantly higher in the extruded diet-fed and in the raw diet-fed rats than in the control diet-fed rats. The only difference between the extruded and the raw groups was higher total kidney zinc in the raw diet-fed rats. These results confirmed in vitro results showing extrusion processing had no effect on zinc bioavailability. The results also indicated that phytate and differences in protein source may have influenced zinc bioavailability.

Tissue concentration of copper. Tissue total copper in liver and kidney at the end of the zinc deficient basal period was lower compared to that at wk 1 (Table 11). The concentration of copper and total copper in tissues were unaffected by the two dietary treatments after 1 week of the experimental period. Inconsistent changes in tissue copper were observed at the end of week 3. Rats fed the extruded and the raw diets had significantly higher concentration of copper in liver and significantly lower concentration in kidney than rats fed the control diet at wk 3. Rats fed the extruded diet had significantly higher femur copper concentration than in rats fed the raw diet. Total liver copper was statistically higher in rats fed the extruded and raw diets than in rats fed the control diet, and the total kidney copper was statistically higher in rats fed the extruded diet than in rats fed the raw diet. The femur total copper was statistically higher in rats fed the extruded diet than in rats fed the control diet.

TABLE 11

Concentration and total content of copper in tissues[1]

Diets	Liver	Kidney	Femur	Tissue total copper (μg Cu)		
	(μg Cu/ g dry wt)		(μg Cu/ g defatted dry wt)	Liver	Kidneys	Femur
			Zn deficient			
Basal	11.8±0.8	22.5±1.3	4.86±0.55	19.3±1.23	3.20±0.25	1.15±0.12
			Week 1[2]			
Extruded	12.5±0.6	28.1±2.3	3.49±0.40	25.9±1.27	4.67±0.62	1.02±0.11
Control	11.5±0.4	24.3±1.0	4.16±0.42	27.1±1.53	4.40±0.26	1.22±0.12
			Week 3[3]			
Extruded	12.6±0.4^{b}	81.9±1.2^{a}	3.46±0.12^{b}	36.2±1.0^{b}	35.1±0.8^{a}	1.38±0.04^{b}
Raw	12.6±0.4^{b}	85.4±1.7^{a}	2.84±0.05^{a}	38.6±1.3^{b}	39.0±0.7^{b}	1.18±0.03^{ab}
Control	11.1±0.2^{a}	94.0±1.2^{b}	3.05±0.29^{ab}	29.6±1.7^{a}	35.9±1.3^{ab}	1.17±0.11^{a}

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's t-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

Tissue concentration of iron. Concentration of iron in tissues at the end of the zinc deficient diet period was higher but tissue total iron was lower compared to values at wk 1 (Table 12). After 1 week of the experimental diet period, the concentration and the total content of iron in the femur were significantly higher in rats fed the control diet than rats fed the extruded diet. After wk 3, there were no significant differences in the concentration and the total content of iron in liver and femur. Kidney iron concentration was significantly higher in the control diet-fed rats than in the extruded diet-fed rats, whereas the total kidney iron was significantly lower in the control diet-fed rats than in the raw diet-fed rats.

Feces weights and concentrations of zinc, copper and iron. Fecal dry weights were significantly different among groups : raw > extruded > control (Table 13). The fecal concentration of zinc was higher in the extruded diet-fed group but total fecal zinc excretion was lower compared to the raw diet-fed group because of lower total fecal weight. Significant differences in fecal zinc concentration and total zinc were found only between the raw and the control diet group. Rats fed the raw diet had a significantly lower zinc concentration compared to control rats. However, total zinc was significantly higher in rats fed the raw diet compared to control rats. Fecal copper concentration was significantly higher in rats fed the control diet than rats fed the extruded and raw diets while total copper was significantly higher in rats fed the raw diet than rats fed the extruded and control diets. A similar trend was found in fecal iron concentration and total iron. Control rats had a significantly higher iron concentration but raw diet-fed rats had a significantly higher total iron.

TABLE 12

Concentration and total content of iron in tissues[1]

Diets	Liver (μg Fe/ g dry wt)	Kidney (μg Fe/ g dry wt)	Femur (μg Fe/ g defatted dry wt)	Tissue Total Iron (μg Fe) Liver	Kidneys	Femur
			Zn deficient			
Basal	424±45	301±10	113±3	680±31	84.2±2.8	57.5±3.0
			Week 1[2]			
Extruded	358±18	289±16	81.6±3.9[a]	751±60	95.9±3.0	45.9±1.9[a]
Control	310±14	263± 4	99.0±5.6[b]	735±57	87.0±4.2	56.2±3.5[b]
			Week 3[3]			
Extruded	331±18	279±6[a]	66.6±4.4	946±36	119.6±4.1[ab]	47.3±3.5
Raw	311±22	282±6[ab]	62.4±4.2	953±70	128.6±3.9[b]	45.1±3.2
Control	334±23	302±9[b]	78.3±6.7	858±47	115.0±3.8[a]	51.4±3.9

[1]Each value represents mean ± SEM (after 1wk, n=6 in extruded and control; after 3wk, n=7 in extruded, control and n=6 in raw)
[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by Student's-test.
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

TABLE 13

The weight of feces and concentrations and total contents of zinc, copper, iron in feces[1,2]

Diets	Fecal weight (g dry wt/d)	Zinc	Copper	Iron	Total Mineral Content (μg/daily) Zinc	Copper	Iron
			(μg/ g dry wt)				
Extruded	1.24±0.04[b]	123±12[ab]	51.9±1.1[a]	469±10[a]	152.5±15.7[ab]	64.0±1.9[a]	579.8±19.9[a]
Raw	1.52±0.06[c]	117± 6[a]	52.1±1.2[a]	452±17[a]	177.8±10.7[b]	79.3±2.0[b]	687.3±27.8[b]
Control	0.99±0.06[a]	131± 9[b]	68.4±1.2[b]	551± 7[b]	131.3±15.2[a]	65.1±4.1[a]	549.1±38.4[a]

[1]Each value represents mean ± SEM (n=7 in extruded and control; n=6 in raw)

[2]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.

