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DETERMINATION OF IRON IN COCOA BASED FOOD PRODUCT

Dissertation submitted in partial fulfillment for the Degree of Bachelor of Science in Forensic

MOHD IZHAR ABU BAKAR

School of Health Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan
Malaysia

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CERTIFICATE

This is certify that dissertation entitled

"Determination of iron in cocoa based food products"

is the bonafide record of research work done by

Mr. Mohd Izhar Abu Bakar

during the period from January 2006 to April 2006

under my supervision

Signature of Supervisor :

Name and Address of Supervisor : Dr. Che Wan Zanariah Che Wan Ngah

: School of Health Science,
University Sains Mlaysia,
Health Campus,
16150 Kubamg Kerian
Kelantan, Malaysia

Date : 18/05/06

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ABBREVIATIONS

AAS	: Atomic Absorption Spectrophotometry
Fe	: Ferum or iron
H_2O_2	: Hydrogen peroxide
HCl	: Hydrochloric acid
HNO_3	: Nitric acid
IC	: Ion Chromatography
mg/L	: milligram per liters
mL	: milliliters
nm	: nanometer
ppm	: part per million
RDA	: Recommended Dietary Allowances

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ABSTRACT

Determination of iron in cocoa based food product

The concentrations of iron in the cocoa based products available in local market around Kota Bharu were determined. Out of 15 samples analysed, three were nutritious chocolate drink powder, seven cereal meal product, two chocolate bars, two chocolate flavored beverages and one sample was pure cocoa powder. 1:1 dilution of concentrated nitric acid digestion followed with analysis by flame atomic absorption. The samples were homogenized and were heat to boiling with nitric acid for 30 minutes prior to the analysis. The iron obtained in this study is about 0.6 to 1.0 ppm for powder sample, 0.2 to 0.4 for bar sample and 0.2 ppm for liquid sample. The overall result shows the concentration of iron in the cocoa based food product within the range of 0.2 to 1.3 ppm.

ABSTRAK

Penentuan zat besi dalam produk makanan berasaskan koko

Kepekatan zat besi di dalam produk berasaskan makanan yang boleh didapati di kedai sekitar Kota Bharu telah ditentukan. Sebanyak 15 sampel dianalisa iaitu tiga sampel merupakan serbuk minuman coklat berkhasiat, tujuh sampel pula adalah produk makanan bijirin, dua coklat berbentuk bar, dua sampel minuman berperisa coklat dan satu sampel serbuk koko. Proses penyediaan sample menggunakan 1:1 cairan asid nitrik pekat diikuti dengan penentuan besi menggunakan AAS. Perolehan zat besi dalam kajian ini adalah kira-kira 0.6 hingga 1.0 ppm untuk sampel bentuk serbuk, 0.2 hingga 0.4 untuk sampel berbentuk bar dan 0.2 ppm untuk sampel berbentuk cecair. Hasil keseluruhan menunjukkan kepekatan zat besi di dalam produk makanan berasakan koko berada dalam lingkungan 0.2 hingga 1.3 ppm.

1. Introduction

Metals are natural constituents in the environment. At high concentrations, trace metals can become toxic for living organisms and behave as conservative pollutants. Metals may enter the environment mainly by two means. First processes are occurred naturally such as erosion of rocks, volcanic activity and forest fires while second processes are due to human activities. An anthropogenic activity may add considerable amounts of polluting compounds, which will influence the existing natural aquatic system. Metals are frequently released in large quantities during different processes derived from human activities and may lead to health disorder in human being. Moreover, human beings located at places contaminated by heavy metals could be especially sensitive to these contaminants due to bioaccumulation. The metals can accumulate in bone, hair and in some soft tissues, such as the liver, kidney, brain or lungs.

Iron (Fe) plays an important role in metabolic and fermentation processes, as an enzyme activator, stabilizer and functional component of proteins at low concentrations. Above trace levels iron has other roles. Iron also exists a fairly narrow “concentration window” between the essential and toxic levels. Iron is a moderately toxic element when compared with other transition metals. However, the toxic doses of iron and its compounds can lead to serious problems, including depression, rapid and shallow respiration, coma, convulsions and cardiac arrest.

Excess iron stores may also promote cancer and increase the cardiovascular risk, though the latter is a subject of current debate (Martınez et. al, 2002).

