DEVELOPMENT OF IRON-RICH FOOD PRODUCTS FROM ANIMAL SOURCES

POONSUB INSUNG

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Chicken and porcine blood curds are the iron-rich foods from animal, which have the lowest costs per mg of iron. The blood curds were pressed in order to remove certain amount of water before using in the developments of blood-fortified cookies and traditional Thai fish chips (Kow-krieb). The fortification was performed by adding blood curds into the original recipes at 10, 20, 30 and 40%. Total, heme and nonheme iron contents were analyzed. Iron contents of all forms were not significantly different in both kinds of blood curd ($p>0.05$). Iron contents of the blood curds collected from 6 different markets and/or at different times were not much different and on average about 15 mg/100 g (about 230 mg/100 g dry basis). Pressed blood curds contained about 190 mg of iron/100 g dry basis. Addition of either chicken or porcine blood curd affected sensory qualities of cookies and chips. The sensory scores for general appearance, color, overall acceptability, odor, taste, texture, crispness and off-flavor of the fortified products: chicken blood fortified cookies (CkC)and chips (ChC), and porcine blood fortified cookies (CkP) and chips (ChP) were significantly better at the 10% fortification level ($p<0.05$). The overall acceptability scores of the 40%CkC, 40%ChC, 40%ChP, and the 30%CkP products were higher than 5 on 9-point hedonic scale (neither like nor dislike, to like slightly); the products were studied for shelf stability along with the 10% fortification samples. The iron contents in the CkC and ChC products at 10 and 40% fortification levels were 3.07, 8.62 and 2.39, 5.15 mg/serving, respectively, and were 3.68, 7.24 and 2.57, 5.06 mg/serving in the CkP and ChP at 10, 30 and 10, 40% fortification levels, respectively. Upon processing, heme iron of fortified blood curds significantly changed into nonheme iron ($p<0.05$). During the 30-day storage, the sensory acceptability scores of the blood-fortified cookies did not significantly change ($p>0.05$). However, the scores for general appearance of deep-fried blood-fortified chips significantly changed during storage ($p<0.05$). Nonheme iron contents in the products of higher fortification levels (30,40%) significantly increased ($p<0.05$). The water activities of the products were in the range of 0.3-0.4 and remained under 0.6 during storage. The peroxide values of the cookies at 10% fortification levels were lower than of the cookies at 30% and 40% fortification levels during storage. However, such difference was not observed in the deep-fried chips. Costs of raw materials for fortified cookies were about 56 and 65 Baht/kg in 10% and 30-40%fortification, respectively. While, the costs of deep-fried chips were about 50 and 60 Baht/kg in 10% and 40% fortification.

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พูลทรัพย์ อินทร์สังข์ : การพัฒนาผลิตภัณฑ์อาหารเสริมธาตุเหล็กโดยใช้แหล่งอาหารที่มีธาตุเหล็กสูงจากสัตว์ (DEVELOPMENT OF IRON-RICH FOOD PRODUCTS FROM ANIMAL SOURCES). คณะกรรมการควบคุมวิทยานิพนธ์ : วิสิฐ จะวะสิต, Ph.D., รัชนี คงคาฉุยฉาย, Ph.D., สมศรี เจริญเกียรติกุล, D.Sc., พัตธนี วินิจจะกูล, Ph.D., ประไพศรี ศิริจักรวาล, Ph.D. 122 หน้า. ISBN 974-663-655-3