Apparent digestibility of protein, zinc, copper and iron. Apparent protein digestibility was lower in rats fed the raw diet compared to rats fed the extruded or control diet (Table 14). Although the mean apparent digestibility of zinc was 30 to 50% higher in rats fed the control egg white diet compared to the semolina/soy protein diets, the differences were not significant due to large individual variations. In contrast, copper digestibility was significantly higher in the semolina/soy diet-fed groups than in the control diet-fed group. However, iron digestibility was higher in rats fed the control diet than in rats fed the extruded diet.

TABLE 14

Apparent digestibility of protein, zinc, copper and iron[1, 2, 3]

Diets	Protein (%)[4]	Zinc (%)[5]	Copper (%)[5]	Iron (%)[5]
Extruded	91.2±0.6[b]	34.4±7.3	24.8±1.5[b]	8.8±1.7[a]
Raw	89.3±0.6[a]	29.9±4.3	20.4±1.4[b]	12.6±2.6[ab]
Control	92.7±0.4[b]	44.6±5.5	11.8±2.7[a]	24.2±3.1[b]

[1]Each value represents mean ± SEM (n=7 in extruded, control; n=6 in raw)
[2]All data were log-transformed prior to statistical analysis
[3]Different letters within a column indicate significant differences at $p<0.05$ as determined by ANOVA and LSD.
[4]Apparent protein digestibility = (N consumed/d - N feces/d) / N consumed/d x 100
[5]Apparent mineral digestibility
= (μg mineral consumed/d - μg mineral in feces/d) / μg mineral consumed/d x 100

DISCUSSION

Limited data for the effects of extrusion processing on zinc bioavailability have been reported. Kivisto et al (1986) reported an adverse effect of extrusion processing on zinc absorption. Extrusion cooking of a high fiber cereal product decreased apparent absorption of zinc in humans, but the mechanism of action was not clearly defined. Fairweather-Tait (1989), however, showed no effect of extrusion processing on zinc isotope retention from a mixed meal of wheat bran and flour in human subjects. Undoubtedly there are chemical changes associated with extrusion processing and these may alter mineral bioavailability. Anderson et al (1981) reported a reduction in phytate content in an extruded bran product. Because phytate reduction in soy protein has been shown to increase zinc bioavailability in rats (Zhou et al, 1992), one might speculate that extrusion processing of a product containing phytate would enhance zinc bioavailability.

Our results in rats showing no differences in plasma, liver and femur zinc concentrations between the extruded- and raw diets-fed groups suggest that the extrusion conditions used in the present study did not affect zinc bioavailability. These results are consistent with results in the study in humans by Fairweather-Tait et al (1989). Because the extruded product was fed in their study as part of a test meal containing milk, the possible modifying effect of milk on zinc bioavailability may have influenced their results. There also was little change in phytate content between extruded and nonextruded

(raw) products. Kivisto et al (1986) argued that the lower absorption of zinc in ileostomy patients given an extruded cereal compared with a nonextruded product may have been indirectly due to differences in phytase levels in the cereals. They suggested that extrusion processing may deactivate the phytase that is naturally present in cereal thereby leading to higher intestinal phytate levels and hence greater zinc binding. Sandberg et al (1986) also showed no change in phytate on extrusion cooking of a high fiber cereal product. Anderson and Wolf (1995) in a recent review concluded that phytic acid is generally stable to processing of soybeans including extrusion processing. Therefore, it should be noted that the extruded and raw diet had similar amount of phytic acid, which is thought to form insoluble complexes with proteins and zinc. Formation of phytic acid-protein-zinc complexes has been suggested as an explanation for observed differences in zinc absorption with different methods for processing of soy protein (Erdman et al, 1980). Even though total phytic acid content may not be altered by extrusion processing, interactions among phytic acid, protein and zinc may still occur. Conflicting results in the various studies can be explained in terms of variations in extrusion conditions together with the use of different raw materials.

Zinc was less bioavailable in the semolina/soy protein diet that contained phytate than in the control egg white diet that did not contain phytate. These results are in agreement with the observations of numerous investigators (Zhou and Erdman, 1995). The phytate to zinc molar ratio has been suggested as a parameter to predict zinc bioavailability. Values greater than 12 have been reported to reduce accumulation of zinc in femurs in rats but not depress growth (Davies and Olpin, 1979; Morris and Ellis, 1980a). Estimated phytate to zinc molar ratio of the extruded diet and raw diet were 11.7

and 13.9, respectively, and the egg white-based control diet was assumed to contain no phytate. It is concluded that zinc bioavailability is not inhibited when the phytate to zinc molar ratio is less than 12. The extent of formation of phytate-protein-zinc complexes is dependent upon the presence of other minerals, particularly calcium. Graf and Eaton (1984) indicated that a low calcium content in the diet (0.75%) had no effect on zinc bioavailability when the phytate to zinc molar ratio was 12 or less, whereas when the calcium content of the diet was increased to 1.75%, growth of rats was significantly depressed. In the present study, estimated calcium concentrations were lower than the level shown to have a negative effect on zinc bioavailability, and similar in all diets: 0.55, 0.55 and 0. 52% in the extruded, raw and control diets, respectively.

Another dietary component which influences zinc absorption is type and amount of protein. Davisson et al (1996) found no significant differences in the zinc absorption in adult humans from semi-synthetic liquid meals containing 30% egg albumin when compared with those without added protein. However, when dephytinized soybean-protein isolate (< 0.01 g phytic acid/kg) was added instead of albumin, a significant reduction in the absorption of zinc was observed. These results demonstrate that zinc absorption is inhibited by certain protein sources such as dephytinized soybean-protein isolate, while other proteins have little or no effect.

