IRON AVAILABILITY FROM WEANING FOODS

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Thesis

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IRON AVAILABILITY FROM WEANING FOODS

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ABSTRACT

Iron deficiency is the most common nutritional disorder in infancy. Infants aged 6-24 months are at high risk. Rapid growth of infants during this period of life requires an adequate supply of iron that cannot be covered by breast milk. Weaning food with high bioavailable iron has to be given to the child during this critical period. In this study, three homemade infant foods were formulated from locally available food materials: rice-based meals with vegetable and protein, fruit blends or mixed fruit and fruit juice. These foods and some identical commercial infant foods based on cereals (wheat and rice), milk products, vegetables and fruits were measured for iron availability by in vitro determination.

The commercial weaning mixtures had significantly higher iron content (5.26±4.0 mg/meal) than the weaning foods locally formulated (1.52±0.94 mg/meal), $p<0.001$. This was expected because industrially produced infant foods are commonly fortified with insoluble iron compounds sufficient to meet the iron requirements of infants. The mean percentage of ionizable iron observed in the commercial groups was lower than the homemade groups (10.7% vs 15.7%) because of the higher phytate content (7.7 vs 4.2 mg/meal, $p<0.05$) and the iron compounds used by most producers have low iron bioavailability. The 95% confidence intervals were 11.00 to 20.25% and 6.6 to 14.83% for homemade and commercial meals. However, as the commercial foods are iron-fortified and the mean iron content was about 3.5 times of the homemade formulas, the estimated iron availability (EIA, 0.559 mg/meal) was found to be 2.5 times of the average EIA (0.23 mg/meal) from homemade mixtures ($p<0.005$). Homemade foods with pork liver were found to be as good as the commercial wheat and rice-based formulas. Some selected rice-based meals with iron-rich vegetables (Ivygourd leaves), and chicken or pork liver, contributed up to 0.76 and 0.78 mg of the EIA per meal from more than 3 mg of iron intake per meal. These amounts can fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron for infants.

In conclusion, the commercial products are good at meeting the nutritional and development needs for infants, but they are very expensive compared to similar homemade foods. Nutritious and economical weaning foods can be simply prepared from locally available food materials. Supplementary iron is not required if iron containing foods such as meats, some vegetables,or legumes are used in the preparation.

KEY WORDS : IRON ABSORPTION / AVAILABILITY / WEANING FOODS / COMMERCIAL INFANT FORMULAS

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ปริมาณธาตุเหล็กที่จะถูกดูดซึมได้จากอาหารเสริมทารกวัยหย่านม (IRON AVAILABILITY FROM WEANING FOODS)

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บทคัดย่อ

ภาวะขาดธาตุเหล็กในเด็กทารกเป็นปัญหาที่พบมาก และพบความเสี่ยงสูงในเด็กทารกวัย 6-24 เดือน การเจริญเติบโตอย่างรวดเร็วของเด็กในช่วงวัยนี้ ทำให้มีความต้องการธาตุเหล็กมากขึ้น ซึ่งในน้ำนมแม่มีปริมาณธาตุเหล็กไม่เพียงพออาหารเสริมสำหรับทารกจึงมีส่วนช่วยให้ทารกได้รับธาตุเหล็กเพียงพอในช่วงเวลานี้ การศึกษาเพื่อหาปริมาณธาตุเหล็กในอาหารเสริมทารก ซึ่งแบ่งการศึกษาเป็น อาหารเสริมทารกจากครัวเรือน ได้แก่ ข้าวบดผสมกับผักและโปรตีนชนิดต่างๆ, ผลไม้บด, น้ำผลไม้ และในอาหารเสริมสำเร็จรูปที่วางขายตามท้องตลาด ได้แก่ อาหารเสริมจากธัญพืช, ข้าวบดผสมผักและโปรตีน, ผลไม้บด, และน้ำผลไม้สำเร็จรูป

ปริมาณธาตุเหล็กในอาหารเสริมสำเร็จรูป (5.26±4.0 มก./มื้อ) ซึ่งมีปริมาณสูงกว่าในอาหารเสริมแบบครัวเรือน (1.52±0.94 มก./มื้อ) อย่างมีนัยสำคัญ ($p<0.001$) ที่เป็นเช่นนี้ เนื่องจากในอาหารเสริมสำเร็จรูปมีการเติมธาตุเหล็กให้ได้ในปริมาณที่ทารกต้องการ แต่ค่าเฉลี่ยของร้อยละการดูดซึมธาตุเหล็ก ระหว่างสองกลุ่มพบว่าในกลุ่มอาหารเสริมสำเร็จรูปต่ำกว่าในกลุ่มอาหารเสริมครัวเรือน (10.7% กับ 15.7%) เนื่องจากในกลุ่มอาหารเสริมสำเร็จรูปพบปริมาณฟัยเตทสูงกว่า (7.7 กับ 4.2 มก./มื้อ) ($p<0.05$) ซึ่งมีผลในการยับยั้งการดูดซึมธาตุเหล็ก ที่ระดับความเชื่อมั่น 95%มีค่าร้อยละการแตกตัวของเหล็กอยู่ระหว่าง 11.00 ถึง 20.25% และ 6.6 ถึง 14.83% ในกลุ่มอาหารเสริมครัวเรือน และอาหารเสริมสำเร็จรูปตามลำดับ อย่างไรก็ตามอาหารเสริมสำเร็จรูปที่มีการเติมธาตุเหล็ก พบธาตุเหล็กที่มากกว่าอาหารเสริมครัวเรือน 3.5 เท่า และมีปริมาณธาตุเหล็กที่คาดว่าจะดูดซึมได้จริงในอาหารเสริมสำเร็จรูป (0.559 มก./มื้อ) ซึ่งเป็น 2.5 เท่า ของอาหารเสริมครัวเรือน (0.23 มก./มื้อ) ($p<0.05$) อาหารเสริมครัวเรือนที่มีตับหมูเป็นส่วนประกอบให้ปริมาณธาตุเหล็กได้เทียบเท่ากับกลุ่มอาหารเสริมสำเร็จรูป ในรูปแบบธัญพืชและข้าวบดสูตรต่างๆ การเลือกผักที่มีธาตุเหล็กสูง (ผักตำลึง) และ โปรตีนที่มีธาตุเหล็กสูง เช่น ตับไก่, ตับหมู จะมีปริมาณธาตุเหล็กในอาหาร 3 มก. และดูดซึมได้ 0.76 และ 0.78 มก./มื้อ ตามลำดับ ซึ่งปริมาณเท่านี้เพียงพอต่อความต้องการของทารกที่ต้องการธาตุเหล็ก 0.7 ถึง 1.0 มก.ต่อวัน

สรุป อาหารเสริมสำเร็จรูปมีโภชนาการที่ดีเหมาะแก่การเจริญเติบโตของทารก แต่มีราคาสูงมากเมื่อเปรียบเทียบกับอาหารเสริมครัวเรือน ดังนั้นการให้อาหารเสริมครัวเรือนแก่ทารกโดยเลือกกลุ่มอาหารที่มีธาตุเหล็กสูง นอกจากจะให้ธาตุเหล็กแก่ทารกที่เพียงพอแล้ว จะช่วยให้ประหยัดค่าใช้จ่ายได้มากกว่า

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LIST OF ABBREVIATIONS

Abbreviation	Term
conc.	concentrate
cpm	count per minute
EIA	Estimated Iron Availability
FAO	Food and Agriculture Organization
gm	gram
kcal	kilocalorie
kg	kilogram
M	Mole
mCi	millicurie
mg	milligram
mgFe	milligram of iron
min.	minutes
ml	millilitre
MW	Molecular Weight
µCi	microcurie
µg	microgram
µgFe/gm	microgram of iron per gram
µgP/ml	microgram of phosphorus per millilitre
µl	microlitre
nm	nanometre
r.p.m.	revolution per minute
RDA	Recommended Dietary Allowances
STD	Standard
WHO	World Health Organization

CHAPTER I
INTRODUCTION

1.1 Rationale

Iron is an essential nutritional mineral for life, particularly during the age of six months to two years or the weaning period. It is important because this is a period of rapid brain growth and the development of cognitive and motor skills. In both industrialized and the developing world, low iron will put a child at risk for developing iron deficiency anemia which has been strongly associated with poor growth and development and impaired learning ability. At birth, the infant contains about 80 mg of iron per kilogram of body weight [1]. Of this total, 50 mg per kilogram is in the blood as circulating hemoglobin and 25 mg per kilogram is present as storage iron divided equally between the liver and other body tissues. Another 5 mg per kilogram is found in the erythroid cells of the marrow, in myoglobin, and in various intracellular enzymes. The newborn full-term infant, iron stores are adequate to support normal growth to about 6 months of life, thereafter when iron stores are exhausted the iron requirements are very high because of the rapidly growth. So they need are appropriate complementary feeding, particularly iron. It was suggested that food iron intake should be about 9.3 mg a day at the end of the first year [2]. There is no time in life when dietary iron supplies are as critical as in infancy. Because of the limited diversity of diet and the majority of infant diets are marginal in iron content, all infants are at risk of developing iron deficiency. Iron deficiency anemia in infant has been shown to be associated with psychomotor delays [3]. The areas most involves are language and body balance [4]. In most cases iron therapy was not sufficient to reverse this psychological effects even after complete correction of hematological measures [5,6,7]. Because of the irreversible adverse effects the only feasible way to approach the problem is by prevention of iron deficiency in infancy. In Thailand iron deficiency anemia in infants has been conclusively seen to delay psychomotor development and impair cognitive performance [8]. However, no work

has been done on iron availability from the Thai weaning foods. The purpose of this study was to determine the non-heme iron ionizability in various kind of Thai weaning foods by using an invitro ^{59}Fe radiometric method. It is hoped that results from this study may be useful for iron nutrition in Thai weaning food to ensure optimal health and development.

1.2 Objectives

General objective:

- To determine the non-heme iron ionizability in various kind of Thai weaning foods by using an invitro ^{59}Fe radiometric method.

Specific objectives:

(1) To compare the amount of total iron between homemade and the commercial infant foods.

(2) To compare the amount of inhibitor (Phytate) between homemade and the commercial infant foods.

(3) To compare the percentage of iron ionizability and the EIA between home-made and the commercial infant foods.

1.3 Expected outcome

It is hoped that information from this study may by useful in prevention of iron deficiency in infancy by guiding an adequate meals or proper recipe to meet the recommended daily iron requirements for healthy infants.

CHAPTER II
LITERATURE REVIEW

2.1 Iron

Iron is an essential trace mineral in human nutrition. It is present in all cells and has several vital function in human body. As a component of heme, it carries oxygen to the tissues from the lungs in the form of hemoglobin, and acts as a facilitator of oxygen use and storage in the muscle as myoglobin [9]. Iron is also a component of heme-containing enzymes (cytochromes, catalase, and peroxidase) and nonheme-containing enzymes (iron-sulfur proteins and metalloflavoproteins) involved in oxidative metabolism. Several iron-containing nonheme-containing enzymes are involved in physiologic functions other than oxidative metabolism such as (ribonucleotide reductase which is essential for DNA synthesis) and as a cofactor for tyrosine hydroxylase, the rate-limiting enzyme for biosynthesis of the catecholamine. In addition, it is also involved in the synthesis of collagen, serotonin, dopamine and norepinephrin.

In the normal adult male, 65% or more of total body iron is present in hemoglobin, approximately 10% in myoglobin 3% in iron-containing enzymes, and the remainder in storage compounds [9]. At birth a high percentage of total body iron is present in hemoglobin, but there is also a considerable amount of iron in body stores. The further rise in storage iron during the first few days of life is the result of rapid degradation of hemoglobin when the high red cell mass required for intrauterine oxygen transport is no longer needed for the extra uterine respiratory function. This iron from the degraded red cells further adds to the high iron stores at birth. Storage iron together with that present in breastmilk, is usually sufficient for the first 4 to 6 months of life. Thereafter, the rapid expansion of hemoglobin mass and myoglobin mass nearly exhausts iron stores, and the great proportion of total body iron during the remainder of the first year is present in hemoglobin and myoglobin. This rapid decline

of body iron stores can be viewed as a dilution effect, the existing iron in stores becomes distributed to a greater mass of tissue including red blood cells.[10]

2.1.1 Iron Metabolism

Iron metabolism can be summarized as the maintenance of iron-related physiological function through the balance of intake, transport, stores and loss of iron. Total body iron averages approximately 3.8 g in men and 2.3 g in women, which is equivalent to 50 mg/kg body weight for a 75-kg man [11,12] and 42 mg/kg body weight for a 55-kg woman [13], respectively. When the body has sufficient iron to meet its needs, most iron (>70%) may be classified as functional iron; the remainder is storage or transport iron. More than 80% of functional iron in the body is found in the red blood cell mass as Hb, and the rest is found in myoglobin and intracellular respiratory enzymes (e.g., cytochromes) (Table 1). Iron is stored primarily as ferritin, but some is stored as hemosiderin. Iron is transported in blood by the protein transferrin. The total amount of iron in the body is determined by intake, loss, and storage of this mineral [11].

Table 1. Normal distribution of iron-containing compounds in men [12] and women[13] (milligrams of iron per kilogram of body weight).

Compound	Men	Women
Storage complexes		
Ferritin	9	4
Hemosiderin	4	1
Transport protein		
Transferrin	<1	<1
Functional compounds		
Hemoglobin	31	31
Myoglobin	4	4
Respiratory enzymes	2	2
Total	50	42

2.1.2 Iron Intake

The body can get the iron from the diets. Diets can be divided into those of low, intermediate and high iron bioavailability. These correspond to absorptions of about 5 percent, 10 percent and 15 percent in subjects with depleted iron stores.[13] A diet of low bioavailability (<5 %) is typically consumed in many developing countries. It contains negligible quantities of meat and has a high inhibitor content from cereals and legumes. A diet of intermediate bioavailability (±10%) is almost similar but has more amount of iron absorption enhancers (meat, fish and/or ascorbic acid). For high bioavailability diet (>15%), it is typically eaten by people in industrialized countries, where there is a low prevalence of iron deficiency anemia. The recommended daily iron intake as a function of estimated dietary iron bioavailability in various categories by age and sex is shown in Table 2.

Table 2. Calculated daily iron requirements and recommended daily iron intake as a function of estimated dietary iron bioavailability in various categories by age and sex.Values are based on 95th percentile calculations [14]

Group	Age (yrs)	Requirements (µg/kg/day)	Recommended intake (mg/day)		
			Low (5%)	Intermediate (10%)	High (15%)
Children	0.25-1	120	21	11	7
	1-2	56	12	6	4
	2-6	44	14	7	5
	6-12	40	23	12	8
Boys	12-16	34	36	18	12
Girls	12-16	40	40	20	13
Adult men		18	23	11	8
Adult women:					
menstruating*		43	48	24	16
post-menopausal		18	19	9	6
lactating		24	26	13	9

*Recommended intakes for Low and Intermediate bioavailability are unlikely to be achieved with conventional diet

For infant, the first year of life is a time of more rapid growth and development than any other time of life. A baby usually doubles its birth weight with in the first 4 months and triples birth weight by the first birthday. For this amazing growth, the infant requires an adequate intake of calories and essential nutrients. Breast milk is the main source of nutrition for a baby's first 6 months. Evidence is not available to establish a RDA (The Recommended Dietary Allowance) for iron for infants from birth through 6 months of age. The Recommended iron intake for infants from 0 to 6 months is based on an Adequate Intake (AI) of 0.27 milligrams per day that reflects the average iron intake of breast fed infants (Table 3).