การศึกษานี้ใช้ เลือดหมู และเลือดไก่ต้มสุกเป็นแหล่งธาตุเหล็กจากสัตว์ เนื่องจากมีราคาธาตุเหล็กต่อน้ำหนักเป็นมิลลิกรัมต่ำที่สุด โดยนำเลือดสุกมาบีบเอาน้ำออกก่อนนำไปเสริมในผลิตภัณฑ์คุกกี้และข้าวเกรียบในระดับร้อยละ 10 20 30 และ 40 ทั้งนี้ได้ทำการวิเคราะห์หาปริมาณธาตุ เหล็กทั้งหมด ธาตุเหล็กรูป ฮีม และธาตุเหล็กไม่ใช่ฮีม ปริมาณธาตุเหล็กทั้งหมดจากเลือดหมูและเลือดไก่มีค่าไม่แตกต่างกันทางสถิติ ($p>0.05$) เลือดสุกที่ได้รวบรวม จาก 6 แหล่ง และในช่วงเวลาต่างกันมีปริมาณธาตุ เหล็กที่ไม่ต่างกันมากโดยมีค่าเฉลี่ยประมาณ 15 มก.ต่อ 100 กรัม (ประมาณ 230 มก.ต่อ 100 กรัม ของนน.แห้ง) ส่วนเลือดสุกที่นำมาบีบเอาน้ำออกมีปริมาณธาตุ เหล็กประมาณ 190 มก. ต่อ 100 กรัม ของนน.แห้ง การเสริมเลือดหมูและเลือดไก่ในผลิตภัณฑ์คุกกี้และข้าวเกรียบมีผลกระทบต่อคุณภาพทางประสาทสัมผัส ทั้งนี้พบว่าคะแนนการยอมรับในด้านลักษณะทั่วไป สี การยอมรับโดยรวม กลิ่น รส ชาติ เนื้อสัมผัส ความกรอบ และกลิ่นผิดปกติ ของผลิตภัณฑ์ที่เสริมเลือดที่ระดับการเสริมร้อยละ 10 ดีกว่าผลิตภัณฑ์ที่เสริมในระดับอื่นๆอย่างมีนัยสำคัญทางสถิติ($p<0.05$) อย่างไรก็ตามค่าคะแนนการยอมรับโดยรวมของคุกกี้และข้าวเกรียบเสริมเลือดไก่ที่ระดับร้อยละ 40 และของคุกกี้และข้าวเกรียบเสริมเลือดหมูที่ระดับร้อยละ 30 และ 40 ตามลำดับ มีค่าคะแนนการยอมรับโดยรวมมากกว่า 5 จากตารางความพอใจ 9 จุด ผลิตภัณฑ์กลุ่มดังกล่าวและผลิตภัณฑ์ที่มีการเสริมที่ระดับร้อยละ10 จึงถูกใช้ในการศึกษาอายุการเก็บของผลิตภัณฑ์คุกกี้และข้าวเกรียบเสริมเลือดไก่ที่ระดับการเสริมร้อยละ 10 และ 40 มีปริมาณธาตุเหล็กเท่ากับ 3.07, 8.62 และ 2.93, 5.15 มก.ต่อหน่วยบริโภค (30 กรัม) ตามลำดับ ส่วนการเสริมเลือดหมูในคุกกี้ที่ระดับการเสริมร้อยละ 10 และ 30 และในข้าวเกรียบที่ระดับการเสริมร้อยละ 10 และ 40 มีปริมาณธาตุเหล็ก 3.68, 7.24 และ 2.57, 5.06 มก.ต่อหน่วยบริโภค ตามลำดับ กระบวนการผลิตมีผลต่อรูปของธาตุเหล็กโดยปริมาณธาตุ เหล็กในรูปฮีม เปลี่ยนเป็นธาตุเหล็กรูปไม่ใช่ฮีม อย่างมีนัยสำคัญทางสถิติ($p<0.05$)ในระหว่างการศึกษาอายุการเก็บเป็นเวลา30วันพบว่าคุกกี้มีค่าคะแนนการยอมรับทางประสาทสัมผัสไม่เปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิต($p>0.05$)แต่คะแนนความชอบลักษณะทั่วไปของข้าวเกรียบมีการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติ($p<0.05$)นอกจากนี้ผลิตภัณฑ์ที่มีการเสริมในระดับสูง(ร้อยละ 30-40) มีการเพิ่มปริมาณธาตุ เหล็กในรูปไม่ใช่ฮีมอย่างมีนัยสำคัญทางสถิติ($p<0.05$) ค่า A_wของผลิตภัณฑ์อยู่ในช่วง 0.3-0.4 และในระหว่างการเก็บอยู่ในระดับต่ำกว่า 0.6 ส่วนค่าเปอร์ออกไซด์ของคุกกี้ที่มีการเสริมในอัตราร้อยละ 10 มีค่าต่ำกว่าที่มีการเสริมร้อยละ 30 และ 40 แต่ไม่พบความแตกต่างในผลิตภัณฑ์ข้าวเกรียบ ราคาต้นทุนวัตถุดิบของคุกกี้ที่มีการเสริมในอัตราร้อยละ 10 และ30-40 คิดเป็นกิโลกรัมละ 56 และ 65 บาท ตามลำดับ ส่วนราคาต้นทุนวัตถุดิบของข้าวเกรียบที่อัตราการเสริมร้อยละ 10 และ 40 คิดเป็นกิโลกรัมละ 50 และ 60 บาทตามลำดับ