When food intakes were not controlled, rats fed the semolina/soy protein diets tended to ingest more food than the control group fed the egg white-based diet. The factor(s) responsible for this apparent preference for the semolina/soy diets are not clear, but may have included differences in palatability of the diets. Although all diets contained egg white, the amount in the extruded and raw diets was approximately half

that in the control diet. Zhou et al. (1992) showed that soy protein-fed rats consumed greater amounts of diet than egg white-fed rats. In our experiment, higher total weight gains in the semolina/soy diets were also observed, and this difference was related to higher food intakes. If food intakes are controlled, theoretically the total weight gains should be the same in all groups. Although diets were planned to be as similar as possible, small compositional differences in the diets may have contributed in part to the differences in weight gain.

A feeding trial of 1 week following a 10 d depletion period was found to be long enough to show significant differences in femur zinc content in response to differences in zinc bioavailability of the extruded semolina/soy and the control egg white diets. Concentrations of zinc and total zinc in the femur of rats in the control group were significantly higher than in rats fed the extruded diet after 1 week. Plasma zinc concentrations also were higher in the control group but the difference was not statistically significant. After week 3 of the experimental period, there was a significant difference in plasma zinc between rats fed the extruded and the control diet. Therefore, a feeding trial more than three weeks may be needed to show significant differences in plasma concentrations of zinc in response to differences in bioavailability.

Zinc digestibility was higher in the extruded diet-fed group than in the raw diet-fed and higher in the control diet-fed group than the semolina/soy protein diet-fed group, but the differences were not statistically significant. Apparent protein digestibility for all three groups was ~90%. Godinez et al (1992) reported that the values for apparent protein digestibility in rats are considerably higher than the ones obtained in humans. Apparent protein digestibility of black beans cooked at 120 °C with or without seedcoat varied

from 67-73% in humans and 70-81% in rats. There was a significant negative correlation between phytic acid and in vitro protein digestibility of soybean and other legumes (Chitra et al, 1995). It appeared that phytic acid reduced the protein digestibility by interfering with protease enzymes (Singh et al, 1991). However, in our study, apparent protein digestibility was low in rats fed the raw diet containing phytate whereas digestibility in rats fed the extruded diet was similar to that in rats fed the control diet. This result may be related to the heating of protein during processing, which has been shown to cause protein denaturation, destruction of protease inhibitors and consequently increased digestibility (Wapnir, 1990). Rats fed the raw diet also had higher fecal protein output. This result is consistent with a report by Wu et al (1995). They observed high fecal protein (based on Kjeldahl N) relative to low protein digestibility in a rat balance study.

Results of the in vitro measures to assess zinc bioavailability showed no differences in soluble and dialyzable zinc between extruded and raw products. These results are consistent with our in vivo results showing that extrusion processing has no effect on zinc bioavailability. Other researchers reported contradictory results with respect to the correlation between in vitro and in vivo bioavailability when they investigated the solubility of minerals as a measure of in vivo bioavailability (Narasinga Rao and Prabhavathi, 1978; Wien and Schwartz, 1983; Hunt et al, 1987; Sandberg et al, 1989; Schwartz and Nevins, 1989; Turnland et al, 1990). The in vitro method developed by Miller et al (1981), which is a so-called " equilibrium" in vitro method, has been modified by several investigators for estimation of the bioavailability of iron and zinc. The equilibrium method is based on equilibrium dialysis of minerals across a

semipermeable membrane as a model for the passage across the intestinal wall . Promising correlations between in vitro dialyzability and in vivo bioavailability were reported (Schricker et al, 1981; Hazell and Johnson, 1987; Hurrell et al, 1988; Sandstrom and Almgren, 1989). Because the absorption of minerals and trace elements is taking place in the complex environment of the small intestine, simulation of the conditions prevailing in the small intestine is probably the most critical step for in vitro methods aiming at prediction of the bioavailability of minerals and trace elements. Therefore, Wolters et al (1993) developed a continuous in vitro method by which dialyzable components are continuously removed from pancreatic digestion mixture. They compared the results of the continuous in vitro method with the results of the equilibrium in vitro method with respect to the bioavailability of zinc and other minerals from different types of bread. The dialyzability of zinc was comparable for the two methods.

No studies determining the effect of extrusion processing on copper bioavailability were identified. Data for effects of extrusion processing on iron bioavailability are conflicting. Absorption of iron was not altered in the same experiment by Kivisto et al (1986) that showed absorption of zinc was decreased by 41%. Extrusion processing had no effect on the amount of iron absorbed from potato or maize products by rats (Fairweather-Tait, 1987). There also was no effect of extrusion on the retention of iron as well as zinc using test meals in human subjects (Fairweather-Tait et al, 1989). However, results in a study by Ummadi et al (1993) showed decreased bioavailability of iron in an extruded semolina product. Our results showed a pattern that total contents of copper and iron in liver and kidney were higher in rats fed the raw diet due to higher total copper and iron intake. This pattern was not found in total copper and iron in femur.

Copper digestibility was significantly higher in semolina/soy diet-fed groups compared to the control diet-fed group, whereas iron digestibility was higher in rats fed the control diet compared to rats fed the extruded semolina/soy diet. Consequently, no conclusion can be made related to the effect of extrusion processing on copper and iron bioavailability in this product.

CONCLUSION

The overall results of this study indicate that zinc bioavailability is not affected by extrusion processing. Using in vitro techniques to evaluate zinc bioavailability, when soluble and dialyzable zinc were measured there was no differences between the extruded and raw products. In vitro results were confirmed in the animal study showing extrusion processing had no effect on the bioavailability of zinc. There were no significant differences in plasma and tissue zinc concentrations between the two groups. The fecal concentration of zinc was higher in the extruded diet-fed groups but total fecal zinc excretion was lower because of lower total fecal excretion. Zinc digestibility was similar for the two group. Apparent protein digestibility was lower in rats fed the raw diet. Results for copper and iron showed minor and inconsistent differences.

Compared to the rats fed semolina/soy protein diets, rats consuming the control diet based on egg white showed a lower total food intake and lower body weight. Plasma and femur zinc concentrations were higher than those in rats fed the semolina/soy protein diet. The apparently better utilization of zinc by rats fed this diet might be explained by the absence of phytate in the diet, although the phytate/zinc molar ratios in the extruded and raw diets were only 11.7 and 13.9, respectively. Differences in protein source may also have influenced zinc bioavailability. Although all diets contained egg white, the amount in the extruded and raw diets was approximately half that in the control diet.