Table 3. Adequate iron intake for infants (0-6 months) [15]

Age (months)	Males and Females (mgFe/day)
0 to 6	0.27

2.1.3 Iron Turnover and Loss

Red blood cell formation and destruction is responsible for most iron turnover in the body. For example, in adult men, approximately 95% of the iron required for the production of red blood cells is recycled from the breakdown of red blood cells and only 5% comes from dietary sources. In contrast, an infant is estimated to derive approximately 70% of red blood cell iron from the breakdown of red blood cells and 30% from the diet [16]. In adults, approximately 1 mg of iron is lost daily through feces and desquamated mucosal and skin cells [17]. Women of childbearing age require additional iron to compensate for menstrual blood loss (an average of 0.3–0.5 mg daily during the childbearing years) [13] and for tissue growth during pregnancy and blood loss at delivery and postpartum (an average of 3 mg daily over 280 days' gestation) [18]. In all persons, a minute amount of iron is lost daily from physiological gastrointestinal blood loss. Pathological gastrointestinal iron loss through gastrointestinal bleeding occurs in infants and children sensitive to cow's milk and in adults who have peptic ulcer disease, inflammatory bowel syndrome, or bowel cancer. Hookworm infections, although not common in the United States [19], are also associated with gastrointestinal blood loss and iron depletion [20].

2.1.4 Iron Stores

Iron present in the body beyond what is immediately needed for functional purposes is stored as the soluble protein complex ferritin or the insoluble protein complex hemosiderin [11,12]. Ferritin and hemosiderin are present primarily in the liver, bone marrow, spleen, and skeletal muscles. Small amounts of ferritin also circulate in the plasma. In healthy persons, most iron is stored as ferritin (an estimated 70% in men and 80% in women) and smaller amounts are stored as hemosiderin (Table 1). When long-term negative iron balance occurs, iron stores are depleted before iron deficiency begins. Men store approximately 1.0–1.4 g of body iron [12, 21], women approximately 0.2–0.4 g [13, 21], and children even less [16]. Full-term infants of normal or high birthweight are born with high body iron (an average of 75 mg/kg body weight), to which iron stores contribute approximately 25% [16]. Preterm or low-birth weight infants are born with the same ratio of total body iron to body weight, but because their body weight is low, the amount of stored iron is low too.

2.1.5 Iron requirements

Iron is needed to replace daily endogenous losses, the figures for adults include hemolysed red cells (0.38 mg), bile (0.24 mg), desquamated gastrointestinal cells (0.14 mg), and urine (0.1 mg). This amounts to a mean total daily loss of 0.9 mg. For female the additional losses via menstruation is about 0.5 mg per day. The daily requirement is therefore usually about 1.4 mg. For the newborn, full-term infant, iron requirements during the first 4-6 months of life are negligible, because of iron stores covers iron needs for this period. After about 6 months, when iron stores are exhausted, the iron requirements are very high, especially during the following 18 months, the weaning period. Iron requirements may amount to about 100 µg/kg/day, which is about four times more than for an average adult menstruating woman. All the iron requirements during infancy are for growth and replace losses. It is estimated to be 0.5 mg/d for infants from 1 to 6 months of age and 0.9 mg/d for infants 7 to 12 months of age, as shown in Table 4

Table 4: Iron requirements of breastfed infants.

Age (months)	Faecal and skin losses (mg/day)	Iron gain (mg/day)	Total iron requirments (mg/day)
1-6	0.24	0.25	0.49
7-12	0.37	0.53	0.90

Source: Fomon SJ. Nutrition of Normal infants. st. Louis, Mosby, 1993

For the first 6 months of age, the iron intake from breastmilk is adequate because it is clear that breastfed infants subsidize their requirements from iron body reserves. At the second half six months of age, this greater iron requirement in infancy is the most difficult period because of the limited diversity of diet and the majority of infant diets are marginal in iron content. For this reason, late infancy and early childhood is a period of high risk for iron deficiency. It have been shown that iron deficiency anemia during infancy was associated with irreversible adverse effects on cognitive performance. Because this is a particularly critical time for brain growth, and iron deficiency anemia can have adverse effects on cognitive and motor development that may not be reversible even after the anemia is corrected. Prevention of iron deficiency is crucial.

Primary prevention of iron deficiency in infants and preschool children should be achieved through diet. Information on diet and feeding is available in the Pediatric Nutrition Handbook [22], Guide to Clinical Preventive Services [23], Nutrition and Your Health: Dietary Guidelines for Americans [24], Breastfeeding and the Use of Human Milk [25], and Clinician's Handbook of Preventive Services: Put Prevention into Practice [26]. For secondary prevention of iron deficiency in this age group, screening for, diagnosing, and treating iron-deficiency anemia are recommended.
The estimates iron requirements and recommended daily iron intakes for the various groups stratified by age and sex for healthy Thai [27] are summarized in Table 5.

Table 5. Recommended daily dietary iron allowances for Thai in various categories by age and sex.

Subjects	Age	Weight(kg)	Height(cm)	Iron (mg)
	(months)			
Infants	under 3	4	55	(breast feeding)
	3-5	6	59	6
	6-8	7	67	7
	9-11	8	70	8
	(years)			
Children	1-3	12	84	10
	4-6	16	106	10
	7-9	22	121	10
Boys	10-12	29	135	12
	13-15	42	154	12
	16-19	54	166	12
Girls	10-12	31	138	15
	13-15	44	152	15
	16-19	48	155	15
Men	20-29	58	166	10
	30-39	58	166	10
	40-49	58	166	10
	50-59	58	166	10
	60+	58	166	10
Women	20-29	50	155	15
	30-39	50	155	15
	40-49	50	155	15
	50-59	50	155	10
	60+	50	155	10
	Pregnant			+30
Lactating	0-5 months postpartum			15
	6+ months postpartum			15

The allowances are intended for healthy Thais under usual conditions.

The Recommended Daily Allowance (RDA) is the daily dietary intake level for iron that is sufficient for most health people. Infants, adolescents, menstruating women and pregnant women have the greatest iron needs, and are therefore at risk of low iron intake

2.1.6 Iron Absorption

Iron absorption occurs predominantly in the duodenum and the proximal jejunum. Absorption takes place from two distinct pools in the gastrointestinal tract: (1) the heme iron pool, which is made up of iron in hemoglobin, myoglobin, and a small quantity of heme-containing enzymes; and (2) the nonheme iron pool, which includes all other forms of iron. Different mechanisms are involved in absorption of iron from the two pools.

2.1.6.1 Absorption of nonheme iron

Most dietary iron is in the form of nonheme iron. The percentage of ingested nonheme iron that is absorbed depends on the quantity consumed, the iron nutritional status of the individual, and the presence of inhibitors or enhancers of iron absorption. Nonheme iron must be delivered to the intestinal mucosa in an ionic form, and it is therefore subject to interaction with a large number of dietary components that inhibit or, less commonly, enhance its absorption. Because the effects of food components on iron absorption result primarily from their interaction with iron in the gastrointestinal tract, effects are greatest when iron and the inhibitory or enhancing components are fed in the same meal [28]

2.1.6.2 Absorption of heme iron

Heme iron is absorbed as an iron-porphyrin complex directly into the mucosal cells [29]. The mechanism for absorption therefore is different from that for nonheme iron. The quantity of heme iron in a meal exerts little influence on its percentage absorption [30]; in general, 20% to 25% of the hemeiron in a meal is absorbed. Only one inhibitor and one enhancer of heme iron absorption have been identified. Calcium inhibits the absorption of heme (and also nonheme) iron. The inhibitory effect of

calcium on the absorption of heme iron does not occur in the intestinal lumen, nor at the point of entry of heme into the enterocyte, but in the intracellular transport of iron [31]. With less transport through the enterocyte, less iron reaches the circulation. The only known enhancer of absorption of heme iron is meat [32].

Data from two publications [33, 34] concerning total iron concentration and heme iron concentration of various meats are in good agreement. Hazall(1982) reported total iron concentrations of uncooked beef, lamb, pork, and chicken to be 2.4, 1.9, 0.7 and 0.9 mg per 100 g of wet weight, respectively, with 78%, 56%, 45% and 26%, of the iron present in heme, respectively.

Extensive studies of adult subjects have demonstrated that heme iron is much more bioavailable than nonheme iron. Studies of iron absorption from meals consumed by adult subjects indicate that those with moderate iron stores (500 mg) generally absorb less than 5% of nonheme iron and approximately 25% of heme iron [35]. Although heme iron provides only 5% to 10% of the iron in the Western adult's diet, it accounts for more than one third of the iron absorbed [36].

2.1.7 Iron balance

Regulation of iron balance occurs mainly in the gastrointestinal tract through absorption. When the absorptive mechanism is operating normally, a person maintains functional iron and tends to establish iron stores. The capacity of the body to absorb iron from the diet depends on the amount of iron in the body, the rate of red blood cell production, the amount and kind of iron in the diet, and the presence of absorption enhancers and inhibitors in the diet. The percentage of iron absorbed (i.e., iron bioavailability) can vary from <1% to>50% [37]. The main factor controlling iron absorption is the amount of iron stored in the body. The gastrointestinal tract increases iron absorption when the body's iron stores are low and decreases absorption when stores are sufficient. An increased rate of red blood cell production can also stimulate iron uptake several folds [11,38]. Among adults, absorption of dietary iron averages approximately 6% for men and13% for nonpregnant women in their childbearing years [37]. The higher absorption efficiency of these women reflects primarily their lower iron stores as a result of menstruation and pregnancy. Among iron-deficient

persons, iron absorption is also high [39]. Absorption of iron increases during pregnancy, but the amount of the increase is not well defined [40]; as iron stores increase postpartum, iron absorption decreases. Iron bioavailability also depends on dietary composition. Heme iron, which is found only in meat, poultry, and fish, is two to three times more absorbable than nonheme iron, which is found in plant-based foods and iron-fortified foods [37, 38]. The bioavailability of non-heme iron is strongly affected by the kind of other foods ingested at the same meal.

Enhancers of iron absorption are heme iron (in meat, poultry, and fish) and vitamin C; inhibitors of iron absorption include polyphenols (incertain vegetables), tannins (in tea), phytates (in bran), and calcium (in dairy products) [11, 41]. Vegetarian diets, by definition, are low in heme iron. However, iron bioavailability in a vegeterian diet can be increased by careful planning of meals to include other sources of iron and enhancers of iron absorption [24]. In the diet of an infant, before the introduction of solid foods, the amount of iron absorbed depends on the amount and bioavailability of iron in breast milk or formula [22] (Table 6).

TABLE 6. Iron absorption by infants fed formula or milk [22]

Substance	Iron content (mg/L)	Bioavailable iron (%)	Absorbed iron (mg/L)
Nonfortified formula	1.5–4.8*	~10	0.15–0.48
Iron-fortified formula†	10.0–12.8*	~ 4	0.40–0.51
Whole cow's milk	0.5	~10	0.05
Breast milk	0.5	~50	0.25

*Values are given for commonly marketed infant formulas.

†Iron-fortified formula contains >1.0 mg iron/100 kcal formula. Most iron-fortified formulas contain approximately 680 kcal/L, which is equivalent to >6.8 mg iron/L.

2.2 Iron through breast feeding

Human breast milk is the best food for newborn babies. The iron through breast feeding is about 0.3 – 0.5 mg/L, even it is low concentration but it is in the

form of high bioavailability and extremely easily absorbed. Because of the high lactose and vitamin C levels in human milk aid the absorption of iron. The infant could have sufficient iron from breast feeding since iron from breast feeding could be absorbed for 50%. Term infants who are breastfed exclusively for the first 6 months may not be at risk for iron depletion or for the development of iron deficiency. If they are not properly breast feeding like supplying of other feeding, the iron absorption through breast feeding may be blocked or reduced. Because the specialized breast milk proteins, lactoferrin and transferrin, which are the major-iron binding protein and facilitate the uptake of iron in the small intestine of infants, are diminished. The results from improper breast feeding have shown that not only increasing the prevalence of iron deficiency anemia in early infant but also the high incidence of gastrointestinal problems according to the decreased of lactoferrin (an antimicrobial protein).

2.2.1 Amount of milk through breast feeding

At present, Thailand has suggested mothers to solely raise their babies through breast feeding for 6 months before any feeding is provided because the data have shown that the infants with sole breast feeding during their first 6 months could grow better with less risk of sickness and allergy than those with 4 month of breast feeding. The study shows that the mothers who have properly feed their babies through breast feeding could have sufficient milk for six months after their delivery. If the infant is fed with other feeding that may disturb the milk creation which will be reduced. After the infant has reached six months, the mother’s milk is running low so the infant could be fed with other kind of feeding.

The amount of breast feeding milk which provided to infants after their delivery to six months in developed and developing countries is shown in Table 7. The result shows that the amount of breast feeding milk in developing countries during the first three months is less than the developed countries but it is still sufficient for the infant. However, the amount of milk for developing and developed countries during the last three months is quite similar.

Table 7. Human milk intake of infants from developed and developing countries.[41]

Countries of study		No. of months of breast feeding					
		1	2	3	4	5	6
Developed countries	No.of study groups	11	14	17	13	10	8
	No. of mothers studied	186	354	376	257	131	93
	Amount of mother milk (ml./day)	699±134*	731±132	751±134	780±138	796±141	854±118
Developing countries	No.of study groups	3	3	2	4	4	3
	No. of mothers studied	61	62	34	95	97	64
	Amount of mother milk (ml./day)	562±92	634±110	582±42	768±63	778±83	804±76

* Mean ± SD

2.3 Weaning food.

2.3.1 Definition and important of weaning food.

Weaning means ‘to accustom a baby to take food other than milk’. It is generally used to mean the process of introducing solid foods into a baby’s diet in order to fulfill their growing nutritional needs. Nutrition in the early years of life is a major determinant of healthy growth and development throughout childhood and good health in adulthood. Breast milk is the ideal food for infants during the first 6

months of life, after that milk alone cannot provide all of the nutrients (particularly iron) and calories for infants who need other more nutritious foods in addition to milk to meet nutrient requirement and adequate growth called weaning or complementary food.

The first weaning food of a baby is usually soft or semi-liquid food which made from a starchy simple (e.g. maize, miller, cassava). Plain rice cereal is also usually given as the first solid food. Vegetables as cauliflower, carrots and potatoes can be boiled, mashed or pureed and added to the cereal or rice porridge. Fruits such as bananas, papaya and other soft, ripe fruits can be mashed and added to the cereal or scraped and fed to the baby directly.

Weaning too early, at 2-3 months, could result in digestive disorders and there is also a high risk of developing food allergy. In the other hand, if the baby is weaned too late, an inadequate nutrition will lead to growth retardation. It has been shown that late weaning is associated with iron deficiency anemia. [43, 44]

Infants with iron deficiency anemia are easily fatigued, more irritable, and have shorter attention spans. They also do less well in tests of psychomotor development during late childhood than those who were not iron-deficient infancy. This effect is apparent even after the iron- deficiency anemia has been reversed. Thus, iron- deficiency anemia during infancy may have long-term and irreversible adverse effects on cognitive development.[45] Preventive and controlling of iron deficiency during infancy are very important, especially in the weaning period, since infants need an additional source of iron to maintain adequate iron nutrition and prevention of iron deficiency anemia. The World Health Organization (WHO) recommends that normal infants should be exclusively breast-fed for 6 months and continue breast feeding with nutritionally adequate complementary food up to2 years of age. [46]

2.3.2 Good weaning food.

Good weaning foods should be:

- Rich in energy, protein and micronutrients (particularly iron, zinc, calcium, vitamin A, vitamin C and folate).
- Clean and safe: no disease-causing bacteria or other harmful

organisms, no harmful chemicals or toxins, no bones or hard bits that may choke a child.