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CHAPTER I

INTRODUCTION

Iron deficiency anemia (IDA) is one of the uttermost severe malnutrition problems found in Thailand. Primary school children and pregnant women are among the prevalent groups, which account for 12.6 and 12.9%, respectively (1). About 30% of pregnant women in the south of Thailand are confronting with the iron deficiency anemia (2). IDA in pregnant women can lead to low birth weight baby, fetal morbidity and mortality, maternal morbidity and mortality (3). While IDA in general population can lead to decreases in learning ability, attention span, work performance and immune status (3-4), and finally lowers the efficiency of people in the nation. The main cause of IDA is normally based on the fact that the amount of iron consumption per day of Thai people is lower than the requirement of each specific age, gender and stage of development (5). Thai RDI recommends iron intake of 15 mg (6), however the average iron intake for the Thais was only 11.8 mg. and the consumption of iron from animal sources, which is the best bioavailability, contributes only 3 mg in one day (5). The problem can be worsening in pregnant women since their iron requirement is as high as 45 mg/day (7). Supplementation with iron tablet is an important strategy used for preventing IDA especially in pregnant women. However, iron tablet intake sometimes causes unacceptable symptoms such as vomiting, constipation, epigastric discomfort, diarrhea (3). Dietary modification is therefore another suitable and practical strategy, especially in the country like Thailand, which is one of the biggest food producers in the world (8). Food sources for iron can be from both plants and animals. The disadvantage of this food-based strategy, however,

is based on the fact that the availability of iron, especially from plant sources, is usually low due to natural inhibitors found in plant itself. It has been known that iron from animal source is the best in term of bioavailability.

Animal blood, which is a by-product from slaughterhouse, is widely consumed among Thai population. Animal blood is therefore a potential source of iron from animal, which can reach the target population. Many researchers had performed studies on the uses of animal blood for fortifying food products such as chip, biscuit. However, most product developments were based on the use of heme iron concentrate, which cannot be locally produced. Products developed by using cooked blood curd did not contain iron in the amount that could have significant contribution. Furthermore, the change in heme iron content during processing of blood curd also needed to be considered, since such change can decrease the bioavailability. Very often, this has not been investigated.

This research was designed to develop the iron-rich food products, which can be produced by using locally available animal source and technology. The products should be acceptable and affordable by general consumers, and have a reasonable shelf life.

Objective of the study

General objective

To develop iron-rich food products from animal sources, which are acceptable to consumers in Thailand.

Specific objective

1. To select the appropriate animal sources of iron for product development.
2. To develop iron-rich food products.
3. To evaluate the nutritive value of animal blood curd and products.
4. To evaluate the shelf stability of the products.
5. To determine cost of the products.

CHAPTER II

LITERATURE REVIEW

2.1 Overview of Iron

Iron is the 26th element of the periodic table and has an atomic weight of 55.85 (9). Iron is the fourth most common elements after oxygen, silicon and aluminum (10). Element iron, Fe^0, is rarely found in biological environment but does occur in food, as it is a common food additive (11). Dietary iron is present in food both in inorganic forms as ferrous (Fe^{2+}) and ferric (Fe^{3+}) compounds and in organic forms, which the most important of these being heme iron (figure.1) (12). Another organic form of iron existed in bacteria, plants and animals is nonheme iron protein which iron is incorporated not to nitrogen but to sulfur of some sulfur-containing amino acid such as cysteine (figure 2) (13). The major function of iron in the body is oxygen carrier in blood and muscle tissue, electron carrier in cytochromes and enzyme catalysis (13). In differing chemical environments, several oxidation states of iron ranging from Fe^{6+} to Fe^{2+} were found (14, 11). In aqueous solution, iron exists mostly in two oxidation states, Fe^{2+}, the ferrous form and Fe^{3+}, the ferric form (9). A special properties of iron is how easily it changes between these two forms which enables iron to serve as a catalyst in redox reactions by donating or accepting electrons (9). The importance of iron as an element necessary for life derives from its redox reactivity as it exists in two stable, interchangeable forms of the

ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms (12). The importance of iron to the world's population in the nutritional point of view is that about 2,150 million people worldwide are confronting with the iron deficiency and anemia (15). The research coped with searching the way to alleviate iron deficiency is therefore undertaken worldwide.

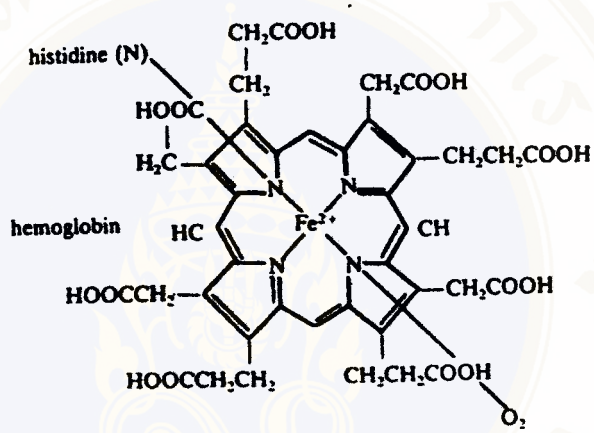

Figure 1 Structure of heme-iron in hemoglobin/myoglobin/cytochrome
Source: Spallholz et al. 1999 (13)