FUTURE RESEARCH

The nutritional consequences of extrusion processing as a food preparing process are not fully elucidated. Extrusion processing under conditions we used did not lead to negative or positive effects on the bioavailability of zinc. The possibility of negative or positive effects cannot be excluded, however, if products were extruded under conditions of different pressure and temperature. Therefore, more research is needed to examine the effects of extrusion processing on mineral bioavailability under different processing conditions. These efforts could contribute to determine optimum extrusion processing conditions required for improving mineral bioavailability in a wide variety of cereals and legumes.

Another aspect of zinc bioavailability is the adverse effect of phytic acid in forming phytate-protein-zinc complexes. Different compositions of test diets, e.g. higher content of phytic acid, may induce different results in zinc bioavailability because extrusion processing may cause changes in phytate-protein-zinc interactions. Future study to identify and develop a procedure to quantify phytate-protein-zinc complexes in foods is also recommended.

LIST OF REFERENCES

LIST OF REFERENCES

AKTAN, B. and KHAN, K. 1992. Effect of high-temperature drying of pasta on quality parameters and on solubility, gel electrophoresis, and reversed-phase high-performance liquid chromatography of protein components. Cereal Chem. 69: 288-295.

ANDERSON, R. L. and WOLF, J. W. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J. Nutr. 125: 581S-588S.

ANDERSON, Y., HEDLUND, B., JONSSON, L. and SVENSSON, S. 1981. Extrusion cooking of a high-fiber cereal product with crispbread character. Cereal Chem. 58: 370-374.

AOAC. 1984. Association of Official Chemists Official Methods of Analysis. Pages 1-90 14th Ed. The Association: Arlington, VA.

BINDRA, G. S., GIBON, R. S. and THOMPSON, L. 1986. (Phytate) (calcium)/(zinc) molar ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr. Res. 6: 475-479.

BIRKENMEIER, E. H. and GORDON, J. I. 1986. Developmental isolation of a gene that encodes a cysteine-rich intestinal protein and maps near the murine immunoglobulin heavy chain location. Proc. Natl. Acad. Sci. 83: 2516-2520.

BJÖRCK, I and ASP, N. G. 1983. The effects of extrusion cooking on nutritional value-a literature review. J. Food Engineering 2: 281-308.

BLAKEBOROUGH, P., SALTER, D. N. and GURR, M. I. 1983. Zinc binding in cow's milk and human milk. Biochem. J. 209: 505-512.

BREMNER, I. and BEATTIE, J. H. 1990. Metallothionein and the trace minerals. Annu. Rev. Nutr. 10: 63-83.

BREWER, G. J., HILL, G. M., DICK, R. D., NOSTRANT, T. T., SAMS, J. S., WELLS, J. J. and PRASAD, A. S. 1987. Treatment of Wilson's disease with zinc. III. Prevention of reaccumulation of hepatic copper. J. Lab. Clin. Med. 109: 526-530.

CASTILLO-DURAN, C. and SOLOMONS, N. W. 1991. Studies on the bioavailability of zinc in humans. IX. Interactions of beef-zinc with iron, calcium and lactose. Nutr. Res. 11: 429-438.

CHERYAN, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13: 297-335.

CHITRA, U., VIMALA, V., SINGH, U. and GEERVANI, P. 1995. Variability in phytic acid content and protein digestibility of grain legumes. Plant Foods Human Nutr. 47: 163-172.

COUSINS, R. J. 1989. Theoretical and practical aspects of zinc uptake and absorption. Pages 3-12 in: Mineral Absorption in the Monogastric GI Tract: Chemical, Nutritional and Physiological Aspects. F. R. Dintizis and J. A. Laszlo eds. Plenum Press: New York. NY.

COUSINS, R. J. and SMITH, K. T. 1980. Zinc-binding properties of bovine and human milk in vitro: Influence of changes in zinc content. Am. J. Clin. Nutr. 33: 1083-1087.

DAHLIN, K. and LORENZ, K. 1993. Protein digestibility of extruded cereal grains. Food Chem. 48: 13-18.

DAVIES, N. T. and OLPIN, S. E. 1979. Studies on the phytate: zinc molar contents in diets as a determinant of Zn availability to young rats. Br. J. Nutr. 41: 590-596.

DAVIES, N. T., CARSWELL, A. J. P. and MILLS, C. F. 1985. The effects of variation in dietary calcium intake on the phytate-zinc interaction in rats. Pages 456-489 in: Trace Elements in Man and Animals. C. F. Mills, I. Bremner and J. K. Chesters eds. Aberdeen, Scotland.

DAVISSON, L., ALMGREN, A., SANDSTROM, B. JUILLERAT, M-A. and HURRELL, R. F. 1996. Zinc absorption in adult humans: the effect of protein sources added to liquid test meals. Br. J. Nutr. 75: 607-613.

DE-STEFANIS, E. and SGRULLETTA, D. 1990. Effects of high-temperature drying on technical properties of pasta. J. Cereal Sci. 12: 97-104.

DEXTER, J. E. and MATSUO, R. R. 1977. Changes in semolina proteins during spaghetti processing. Cereal Chem. 54: 882-894.

EDWART, J. A. D. 1988. Studies on disulfide bonds in glutenin. Cereal chem. 65: 95-99.

ELLIS, R. and MORRIS, E. R. 1981. Relation between phytic acid and trace metals in wheat bran and soybean. Cereal Chem. 58: 367-370.

ERDMAN, J. W. 1979. Oilseed phytate; nutritional implications. J. Am. Oil Chem. Soc. 56: 736-739.

ERDMAN, J. W., WEINGARTNER, K. E., MUSTAKAS, G.C., SCHMUTZ, R. D., PARKER, H. M. and FORBES, R. M. 1980. Zinc and magnesium bioavailability from acid-precipitated and neutralized soybean protein products. J. Food Sci. 45: 1193-1199.