1.1 Classification of Iron

Iron compounds have been classified as:

1. Water soluble such as ferrous sulfate, ferrous gluconate, ferrous lactate, and ferric ammonium citrate.
2. Poorly water soluble but soluble in diluted acids, for example ferrous fumarate, ferrous succinate, and ferric sacharate.
3. Poorly soluble in water or acid solutions like ferric pyrophosphate, ferric orthophosfate, elemental iron
4. Protected compounds like hemoglobin, NaFeEDTA, and iron-bisglycine chelate.

(Nieves et. al, 2003).

1.2 Iron in Food

Iron found in foods is either in the form of heme or non-heme iron. Heme iron is better absorbed than non-heme iron. Foods containing heme iron are the best for increasing or maintaining healthy iron levels. Such foods include (in order of iron-richness) clams, oysters, organ meats, beef, pork, poultry, and fish. Non-heme iron is less well absorbed. About 60% of iron in meat in non-heme.

Eggs, dairy products, and iron-containing vegetables only have the non-heme form. Such vegetable products include dried beans and peas, iron-fortified cereals, bread, and pasta products, dark green leafy vegetables, dried fruits, nuts, and seeds. Increasing intake of vitamin-C rich foods can enhance absorption of non-heme iron during a single meal.

1.3 Iron Defiency

A deficiency of iron makes individual feels tired and apathetic. This condition is known as iron deficiency anemia (IDA). Anemia is characterized by low levels of hemoglobin causing oxygen starvation in living tissues. Iron deficiency anemia (IDA) has a large impact on productivity, mental performance, child growth, immunity and pregnancy outcome (Benny, 1998). Iron deficiency anemia is a major nutritional problem in the world, particularly in vulnerable population groups such as infants, children, and women of childbearing age. An iron defiency leads to a reduced synthesis of haemoglobin and therefore, lower than normal haemoglobin in the red cells (Anzano et al, 2000). Hence there is an effort to develop strategies to combat Fe deficiency. Recently, the strategies include supplementation with medicinal Fe along with fortification of foods with iron. Food fortification programs are generally considered the most cost-effective approach (Hurrell 1997, 1998). For example iron fortification of infant formula has been successful in reducing the prevalence of iron deficiency in infants. On the other hand lack of iron may lead to unusual tiredness, shortness of breath, a decrease in physical performance, and learning problems in children and adults, and may increase your chance of getting an infection (Martınez *et. al*, 2002). Human milk contains about 0.35 mg/L of Fe and it is very well absorbed(Lynch et al, 2003). However, the quantity is insufficient to meet the need of infants after age 6 month. Other source of iron is animal flesh. It is also well absorbed and promotes the absorption of other dietary iron because it contains heme.

The level of essential minerals usually changes and may vary due to several factors. Some of the sources of variation are biological like variety species of the plant, different fertilization of the crop or different feeding principles for animal. These variations also influence by annual and seasonal factor. Other reasons are related to differences in harvest, storage and processing (Nilva *et. al*, 2004). As a result some companies may add fortified minerals such as iron and other substances like vitamins to their product. The main objective is to increase the nutritional value in the food.

The relationship between iron bioavailability and the organoleptic is important in the success of fortified food program. For instance, water-soluble Fe compounds such as ferrous sulfate have high relative bioavailability but may have an undesirable organoleptic impact on the product, i.e. provoking rancidity or undesirable color and flavor changes during storage or food preparation. In order to minimize such organoleptic changes, many fortification programs use less soluble, and hence less bioavailable Fe compounds, such as elemental Fe and Fe phosphate compounds (Forbes *et al.* 1989)

Thus, there is a need for alternative Fe compounds that possess high relative bioavailability which do not provoke unacceptable organoleptic changes in cereal foods. Ferrous fumarate has been proposed as an alternative for Fe fortification of infant cereals since this compound was shown to be absorbed as well as ferrous sulfate in adults (Hurrell *et al.* 1989). (Ferrous sulfate is the iron compound generally used as the standard by which to measure the relative bioavailability of other iron forms). Ferrous fumarate is less soluble than ferrous sulfate in water but is readily soluble in dilute acid such as gastric juice. Further, ferrous fumarate does not provoke unacceptable organoleptic changes in the food during storage to the same extent as does ferrous sulfate and it has a high relative bioavailability. Therefore, ferrous fumarate could be a very promising Fe fortificant for food fortification of cereal products such as corn masa flour.