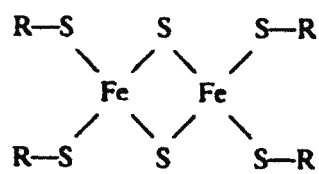

Figure 2 Structure of Iron-sulfur protein
Source: Spallholz et al. 1999. (13)

2.2 Distribution of Iron in the Body

Iron is present in all cells of the body (16). It accounts for 0.004 percent of body weight (17) which is equivalent to 2.5 to 4 g of iron. The precise amount of iron in an individual depends on gender, age, size, nutrition status and health (18). Approximately two thirds of body iron is utilized as functional iron such as hemoglobin (in the blood), myoglobin (in the muscles), and the rest of functional iron as a component of enzymes in the cells. The remaining one-third of body iron exists as storage iron such as ferritin and hemosiderin, which are found in the liver, spleen and bone marrow (18), and as iron in transit bound to the protein transferrin within the blood or the cells of the intestinal wall (18,12). The amount of stored iron in the body is variable, being more in men (approximately 1000 mg) than in women (approximately 400 mg) (18). Up to 40 mg of iron is transported each day while only about 4 mg of iron is being transported within the blood at any time (18).

2.3 Nutritional Aspects of Iron

Iron is among the minerals known to be dietary essentials that are required in exceedingly small or trace amount (19). It is the fourth most abundant terrestrial element, comprising approximately 4.7% of the earth's crust (10). This great abundance of iron in nature makes it surprising that iron deficiency is the most common nutritional deficiency in the world. The clinical sign of iron deficiency is known as the nutritional anemia.

2.4 Function of Iron

The major function of iron is oxygen transport (17). However, the known function in general is the constituent of (a) hemoglobin for oxygen transport, (b) myoglobin for muscle storage of oxygen, (c) cytochromes for oxidative production of cellular energy in the form of ATP (9), and (d) formation of red blood cell.(16)

2.4.1 Hemoglobin for oxygen transport

Hemoglobin is the oxygen-carrying protein of vertebrate blood (20). It is a multi-subunit of protein comprising with four subunits. Each hemoglobin molecule binds to four oxygen molecules. When the red blood cells pass through the capillaries of the lungs, oxygen from the lungs binds to the hemoglobin molecules in the cells. Then, the red blood cells transport oxygen-bearing hemoglobin through the body. In the tissues, the oxygen is released from the hemoglobin to diffuse out of the red blood cells, out of the blood and into the tissues cells (18). Hemoglobin also transports carbon dioxide, a by-product of cellular metabolism back to the lungs for excretion (17).

2.4.2 Myoglobin for muscle storage of oxygen

Myoglobin is a single-subunit protein consisted of a single heme with a single globin chain (18). It functions as a temporary oxygen reserve in muscle metabolism (17). The oxygen is needed to combine with nutrient molecules and to release the energy to meet the needs during muscle concentration (9). If the rate of diffusion of oxygen from the red blood cells to the cells is limited, the concentration of muscle myoglobin is drastically reduced. This is due to the rate of diffusion of dioxygen from erythrocytes to mitochondria is limited (10).

2.4.3 Cytochromes for oxidative production of cellular energy

Cytochromes are heme-containing compounds. Their role in respiration and energy metabolism is through mitochondrial electron transport. Cytochromes are essential to the production of cellular energy by oxidative phosphorylation. They serve as electron carriers in the transforming of adenosine triphosphate (ATP), which is the primary energy storage compound. Cytochrome C is found highest in the heart muscle that has a high rate of oxygen utilization (9).

2.4.4 Iron containing enzymes

Some nonheme iron containing enzymes such as the iron-sulfur complexes of NADH dehydrogenase and succinate dehydrogenase are also involved in energy metabolism. These enzymes are required for electron transport chain (ETC) (9).

2.4.5 Formation of red blood cell

Because the iron-containing protein hemoglobin is a major component of the red blood cells (erythrocytes), iron is obviously required for the formation of red blood cells. This can be expressed in other way that iron is essential for formation of red blood cells which is formed in the bone marrow in the process known as erythropoiesis. During the erythropoiesis process, the heme groups become bound to globin molecules, also synthesized by the erythroblast, to form a completed hemoglobin molecules. This process requires the help of vitamin B_6 and the mineral copper (18). When the red blood cell production starts to deteriorate, plasma iron concentration begin to fall. If the situation continues, the effects of the reduced supply of the oxygen to the cells become evident in the symptoms of nutritional or iron deficiency anemia (19).

2.5 Metabolism of Iron

2.5.1 Digestion

Iron is present in food in two forms i.e. the heme and nonheme iron. Most nonheme iron in food is present as insoluble ferric iron (Fe^{3+})(10). In the stomach, insoluble ferric iron is solubilized by hydrochloric acid and is reduced to the ferrous form (Fe^{2+}) (21). At the same time, peptic digestion release heme iron from its globin bound form, but is not degraded (22). However, iron availability is not entirely dependent on factors within the stomach alone. Various dietary components in the intestine also influences iron bioavailability.