EVANS, G.W. and JOHNSON, E. C. 1981. Effect of iron, vitamin B-6 and picolinic acid on zinc absorption in the rat. J. Nutr. 116: 68-72.

FAIRWEATHER-TAIT, S. J. 1992. Bioavailability of trace elements. Food Chem. 43: 213-217.

FAIRWEATHER-TAIT, S. J., SYMSS, L. L., SMITH, A. C. and JOHNSON, I. T. 1987. The effect of extrusion cooking on iron absorption from maize and potato. J. Sci. Food Agric. 39: 341-348.

FAIRWEATHER-TAIT, S. J., PORTWOOD, D. E., SYMSS, L. L., EAGLES, J. and MINSKI, M. J. 1989. Iron and zinc absorption in human subjects from a mixed meal of extruded and nonextruded wheat bran and flour. Am. J. Clin. Nutr. 49: 151-155.

FAN, M. Z., SAUER, W. C. and LANGE, C. F. M. 1995. Amino acid digestibility in soybean meal, extruded soybean and full-fat canola for early-weaned pigs. Anim. Feed Sci. Tech. 52: 189-203.

FESTA, M. D., ANDERSON, H. L., DOWDY, R. P. and ELLERSIECK, M. R. 1985. Effect of zinc on intestinal copper excretion and retention in men. Am. J. Clin. Nutr. 41: 285-291.

FORBES, R. M. and ERDMAN, J. W. 1983. Bioavailability of trace mineral elements. Annu . Rev. Nutr. 3: 213-231.

FORBES, R. M. and PARKER, H. M. 1977. Biological availability of zinc in and as influenced by whole fat soy flour in rat diets. Nutr. Rept. Intl. 15: 681-688.

FOX, T. E., FAIRWEATHER-TAIT, S. J., EAGLES, J. and WHARF, S. G. 1994. Assessment of zinc bioavailability: studies in rats on zinc absorption from wheat using radio- and stable isotopes. Br. J. Nutr. 71: 95-101.

FUKUSHIMA, D. 1991. Recent progress of soybean protein foods: chemistry, technology, and nutrition. Food Rev. Intl. 7: 323-351.

GIFFORD-STEFFEN, S. R. and CLYDESDALE, F. M. 1993. Effect of varying concentrations of phytate, calcium, and zinc on soy protein concentrate. J. Food. Proc. 56: 42-46.

GODINEZ, C. M., BRESSANI, R. and MELGAR, M. 1992. Apparent digestibility of bean protein evaluated in humans, rats and in vitro assays. Nutr. Res. 12: 235-246.

GOLDEN, B. E. and GOLDEN, M. H. N. 1981. Plasma zinc, rate of weight gain and energy cost of tissue deposition in children recovering from severe malnutrition on cow's milk or soy protein based diet. Am. J. Clin. Nutr. 34: 892-899.

GORDON, D. T. 1983. Interaction of iron and zinc on bioavailability of each element in the rat. Fed. Proc. 42: 1184-1188.

GRAF, E. and EATON, J. W. 1984. Effects of phytate on mineral bioavailability in mice. J. Nutr. 114: 1192-1198.

GREGER, J. L. and MULVANEY, J. 1985. Absorption and tissue distribution of zinc, iron and copper by rats fed diets containing lactalbumin, soy and supplemental sulfur-containing amino acids. J. Nutr. 115: 200-210.

GREGER, J. L. and SNEDEKER, S. M. 1980. Effect of dietary protein and phosphorous levels on the utilization of zinc, copper and manganese by adult males. J. Nutr. 110:2243-2253.

GREGER, J. L. 1988 Effect of variations in dietary protein, phosphorous, electrolytes, and vitamin D on calcium and zinc metabolism. Pages 205-228 in: Nutrient Interactions. C. E. Bodwell and J. W. Erdman eds. Marcel Dekker: New York, NY.

GREGER, J. L., GUTKOWSKI, C. M. and KHAZEN, R. R. 1989. Interactions of lactose with calcium, magnesium and zinc in rats. J. Nutr. 119: 1691-1697.

GUJSKA, E. and KHAN, K. 1991. High temperature extrusion effects on protein solubility and distribution in navy and pinto beans. J. Food Sci.: 1013-1016.

GUTHRIE, B. E. and ROBINSON, M. F. 1978. Zinc balance studies during wheat bran supplementation. Fed. Proc. 37: 254-260.

HAGER, D. F. 1984. Effects of extrusion upon soy concentrate solubility. J. Agric. Food Chem. 32: 293-296.

HAMBIDGE, K. M., CASEY, C. E. and KREB, N. F. 1986. Zinc. Pages 1-137 in: Trace Elements in Human and Animal Nutrition, 5th Ed. W. Mertz ed. Academic Press: Orlando, FL.

HARLAND, B. F. and OBERLEAS, D. 1986. Anion-exchange method for determination of phytate in foods: Collaborative study. J. Assoc. Offic. Anal. Chem. 69: 667-670.

HARPER, J. M. 1989. Food extruders and their applications. Pages 1-16 in: Extrusion Cooking. C. Mercier, P. Linko and J. M. Harper eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

HARPER, J. M. 1979. Food extrusion. Crit. Rev. Food Sci. Nutr. 11: 155-215.

HARPER, J. M. 1986. Extrusion texturization of foods. Food Tech. 40: 70-76.

HARRIS, W. R. and KEEN, C. L. 1989. Calculations of the distribution of zinc via computer model of human serum. J. Nutr. 119: 1677-1682.

HARZER, G. and KAUER, H. 1982. Binding of zinc to casein. Am. J. Clin. Nutr. 35: 981-987.

HAZELL, T. and JOHNSON, I. T. 1987. In vitro estimation of iron availability from a range of plant foods: influence of phytate, ascorbate and citrate. Br. J. Nutr. 57: 223-233.

HEMPE, J. M. and COUSINS, R. J. 1989. Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. J. Nutr. 119: 1179-1187.