Bioavailability of dietary iron (including added Fe) depends on the overall composition of the meal including the presence of enhancers and inhibitors of Fe absorption. Corn masa flour contains relatively high levels of phytic acid, a potent inhibitor of Fe absorption. Therefore Fe bioavailability from fortified corn masa is relatively low, unless an absorption enhancer is added. Ascorbic acid is one of the major enhancers of Fe absorption as well as an antioxidant in processed foods. However acid ascorbic is unstable to heat and oxygen especially during storage and preparation.

Iron compounds are difficult to add to cereal-based foods since they can provoke rancidity and adverse color and flavor changes (Hurrell 1997). SUSTAIN (2000) performed storage studies in order to evaluate iron compounds in fortified corn masa flour as well as further explore the reactions between iron fortificants and cereals.

2.0 Review of literature

2.1 Importance of Iron

The iron present in body at birth is sufficient for physiological requirements of babies of normal age. After this the infant becomes dependent on an adequate supply of readily absorbed dietary iron. Body iron content should increase by about 70% between ages 4 and 12 month (Lynch et al, 2003). Institute of medicine in Washington (2001) recommended based on a factorial model, the estimated average daily requirement for absorbed iron from age 7 to 12 month has been estimated to be 0.69 mg, less iron is needed after age 12 month, about 0.63 mg per day for a child aged 18 month. Normal birth weight breast fed infants rarely develop iron deficiency before age 6 month. It is suggested, that iron is not needed in the formula consumed during the first six months of life (Hemminki et. al, 1995). Because the bioavailability of iron from breast milk decreases when weaning foods are introduced, exclusive breastfeeding is recommended till the age of 4 to 6 month (Kodyat et al, 1998). However, the risk increases rapidly during the next 3 month in those who continue to be breast fed if other dietary items do not include a rich source of highly bioavailable iron (Lynch et al, 2003).

Iron is essential for all living organisms since iron-containing proteins have a crucial role in both oxygen transport in the respiratory chain and in DNA synthesis. Iron is, however, a potentially toxic element, being a catalyst in the development of the highly poisonous free oxygen radicals (Hemminki et. al, 1995).

Recommended Dietary Allowances (RDA) is published by Food and Nutrition Board of the national Research Council. The allowances are to provide for individual variations among most normal persons as they live under their usual environmental conditions. Diets should be based on a variety of foods, both to cover known requirement and to provide other nutrients for which human requirements have been less well defined (Murray et al, 1990). Table 2.1 below shows recommended daily dietary allowance for iron.

Table 2.1: RDA for Iron (Murray et. al, Harper's Biochemistry 22nd edition)

	Ages (years)	Iron (mg)
Infants	0.0-0.5	6
	0.5-1.0	10
Children	1-3	10
	4-6	10
	7-10	10
Males	11-14	12
	15-18	12
	19-24	10
	25-50	10
	51+	10
Female	11-14	15
	15-18	15
	19-24	15
	25-50	15
	51+	10
Pregnant	-	30
Lactating	-	15

2.2 Iron Deficiency Anemia (IDA)

Inadequate consumption of iron may lead to Iron Deficiency Anemia (IDA). Iron deficiency anemia is a major nutritional problem in the world, particularly in vulnerable population groups such as infants, children, and women of childbearing age. An iron defiency leads to a reduced synthesis of haemoglobin and therefore, lower than normal haemoglobin in the red cells (Anzano et al, 2000). IDA has a large impact on physical performance, productivity and economic growth, on mental performance and learning ability, on child growth, on the immune system, and on pregnancy outcome. Severe anaemia during pregnancy can result in miscarriages and premature deliveries (Kodyat, 1996).

The direct causes of anemia are a too low iron intake and a too low bioavailability of dietary iron. The main staple in South East Asia is rice which contains little iron and is rich in phytate, which reduces iron bioavailability. The consumption of animal food, a good source of iron with relatively high bioavailability, is low. The consumption of greenleafy vegetables is high, but the bioavailability of their iron is low due to inhibitors such as phytate. In addition, anemia can be associated with chronic diseases or recent infection such as worm infestation, especially hookworm, malaria and tuberculosis (Kodyat, 1998).