2.5.2 Absorption

The majority of iron absorption takes place in the duodenum and upper jejunum (10). Absorption involves at least two steps: (i) uptake of iron from the intestinal lumen into the mucosa and (ii) transfer across the mucosal cell and serosal membrane into the circulation (12). The ferrous form (Fe^{2+}) must first traverse in the mucous layer to reach the brush border of intestinal epithelial cells. Ferrous form (Fe^{2+}) must be oxidized to ferric form (Fe^{3+}) before it enters the epithelial cell. At the brush border of the epithelial cells, the ferric form (Fe^{3+}) is bound to a receptor protein, which then transfers iron into the cellular apotransferrin. The receptor protein plays a regulatory role by facilitating iron absorption when the body's needs for iron are augmented. The final step in iron absorption is the release of iron from the mucosal cell to plasma (22).

The body iron store as reflected by the hemoglobin level is a strong determinant of intestinal uptake of nonheme iron (9). The absorption of nonheme iron from food is about 1-8% of that consumed (23), and its absorption depends on the

individual's iron status, and on the presence of enhancers and inhibitors consumed during the same meal (23). In the case of heme iron, after iron is liberated from heme by the enzyme heme-oxygenase (21), it traverses into the cell for transferring to plasma as ferric iron (Fe^{3+}). However, a small portion of the heme absorbed by mucosal cells enters the portal blood as heme (21). Although heme iron accounts for a smaller proportion of iron in the diet than nonheme iron, it is absorbed readily, 2-3 times more than nonheme iron. About 20-25% of heme iron consumed is absorbed, independent of the iron status and unaffected by the other components of the diet (23).

2.5.3 Transportation

Transferrin is a plasma transport protein, which transports iron from the intestine to the tissue (9). Transferrin delivers iron to the tissues by cell membrane receptors specific for transferrin. The receptors bind the transferrin iron complex at the cell surface and carry it into the cell, where iron is released. Tissues such as the erythroid precursors, the placenta and the liver that have a high iron uptake, contain large numbers of transferrin receptor (9). When cells are in an iron-rich environment, the number of transferrin receptors decreases. Conversely, when iron supply to the cells is inadequate because of iron deficiency or increased iron demand related to high cell turnover, the numbers of transferrin receptors increases (9).

2.5.4 Storage

The iron stored in the body is in the range of 200 to 1500 mg body weight (24). The amount of iron stored in the body is 30% in the liver, 30% in the bone marrow, and the rest is found in the spleen and muscles (24). The iron storage compounds are ferritin (about 20 %) and hemosiderin (10 %) and both of these are protein-iron complexes (10). The iron bound to ferritin is more readily mobilized

than iron bound to hemosiderin (9). However, the storage iron concentration in the body varies depending on gender and iron status (10). Generally it is higher in healthy adult males than in premenopausal women (18,19). Stored iron serves as a reservoir to supply cellular iron needs, which is used for hemoglobin production (9,24). However, storage iron can be mobilized up to 50 mg/day and of this, only 20 to 25 mg is used for hemoglobin production (21, 24). A small amount of circulating ferritin are detectable and closely correlate with body iron stores (24). More than 90% of hemoglobin iron is repeatedly recycled (21). In general, poor iron status improves absorption (19).

2.5.5 Excretion

The basal excretion of iron from the body are occurred primarily in the feces, desquamated mucosal cells, blood, desquamated skill cells, sweat and urinary (9). The total losses average 1.0 mg/day and 0.8 mg/day in adult men and women, respectively (12). For premenopausal women, the iron lost in the menstrual blood average 0.4-0.5 mg/day (9).

2.6 Recommended Daily Dietary Allowances of Iron

The amounts of iron that must be received each day to compensate for the normal losses and meet the body's needs for good health is what is described as daily recommendation. Iron requirements are increased during pregnancy and period of rapid growth (23). The current recommended dietary allowances (RDA) for iron is based on average iron stores of 300 mg and an average daily iron losses of 1 mg for men and 1.5 mg for women (9,25). In 1989, Ministry of Public Health established the

recommended daily dietary allowance (RDA) for healthy Thai people (7). According to Thai RDA, the iron requirement depends on age and gender (Table 1). Later, in the year 1995, Thai recommended daily intake (Thai RDI) was established by Thai Food and Drug Administration to be used for nutrition labeling and as a guideline for the development of fortified product (6). The Thai RDI for dietary iron is 15 mg/day for people aged 6 years old and over(6).