- Locally available and easy to prepare.
- Liked by the child, soft and easy to chew.
- Not too sweet, salty or peppery.

2.3.3 Appropriate complementary feeding.

Weaning or complementary feeding refers to the period during which an infant gradually becomes accustomed to food other than milk. Infants should not be given solid foods before the age of four months because baby may not able to digest the food properly. A mixed diet should be offered by the age of 6 months, or which stage babies need a source of iron in their diet as breast milk can no longer provide enough. An adequate iron containing food must be consumed at this time. It is best to introduce solid foods one at a time and in small amounts at the beginning. Gradually, the amount and number of servings and variety of foods can be increased. The World Health Organization (WHO) recommended that infants start receiving complementary foods at 6 months of age in addition to breast milk, initially 2-3 times a day between 6-8 months, increasing to 3-4 times daily between 9-11 months and 12-24 months with additional nutritious shacks offered 1-2 times per day, as desired. It is necessary to consider the appropriate timing and quality of weaning foods. By general, the appropriate complementary feeding is:

1. *Timely*-meaning that foods are introduced when the need for energy and nutrients exceeds what can be provided through exclusive and frequent breast feeding.
2. *Adequate*-meaning that foods provide sufficient energy, protein and micronutrient to meet a growing child's nutritional needs.
3. *Safe*-meaning that foods are hygienically stored and prepared, and fed with clean hands using clean utensils and hot bottles.
4. *Properly fed*-meaning that foods are given consistent with a child's signals of appetite and satiety, and that meal frequency and feeding method are suitable for age.

2.3.4 Guiding principles [47]

The adequacy of complementary feeding (adequacy in short for timely, adequate, safe and appropriate) not only depends on the availability of a variety of foods in the household, but also on the feeding practices of caregivers. There are many feeding guidelines; some are based more on tradition and speculation than on scientific evidence. Thus, a set of unified, scientifically based guidelines is needed, which can be adapted to local feeding practices and conditions. The guidelines described herein were developed from discussion at several technical consultations and documents on complementary feeding. [48, 49, 50] The summary of the guideline principles are:

1. Duration of exclusive breastfeeding and age of introduction of complementary foods.
   Practice exclusive breastfeeding from birth to 6 months of age, and introduce complementary food at 6 months of age(180 days) while continuing to breastfeed.
2. Maintenance of Breastfeeding.
   Continue frequent, on-demand breastfeeding until 2 years of age or beyond.
3. Responsive feeding.
   Practice responsive feeding, applying the principles of psychosocial care. Specifically:
   a) feed infants directly and assist older children when they feed themselves, being sensitive to their hunger and satiety cues;
   b) feed slowly and patiently, encourage to eat, do not force them;
   c) if children refuse many foods, experiment with different food combinations, taste, textures and methods of encouragement ;
   d) minimize distractions during meals if the child loses interest easily;
   e) remember that feeding times are periods of learning and love-talk to children during feeding, with eye to eye contact.
4. Safe preparation and storage of complementary foods.
   Practice good hygiene and proper food handling by

a) washing caregivers' and children's hands before food preparation and eating,
b) storing foods safely and serving foods immediately after preparation,
c) using clean utensils to prepare and serve food,
d) using clean cups and bowls when feeding children, and
e) avoiding the use of feeding bottles, which are difficult to keep clean.

5. Amount of complementary food needed.

Start at 6 months of age with small amounts of food and increase the quantity as the child get older, while maintaining frequent breast feeding. The energy needs from complementary foods for infants with average breast milk intake in developing countries are approximately 200 kcal per day at 6-8 months of age, 300 kcal per day at 9-11 months of age, and 550 kcal per day at 12-23 months of age. In industrialized countries these estimates differ somewhat (130, 310 and 580 kcal/d at 6-8, 9-11 and 12-23 months, respectively) because of differences in average breast milk intake

6. Food consistency.

Gradually increase food consistency and variety as the infant gets older, adapting to the infant's requirements and abilities. Infants can eat pureed, mashed and semi-solid foods beginning at six months. By 8 months most infants can also eat 'finger foods' (snacks that can be eaten by children alone). By 12 months, most children can eat the same types of foods as consumed by the rest of the family. Avoid foods that may cause choking.

7. Meal frequency and energy density.

Increase the number of times that the child is fed complementary foods when he/she gets older. For the average health breastfed infant, meals of complementary foods should be provided 2-3 times per day at 6-8 months of age and 3-4 times per day at 9-11 and 12-24 months of age, with additional nutritious snacks offered 1-2 times per day, as desired. Snacks are defined as foods eaten between meals-usually self-fed, convenient and easy to prepare. If energy density or amount of food per meal is low, or the

child is no longer breastfed, so more frequent meals may be required.

8. Nutrient content of complementary foods.

   Feed a variety of foods to ensure that nutrient needs are met. Meat, poultry, fish or eggs should be eaten daily, or as often as possible. Vegetarian diets cannot meet nutrient needs at this age unless nutrient supplements or fortified products are used. Vitamin A-rich fruits and vegetables should be eaten daily. Provide diets with adequate fat content. Avoid giving drinks with low nutrient value, such as tea, coffee and sugary drinks such as soda. Limit the amount of juices offered so as to avoid displacing more nutrient-rich foods.

9. Use of vitamin-mineral supplements or fortified products for infant and mother.

   Use fortified complementary foods or vitamin-mineral supplements for the infant, as needed. In some populations, breastfeeding mother may also need vitamin-mineral supplements or fortified products, both for their own health and to ensure normal concentrations of certain nutrients (particularly vitamins) in their breast milk.

10. Feeding during and after illness.

    Increase fluid intake during illness, including more frequent breastfeeding, and encourage the child to eat soft, varied, appetizing, favorite foods. After illness, give food more often than usual and encourage the child to eat more.

2.4 Method for measuring the bioavailability of iron from food

There are two major methods for measuring bioavailability from food, in vivo and in vitro method.

2.4.1 In vivo method

The radioactive iron (^{55}Fe or ^{59}Fe) is simply added to a food or to a complete meal as an extrinsic label. After the test meal has been labeled, iron absorption is measured by the incorporation of the isotope into whole blood or hemoglobin. This

method involves drawing of basal blood sample before the administration of a radiolabeled meal, followed by another blood sample 14 days later to determine the amount of radioiron in hemoglobin. The approach is based on the fact that most of absorbed iron is normally incorporated into erythrocytes within 10 to 14 days of ingestion. Thus, the percent of radioiron absorbed can be calculated from the radioactivity present in red cells after 14 days, assuming that 80 percent of absorbed iron is incorporated in normal subjects. An important limitation of this method is the need to use radioactivity in human subjects. While the dose of radiation is very small, and would be approved by Human Subjects Committees, the radioactivity may make it more difficult to recruit participants, and radiolabeled foods are forbidden to children, pregnant, lactating woman or woman at risk of becoming pregnant.

2.4.2 In vitro method

Several methods have been made to design in vitro system that will accurately predict absorption in humans. In this thesis, an in vitro method is analyzed by adding radioisotope tracer (^{59}Fe) into the homogenized test meal. Under simulated gastrointestinal digestion include acid hydrolysis, proteolytic digestion by pepsin and pancreatin in bicarbonate buffer as in duodenal environment, the sample was incubated at 37°C in the water bath. The amount of ionizable iron is then measured by determination of the extractability of iron from food by chelating agents.The major use of an in vitro method is in predicting trends rather than absolute levels of iron absorption. It has an advantage in the elimination of variation in iron absorption which occur in vivo due to difference in iron status and day to day variability. It can be evaluated the effect of promoter or inhibitor for the amount of iron that will be available for absorption. Moreover it suits for laboratory facility.

2.4.3 Radioisotope iron

Physical characteristic [51]: Radioisotope iron, Fe^{59}, has a physical half life of 45.1 days, decaying with emission of beta and gamma emissions which are:

ß¯ energy (MeV)	ϒ energy (MeV)
0.13 (1 %)	0.14 (0.9 %)
0.27 (46 %)	0.19 (2.4 %t)
0.46 (53 %)	0.34 (0.3 %)
1.56 (0.3 %)	1.10 (56 %)
	1.29 (44 %)

Preparation: Fe-59 is produced by neutron irradiation of an enriched target of Fe-58 according to the reaction: Fe-58 (n,γ) Fe-59 [52].

Quality control: Fe-55 may be present as an impurity but Fe-59 should be greater than or equal to 99 percent.

Rationale of use in diagnostic of Anemia: Radioactive iron has been used to study the absorption of iron from the gastrointestinal tract, the transport of iron in the plasma, the disappearance of iron from the plasma, its storage and utilization to form red blood cells and its excretion from the body. Determination of the rate of red cell destruction using a radioactive iron labeling technique may be less accurate than the Cr51 technique because the iron released by RBC breakdown is reutilized in erythropoiesis. The life span of newly formed erythrocytes can be measured with radioiron, however, by blocking the reutilization of radioiron by giving large quantities of non-radioactive iron. In general, radioiron is most helpful in studying the rate of red cell production, and radiochromium is more useful than iron in determining the rate of red cell destruction [53].

The radioactive isotope Fe-59 is a suitable tracer for studying the metabolism of iron. It emits both beta particles and gamma rays, so is readily measured in small quantities either in blood samples or from outside the body. Its radioactive half-life is 45 days, which turns out to be long enough to enable measurements to be made over the life period of the red blood corpuscles without leaving radioactivity unnecessarily hanging about in the body after the completion of the test [54].

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

Test meals: The weaning food samples were divided into 2 groups; the home made weaning foods and the commercial infant foods.

1. The home made weaning foods.

Three kinds of home made weaning foods were used in this study.

1.1 Rice with protein and vegetable.

Meal preparation : Cooked rice protein and vegetable were blended into a soft semi-liquid food.

Vegetables : Chinese convolvulus, ivygourd, pumpkin, carrot and spineless amaranth.

Proteins : White soybean curd, egg yolk, fish, chicken liver and pork liver.

1.2 Fruit.

Meal preparation : Ripe fruit was mashed and sieved with a metal spoon.

Fruit : Banana, papaya, mango, tomato and apple.

1.3 Juice.

Meal preparation : Fruits were crushed for juice with a squeeze tool.

Juice: Orange juice, apple juice, water melon juice and tomato juice.

2. The commercial infant foods.

The commercial infant foods were purchased from the general supermarket.

They were divided into 4 subgroups.

2.1 Wheat-based with protein, vegetable or fruit. The 4 types of wheat-based were : Wheat and milk, Wheat and soya chicken 6%, Wheat and mixed vegetables 4.5% and Wheat and mixed fruits 4%.

2.2 Rice-based with protein, vegetables or fruits. The 6 types of rice-based

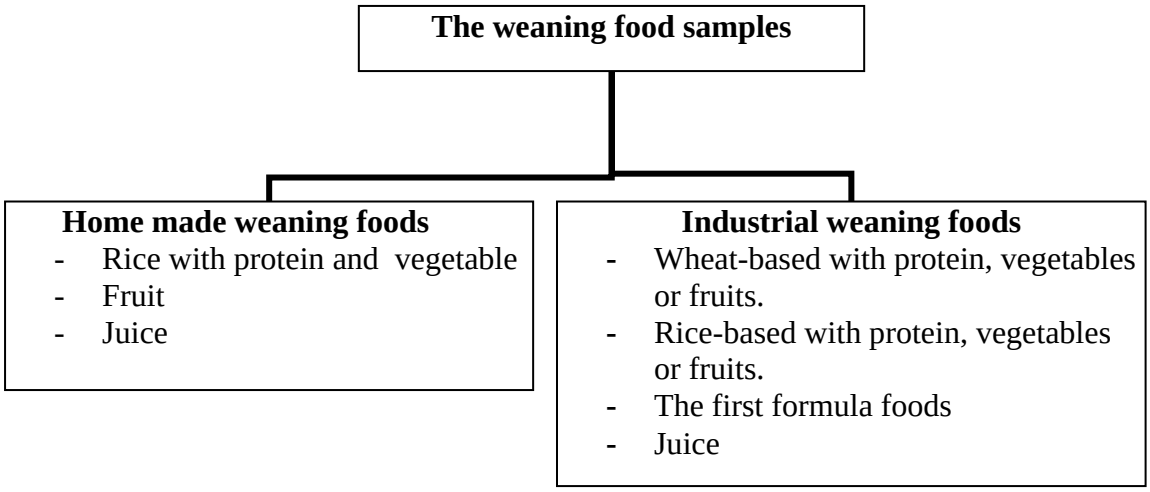
were : Rice and vitamins, Rice and banana, Rice and mixed vegetables, Rice and fish 4%, Rice and chicken 1.8% and Rice and mixed fruits.

2.3 The first formula foods: Applesauce, carrots, bananas and prunes

2.4 Juice: Apple juice, apple prune juice and mixed fruits juice

Name and their components were shown in the Table in Appendix D.

Diagram of the testmeals is shown below.



Radioactive iron : Iron-59 (NEZ 037) as ferric chloride in 0.5 M Hydrochloric acid with specific activity of 10-20 mCi/mgFe obtained from DuPont NEN® Research Products, Boston, Massachusetts, USA, was used in this study.

Reagents

Reagents for determination of ionizable iron in sample

0.17 N HCl

Add 14.17 ml conc. HCl to a 1 litre volumetric flask and bring to volume with iron free water.

^{59}Fe radioactive

^{59}Fe as $FeCl_3$ was prepared 1 μCi /100 μl in 0.17 N HCl.

1% Pepsin in 0.17 N HCl

0.27g pepsin (Sigma G-7000) was dissolved in 27 ml of 0.17 N HCl.

0.1M $NaHCO_3$

4.2 g $NaHCO_3$ (Sigma S-8875) was prepared in 500 ml iron free water.

1% Pancreatin in 0.1 M $NaHCO_3$

0.32 g Pancreatin (Sigma P –7545) was dissolved in 32 ml of 0.1 M $NaHCO_3$.

Bathophenanthroline solution

Dissolve 0.0083 g of 4,7 – diphenyl –1,10- phenanthroline in 40 ml ethyl alcohol and brought to 100 ml with iron free water.

Acetate solution

Dissolve 55.1 g sodium acetate granular ($NaC_2H_3O_3$.$3H_2O$) in 100 ml iron free water.

4N Acetic acid

Dilute 23.5 ml concentrated acetic acid to 100 ml with iron free water.

Acetate buffer solution

4 ml of 4N a acetic acid was mixed with 96 ml acetate solution.

Reagents for determination of total iron in sample

Acetate buffer pH 4.75

450 g sodium acetate ($C_2H_3NaO_2.3H_2O$) was dissolved in deionized water, the pH adjusted to 4.75 using glacial acetic acid, the final volume was made up to one litre with deionized water.

1 % Paranitrophenol

Dissolve 1g paranitrophenol in 100 ml iron free water.

Chromogen solution

200 mg disodium 4, 7 diphenyl 1, 10 – phenanthroline disulphonate was dissoved in deionized water, after adding 1 ml of thioglycolic acid solution (MW 92.12) the volume was adjusted to 100 ml.

Stock iron standard 1 mg/ml

100 mg of iron wire was dissolved in 2 ml concentrated hydrochloric acid, the volume was then adjusted to exactly 100 ml with deionized water.

Working iron standard 1 ug / ml

1 ml of stock iron standard was transferred into a volumetric flask and the volume was adjusted to one litre with deionized water.