2.3 Bioavailability of iron

A major cause of iron deficiency is low bioavailability of dietary iron, especially in populations dependent on monotonous diets high in cereals and legumes (Perks and Miller, 1996). The bioavailability of dietary iron (including fortification iron) depends on the overall composition of the meal, including the presence of the enhancers and inhibitor (Sean R. Lynch et al, 2003). It is generally accepted that the bioavailability of nonheme iron is enhanced by ascorbic acid and meat and inhibited by phytate, polyphenolics, calcium, and other food components (Perks and Miller, 1996). Corn masa flour, for example, has a high phytate level that comprises the body's ability to absorb dietary iron, including fortification iron (SUSTAIN, 2000). There was a small, but significant, inhibition of iron absorption from a milk-containing meal given with the beverage. These findings suggest that the beverage can be provided with or without a small meal (Ana et al, 2004). One study shows that the addition of 5 ounces of orange juice (75 mg ascorbic acid) to a meal of low iron availability would increase iron absorption four-fold from 5 % to 20 % (Perks and Miller, 1996).

Ascorbic acid is the most efficient promoter of nonheme iron absorption. The enhancing effects of ascorbic acid on the absorption of nonheme iron have been demonstrated in a large number of human studies using radioisotope to measure iron absorption (Lynch et al, 2003). On the other hand Perks (1996) suggests that supplementation with ascorbic acid may not enhance iron absorption over the long term.

Lena Davidsson et al (2002) evaluated the bioavailability of iron from iron-fortified Guatemalan meals based on corn tortillas and black bean paste. These meals were fortified with ferrous fumarate, ferrous sulfate or Na_2EDTA. They also investigated the potential of Na_2EDTA to increase the bioavailability of iron from ferrous fumarate. The result showed that from ferrous fumarate was not improved by the addition of Na_2EDTA. However, the bioavailability of iron was enhanced when Na_2EDTA replaced ferrous sulfate.

According to Garcial-Casal (2003), there are some important steps for a fortification program to be successful. Two of the key issues are the food vehicle and the iron compound. The food vehicle selected should reach the entire population and deliver most of the calories of the diet. It has to be consumed daily but at the same time with no risk of excessive consumption. In underdeveloped countries cereal flours, especially wheat and corn, are frequently used as fortification vehicles, because grain products are the staple foods for those populations. Other common fortified foods are ready-to-eat and infant cereals, which in industrialized countries could provide a significant amount of iron and its bioavailability could be significantly increased with the inclusion of vitamin C and, as reported recently, of vitamin A in the fortification formula.

The relationship between iron bioavailability and the organoleptic is important in the success of fortified food program. For instance, water-soluble Fe compounds such as ferrous sulfate have high relative bioavailability but may have an undesirable organoleptic impact on the product, i.e. provoking rancidity or undesirable color and flavor changes during storage or food preparation. In order to minimize such organoleptic changes, many fortification programs use less soluble, and hence less bioavailable Fe compounds, such as elemental Fe and Fe phosphate compounds (Forbes *et al.* 1989)

On the other hand Ronny (2005) also described that stable iron isotopes have been predicted to change their relative abundances in a mass-dependent way by physical transport processes and chemical reactions. Iron isotope fractionation of up to a few permil per atomic mass unit may have been observed in a number of natural and synthetic materials. Instrument like thermal ionisation mass spectrometry (TIMS) was predicted to produce unpredictable instrumental mass discrimination between independent runs of the same sample.

2.4 Storage and Sensory Evaluation of Iron Fortified Food

The selection of the iron compound for fortification is important in order to avoid interactions of iron with the food vehicle or the total meal, because a minor change in organoleptic characteristics of the food will result in consumers' rejection. When the iron compound is added, it is necessary to evaluate possible changes in food color, taste or appearance with time and storage on adverse temperature and humidity conditions. Solubility, chemical reactivity, bioavailability and cost are other important issues when selecting an iron compound. For instance, ferrous sulfate is a highly bioavailable and relatively inexpensive compound, but because of its reactivity produces undesirable changes in some fortified foods. On the other hand, elemental iron (reduced, electrolytic or carbonyl) is also inexpensive but it has been reported to have a low bioavailability depending on particle size and the food vehicle to be fortified (M. Nieves Garcial-Casal et. al, 2003).