HETTIARACHCHY, N. S. and ERDMAN, J. W. 1984. Bioavailability of zinc and iron from mature winged bean seed flour. J. Food. Sci. 49: 1138-1142.

HILL, C. H. and MATRONE, G. 1970. Chemical parameters in the study of in vivo and in vitro interaction of transition elements. Fed. Proc. 29: 1474-1488.

HONIG, D. H. and WOLF, W. J. 1991. Phytate-mineral-protein composition of soybeans: Gel filtration studies of soybean meal extracts. J. Agric. Food Chem. 39: 1037-1042.

HORTIN, A. E., BECHTEL, P. J. and BAKER, D. H. 1991. Efficacy of pork loin as a source of zinc and effect of added cysteine on zinc bioavailability. J. Food Sci. 56: 1505-1507.

HUBER, G. R. 1991. Carbohydrates in extrusion processing. Food Tech. 3: 160-168.

HUNT, J. R., JOHNSON, P. E. and SWAN, P. B. 1987. Dietary conditions influencing relative zinc availability from foods to the rats and correlations with in vitro measurements. J. Nutr. 117: 1913-1923.

HURRELL, R. F., LYNCH, S. R., TRINIDAD, T. P., DASSENKO, S. A. and COOK, J. D. 1988. Iron absorption in humans: bovine serum albumin compared with beef muscle and egg white. Am. J. Clin. Nutr. 47: 102-107.

ISTFAN, N. W., JANGHORBANI, M. and YOUNG, V. R. 1983. Absorption of stable ^{70}Zn in healthy young men in relation to zinc intake. Am. J. Clin. Nutr. 38: 187-194.

KING, J. C. and KEEN, C. L. 1994. Zinc. Pages 214-230 in: Modern Nutrition in Health and Disease, 8th Ed. M. E. Shils, J. A. Olson and M. Shike eds. Lea & Febiger: Malvern, PA.

KIVISTÖ, B., ANDERSSON, H., CEDERBLAD, G., SANDBERG, A.-S. and SANDSTRÖM, B. 1986. Extrusion-cooking of a high fiber cereal product: II. Effects on apparent absorption of zinc, iron, calcium, magnesium and phosphorus in humans. Br. J. Nutr. 55: 255-260.

KNABE, D. A., LARUE, D. C., GREGG, E. J., MARTINEZ, G. M. and TANKSLEY, JR., T. D.1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci., 67: 441-458.

KOH, B. K. and SCHAICH, K. M. 1994. Effects of extrusion process on wheat flour protein and texture characteristics. Page 215 in: IFT Annual Meeting Technical Program: Book of Abstracts.

KOO, S. I., DODDS, S. J. and MERCER, L. P. 1986. Optimization of the dietary level of histidine in relation to the serum concentrations of zinc and copper in the weaning rat. Nutr. Res. 6: 967-970.

LÁSZTITY, R. and LÁSZTITY, L. 1990. Phytic acid in cereal technology. Pages 309-371 in : Advances in Cereal Science and Technology. Vol X. Pomeranz ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

LI, M. and LEE, T-C. 1994. Effect of extrusion temperature on changes of protein solubility and disulfide bond and sulfydryl group and functionality of extrudates. Page 214 in: IFT Annual Meeting Technical Program: Book of Abstracts.

LO, G. S., SETTLE, S. L., STEINKE, and HOPKINS, D. T. 1981. Effect of phytate: zinc molar ratio and isolated soybean protein on zinc bioavailability. J. Nutr. 111: 2223-2235.

LÖNNERDAL, B., CEDERBLAD, A., DAVIDSSON, L. and SANDSTRÖ, B. 1984. The effects of individual components of soy formula and cow's milk formula on zinc bioavailability. Am. J. Clin. Nutr. 40: 1064-1070.

LÖNNERDAL, B., HOFFMAN, B. and HURLEY, L. S. 1982. Zinc and copper binding proteins in human milk. Am. J. Clin. Nutr. 36: 1170-1176.

LÖNNERDAL, B., KEEN, C. L. and HURLEY, L. S. 1985. Manganese binding proteins in human and cow's milk. Am. J. Clin. Nutr. 41: 550-559.

LÖNNERDAL, B., STANISLOWSKI, A. G. and HURLEY, L. S. 1980. Isolation of a low molecular weight zinc binding ligand from human milk. J. Inorg. Biochem. 12: 71-78.

LUCAS, E.W. and RIAZ, M. N. 1995. Soy protein products: Processing and use. J. Nutr. 125: 573S-5890S.

MAGEE, A. C. and LUGEYE, K. K. 1985. Effect of zinc on growth and trace mineral status of rats fed low protein diets supplemented with amino acids. Fed. Proc. 44: 453-458.

MEADOWS, N. J., GRAINGER, S. L., RUSE, W., KEELING, P. W. N. and THOMPSON, R. P. H. 1983. Oral iron and the bioavailability of zinc. Br. Med. J. 287: 1013-1014.

MILLER, D. D., SCHRICKER, B. R., RASMUSSEN, R. R. and VANCAMPEN, D. 1981. An in vitro method for estimation of iron availability from meals. Am. J. Clin. Nutr. 34: 2248-2256.

MOMCILOVIC, B., BELONJE, B., GIROUX, A. and SHAH, B. G. 1975. Total femur zinc as the parameter of choice for a zinc bioassay in rats. Nutr. Rep. Int. 12: 197-203.

MORRIS, E. R. and ELLIS, R. 1980a. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J. Nutr. 110: 1037-1045.

MORRIS, E. R. and ELLIS R. 1980b. Bioavailability to rats of iron and zinc in wheat bran. Response to low-phytate bran and the effect of the phytate/ zinc molar ratio. J. Nutr. 110: 2000-2010.

NARASINGA RAO, B. S. and PRABHAVATHI, T. 1978. An in vitro method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. 31: 169-175.

OBERLEAS, D. and HARLAND, B. F. 1981. Phytate content of foods: effect on dietary zinc bioavailability. J. Am. Diet. Assoc. 79: 433-436.