Reagents for determination of phytate

Hydrochloric acid (HCL) 2.4 %

Add 54 ml conc HCl to a 1 litre volumetric flask and bring to volume with iron free water.

Sodium chloride solution (NaCL) 0.1 M and 0.7 M

NaCL 0.1 M: Dissolve 5.8443 g NaCl in 1 litre of iron free water.

NaCL 0.7 M: Dissolve 40.9101 g NaCl in 1 litre of iron free water.

Phosphate standard solution (80 ug P/ml)

Weight 0.350 g of dried dessicated potassium acid phosphate (primary standard) into a 1 litre volumetric flask, add approximatety 500 ml of iron free water and 10 ml of 10 N sulfuric acid (H_2SO_4) dilute to mark with iron free water.

Molybdate solution

Dissolve 12.5 g ammonium hepta molybdate in 200 ml of iron free water. Transfer to 500 ml volumetric flask, add 50 ml of N sulfuric acid make to volume with iron free water.

Sulfonic acid reagent

Dissolve 0.16 g of 1 – amino –2- napthal –4- sulfonic acid, and 1.92 g sodium sulfite (Na_2SO_3) and 9.60 g sodium bisufite ($NaHSO_3$) in 90 ml iron free water. Transfer quantitatively to 100 ml volumetric flask. Heat to dissolved if necessary. Make to volume. (Freshly prepared weekly and store in brown bottle in refrigerator).

Na_2EDTA – NaOH reagent

Weigh 10.23 g disodium-ethylenediaminetetra – acetate (Na_2EDTA) and 7.5 g NaOH into 250 ml flask bring to volume with iron free water.

Standard phytic acid

Dissolve 26.6 mg of phytic acid in 25 ml iron free water.

Instruments and special glasswares

Instruments and special glasswares were listed as follows:

- Food Homogenizer, Moulinex France.

- Shaker Bath, Cat No 6250, Eberbach Corporation, Ann Arbor, Michigan.

- Shaker, New Brunswick Scientific, Model R–2, Serial No.382260, Edison, N-J, USA.

- Vortex –mixer, Vortex-Genic 2, Scintific Industries, Inc., Bohemia, N.Y. 11716, USA.

- IEC Centra-8 Centrifuge, Model 2477, International Equipment Company, 300 second Avenue, Needharm Heights, MA 02194, USA.

- Spectrophotometer Spectronic 21, Milton Ray Company, Analytical Products Division (Formerly a division of BAUSCH & LOMB) 820 Lindess Avenue,

Rochester, N.Y. 14625.

- Digital pH meter, Model SP-7, Suntex Instruments Co., Ltd., TAIWAN.

- Magnetic stirer, Ikamag REC-G Janke & Kunkel Gmb H.U.CoKG, IKA-Werk7813 staufen.
- CompuGamma Gamma Counter, Model 1282, LKB Wallac, Wallac Oy, P.O. Box 10, 20101 Turku 10, Finland.

- Mettler Electric Balance, Metter P1210, Metter, Zurich, Switzerland.

- Sauter Electric Balance, August Sauter GmbH D- 7470 Albstadt 1-Ebingen.

- HAVARD TRIP Balance,OHAUS, USA.

- Econo-column, Chromatography Columns, Catalog No. 737-1232 Bio-Rad Laboratories, 32nd & Griffin Avennue, Richmond, California 94804.

- Labconco Micro-Kjeldahl Digestors, Model 60301-01, Labconco Corporation, 8811 Prospect, Kansas City. Mo. 64132.

- The stainless steel Perchloric Acid Hood. Labconco Model 47807, Labconco Corporation, 8811 Prospect, Kansas City, Mo. 64132.

- Eppendorf Pipette 300, Eppendorf Geratebau, Netheler +Hinz GmbH, Postfach 650670,2000 Hamburg 65.

- Anion Exchange resin AG –1 X4. 100-200 mesh ,chloride form, Biorad Lab. Cat No.140-1341.

- Micro Kjeldahl Flask (100 ml), Labconco Model 60375.

- Whatman filter paper No. 541, cat.No.1544425.

3.2 Methods

Chemical composition of meals

Before homogenizing with iron free water to a creamy consistency in a food homogenizer, each individual ingredient in the test meal was weighted and recorded. After blending, weighted amounts of homogenized samples to be analyzed for total phosphorus, phytate, total iron and ionizable iron.

Determination of ionizable iron

An in vitro method for the determination of availability of non heme iron from food described by Sritongkul. [55] was used in this study.

Detailed in Appendix A.

Determination of total iron

The total iron content in meal was determined by the recommended wet digestion method [56].

Detailed in Appendix B.

Determination of total phosphorus and phytate

Phosphorus and phytate were analyzed using an ion exchange procedure as described by Harland and Oberleas [57].

Detailed in Appendix C.

3.3 Statistical analyses

All calculation for descriptive statistics, graphic presentation and statistical analyses were performed with SPSS (version 11.0). Data are presented as the mean ± SD. The P-value less than 0.05 was considered statistically significant. Differences in total iron, phytate, ionizable iron estimated iron and absorption between the test meal groups were evaluated by ANOVA.

CHAPTER IV
RESULTS

4.1 Test meal characteristics

4.1.1 Homemade weaning foods

Energy, Phytate phosphorus and total iron contents of three formulated weaning foods are shown, rice based with vegetable and protein in Table 4.1 and 4.2, fruit blend in Table 4.3 and fruit juice in Table 4.4.

(a) Rice-based meals with vegetable and protein

Twenty five meals were analyzed, as shown in Table 4.1, food energies of the meals ranged from 63.30 to 95.70 kcal (76.8 ± 8.13). As for phytate, it is one of the major anti-nutritional factors in cereals acting as a strong chelating agent by forming mineral-phytate complexes that reduce many mineral unavailable for absorption particularly iron. It is therefore important to determine the phytate in all weaning food recipes. The phytate contents of different weaning foods varied from 2.4 to 12.2 mg/meal. The highest content was found when White soybean curd was added to the meal (Meal no. 21). The mean ± SD among all meals was 5.240 ±2.320 mg/100g.

Iron content of all 25 meals ranged from 0.328 to 3.400 mg/meal (1.727 ± 0.972). The highest concentration was found in meals containing pork liver 3.216, 3.308, 3.400, 2.872 and 3.122 mg/meal in meal number 5, 10, 15, 20 and 25, respectively. Meals with fish and white soybean curd meals contained lower native iron, 0.606 to 1.316 mg/meal and 0.578 to 1.8 mg/meal, respectively.

In Table 4.2 it was obviously seen that iron contents in meals containing fish and white soybean curd were approximately 22% and 30% of meals containing pork liver. Meals with chicken liver contributed approximately 70% of meals with pork liver while meals with egg yolk contributed about 50%.Distribution of iron content by group of vegetables is shown in Figure 4.1 and by group of protein in Figure 4.2.

Table 4.1 Total energy, phytate phosphorus and total iron contents in homemade weaning foods (45 g sample of rice-based meals with vegetable and protein).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
1.	Rice and Chinese convolvulus, White soybean curd	63.3	0.882	9.6
2.	Rice and Chinese convolvulus, Egg yolk	80.4	1.460	4.8
3.	Rice and Chinese convolvulus, Fish	73.8	0.606	4.8
4.	Rice and Chinese convolvulus, Chicken liver	73.1	2.262	4.8
5.	Rice and Chinese convolvulus, Pork liver	74.0	3.216	7.2
6.	Rice and Ivygourd, White soybean curd	65.3	0.746	7.2
7.	Rice and Ivygourd, Egg yolk	82.4	1.612	4.8
8.	Rice and Ivygourd, Fish	75.8	0.676	2.4
9.	Rice and Ivygourd, Chicken liver	75.0	2.334	2.4
10.	Rice and Ivygourd, Pork liver	75.9	3.308	4.8
11.	Rice and Pumpkin, White soybean curd	78.6	0.662	4.8
12.	Rice and Pumpkin, Egg yolk	95.7	1.520	2.4
13.	Rice and Pumpkin, Fish	89.1	0.606	4.8
14.	Rice and Pumpkin, Chicken liver	88.4	2.262	4.8
15.	Rice and Pumpkin, Pork liver	89.3	3.400	3.6
16.	Rice and Carrot, White soybean curd	65.6	0.578	4.8
17.	Rice and Carrot, Egg yolk	82.7	1.158	2.4
18.	Rice and Carrot, Fish	76.1	0.328	4.8
19.	Rice and Carrot, Chicken liver	75.3	1.892	3.6
20.	Rice and Carrot, Pork liver	76.2	2.872	3.6
21.	Rice and Spineless amaranth, White soybean curd	63.3	1.860	12.2
22.	Rice and Spineless amaranth, Egg yolk	80.4	2.124	7.2
23.	Rice and Spineless amaranth, Fish	73.8	1.316	4.8
24.	Rice and Spineless amaranth, Chicken liver	73.1	2.370	7.2
25.	Rice and Spineless amaranth, Pork liver	74.0	3.122	7.2
Mean		76.8	1.727	5.2
SD		8.1	0.97	2.3

Table 4.2 Comparison of total iron contents between rice-based meals with different types of proteins.

Type of protein	Meal No.	Total iron (mg/meal)	Total iron % of Pork liver
White soybean curd	1	0.882	
	6	0.746	
	11	0.662	29.7
	16	0.578	
	21	1.860	
	Mean ± SD	0.946 ± 0.52	
Egg yolk	2	1.460	
	7	1.612	
	12	1.520	49.5
	17	1.158	
	22	2.124	
	Mean ± SD	1.575 ± 0.35	
Fish	3	0.606	
	8	0.676	
	13	0.606	22.2
	18	0.328	
	23	1.316	
	Mean ± SD	0.706 ± 0.37	
Chicken liver	4	2.262	
	9	2.334	
	14	2.262	69.8
	19	1.892	
	24	2.370	
	Mean ± SD	2.224 ± 0.19	
Pork liver	5	3.216	
	10	3.308	
	15	3.400	100
	20	2.872	
	25	3.122	
	Mean ± SD	3.184 ± 0.20	

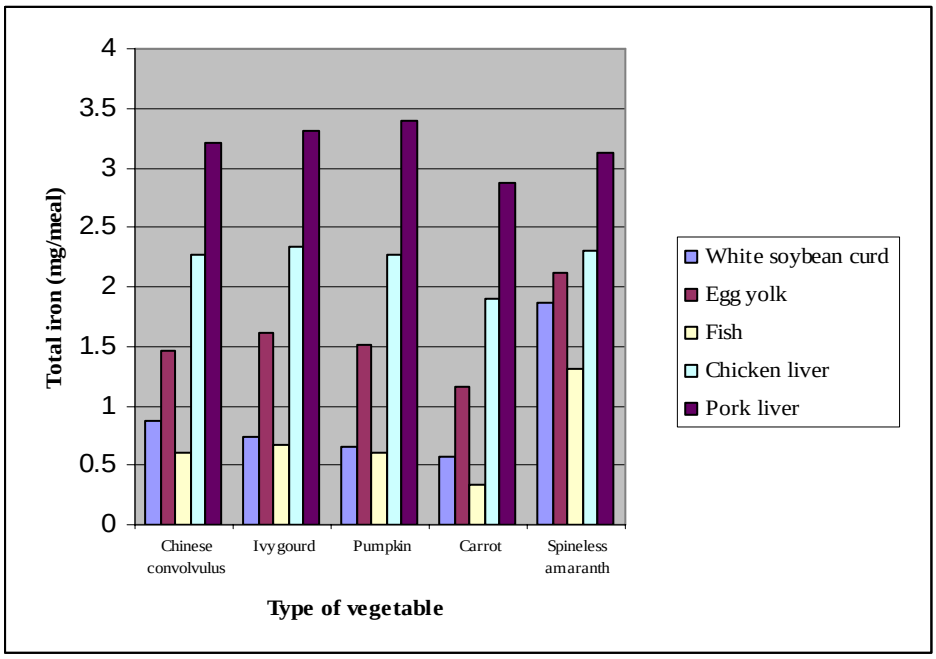


Figure 4.1 Distribution of total iron contents by group of vegetable

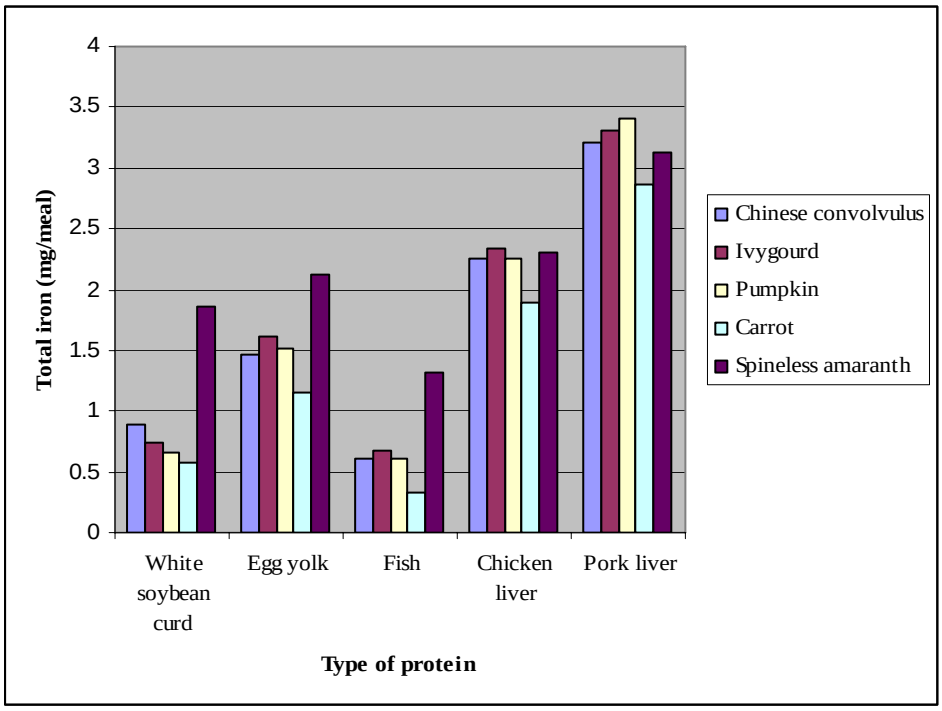


Figure 4.2 Distribution of total iron contents by group of protein

Table 4.3 Total energy, phytate phosphorus and total iron contents in homemade weaning foods (71 g sample of fruit blend).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
26.	Banana	98.7	1.006	2.4
27.	Papaya	36.2	0.854	-
28.	Mango	52.5	0.985	-
29.	Tomato	15.6	1.022	2.4
30.	Apple	21.3	0.234	2.4
Mean		44.9	0.820	1.4
SD		33.3	0.33	1.31

(b) Fruit blend

Fruit blend contributed small amount of total energy, ranged from 15.62 to 98.69 kcal (44.9 ± 33.32). The phytate contents in different fruits varied from 0 to 2.4 mg/meal and it was undetected in papaya and mango. The contents of native iron in these fruit blend ranged from the lowest 0.234 mg/meal in apple blend to the highest 1.022 mg/meal in tomato blend.

Table 4.4 Phytate phosphorus and total iron contents in homemade weaning foods (150 g sample of fruit juice).

No.	Sample	Total iron (mg/meal)	Phytate (mg/meal)
31.	Orange juice	0.887	-
32.	Apple juice	0.478	-
33.	Water melon juice	0.374	2.4
34.	Tomato juice	0.578	2.4
Mean		0.579	1.20
SD		0.22	1.39

(c) Fruit juice

The phytate contents in different fruit juice varied from 0 to 2.4 mg/meal and it was undetected in orange juice and apple juice. The contents of native iron in these fruit juice ranged from the lowest 0.374 mg/meal in water melon juice to the highest 0.887 mg/meal in orange juice. Total energies for fruit juice are not available from food tables.