SUSTAIN (2000) reported storage and sensory studies for evaluation of iron fortified corn masa flour. The authors used two methods for oxidation measurement which are pentane test and hexanal test.

After one month, the sample was strongly oxidized and had unacceptable sensory attributes. The control sample with no added iron was stable for 2 months but had oxidized at 3 months and 4 months, generating pentane and giving unacceptable odor scores. All the iron fortified samples behaved in a similar way to the control sample, oxidizing between 2 and 3 months of storage. There were no differences between ferrous sulfate, ferrous fumarate, both with and without Na_2EDTA, and NaFeEDTA.

In hexanal examination, the same samples are used and heated at 65°C for 16 hours. After that they stored the samples for 3 months at room temperature. They notified that there is no evidence that any of the added iron fortification compounds or Na_2EDTA will influence fat oxidation or reduce the shelf life of stored corn masa flour.

The ferric nitrate-fortified corn masa produced 3.2 times more hexanal than the control sample with no added iron. The levels of hexanal produced from the iron-fortified corn masa flours were somewhat inconsistent and ranged from 40 to 140% of the control value. There was no clear evidence, however, that any of the test iron compounds, or Na_2EDTA, influenced hexanal formation. Similarly, after the samples had been stored for 3 months at room temperature, no hexanal was detected in any of the stored corn masa flours (except that containing ferric nitrate) or in the control flour. These results are not consistent with the pentane data after storage, for unknown reasons. Hexanal was detected in the stored corn masa flour with added ferric nitrate at a level similar to that generated when the same sample was heated for 16 hours at 65°C. This indicates that in a highly pro-oxidant form, iron readily catalyzes fat oxidation of corn masa. The author concludes that all corn masa flour used in this study had poor stability. It was stable for only 2 months at 37°C, which can be extrapolated to 4 months at 30°C or 8 months at 20°C under these standardized conditions. The iron compounds tested did not affect keeping quality.

2.5 Pretreatment and Sample Preparation

It is important to understand pretreatment procedure in the sample analysis. Most analytical instruments especially Atomic Absorption spectroscopy does not permit the whole sample to be introduced for direct measurement. Pretreatment of food for atomic absorption spectrometry usually involves ashing of the sample and subsequent dissolution of the ash in an acid medium or, alternatively, direct acid treatment (Azano et. al, 1999). On the other hand, acid digestion of food requires the acid to have an oxidizing character or contain an external oxidant. Silva et al (2003) investigated four micro methods which were i) micro-digestion with nitric acid, ii) slurry procedure using tetramethylammonium hydroxide (TMAH) tissue stabilizer, iii) a slurry prepared in 1.4 Mol L^{-1} nitric acid and treated in a ultrasonic bath, and iv) the direct analysis of the solid sample for the determination of the iron in roots and leaves of rice plants. The objective was to study the best method for iron determination from cultivation experiment using graphite furnace atomic absorption spectrometry. The slurry technique with ulrasonic treatment of sample, suspended in dilute nitric acid, was finally adopted as the method of choice.

Sudhir Dahiyaa et al (2004) determined nickel, lead and cadmium content in 69 diffrernt brands of chocolates and candies available in local market of suburban areas of Mumbai, India. The sample preparation used in this literature was wet oxidation with mixture of nitric acid and perchloric acid in the ratio 3:1 for decomposition. Elemental concentration is then quantified by means of atomic absorption spectrophotometer.

Like other author, S. Anzalone, E. Bottari and M.R. Festa (1996) also utilized atomic absorption to characterize the heavy metal in wheat. The amount of barium, iron, manganese, nickel, cobalt, copper, lead and zinc has been determined by using AAS. Trace quantities (up to 0.5 mg/L) of alkaline and alkaline-earth metals have also been detected by Ion Chromatography. Each sample was ignited in a muffle furnace at 600°C until constant weight to obtain ashes. A known amount of ashes was solubilised in hydrochloric acid, filtered through a gooch crucible and diluted to a selected volume. The obtained solution was divided into two parts for AAS and ion chromatography (IC) analysis, respectively.