Table 1 Recommended dietary allowance of iron (Thai RDA)

Group	Age (months)	Iron (mg)
Children	3-5	6
	6-8	7
	9-11	8
	(year)	
Boy-Girl	1-9	10
Boy	10-15	12
Male	16	10
Female	10-49	15
	50-	10
Pregnant		30
Lactating		15

Source: Thai RDA (1989), (7)

2.7 Sources of Iron in Foods

Iron in food can be found in three forms i.e. food iron such as animal and plant products, fortification iron, and contamination iron (3).

2.7.1 Food Iron

One of the best food iron sources is the animal product such as meat, fish and poultry (MFP). These products contained between 30 to 60 percent iron in the heme

form and the rest is appeared in the nonheme form (26). The study of Kangsadalampai and Wattanapenpaiboon, (1987) depicted that beef, pork and chicken were good sources of iron with regard to their high total and heme iron contents (27). Liver and heart contained higher total and heme iron than that of the meat (27). Among the animal products, it is well accepted that cooked blood curd contained the highest in both the total and heme iron contents. Wang et al. (1994) showed that the average total iron content in porcine blood curd was 224 mg/kg. The content of iron from animal sources, however, varies between animals and products. The total and heme iron contents of cooked porcine blood curd were found to be 161.79 and 142.33 μg/g. Total and heme iron contents in cooked chicken blood curd were only 99.94 and 80.67 μg/g (27). A study indicated that the quantities of heme and nonheme iron in porcine blood were 182 and 43 mg/kg, respectively (28). Whereas the total iron content of heme iron concentrate was found to be 2.5-3.3 mg/g (29), dry pig blood powder contained about 2.3-2.6 mg/g of total iron (30). On this ground, it can be concluded that cooked or dried blood may be a good source of iron for fortification in other food products.

Another source of food iron is iron from plants. Plant iron can be a fairly good source of iron, but most of their iron is present in the nonheme form (18). Potatoes, white sesame seeds, green stalks, dark green leaf are good sources, and whereas high amount of iron is also found in legumes, especially soybean hulls (18,26,31). However, certain dietary factors such as phytate and fiber, decrease the absorption of iron from plants (26).

Table 2 Total iron, heme and nonheme iron contents of animal sources in Thailand

Sources	Iron content (μg/g wet basis)		
	Total Iron	Heme Iron	Nonheme Iron
Beef round	41.63	27.42	14.22
Beef tenderloin	42.77	22.71	20.06
Pork round	11.52	6.85	4.67
Pork tenderloin	8.82	3.24	5.58
Chicken leg	11.41	3.48	7.93
Chicken breast	12.86	1.27	11.70
Liver			
Beef	122.28	51.53	70.76
Hog	214.37	28.95	185.42
Chicken	99.19	18.56	80.73
Heart			
Beef	52.48	27.15	25.28
Hog	57.98	24.88	33.10
Chicken	36.99	15.83	21.16
Cooked blood			
Hog blood	161.79	142.33	19.46
Chicken blood	99.94	80.67	19.32

Sources: Kangsadalampai O and Wattanapenpaiboon N, 1987 (27)

2.7.2 fortification iron

There are several examples of iron fortification applications that are successful on a large scale such as wheat flour, cereal products, pasta products, milk powder, infant formulas, rice flour, salt and curry powder (32). Most of these iron criterions depicted that iron can be fortified successfully by using these food vehicles.

2.7.3 Contamination iron

An additional iron source is iron cooking utensils. Most of foods (90%) contained significantly more iron when cooked in iron utensils than when cooked in non-iron utensils (33). According to a study of Liu et al (1986), it was found that the amount of dissolved iron in food cooked in Chinese iron pots was two to five times higher than that in food cooked in the other types of pots (34). Moreover, the iron added to food cooked in iron pots is bioavailable (35).

2.8 Dietary Factors Affecting Iron Bioavailability

The bioavailability of iron from diets depends on the interaction of the enhancers and inhibitors of iron absorption present in the meal or consumed immediately after eating (36). The factors enhancing the bioavailability of dietary iron are animal tissue and ascorbic acid, whereas several potential inhibitors have been identified, including phytate, polyphenols (tannins) and calcium (12,37-41).

2.8.1 Enhancers

(a) Meat

The heme iron in meat is absorbed as intact metalloporphyrin via specific and high-affinity mucosal brush border heme-binding site (42). The absorption of heme iron is

unaltered by dietary composition (12). Moreover, meat is an excellent source of bioavailable food iron and it also enhances absorption of nonheme iron from a variety of staple foods of low bioavailability. Cook and Monsen (1976) showed that the substitution of beef, pork, liver, fish and chicken in the semi-synthetic meal resulted in an increased rate of iron absorption between two to four folds. Whereas substitution of milk, cheese or egg will not increases iron absorption (42). Hallberg and Rossander (1984) found that adding 75 g of lean meat (15 g protein) in the basal meal (maize, black bean and rice) can increase the absorption of nonheme iron by about 2.5 times (43). Hallberg et al. (1978) studied on iron absorption from Southeast Asia diets and found that the addition of fish to the basal meal doubled the absorption of iron while the iron absorption from the simple meal was negligible (37). Hulten et al. (1995) measured on iron absorption from the whole diet to compare with types of diets. The result showed that iron absorption was increased from the diet with high iron bioavailability, which usually contained more meat and less phytate (44).