4.1.2 The commercial infant foods.

Energy, Phytate phosphorus and total iron contents of four formulated weaning foods are shown, wheat and vegetable-protein blend in Table 4.5, rice-based with vegetable or protein mixed in Table 4.6, the first formula foods (fruit blend) in Table 4.7 and fruit juice in Table 4.8.

Table 4.5 Total energy, phytate phosphorus and total iron contents in commercial infant foods (40 g sample of wheat and vegetable-protein blend).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
35.	Wheat and Milk	164.4	4.959	14.8
36.	Wheat and Soya Chicken 6%	167.6	4.811	22.4
37.	Wheat and Mixed Vegetables 4.5%	163.2	4.667	27.6
38.	Wheat and Mixed Fruits 4%	164.0	5.108	19.8
Mean		164.8	4.886	21.2
SD		1.9	0.19	5.33

(a) Wheat and vegetable-protein blend

Total energies of wheat meals ranged from 163.2 to167.6 kcal (164.8 ± 1.9). The phytate contents in different wheat meals varied from 14.8 to 27.6 mg/meal. The contents of native iron in these wheat and vegetable-protein blend ranged from the lowest 4.667 mg/meal in wheat and mixed vegetables 4.5% to the highest 5.108 mg/meal in wheat and mixed fruits 4%.

Table 4.6 Total energy, phytate phosphorus and total iron contents in commercial infant foods (40 g sample of rice-based with vegetable or protein mixed).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
39.	Rice and Vitamins	77.0	3.720	2.4
40.	Rice and Banana	162.8	5.578	9.6
41.	Rice and Mixed Vegetables	161.6	5.263	7.2
42.	Rice and Fish 4%	161.2	4.667	7.2
43.	Rice and Chicken 1.8%	161.6	4.811	9.6
44.	Rice and Mixed Fruits	162.8	5.263	9.6
Mean		147.8	4.883	7.6
SD		34.7	0.66	2.80

(b) Rice-based with vegetable or protein mixed

Food energy of rice-based with vegetable or protein mixed ranged from 77.0 to 162.8 kcal (147.8± 34.7). The phytate contents in different meals varied from 2.4 to 9.6 mg/meal. The contents of native iron in these rice-based with vegetable or protein mixed ranged from the lowest 3.720 mg/meal in rice and vitamins to the highest 5.578 mg/meal in rice and banana (Meal number 40).

Table 4.7 Total energy, phytate phosphorus and total iron contents in commercial infant foods (71 g sample of the first formula foods (fruit blend)).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
45.	Bananas	68.5	9.600	-
46.	Carrots	20.3	9.600	-
47.	Applesauce	36.3	2.400	-
48.	Prunes	70.0	17.200	-
Mean		48.8	9.700	-
SD		24.54	6.04	-

(c) The first formula foods (fruit blend)

The first formula foods (fruit blend) contributed small amount of total energy,

ranged from 20.3 to 70.0 kcal (48.8 ± 24.54). The phytate contents in different fruits were undetected. The contents of native iron in these the first formula foods ranged from the lowest 2.400 mg/meal in applesauce to the highest 17.200 mg/meal in prunes**.**

Table 4.8 Total energy, phytate phosphorus and total iron contents in commercial infant foods (150 g sample of fruit juice).

No.	Sample	Energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
49.	Apple juice	67.97	0.124	-
50.	Apple Prune juice	77.27	0.806	-
51.	Mixed Fruits juice	70.78	0.970	-
Mean		72.0	0.633	-
SD		4.77	0.45	-

(d) Fruit juice

Fruit juice contributed small amount of total energy, ranged from 67.97 to 77.27 kcal (72.0± 4.77). The phytate contents in different fruits were undetected. The contents of native iron in these fruit juice ranged from the lowest 0.124 mg/meal in apple juice to the highest 0.970mg/meal in mixed fruits juice**.**

4.1.3 Test meal characteristics, summarized.

Descriptive characteristics of homemade weaning foods and industrially produced infant formulas are shown in Table 4.9. Total iron contents in commercial infant formulas were significantly higher than the homemade formulas, 5.27 ± 4.0 vs 1.52 ± 0.94 mg/meal ($p<0.001$).

Table 4.9 Total energy, phytate phosphorus and total iron contents in homemade weaning foods and commercial infant formulas.

Food supplements	n	Total energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
Homemade				
- Rice-based meals with vegetable and protein	25	76.8 ± 8.13	1.727 ± 0.97	5.2 ± 0.5
- Fruit blend	5	44.9± 33.3	0.820 ± 0.33	1.4 ± 0.6
- Fruit juice	4	0	0.579 ± 0.22	1.2 ± 0.7
Mean±SD		40.6± 13.8	1.520 ± 0.94	4.2 ± 2.7
Food supplements	n	Total energy (kcal)	Total iron (mg/meal)	Phytate (mg/meal)
Commercial infant formulas				
- Wheat and vegetable-protein blend	4	164.8 ± 1.9	4.886 ± 0.19	21.2 ± 2.7
- Rice-based with vegetable or protein mixes	6	147.8± 34.7	4.884 ± 0.66	7.6 ± 1.1
- Fruit blend	4	48.8 ± 24.54	9.700 ± 6.04	0
- Fruit juice	3	72.0± 4.77	0.633 ± 0.45	0
Mean±SD		108.3 ± 16.48	5.27 ± 4.0	7.7 ± 8.9

The phytate contents in commercial infant formulas were also significantly higher than the homemade formulas, 7.7 ± 2.2 vs 4.2 ± 0.5 mg/meal ($p < 0.001$).

The commercial infant formulas contributed more energy than the home made formulas, 108.3 ± 16.48 vs 40.6± 13.8 ($p < 0.05$).

4.2 Ionizability of iron and the estimated iron availability

4.2.1 Homemade weaning foods

Ionizability of iron and the estimated iron availability of three formulated weaning foods are shown, rice based with vegetable and protein in Table 4.10, fruit blend in Table 4.11 and fruit juice in Table 4.12.

Table 4.10 The percentage of ionizable iron and estimated iron absorption in homemade weaning foods. (45 g sample of rice-based meals with vegetable and protein)

No.	Sample	ionizable iron(%II)	EIA (mg/meal)
1.	Rice and Chinese convolvulus, White soybean curd	4.63	0.040
2.	Rice and Chinese convolvulus, Egg yolk	5.13	0.074
3.	Rice and Chinese convolvulus, Fish	4.73	0.028
4.	Rice and Chinese convolvulus, Chicken liver	5.03	0.113
5.	Rice and Chinese convolvulus, Pork liver	5.67	0.183
6.	Rice and Ivygourd, White soybean curd	24.87	0.186
7.	Rice and Ivygourd, Egg yolk	19.93	0.321
8.	Rice and Ivygourd, Fish	32.27	0.218
9.	Rice and Ivygourd, Chicken liver	24.40	0.569
10.	Rice and Ivygourd, Pork liver	23.10	0.764
11.	Rice and Pumpkin, White soybean curd	4.13	0.027
12.	Rice and Pumpkin, Egg yolk	7.50	0.114
13.	Rice and Pumpkin, Fish	19.57	0.119
14.	Rice and Pumpkin, Chicken liver	21.33	0.482
15.	Rice and Pumpkin, Pork liver	22.93	0.780
16.	Rice and Carrot, White soybean curd	4.57	0.027
17.	Rice and Carrot, Egg yolk	6.90	0.080
18.	Rice and Carrot, Fish	21.00	0.069
19.	Rice and Carrot, Chicken liver	20.20	0.382
20.	Rice and Carrot, Pork liver	23.30	0.669
21.	Rice and Spineless amaranth, White soybean curd	5.20	0.097
22.	Rice and Spineless amaranth, Egg yolk	5.60	0.119
23.	Rice and Spineless amaranth, Fish	9.90	0.130
24.	Rice and Spineless amaranth, Chicken liver	14.50	0.344
25.	Rice and Spineless amaranth, Pork liver	12.70	0.396
Mean		13.96	0.255
SD		8.81	0.24

(a) Rice-based meals with vegetable and protein

Twenty five meals were analyzed, as shown in Table 4.10, ionizability of iron of all meals ranged from 4.13 to 32.27 %. The highest ionizability was found when ivygourd was added to the meal (Meal no. 8). The mean ±SD among all meals was 13.96±8.814 %.

Estimated iron absorption of all 25 meals ranged from 0.027 to 0.780 mg/meal (0.255 ± 0.239). The highest EIA was found in meals containing pork liver 0.183, 0.764, 0.780, 0.669 and 0.396 mg/meal in meal number 5, 10, 15, 20 and 25, respectively. EIA from meals with fish and white soybean curd was 0.028 to 0.218 mg/meal and 0.027 to 0.186 mg/meal, respectively.

Table 4.11 The percentage of ionizable iron and estimated iron absorption in homemade weaning foods (71 g sample of fruit blend).

No.	Sample	ionizable iron(%II)	EIA (mg/meal)
26.	Banana	1.10	0.011
27.	Papaya	55.70	0.476
28.	Mango	0.70	0.006
29.	Tomato	20.50	0.210
30.	Apple	1.60	0.037
Mean		15.92	0.148
SD		23.77	0.20

(b) Fruit blend

Ionizable iron from fruit juice ranged from 0.70 to 55.70 %. (15.92±23.77). The EIA in different fruits varied from 0.006 to 0.476 mg/meal. The EIA in these fruit blend ranged from the lowest 0.006 mg/meal in mango to the highest 0.476 mg/meal in papaya.

Table 4.12 The percentage of ionizability iron and estimated iron absorption in homemade weaning foods (150 g sample of fruit juice).

No.	Sample	ionizable iron(%II)	EIA (mg/meal)
31.	Orange juice	56.20	0.498
32.	Apple juice	5.60	0.027
33.	Water melon juice	25.20	0.094
34.	Tomato juice	19.20	0.111
Mean		26.55	0.183
SD		21.40	0.21

(b) Fruit juice

Fruit juice was ionizability iron of the meals ranged from 5.60 to 56.20 % (26.55±21.40). The EIA in different fruits varied from 0.027 to 0.498 mg/meal. The EIA in these fruit juice ranged from the lowest 0.027 mg/meal in apple juice to the highest 0.498 mg/meal in orange juice.

4.2.2 Industrially produced infant formulas

Ionizability of iron and the estimated iron availability of four formulated weaning foods are shown, wheat and vegetable-protein blend in Table 4.13, rice-based with vegetable or protein mixed in Table 4.14, the first formula foods (fruit blend) in Table 4.15 and fruit juice in Table 4.16

Table 4.13 The percentage of ionizability iron and estimated iron absorption in commercial infant foods (40 g sample of wheat and vegetable-protein blend).

No.	Sample	ionizable iron (%II)	EIA (mg/meal)
35.	Wheat and Milk	10.20	0.506
36.	Wheat and Soya Chicken 6%	9.70	0.467
37.	Wheat and Mixed Vegetables 4.5%	8.50	0.397
38.	Wheat and Mixed Fruits 4%	9.60	0.490
Mean		9.50	0.465
SD		0.72	0.05

(a) Wheat and vegetable-protein blend

Ionizability of iron from wheat based meals was found to be in the same magnitude ranging from 8.50 to 10.20 %. (9.50 ± 0.72). The EIA in wheat based meals varied from 0.397 to 0.506 mg/meal. The EIA values were also with in the same magnitude ranging from the lowest 0.397mg/meal in wheat and mixed vegetables to the highest 0.506 mg/meal in wheat and milk.(0.465 ± 0.05)

Table 4.14 The percentage of ionizability iron and estimated iron absorption in commercial infant foods (40 g sample of rice-based with vegetable or protein mixed).

No.	Sample	ionizable iron (%II)	EIA (mg/meal)
39.	Rice and Vitamins	23.50	0.874
40.	Rice and Banana	8.50	0.474
41.	Rice and Mixed Vegetables	28.60	1.505
42.	Rice and Fish 4%	17.20	0.803
43.	Rice and Chicken 1.8%	14.80	0.712
44.	Rice and Mixed Fruits	13.50	0.711
Mean		17.68	0.847
SD		7.26	0.35

(b) Rice-based with vegetable or protein mixed.

The percentage of ionizable iron in the commercial rice-based meals ranged from 8.50 to 28.60 %. (17.68 ± 7.26). The EIA in different rice-based meals varied from 0.474 to 1.505 mg/meal. The lowest 0.474 mg/meal was found in rice and banana and the highest 1.505 mg/meal in rice and mixed vegetables.

Table 4.15 The percentage of ionizability iron and estimated iron absorption in commercial infant foods (71 g sample of the first formula foods (fruit blend)).

No.	Sample	ionizable iron (%II)	EIA (mg/meal)
45.	Bananas	1.10	0.106
46.	Carrots	20.20	1.939
47.	Applesauce	2.70	0.065
48.	Prunes	2.00	0.344
Mean		6.50	0.613
SD		9.16	0.89

(c) The first formula foods (fruit blend)

Ionizability of iron from these meals ranged from 1.10 to 20.20 %. (6.50 ± 9.16). The EIA varied from 0.065 to 1.939 mg/meal.

Table 4.16 The percentage of ionizable iron and estimated iron absorption in commercial infant foods (150 g sample of fruit juice)

No.	Sample	ionizable iron (%II)	EIA (mg/meal)
49.	Apple juice	3.80	0.005
50.	Apple Prune juice	2.70	0.022
51.	Mixed Fruits juice	5.60	0.054
Mean		4.03	0.034
SD		1.46	0.037

(b) Fruit juice

Ionizability of iron from fruit juice was very low ranged from 2.70 to 5.60 %. (4.03 ± 1.46). No significant amount of EIA was contributed fruit juice.

4.2 Compare to estimation of iron absorbed

Descriptive characteristics of homemade weaning foods and commercial infant formulas are shown in Table 4.17. Comparison of EIA from 2 groups of weaning foods between homemade and commercial infant formulas($p < 0.05$).

The total iron contents in commercial infant formulas were also significantly higher than the homemade formulas, 5.267 ± 4.01 vs 1.521 ± 0.94 mg/meal ($p < 0.05$).

The total iron contents in commercial infant formulas (rice-based with vegetable or protein mixed) were also significantly higher than the homemade formulas(rice-based meals with vegetable and protein), 4.884 ± 0.66 vs 1.727 ± 0.97 mg/meal ($p < 0.001$).

The total iron contents in commercial infant formulas (fruit blend) were also significantly higher than the homemade formulas (fruit blend), 9.700 ± 6.04 vs 0.820 ± 0.33 mg/meal ($p < 0.001$).

The commercial infant formulas contributed more EIA than the home made formulas, 0.559 ± 0.13 vs 0.230 ± 0.04 mg/meal ($p < 0.05$).

The commercial infant formulas (rice-based with vegetable or protein mixed) contributed more EIA than the home made formulas (rice-based meals with vegetable and protein), 0.867 ± 0.350 vs 0.255 ± 0.239 mg/meal ($p < 0.001$).

The commercial infant formulas (fruit blend) contributed more EIA than the home made formulas(fruit blend) , 0.613 ± 0.892 vs 0.148 ± 0.202 mg/meal($p < 0.05$).

Table 4.17 Total iron contents, ionizable iron (%II) and EIA in homemade weaning foods and Commercial infant formulas.