OESTREICHER, P. and COUSINS, R. J. 1985. Copper and zinc absorption in the rat: mechanism of mutual antagonism. J. Nutr. 115: 159-166.

OSBORNE, T. B. 1907. The proteins of the wheat kernel. No.84. Carnegie Inst. Wash., Publ.: Washington D.C.

PATTERSON, W. P., WINKELMANN, M. and PERRY, M. C. 1985. Zinc-induced copper deficiency: megamineral sideroblastic anemia. Ann. Intern. Med. 103: 385-386.

PHILLIPS, D. R. 1989. Effect of extrusion cooking on the nutritional quality of plant proteins. Pages 219-246 in: Protein Quality and the Effects of Processing. R. D. Phillips and J. W. Finley eds. Marcel Dekker: New York, NY.

PLATT, S. R., NADEAU, D. B., GIFFORD, S. R. and CLYDESDALE, F. M. 1987. Protective effect of milk on mineral precipitation by Na phytate. J. Food Sci. 52: 240-241.

PRUDENCIO-FERREIRA, S. H. and AREAS, J. A. G. 1993. Protein-protein interactions in the extrusion of soya at various temperatures and moisture contents. J. Food Sci. 58: 378-381.

REEVES, P. G., NIELSEN, F. H. and FAHEY, G. C. 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939-1951.

RHEE, K. C., KUO, C. K. and LUCAS, E. W. 1981. Texturization. Pages 51-88 in: Protein Functionality in Foods. J. P. Cherry ed. American Chemical Society: Washington, DC.

RITTER, M. A., MORR, C. V. and THOMAS, R. L. 1987. In vitro digestibility of phytate-reduced and phenolics-reduced soy protein isolates. J. Food Sci. 52: 325-341.

ROCKEY, G. J., HUBER, G. R. and BEN-GERA, I. 1993. Extrusion cooked and texturized defatted soybean flours and protein concentrates. Pages 290-298 in: Proceedings of the World Conference on Oilseed Technology and Utilization. T. H. Applewhite ed. AOCS Press: Champaign, IL .

RUDOLPH, B. C., BOGGS, L. S., KNABE, D. A., TANKSLEY, JR., T. D. and ANDERSON, S. A. 1983. Digestibility of nitrogen and amino acids in soybean products for pigs. J. Anim. Sci. 57: 373-386.

SANDBERG, A.-S., ANDERSSON, H., CARLSON, N. G. and SANDSTRÖM, B. 1987. Degradation products of bran phytate formed during digestion in the human small intestine: effect of extrusion cooking on digestibility. J. Nutr. 117: 2061-2065.

SANDBERG, A.-S., ANDERSON, H., KIVISTO, B. and SANDSTROM, B. 1986. Extrusion cooking of a high-fibre cereal product. I. Effects of digestibility and absorption of protein, fat, starch, dietary fibre and phytate in the small intestine, Br. J. Nutr. 55: 245-254.

SANDBERG, A. S., CARLSSON, N. G. and SVANBERG, U. 1989. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. J. Food Sci. 54: 723-728.

SANDSTEAD, H. H. 1987. Editorial: zinc-copper interaction provides a novel and apparently effective alternative therapy for Wilson's disease. J. Lab. Clin. Med. 109: 521-526.

SANDSTRÖM, B. and ALMGREN, A. 1989. Dialysable zinc after in vitro digestion in comparison with zinc absorption measured in humans. Special publication, Royal Society of Chemistry: Nutrient Availability: Chemical and Biological Aspects. 72: 238-240.

SANDSTRÖM, B., KIVISTÖ, B. and CEDERBLAD, A. 1987. Absorption of zinc from soy protein meals in humans. J. Nutr. 117: 321-327.

SANDSTRÖM, B. KEEN, C. L. and LONNERDAL, B. 1983. An experimental model for studies of zinc availability from milk and infant formulas using extrinsic labeling. Am. J. Clin. Nutr. 38: 420-428.

SANDSTRÖM, B., ANDERSSON, H., KIVISTÖ, B. and SANDBERG, A.-S. 1986. Apparent small intestinal absorption of nitrogen and minerals from soy and meat protein-based diets. A study on ileostomy subjects. J. Nutr. 116: 2209-2218.

SANDSTRÖM, B., ARVIDSSON, B., CEDERBLAD, A. and BJÖRN-RASMUSSEN, E. 1980. Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium and protein content in meals based on bread. Am. J. Clin.. Nutr. 33: 739-745.

SANDSTRÖM, B., DAVIDSSON, L., CEDERBLAD, A. and LONNERDAL, B. 1985. Oral iron, dietary ligands and zinc absorption. J. Nutr. 115: 411-414.

SANDSTRÖM, B. and LONNERDAL, B. 1989. Promoters and antagonists of zinc absorption. Pages 57-78 in: Zinc in Human Biology. C. F. Mills ed. Springer-Verlag: New York, NY.

SCHOFIELD, J. D., BOTTOMLEY, R. C., TIMMS, M. F. and BOOTH, M. R. 1983. The effect of heat on wheat gluten and the involvement of sulphydryl-disulfide interchange reactions. J. Cereal Sci. 1:241-246.

SCHÖLMERICH, J. FREUDMANN, A., KÖTTGEN, E., WIETHOLTZ, H., STEIERT, B., LÖHLE, E., HÄUSSINGER, D. and GEROK, W. 1987. Bioavailability of zinc from zinc-histidine complexes. I, Comparison with zinc sulfate in healthy man. Am. J. Clin. Nutr. 45: 1480-1486.

SCHRICKER, B. R., MILLER, D. D., RASMUSSEN, R. R. and VAN CAMPEN, D. 1981. A comparison of in vivo and in vitro methods for determining availability of iron from meals. Am. J. Clin. Nutr. 34: 2257-2263.

SCHWARTZ, R., APGAR, B. J. and WIEN, E. M. 1986. Apparent absorption and retention of Ca, Cu, Mg, Mn, and Zn from a diet containing bran. Am. J. Clin. Nutr. 43: 444-455.