(b) Ascorbic acid

Ascorbic acid promotes acid conditions within the stomach enhancing the efficiency of solubilization of dietary ferric iron to ferrous forms, thereby precluding the formation of insoluble ferric hydroxides. Ascorbic acid forms chelates with solubilized ferric iron within the stomach and maintain solubility of ferric iron until the chyme enters the alkaline environment of the small intestine (12). Ascorbic acid has a dose related enhancing effect on iron absorption, but the nature of relationship is affected by the composition of the meal (41). Hallberg and Rossander (1984) reported that the addition of boiled cauliflower containing 65 mg ascorbic acid in the meal increased the bioavailability of iron three to four times. While the addition of 50 mg

pure ascorbic acid in the same meal was increased three times (43). Ballot et al. (1987) observed that the absorption of nonheme iron by serving the fruit juice of pawpaw and guava with rice meal was markedly increased. The increase was directly related to the high ascorbic acid contents in both fruit juices, pawpaw and guava (45).

Furthermore, the addition of ascorbic acid to the typical South-East Asian meal (rice, fish-curry, Namprikkapi, Yod Kratin) with high content of iron binding phenolic groups, and a bread meal with high phytate content counteracted the inhibitory effect of these compounds on nonheme iron absorption and improved the bioavailability of iron from these meals (38-39). Hamdaoui et al. (1995) reported that the addition of 20 mg ascorbic acid in the mixed meal with the tea decoction increased the nonheme iron absorption from this meal by more than 100%. However the addition of 5 mg ascorbic acid in the meal was not able to overcome the inhibitory effect on nonheme iron absorption (36). Davidson et al. (1998) evaluated the influence of ascorbic acid on iron absorption from an iron fortified chocolate-flavored milk drink in children. Iron absorption from the milk drink containing the standard commercial amount of ascorbic acid (25 mg/ 25 g dry powder) was significantly greater than that of the chocolate milk drink with no added ascorbic acid. In this study, doubling the amount of ascorbic acid in the chocolate milk drink from 25 to 50 mg increased the iron absorption from 5.4% to 7.7% (45).

2.8.2 Inhibitors

(a) Phytate

Phytate is a strong complex form of phytic acid, which occurs in many plant foods. It is the storage form of phosphorus in most seeds and in cereal grains (46). Normally, the phytate presents in high fiber diets. Insolubility and/or indigestibility of

complexes formed between iron, phytate and protein or fiber is a major cause of inhibition of iron absorption (12). Phytate compounds have been shown to have varying effects on iron availability. However the addition of phytate equivalent to 300 mg of phytic acid to the study meal produced a dramatic reduction in iron absorption from 21.69% to 2.15%. When the no phytate protein sources were compared to those with phytate, the lowest iron absorption was observed when either egg white, meat or soy protein was fed alone with phytate in the meal (47).

(b) Polyphenols

The phenolic compounds found in vegetables, seeds, fruits, cereals, tea, coffee and spices occur in several chemical forms. The form of iron binding with phenolic compounds such as phenolic galloy group, phenolic catechol groups also contribute to the effect of inhibition of iron absorption (48). Cook et al. (1995) studied the effect of red and white wine on nonheme iron absorption in humans. The iron absorption from the meal containing red wine was less than one-half of meal containing white wine or water (49). Similarly, the addition of tea decoction to the study meal reduced the absorption of nonheme iron from standard meal by 50% (36). Davidsson et al. (1998) measured iron absorption of iron-fortified chocolate flavored milk drink in children by stable-isotope technique. The result showed that absorption was significantly lower from the chocolate flavored milk containing no ascorbic acid (45). For coffee, it is well documented that the polyphenols bind to iron in the intestinal lumen, forming an insoluble complex and thereby inhibiting iron absorption (50-51). Tuntawiroon et al. (1991) studied on dose-dependent inhibitory effect of phenolic compounds in food on nonheme iron absorption in men. The presence of increasing amounts of Yod Kratin (*Leucaena glauca*) decreased the iron absorption more (38).