Food supplements	**n**	**Total iron (mg/meal)**	**ionizable iron (%II)**	**EIA (mg/meal)**
Homemade				
- Rice-based meals with vegetable and protein	25	1.727 ± 0.97	13.96 ± 8.81	0.255 ± 0.239
- Fruit blend	5	0.820 ± 0.33	15.92 ± 23.77	0.148 ± 0.202
- Fruit juice	4	0.579 ± 0.22	26.55 ± 21.40	0.183 ± 0.213
Mean±SD		1.520 ± 0.94	15.73 ± 13.53	0.230 ± 0.229
Commercial infant formulas				
- Wheat and vegetable-protein blend	4	4.886 ± 0.19	9.5 ± 0.72	0.465 ± 0.048
- Rice-based with vegetable or protein mixes	6	4.884 ± 0.66	17.68 ± 7.26	0.867 ± 0.350
- Fruit blend	4	9.700 ± 6.04	6.50 ± 9.16	0.613 ± 0.892
- Fruit juice	3	0.633 ± 0.45	4.03 ± 1.46	0.034 ± 0.037
Mean±SD		5.267 ± 4.01	10.71 ± 8.00	0.559 ± 0.523

CHAPTER V
DISCUSSION

Iron deficiency anemia is the most common nutritional disorder in infancy. It affects communities not only in developing countries but also in highly industrialized countries [2]. Studies of prevalence of iron deficiency anemia (IDA) in Thai infants are limited. Tantracheewathorn and Lohajaroensub [58] reported 25.7% IDA in breast-fed infants and 2.9% in iron-fortified formula-fed infants while Chuansumrit et al.[59] reported only 1.4% of IDA in rather high income families.

Infants are at risk for iron deficiency as breast milk or iron-fortified formula which is replaced by semisolid foods during weaning. Because of the greater physiological requirements within the first two years of life, especially from 6 to 12 months, it is rare that the child will manage to ingest the recommended daily amount of iron as tabulated in Table 5.1. Iron nutrition during weaning needs much greater attention.

The average daily iron needs are 70 mg/kg for full-term infants. The total absolute requirements for growth and basal losses are 0.72 mg and 0.46 mg (Table 5.1) for children from five months to one year old and from one to three years old, respectively [2]. Human milk has high iron bioavailability but it has low content of iron (0.26 to 0.73 mg/ml) in mother's milk [60, 61], so breastfeeding alone can not provide enough iron for the infant enter the weaning period. After 6 months of age, breast-fed infants should be received the extra iron in the form of iron-fortified infant cereals or other iron-rich foods [46].

Total iron contents

Varieties of homemade and commercial infant foods were selected for the study. It was expected that the formulated homemade and the commercial available infant meals contained some considerable amount of ascorbic acid that could enhance non-heme iron availability and contained low iron absorption inhibitors such as phytate and tannin. Analysis of food samples have been shown in Table.4.1 to 4.9. Homemade foods were rice mixed with vegetable and protein both of animal and soy protein. The commercial foods were both of wheat and rice based with vegetable, fruits, milk products or protein. Other homemade preparations, fruit mixed and fruit juice which contained low iron contents (0.82?0.33 and 0.579?0.22 mg/meal of 50 g, respectively). They were used as supplement foods and a source of ascorbic acid rather than a source of iron. The commercial fruit blend or fruit mixed was iron-fortified, the iron content was as high as 9.7?6.04 mg/meal of 50 g (Table 4.7). These amounts meet the iron requirements recommended for infants.

Table 5.1 The requirement of iron for infants and children (FAO/WHO, 2001) (2)

Age (year)		Total absolute requirements [c] (median) (mg/day)	Recommended iron intakes to cover requirement of 97.5% of population at the level of 10% iron bioavailability (mg/day)	
			Iron intake	Iron requirement
Infants	0 – 5 M [a]	Breast milk	Breast milk	Breast milk
	6 – 11 M	0.72	9.3	0.93
Children	1 – 3 Y [b]	0.46	5.8	0.58
	4 – 6 Y	0.50	6.3	0.63
	7 – 10 Y	0.71	8.9	0.89

[a] Newborn to age before 6 months.
[b] One year to age before four years.
[c] Total absolute requirements include requirement for growth and basal losses

Homemade infant foods: The iron content in the homemade meals was varied by adding some vegetables and the protein. Of 25 rice based meals, the mean iron content was 1.73 ± 0.0.97mg/meal (Table 4.9). When categorized by type of proteins,

the iron contents were different significantly (P<0.001). Fish and white soybean curd were not found to be the good sources of iron. The content in meals with white soybean (0.95 ± 0.52mg/meal) or fish (0.71 ± 0.37mg/meal) was approximately 29.7 and 22.2 % of meals with egg yolk, chicken liver or pork liver (Table 4.2). Liver is an excellent source of iron because the meals with chicken liver (2.22 ± 0.20mg/meal)and pork liver (3.18 ± 0.20mg/meal) contributed high iron intake. Liver is also a source of vitamin A, eat too much liver will get too much vitamin A. Egg yolk contains both heme and non heme iron (1.57 ± 0.35mg/meal). With a soft texture, it is suitable for weaning infants but it has been shown that iron in egg yolk is poorly absorbed [31]. When categorized by type of vegetables, no significant different in the content of iron was found (P=0.82).

Commercial infant foods: Iron contents in commercial foods were in the high magnitude and significantly higher than the homemade foods. The 95% confidence interval for mean was from 3.21 to 7.33 mg/50 g meal. Commercial wheat and rice based meal contained almost the same content of iron (4.89 ± 0.19 and 4.88 ± 0.66mg/mealm, respectively). Commercial rice-based meal was found to contain iron 2.8 times higher than the identical homemade meals (4.88 vs 1.73mg/meal, P<0.001). Industrially produced infant cereals provide an excellent vehicle for targeted food fortification of infants in societies in which the cost of these foods does not limit their use. The fortification levels of the commercial products are approximately 10 mg Fe/100 g dry product.

Phytate contents

Most weaning diets are mainly based on cereals. The high content of iron absorption inhibitors such as phytate in the cereals, in the long run, will provide low amounts of bioavailable iron and significantly contributed to poor iron nutrition unless enhancing factors are added to the meal. Phytate contents in homemade meals (4.2 ±0.47 mg/meal) were significantly lower than those of the commercial meals (7.55 ±2.16 mg/meal). The highest phytate contents were found in wheat meals (21.2±5.33 mg/meal).

Ionizability of iron and the estimated iron availability (EIA) from the infant foods

The *in vitro* method for determination of food iron availability was first described by Miller et al. [62]. The original method was modified in an attempt to improve this methodology, that was based on an adaptation to the gastrointestinal digestion of food with pepsin hydrochloric acid and the other digestive enzyme, pancreatin, followed by determination of ionizable iron at the intestinal pH of 6.8. The overall results of *in vitro* measurements of the whole series of infant foods showed a wide degree of variability between homemade and commercial foods. The 95% confidence intervals were 11.00 to 20.25% and 6.6 to 14.83% for homemade and commercial meals, respectively. The overall percentage of ionizability for iron in commercial infant foods (10.7 ±8.0 %) was lower than the homemade foods (15.73 ±13.53%) but it is statistically not significant .

Iron available from iron fortification compounds depends not only on the characteristics of the compounds but also on the overall composition of the diet. The possibility for the low availability could be from the presence of considerable amounts of phytate and the fortification iron added. Infant foods are commonly fortified with soluble iron compounds with low relatively availability, such as ferric pyrophosphate. In vivo studies using stable isotope (^{57}Fe pyrophosphate) showed that iron bioavailability was significantly higher from ferrous fumerate (1.7-14%) than from ferric pyrophosphate (0.7-2.7%) [63].

In the present study, the percentage of ionizable iron in the commercial cereals was not greatly inhibited by the content of phytate in the meals. This could result from the ability of the addition of ascorbic acid to overcome the inhibitory effect of phytate. The commercial wheat-based meals were found to contain moderate amounts of phytate, 21.2 ±5.33 mg/meal and ample amount of ascorbic acid, which is a potent enhancer of iron absorption in infants [64, 65].

Liyanage et al (66) showed the mean iron absorption of 2.2% from traditional Sri Lankan weaning foods (Centella gruel) and the result was increased to 5% after adding of 50 mg ascorbic acid.

The low iron availability was also reported by Bosscher et al. [67]. They developed a continuous-flow dialysis method for in vitro determination of iron availability. The availability of iron from infant foods was higher at gastric pH of 2.0 (4.06?0.66%) and 3.01?0.58% at low intestinal pH of 5.5. However, the reproducibility of the method was not acceptable and was explained to be due to the very low availability of iron from infant formula.

Gahlawat and Sehal [68] used the improved HCl-extractaility method to determine the bioavailability of iron from homemade weaning foods formulated from cereals and pulses. The extractable iron was found to range from 14 to 17%. These were in agreement to the percentage of ionizable iron in our homemade weaning foods formulated from rice, vegetable and protein (10.32 to 17.6%) and the commercial formulas prepared from rice and mixed vegetables, fruits, vitamins or protein (10.1 to 25.3%). The percentage of ionizable iron in commercial wheat-based formulas was slightly low (8.36 to 10.6%). No significant difference was found.

Bosscher et al. [69] determined iron availability from weaning food containing a variety of ingredient by in vitro continuous flow dialysis method. Iron availability from vegetables was found to be marginally higher (13.0 ±4.7%) than fruit mixed (10.2 ±1.6%). In this study, the results revealed that fruits mixed had a wide range of iron availability, and the mean±SE was 15.9±10.6% and 6.5±4.6% for homemade and commercial fruit mixed, respectively.

Homemade and commercial fruit juice had similar low contents of iron (0.579±0.22 mg/meal vs 0.633±0.45 mg/meal) but the percentage of ionizable iron in homemade fruit juice was significantly higher than the commercial products (26.55±21.4% vs 4.0±1.5%, $P<0.05$). This could be from the freshly-prepared homemade juice.

Domestic or homemade processing was found to affect the percentage of iron availability in weaing foods, Gahlawat and Sehgal [70] reported that 16 to 32 % increased in iron availability was observed on processing and the effect was more remarkable in malted weaning foods as compared to roasted ones. In this study we did not design to demonstrate these effects.

The in vitro method in this study was found to be simple, rapid and inexpensive for measuring food iron availability. Moreover its precision is well accepted and suitable for predicting the availability for iron from infant foods.

The availability of iron from meals with soy protein added (0.075?0.068 mg of Fe /meal) appeared to be significantly lower than those of added with animal protein, such as chicken liver (0.378?0.17 mg of Fe /meal) and pork liver (0.565?0.27 mg of Fe/meal). This result was in agreement to the in vivo study by Morck el al. [71]. They reported that the iron added to infant food supplement could not fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron which was from the presence of soy protein. However, it is not adequately justified to eliminate soy protein from infant foods because of the poor availability. Low iron availability from soy-based infant foods could be improved by ensuring adequate ascorbic acid content in the meals.

Among the 25 homemade-rice-based meals, categorized by type of vegetables used in the preparations showed that ***Chinese convolvulus*** *(a variety of swamp cabbage)* provided the lowest EIA, 0.086?0.061 mg of Fe/meal. It is possible that factor account for the low EIA was vegetable tannin [72, 73]. Meals with ***Ivygourd*** contributed the highest EIA, 0.412?0.0.245 mg of Fe/meal because of the high iron content in Ivygourd [27].

Many studies have shown that an increased uptake of meat or organ meat is associated with a better iron nutrition [32, 36, 37]. The role of meat to improve the critical iron balance during weaning had been reported by Hallberg et al. [74]. The authors concluded that addition of powder red meat to weaning gruels had markedly increased total iron absorption. And, the total daily iron requirement of weaning infants at 12 months (1mg/day) would be well covered by the combined addition of meat and ascorbic acid.

The requirement of iron for infants and children (FAO/WHO, 2002) has been shown in Table 5.1, and the calculation of estimated iron available from homemade infant foods (rice-based with varieties of vegetables and proteins) is given in Table 5.2. The results of the present study indicated that some selected homemade infant foods could fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron when combined rice, iron-rich vegetable and animal protein in the same meal (Table 5.3).

Fruit juice or fruit-mixed was used to provide needs for vitamin C without increasing substantial amount of caloric intake.

Table 5.2 The calculation of expected iron available from homemade formulas (rice-based with varieties of vegetables and proteins).

Vegetable	Protein				
	Soy bean	Egg	Fish	Chicken liver	Pork liver
Chinese convolvulus	0.040	0.074	0.028	0.113	0.183
Ivygourd	0.186	0.321	0.218	0.569	0.764
Pumpkin	0.027	0.114	0.119	0.482	0.780
Carrot	0.027	0.080	0.069	0.382	0.669
Amaranth	0.097	0.119	0.130	0.344	0.396

Table 5.3 Iron intake, ionizable iron and expected iron availability from rice based meals with pork liver and vegetables.

	Vegetable	Iron intake (mg/meal)	Ionizable iron (%)	EIA (mg/meal)
Pork liver	Chinese convolvulus	3.216	5.67	0.183
	Ivygourd	3.308	23.10	0.764
	Pumpkin	3.400	22.93	0.780
	Carrot	2.872	23.30	0.669
	Amaranth	3.122	12.70	0.396

The commercial products are good at meeting the nutritional and development needs for infants, but they are very high price compared to similar homemade foods. Nutritious and economical foods could be simple prepared from locally available food materials.

In conclusion, effort should be made to improve iron nutrition for the children to ensure that they will grow into healthy adults; breastfeeding should be continued for 4-6 months of age, use of iron-fortified formulas in infants who are not breast-fed, and consumption of iron-rich complementary food after 6 months. For children over 1 year of age, the recommended daily nutrient intake of iron should be given. Iron containing foods such as meats, some vegetables, legumes and cereals provide iron in sufficient amount. Supplement iron would not be required unless the diet is lacking in these foods.

CHAPTER VI
CONCLUSION

Iron availability between homemade and commercial infant foods was compared. The results of the study revealed that the commercial weaning mixtures had significantly higher iron contents (5.26±4.0mg/meal) than those of the weaning foods locally formulated (1.52±0.94mg/meal). The difference was approximately 3.5 times ($p<0.001$). This is because most of the commercial infant foods are iron-fortified.

The mean percentage of ionizable iron observed in the commercial groups was lower than the homemade groups (10.7% vs 15.7%). This could probably due to the slightly higher phytate content (7.7 vs 4.2 mg/meal,$p<0.05$) and the iron fortified compounds used by most producers have relatively low bioavailability. However, because of the high iron intake per serving of the commercial groups, the mean estimated iron availability (EIA, 0.559 mg/meal) was found to be 2.5 times of the mean EIA (0.23 mg/meal) from the homemade groups. Based on the daily requirements of 0.7 to 1.0 mg absorbed iron per day plus a 0.3 mg from mother's milk for 6-12 month infant, EIA from homemade groups would not sufficient to cover the needs of growing infant.

In conclusion, the magnitude of iron available for absorption from cereal-based meals depends on the intake of iron and the contents of different components that enhance or inhibit iron uptake. The results of the present study show that intake of one serving of a homemade rice-based meal would provide a mean of 0.255±0.24 mg available Fe compared with 0.465±0.05 mg from commercial wheat-based meal and 0.847±0.35 mg from commercial rice-based meal, equivalent to 35%, 65% and 118% respectively, of the estimated daily requirement of absorbed iron in infants aged 5 to 12 months (0.72mg/d). These results show that, among the homemade foods with relatively low EIA, adequate ascorbic acid content and food containing iron should be included in the diet to provide adequate quantities of iron to rapidly growing children. Some vegetables, fruits and their juices which are good sources of vitamin C and

minerals should also be given as supplementary to the diet to improve iron nutrition during the weaning period.