SCHWARTZ, R. and NEVINS, P. 1989. Effects of phytate reduction, fat extraction, and level of Ca on Ca and Zn bioavailability: compared in vitro and in vivo. Bio. Trace Elem. Res. 19: 93-106.

SINGH, U., SINGH, B. and SMITH, O. D. 1991. Effect of varieties and processing methods on phytic acid and protein digestibility of groundnut (Arachis hypogaea). J. Food Sci. Tech. 28: 345-347.

SMITH, K. T. 1988. Zinc. Pages 29-67 in: Trace Minerals in Foods. K. T. Smith ed. Marcel Dekker: New York, NY.

SNEDEKER, S. M. and GREGER, J. L. 1981. Effect of dietary protein, sulfur amino acids, and phosphorous on human trace element metabolism. Nutr. Rep. Intl. 23: 865-864.

SNEDEKER, S. M. and GREGER, J. L. 1983. Metabolism of zinc, copper and iron as affected by dietary protein, cysteine and histidine. J. Nutr. 113: 644-652.

SOLOMONS, N. W. 1982. Factors affecting the bioavailability of zinc. J. Am. Diet. Asssoc. 80: 115-121.

SOLOMONS, N. W. 1986. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J. Nutr. 116: 927-935.

SOLOMONS, N. W. and COUSINS, R. J. 1984. Zinc absorption and malabsorption of mineral nutrients. N. W. Solomons and I. H. Ronsenberg eds. Alan R. Liss: New York, NY.

SOLOMONS, N. W. and JACOBS, R. A. 1981. Studies on the bioavailability of zinc in humans. IV. Effect of heme and nonheme iron on the absorption of zinc. Am. J. Clin. Nutr. 34: 475-480.

SOLOMONS, N. W., PINEDA, O., VITERI, F. and SANDSTEAD, H. H. 1983. Studies on the bioavailability of zinc in humans. V. Mechanisms of the intestinal interactions of nonheme iron and zinc. J. Nutr. 113: 337-342.

STRECKER, T. D., CAVALIERI, R. P., ZOLLARS, R. L. and POMERANZ, Y. 1995. Polymerization and mechanical degradation kinetics of gluten and glutenin at extruder melt-section temperatures and shear rates. J. Food. Sci. 60: 532-537.

TURNLUND, J. R., MICHEL, M. C., KEYES, W. R., KING, J. C. and MARGEN, S. 1982. Use of enriched stable isotopes to determine zinc and iron absorption in elderly men. Am. J. Clin. Nutr. 35: 1033-1040.

TURNLUND, J. R., SMITH, R. G., KRETSCH, M. J., KEYES, W. R. and SHAH, A. G. 1990. Milk's effect on the bioavailability of iron from cereal based diets in young women by the use of in vitro and in vivo methods. Am. J. Clin. Nutr. 52: 373-378.

UMMADI, P., CHENOWETH, W. L. and NG, P. K. W. 1995. Changes in solubility and distribution of semolina proteins due to extrusion processing. Cereal Chem. 72: 564-567.

UMMADI, P., CHENOWETH, W. L., UBERSAX, M. A. and OCCEANA, L. 1993. Factors affecting bioavailability of iron in extruded legumes. FASEB J. 7: 1777.

USDA 1989. Agriculture Handbook No. 8 Series-Composition of Foods: Cereal Grains and Pasta (8-20). Page 81 Nutrition Monitoring Division, Human Nutrition Information Service, U. S. Dept. of Agriculture: Washington, D.C.

VALBERG, L. S., FLANAGAN, P. R. and CHAMBERLAIN, M. J. 1984. Effect of iron, tin, and copper on zinc absorption in humans. Am. J. Clin. Nutr. 40: 536-540.

WAPNIR, R. A. 1990. Zinc absorption and sufficiency as affected by protein and other nutrients. Pages 131-164 in: Protein Nutrition and Mineral Absorption. CRC Press: Boca Raton, FL.

WAPNIR, R. A. and STIEL, L. 1986. Zinc intestinal absorption in rats: specificity of amino acids as ligands. J. Nutr., 116: 2171-2179.

WENSINK, J. and VAN DEN HAMPER, C. J. A. 1988. Effect of excess dietary histidine on rate of turnover of ^{68}Zn in brain of rat. Biol. Trace Elem. Res. 16: 137-145. 1985.

WIEN, E. M. and SCHWARTZ, R. 1983. Comparison of in vitro and in vivo measurements of dietary Ca exchangeability and bioavailability. J. Nutr. 113: 388-393.

WOLTERS, M. G. E., SCHREUDER, H. A. W., HEUVEL, G. V. D., LONKHUIJSEN, H. J. V., HERMUS, R. J. J. and VORAGEN, A. G. J. 1993. A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: applications to breads varying in phytic acid content. Br. J. Nutr. 69: 849-861.

WOOD, R. J. and HANSSEN, D. A. 1988. Effect of milk and lactose on zinc absorption in lactose-intolerant postmenopausal women. J. Nutr. 118: 982-986.

WOOD, R. J. and ZHENG, J. J. 1990. Milk consumption and zinc retention in postmenopausal women. J. Nutr. 120: 398-403.

WU, W., WILLIAMS, W. P., KUNKEL, M. E., ACTON, J. C., HUANG, Y., WARDLAW, F. B. and GRIMES, L. W. 1995. True protein digestibility and digestibility-corrected amino acid score of red kidney beans (phaseolus vulgaris L.). J. Agric. Food Chem. 43:1295-1298.

ZHOU, J. R. and ERDMAN, J. W. 1995. Phytic acid in health and disease. Crit. Rev. Food Sci. Nutr. 35: 495-508.

ZHOU, J. R., FORDYCE, E. J., RABOY, V., DICKINSON, D. B., WONG, M. S., BURNS, R. A. and ERDMAN, J. W. 1992. Reduction of phytic acid in soybean products improves zinc bioavailability in rats. J. Nutr. 122: 2466-2473.