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APPENDIX

APPENDIX A

Determination of ionizable iron

Principle

An inorganic radioiron tracer (^{59}Fe) is added to a homogenized food sample. After a two-stage (pepsin and pancreatin) digestion, the ionizable iron was determined by using bathophenanthroline solution. The bathophenanthroline reactive iron was extracted with isoamyl alcohol. The ^{59}Fe activity in isoamy alcohol extracted was calculated as the percentage of ionizable iron against total amount of radioiron added to homogenized food sample.

Procedure

1. Weight 20 gm of homogenized food sample into 125 ml Erlenmeyer flask.
2. Add 20 ml 0.17 N HCl, 5 ml 1% Pepsin and 500 μl ^{59}Fe.
3. Mix and incubate in a waterbath, 37^{o}c for 60 minutes.
4. After incubation, weigh out the digestion mixture 1 gm into counting test tube, it's activity represent the total amount of radioiron added to food sample. Another 4 gm was weighed into 15 ml test tube for the next incubation.
5. Add 3 ml acetate buffer and 1 ml 1% pancreatin in 0.1 M $NaHCO_3$. Mix and then divide into 2 sets, one without adjusting pH and the other adjust pH to 6.8 by using NaOH (conc.).
6. Mix and incubate 37^{o}c for 2 hours.
7. After 2 hours add 1.0 ml chloroform and mix. The tube is then centrifuged 2000 rpm, at room temperature for 10 minutes.
8. Pipette 1 ml supernatant into counting test tube. This represents the half dilution counts. Another triplicate of 0.5 ml was pipetted into 6 ml screw cap vials.
9. Add 1 ml bathophenanthroline solution into screw cap vials. Mix and then shake for 90 minutes at room temperature.

10. Add 1 ml isoamyl alcohol after the shaken period, mix and centrifuge 2000 rpm at room temperature for 5 minutes.
11. The upper isoamyl alcohol layer is pipetted off 1 ml to counting test tube for measuring ^{59}Fe activity as the ionizable iron.
12. Radioactivity in the total, half dilution and ionizable iron tubes were measured in a LKB Wallac Gamma counter.
13. Calculate the percentage of ionizable iron by using the following equation:

$$\text{Ionizable iron } (^{59}Fe)\% = \frac{\text{cpm } ^{59}Fe/\text{ml isoamyl alcohol extract} \times 5.6^{*} \times 100}{\text{cpm } ^{59}Fe/\text{g of meal (Total counts)}}$$

*Sample dilution factor

Figure 1A. Weaning food samples were incubated in water bath, 37 ^{o}C for 60 minutes after added 20 ml of 0.17 HCl, 5 ml of 1 % Pepsin and 500µ of Fe-59.

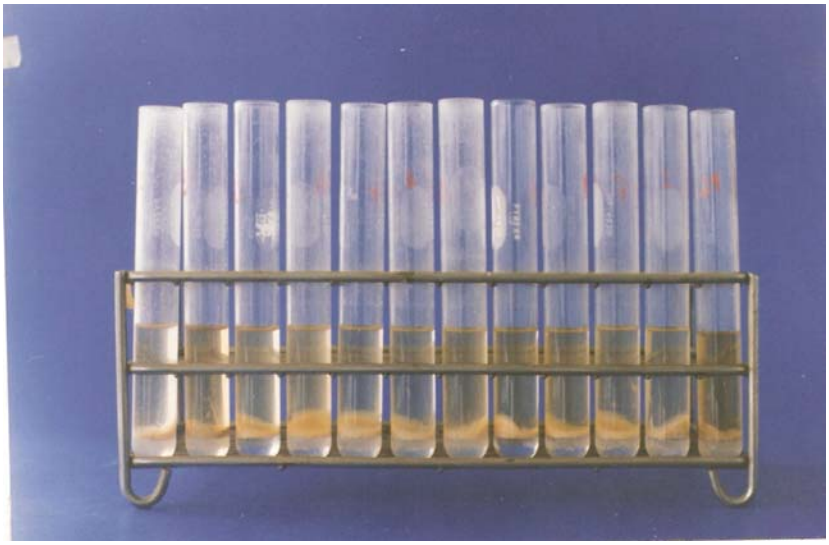

Figure 2A. After two-stage (Pepsin-Pancreatin) digestion of homogenized test meal, added chloroform will settle down the lipid from digestion mixture and separate soluble iron in the supematant.

Figure 3A. Triplicate of 0.5 ml supernatant of digestion mixture was pipetted into 6 ml screw cap vials.

Figure 4A. Bathophenenthroline was added to the supernatant of digestion mixture In a screw cup vial and then shake for 90 minutes by shaker at room temperature.

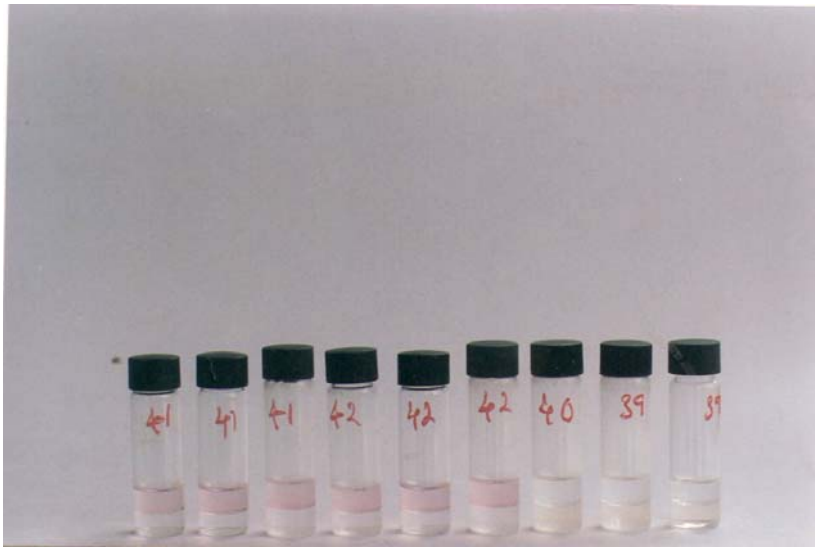

Figure 5A. The upper isoamyl alcohol was the extracted bathophenenthroline reactive iron from the digestion mixture

Figure 6A.Radioactive measurement was counted in the LKB Wallac Gamma counter.

APPENDIX B

Determination of total iron

Principle

The organic material in food sample was ashed by the mixture of acids. Iron content of the ash was estimated by developing color with bathophenanthroline sulphonate.

Procedure

1. Weight 10 gm of homogenized food sample into a 100 ml Kjeldahl flask and add 3 glass beads.
2. Add 2.5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid. Digest with low heat and gradually increase the heat until it become dark brown colour. Allow it to cool.
3. Repeat step 2 again until the solution become orange. Allow it to cool.
4. Add 2.5 ml concentrated nitric acid. Heat until all the orange colour changes to yellow.
5. Once the material has become light yellow, allow the sample to cool and add 2 ml of 30% hydrogen peroxide (H_2O_2). Heat until all the hydrogen peroxide fumes have evolved, and allow it to cool. Repeat this step if the sample is not water white.
6. Transfer the sample quantitively without glass beads to 50 ml volumetric Flask with three to four times washing of iron free water using approximately 5 ml for each wash.
7. Add 1 drop of 1 % paranitrophenol to the volumetric flask and being adding concentrated ammonium hydroxide slowly until the sample just turns yellow.

8. Allow the flask to cool and make up volume (50ml) with iron free water.
9. Include with each analysis an acid blank containing approximately the same volume of acid as was used for the digestion procedure. However, the total volume of acid in this blank should not exceed 15 ml. The acid blank should otherwise be handled in the same manner as the unknown sample.
10. Prepare solutions for colorimetric analysis in iron-free 10 ml glass tubes by placing into each tube 1 ml sodium acetate buffer, 5 ml of sample volume or iron standard with iron free water and 0.2 ml bathophenanthroline sulphonate.
11. The samples were allowed to stand for at least 15 minutes before the measurment of optical density at 535 nm.
12. The iron concentration of sample was calculated in μgFe/gm sample.

Sample volume for colorimetric analysis should be prepared as follows:-

Sample	Acetate Buffer (ml)	DI H_2O (ml)	Iron Std. (ml)	Digest Sample (ml)	Chromogen (ml)
Water blank	1.0	5.0			0.2
Acid blank	1.0			5.0	0.2
Standard Fe					
1 μg	1.0	4.0	1.0		0.2
2 μg	1.0	3.0	2.0		0.2
3 μg	1.0	2.0	3.0		0.2
4 μg	1.0	1.0	4.0		0.2
Unknown	1.0	4.0		1.0	0.2

Figure 1B.Homogenized food sample in Kjeldahl flask.

Figure 2B. Add 2.5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid in to the Kjeldahl flask.

Figure 3B. Digest with low heat and gradually increase the heat unitil it becomes dark brown colour.

Figure 4B. Add 2 ml of 30 % hydrogen peroxide (H_2O_2) into Kjeldahl flask .

Figure 5B. After adding 1 drop of 1% paranitrophenol, slowly adding concentrated ammonium hydroxide until the sample turns yellow.

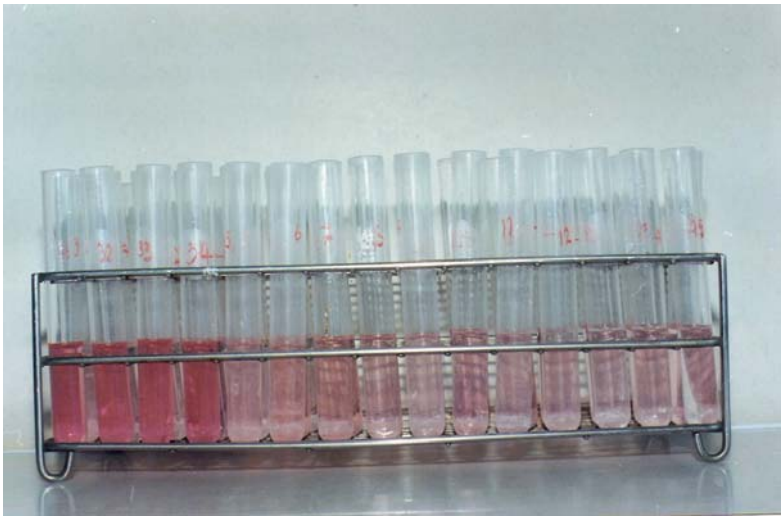

Figure 6B. After adding the chromogen solution it becomes red colour which is directly proportional to the amount of iron in the food sample.

Figure 7B. The optical density was read in spectrophotometer at 535 nm.

APPENDIX C

Determination of total phosphorus and phytate

Principle

Phytate is extracted from food samples using 2.4% HCl. The extracted is mixed with an EDTA/NaOH solution and placed on an ion-exchange column. The phytate is eluted from the column with 0.7 M NaCl and wet digested with a mixture of concentrated nitric/sulfuric acid to release the phosphorus which is then measured colorimetrically.The amount of phytate present in the organic sample is then calculated from the hexaphosphate equivalent by assuming that one molecule of phytic acid contained six molecule of phosphorus.

Procedure

1. Weight caefully 8 gm of homogenized food sample into a 125 ml Erlenmeyer flask.
2. Add 50 ml 2.4% HCl.
3. Cover flask and shake vigorously at room temperature for 3 hours.
4. While sample is being shaken to extract the phytate, prepare the column. Use glass barrell columns (0.7x15 cm) equipped with a valve.
5. Add 3 ml iron free water to empty, clean, mounted column and then pour a water slurry of 0.5 g resin (anion exchange resin, AGI –X4, 100-200 mesh, chloride form Bio-Rad. Cat No.140-1341) into the column.
6. After the resin bed has formed, wash column with three, 15 ml volumes of 0.7 M NaC1.

7. Then wash column with three, 15 ml volumes of iron free water.
8. Remove sample from shaker and filter through Whatman filter paper(No.541). Sample extract is stable for at least one week, after preparation, if refrigerated.
9. Pipette 4.0 ml of filtrate into a 25 ml volumetric flask. Add 4.0 ml Na_2EDTA-NaOH solution. Bring to 25 ml volume with iron free water.
10. Mix and pour quantitatively onto column; discard eluate.
11. Elute with 15 ml iron free water, discard eluate.
12. Elute with 15 ml of 0.1 M NaCl, discard eluate.
13. Elute with 15 ml of 0.7 M NaCl,collect this fraction in digest vessel.
14. Add 0.5 ml concentrated sulfuric acid, and 6.0 ml concentrated nitric acid to flask.

    Note: Before adding the next sample to the column , regenerate the resin by pouring 45 ml of 0.7 M NaCl and 45 ml of iron free water through the columns.
15. Digest under hood on micro-Kjeldahl rack over medium heat until active boiling cease, and a cloud of thick yellow vapor fills the neck of the flask.
16. Heat contents for 5 minutes more on medium heat, 5minutes on low heat, then turn off burner.
17. When the flask is cool,add 10 ml of iron free water,swirl to dissolve salt,or heat tube on low temperature setting if necessary in order to dissolve salt and continue heat flask on low temperature for 10 minutes.
18. Permit solution to cool.
19. Transfer solution quantitively to 100 ml volumetric flask.
20. Add 4.0 ml molybdate solution, mix well.
21. Add 2.0 ml sulfonic acid reagent, mix well.
22. Make to volume,mix well,let stand 15 min and read absorbance at 640 nm.

Preparation of phosphate standard curve

Reagent

1. Phosphate standard solution.
2. Molybdate solution.

3. Sulfonic acid solution.

Procedure

1. Pipette 0.5, 1.0, 1.5, 3.0, 5.0 and 10.0 ml of standard phosphate solution(80 μg P/ml) into each of three ,100 ml volumetric flasks. Add about 20 ml water. Mix throughly.
2. Add 4.0 ml molybdate solution, mix well.
3. Add 2.0 ml sulfonic acid reagent, mix well.
4. Bring to 100 ml volume with iron free water, mix well.
5. Equilibrate for at least 15 minutes.
6. Read in a spectrophotometer at 640 nm.

Standard solution, 80 μg phosphorus/ml.

ml. std.	μg/P	Absorbance(A)	Conc./A (K)
0.5	40	0.0908	440.53
1.0	80	0.1805	443.21
1.5	120	0.2711	442.64
3.0	240	0.516	465.12
5.0	400	0.852	469.48
10.0	800	1.490	536.91
		Mean K	466.48

Calculation of phytate concentration in food

1. Read Absorbance (A).
2. Multiply Absorbance (A) times “Mean K” to get ug phosphorus per sample or dilution.

   (K is derived by dividing concentration of phosphorus in standard by the absorbance obtained for that standard). It represents the calculated

concentration per unit absorbance for each concentration of standard. The "Mean K" obtained from the several standard is comparable to, but a more precise estimate of the mid point on the best straight line of a standard curve.

3. Multiply by 0.781 to obtain as phosphorus per gm of sample. (0.781 is dilution factor derived as follows: 50/(16x4) where 50 is ml of extraction acid, 16 is the weight of the sample in gm and 4 is ml of the extractable filtrate placed on the column).
4. Divide by 1000 to get mg of phosphorus gm of sample.
5. Divide by 0.282 to convert mg of phosphorus per gm of sample to mg of phytate per gm of sample. (The phytate molecule is 28.2% phosphorus).

Calculation

Mg phytate / g sample =Mean K x A x 0.781 / (0.282 x 1000)

Determination of total phosphorus

Total phosphorus in the extracted sample was determined by pipetting 4 ml of filtrate in step 8 into digestion vessel, add 11 ml of 0.7 M NaCl and then follow the procedure from step 14 to 22. Calculate total phosphorus concentration in the sample from following formula:

mg Phosphorus / g sample =Mean K x A x 0.781/1000

Figure 1C. Extract the food phytate with 2.4% HCl by shaking 3 hours at room temperature.

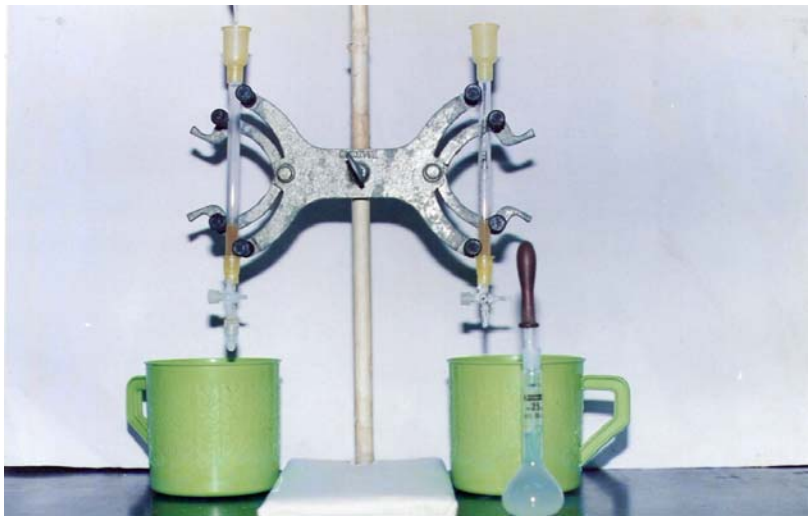

Figure 2C. An anion-exchange resin column using glass barrell (0.7x15 cm.) equipped with a valve.

Figure 3C. Transfer the final eluted from column to Kjeldah flask, added 0.5 ml concentrated sulfuric acid and 6.0 ml concentrated nitric acid to flask.

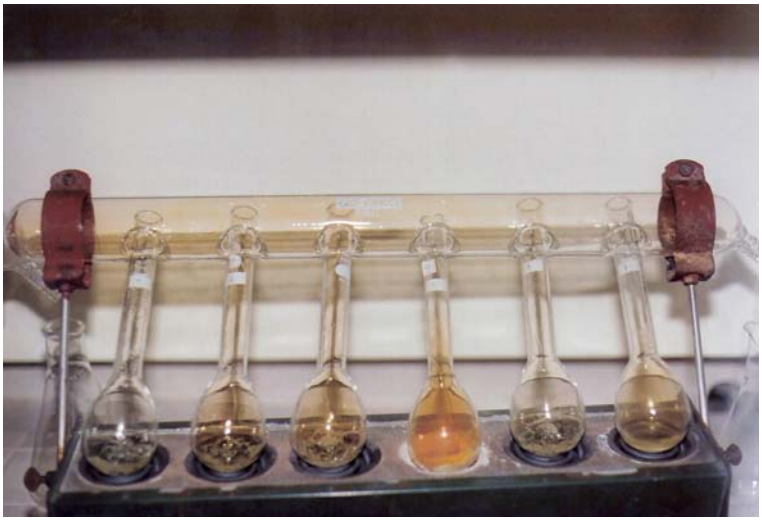

Figure 4C. Digest under hood on micro-Kjeldahl rack over medium heat until active boiling cease , and a cloud of thick yellow vapor fills the neck of the flask.

Figure 5C. After digestion the solution becomes dark brown colour.

Figure 6C. The blue colour was developed according to the amount of phosphate after adding molybdate-sulfonic acid solution to the digested solution which transfers to 100 ml volumetric flask.

Figure 7C. The optical density was read in spectrophotometer at 640 nm after standing for 15 minutes at room temperature.

APPENDIX D

NAME AND THE COMPONENTS OF THE TEST MEALS

Table 1D. Identification and description of ingredients in the commercial infant foods.

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
35	Wheat and milk	40	Wheat 48.483% Skimmed milk powder 26.82% Sugar11.6% Vegetable oil blend 8.1% Prebio 1 (Olegofructose and lnulin 2.5% Minerals 0.34% vitamin 0.15% Nature identical flavour added	Energy 164.4 kcal Fat 3.60 g Linoleaic acid 540 mg Linolenic acid 16 mg Protein 6 g Carbohydrate 27 g Fiber 1.7 g Minerals 1 g Vitamin A 440 IU Vitamin D 80 IU Vitamin E 1.2 IU Vitamin C 14 mg Vitamin B1 320 µg Vitamin B2 120 µg Niacin 1600 µg Vitamin B6 120 µg Folic acid 8.8 µg Pantothenic acid 600 µg Vitamin B12 0.3 µg Biotin 10 µg Torin 16 mg Sodium 60 mg Potassium 212 mg Calcium 81 mg Phosphorus 138 mg Iron 4 mg Iodine 140 µg

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
36	Wheat and soya chicken 6%	40	Wheat 31.45% Genetically modified soya 21.12% Rice Flour 15.57% Sugar 8.253% Chicken Meat 6% Maltodextrix 5.15% Palm Olein 3.75% Vitamins and Minerals 2.49% Skimmed milk powder 2% Onion 1% Carrot 0.5% Spinach 0.5% Lecitin 0.21% Nature identical flavour added	Energy 167.6 kcal Carbohydrate 24.9 g Protein 7 g Fat 4.40 g Linoleaic acid 1340 mg Fiber 2.1 g Minerals 1.2 g Vitamin A 600 IU Vitamin B1 320 µg Vitamin B2 120 µg Vitamin B6 120 µg Vitamin B12 0.3 µg Vitamin C 20 mg Vitamin D 76 IU Vitamin E 1.2 IU Niacin 1600 µg Folic acid 8.8 µg Pantothenic acid 600µg Biotin 10 µg Torin 16 mg Sodium 160 mg Potassium 187 mg Calcium 148 mg Phosphorus 151 mg Iron 4.2 mg Iodine 14 µg Zinc 2.8 mg
37	Wheat and mixed vegetables 4.5%	40	Rice 32.307% Genetically modified soya 21.695% Rice Flour 15.996% Sugar 8.479% Maltodextrix 6% Palm Olein 3.858% Spinach 2.5% Vitamins and Minerals 2.446% Skimmed milk powder 2% Pumkin 1% Carrot 1.0% Lecitin 0.212% Nature identical flavour added	Energy 163.2 kcal Carbohydrate 26.3 g Protein 6 g Fat 3.79 g Minerals 1.3 g Fiber 2.6 g Linoleaic acid 1260 mg Vitamin A 600 IU Vitamin B1 320 µg Vitamin B2 120 µg Vitamin B6 120 µg Vitamin B12 0.3 µg Vitamin C 20 mg Vitamin D 80 IU Vitamin E 1.2 IU Niacin 1600 µg Folic acid 8.8 µg Biotin 10 µg Pantothenic acid 600 µg Calcium 192 mg Phosphorus 128 mg Iron 3.9 mg Sodium 156 mg Potassium 264 mg Zinc 3.4 mg Iodine 24 µg Torin 16 mg

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
38	Wheat and mixed fruits 4%	40	Rice 30.68% Genetically modified soya 20.602% Sugar 16.252% Rice Flour 15.191% Skimmed milk powder 5.6% Palm Olein 3.664% Apple 2.7% Vitamins and Minerals 2.003% Banana 1% Lecitin 0.201% Orange 0.1% Nature identical flavour added	Energy 164 kcal Carbohydrate 26.9 g Protein 6 g Fat 3.60 g Minerals 1.14 g Fiber 2.56 g Linoleaic acid 1180 mg Vitamin A 540 IU Vitamin B1 320 µg Vitamin B2 120 µg Vitamin B6 120 µg Vitamin B12 0.3 µg Vitamin C 20 mg Vitamin D 80 IU Vitamin E 1.2 IU Niacin 1600 µg Folic acid 8.8 µg Biotin 10 µg Pantothenic acid 600 µg Calcium 196 mg Phosphorus 130 mg Iron 3.7 mg Sodium 86 mg Potassium 250 mg Zinc 3.24 mg Iodine 20.4 µg Torin 16 mg
39	Rice and vitamins	20	Rice Flour 71.37% Sugar 15% Rice 10.30% Minerals 1.07% Vitamins 0.25% Nature identical flavour added	Energy 77 kcal Fat 0.08 g Protein 1.23 g Carbohydrate 17.71 g Fiber 0.17 g Minerals 0.22 g Vitamin A 200 IU Vitamin D 40 IU Vitamin E 1.7 IU Vitamin C 17 mg Vitamin B1 160 µg Vitamin B2 134 µg Niacin 1920 µg Vitamin B6 166 µg Folic acid 4 µg Pantothenic acid 584 µg Vitamin B12 0.15 µg Biotin 17 µg Sodium 25 mg Potassium 15 mg Calcium 27 mg Phosphorus 33 mg Iron 2.8 mg Iodine 41.3 µg

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
40	Rice and banana	40	Rice 50.43% Way protein 14.77% Palm Olein 11.14% Sugar 9.12% Vitamins and Minerals 2.58% Banana Flour 5.05% Amylase 0.006%	Energy 162.8 kcal Fat 2.32 g Protein 6.36 g Carbohydrate 29.08 g Fiber 1.08 g Vitamin A 161.20 µg Vitamin B1 0.15 mg Vitamin B2 0.16 mg Vitamin B6 0.26 mg Vitamin C 17.60 mg Vitamin D 2.32 µg Vitamin E 1.32 mg Niacin 2.76 mg Biotin 11.6 µg Pantothenic acid 1 mg Potassium 167.20 mg Phosphorus 198.4 mg Calcium 505 mg Sodium 227 mg Magnesium 32 mg Iodine 49.6 µg Iron 3.2 mg Zinc 1.92 mg
41	Rice and mixed vegetables	40	Rice 56.61% Way protein 14.3% Palm Olein 11.98% Sugar 6.9% Vitamins and Minerals 2.64% Pumkin 1.48% Onion 1.02% Carrot Flour 0.55% Salt 0.5% Green vegetable 0.31% Amylase 0.006%	Energy 161.60 kcal Fat 2.48 g Protein 6.56 g Carbohydrate 28.28 g Fiber 0.32 g Vitamin A 146.40 µg Vitamin B1 0.15 mg Vitamin B2 0.16 mg Vitamin B6 0.23 mg Vitamin C 18.80 mg Vitamin D 2.05 µg Vitamin E 1.52 mg Niacin 3.04 mg Biotin 11.2 µg Pantothenic acid 0.88 mg Potassium 179.60 mg Phosphorus 202.80 mg Calcium 208.40 mg Sodium 170.00 mg Magnesium 30.80 mg Iodine 58.80 µg Iron 3.04 mg Zinc 2.04 mg

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
42	Rice and fish 4%	40	Rice 56.35% Way protein 10.8% Palm Olein 11.82% Sugar 6.9% Fish Flour 4.00% Vitamins and Minerals 2.66% Onion 1.85% Soybean sauce 0.92% Carrot Flour 0.74% Green vegetable 0.15% Amylase 0.006%	Energy 161.20 kcal Fat 2.56 g Protein 6.32 g Carbohydrate 28.28 g Fiber 0.84 g Vitamin A 93.20 µg Vitamin B1 0.15 mg Vitamin B2 0.16 mg Vitamin B6 0.26 mg Vitamin C 19.60 mg Vitamin D 2.82 µg Vitamin E 1.20 mg Niacin 3.20 mg Biotin 10.40 µg Pantothenic acid 1.08 mg Potassium 185.60 mg Phosphorus 228.40 mg Calcium 247.20 mg Sodium 208.80 mg Magnesium 34.00 mg Iodine 51.20 µg Iron 3.12 mg Zinc 2.12 mg
43	Rice and chicken 1.8%	40	Rice 58.56% Way protein 13.88% Palm Olein 11.42% Sugar 6.85% Vitamins and Minerals 2.74% Chicken Flour 1.80% Carrot Flour 0.73% Celery 0.21% Amylase 0.006%	Energy 161.60 kcal Fat 2.20 g Protein 6.12 g Carbohydrate 29.72 g Fiber 0.60 g Vitamin A 233.60 µg Vitamin B1 0.17 mg Vitamin B2 0.16 mg Vitamin B6 0.28 mg Vitamin C 17.60 mg Vitamin D 1.69 µg Vitamin E 1.20 mg Niacin 2.96 mg Biotin 10.80 µg Pantothenic acid 1.00 mg Potassium 161.20 mg Phosphorus 201.60 mg Calcium 213.60 mg Sodium 111.60 mg Magnesium 29.20 mg Iodine 49.20 µg Iron 2.92 mg Zinc 1.92 mg

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
44	Rice and mixed fruits	40	Rice 56.15% Way protein 14.83% Palm Olein 10.94% Sugar 8.97% Vitamins and Minerals 2.67% Banana Flour 1.68% Strawberry Flour 0.65% Orange Flour 0.41% Amylase 0.006%	Energy 162.80 kcal Fat 2.32 g Protein 6.36 g Carbohydrate 29.08 g Fiber 0.28 g Vitamin A 92.80 µg Vitamin B1 0.15 mg Vitamin B2 0.16 mg Vitamin B6 0.25 mg Vitamin C 17.60 mg Vitamin D 0.87 µg Vitamin E 1.52 mg Niacin 3.08 mg Biotin 10.80 µg Pantothenic acid 0.76 mg Potassium 171.20 mg Phosphorus 200.00 mg Calcium 207.60 mg Sodium 89.20 mg Magnesium 30.40 mg Iodine 50.80 µg Iron 3.16 mg Zinc 1.88 mg
45	Bananas	71	Banana 99.8% Vitamin C 0.05%	Energy 68.5 kcal Protein 0.8 g Carbohydrate 16.0 g Fat 0.1 g Vitamin C 25.3µg
46	Carrots	71	Carrot 80.0%	Energy 20.3 kcal Protein 0.5 g Carbohydrate 4.3 g Fat 0.1 g
47	Applesauce	71	Apple 99.95% Vitamin C 0.05%	Energy 36.3 kcal Protein 0.3 g Carbohydrate 8.7 g Fat 0.05 g Vitamin C 9.1µg
48	Prunes	71	Prune 43.1%	Energy 70.0 kcal Protein 0.7 g Carbohydrate 17.0 g Fat 0.1 g

Table 1D. Identification and description of ingredients in the commercial infant foods.(Continued)

No	Sample	weight (g)	Ingredient	Nutrition Facts (% Meal value)*
49	Apple juice	150	Apple juice 99.91% Vitamin C 0.09%	Energy 67.97 kcal Protein 0.09 g Carbohydrate 16.86 g Fat 0.02 g Vitamin C 65.11µg
50	Apple prune juice	150	Prune juice 65% Apple juice 34.8% Vitamin C 0.04%	Energy 77.27 kcal Protein 0.39 g Carbohydrate 18.85 g Fat 0.03 g Vitamin C 15.66 µg
51	Mixed fruits juice	150	Apple juice 69.85% Pineapple juice 16.18% Orange juice 9.6% Banana Puree 3.8% Apricot juice 0.32% Vitamin C 0.04%	Energy 70.78 kcal Protein 0.33 g Carbohydrate 17.29g Fat 0.03 g Vitamin C 27.59 µg

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