(c) Calcium

Barton et al. (1983) concluded that calcium could probably induce iron deficiency by delaying the uptake of iron into the intestinal mucosal cells or by influencing the further transfer of iron from these cells into the circulation (52). When the calcium dose between 40 and 600 mg were added to wheat roll prepared with low-extraction flour. The degree of inhibition appeared to be dose-related with addition up to 300 mg Ca, which decreased in nonheme iron absorption. In the same study, a similar degree of inhibition of iron absorption occurred when comparable amounts of dietary calcium in the form of dairy products were added rather than inorganic calcium (41). In another report, iron inhibition was observed in normal subjects when comparable amounts of supplemental calcium with two different forms were added to meals of either high or low iron availability. The reduction of iron absorption was 28% and 55%, respectively. In another result of the same study, three forms of calcium supplements (calcium carbonate, calcium citrate and calcium phosphate) were shown to inhibit the absorption of iron sulphate if taken with food (40). Gleerup et al. (1995) reported that 30-50% more iron was absorbed when the intake of calcium was low at lunch and dinner compared with the higher intake of calcium at this meal (53).

2.9 Effect of processing on content and form of iron in food

In many steps of food processing has the potential to markedly alter the form of iron in food products. Iron in food or food products may be lost through leaching into processing water. It is found that the losses of iron in food processing are solely due to leaching into cooking or processing water (54-55) or through the removal of iron-rich parts of plants and animals (54,56). The size of pieces, the amount of water

used, the cooking method and the length of cooking time affect the extent of iron loss 55, 57-58). Kimura and Itokawa (1990) found that losses of iron are varied dependent on the type of cooking, size of pieces and time of cooking method. More iron is lost, when the food with thin size were boiled in a large volume of water using slow heating and long cooking time (28, 57). On the other hand, the loss of iron were least with less water cooking (55). However, it was also found that iron in water to used for cooking rice affected in an increase of iron content of rice after it has been cooked (57). Moreover, an increase of iron content in the food containing sour ingredients which is cooked in a cast iron utensil was reported by Torelm et al. (1997). This might mean that iron was extracted from the cooking utensil (59). However, Jansuittivechakul et al. (1985) showed that normal cooking and processing do not appreciably affect the quality of iron in meat (60).

It is well accepted that the food processing influences the change in forms of iron (heme and nonheme) of animal sources (28, 61-63). Moreover, the factors affecting the change of iron form include temperature (28, 60-61, 63-67), method of cooking (60, 64, 66), time (28, 64, 69) and surface area of food source (66, 57). Numerous studies demonstrate dramatic changes in form of iron after food processing. The result indicated that the interaction of heat processing with those factors decreased heme iron content and increased nonheme iron content from animal sources (28, 61, 65-66,68,70). Schricker et al. (1982) showed that heating meat and red blood cells increased the amount of nonheme iron (65). Schricker and Miller (1983) reported that the cooking of fresh beef round using common household methods (braising, roasting, microwave cooking) resulted in an increase of nonheme iron generally less than 10% (66). Jansuittivechakul et al. (1985) reported that the heme iron in beef and bovine

hemoglobin decreased due to cooking method and time of cooking (60). Similarly, Wang and Lin (1994) depicted that the nonheme iron content of the porcine blood curd increased significantly ($p<0.05$) with heating at temperature above 55°C whereas the nonheme iron content was enhanced linearly with heating time at 80°C. In contrast, the heme iron decreased relatively with heating time (28). As the degree of cooking is increased, more heme iron is destroyed, and the iron released into the nonheme form (60 ,66, 68). Slow heating resulted in more release of nonheme iron than fast heating (28, 67). Moreover, the cooking methods either baking (60, 66) or frying (57) had an effect on change of forms of iron.

This conclusion is in the line with the finding of Gall et al. (1983) who concluded that it has small losses of major minerals of low fat fish during baking (71). However, Schricker and Miller (1983) reported that baking increases nonheme iron in meat (66). It is found that the heme iron content in both the product containing bovine hemoglobin and meat was decreased during baking with increasing temperature (60). King et al. (1990) studied the effect of temperature; time and rate of heating on change of nonheme iron concentration of a hemoglobin iron concentrate (HIC). It was found that the baking temperature at 100°C did not increase the nonheme iron content significantly from the original level in spray dried HIC except when heated over 60 minutes. However, baking the product with the temperatures 150°C and 300°C, the nonheme iron content were increased significantly ($p<0.05$) comparing with non baked HIC (70).

Frying has little or no impact on change of the mineral content of fried food (57,72). Fillion et al. (1998) found that in potatoes and fish, the iron content was very little affected by deep-fat frying (72). Whereas, the loss of iron content of thin slice

meat in frying without flour was largest, followed by frying with dry flour and with wet flour, respectively. Cooking loss of minerals is suspected to be caused by outflow of minerals from food material (57). Moreover, cooking such as braising, roasting and microwave cooking resulted in an increase of the non-heme iron content (66, 70).

2.10 Food-based Strategy

The food-based strategies to improve dietary iron intake and its bioavailability can be emerged as the feasible strategy that can be implemented immediately within the existing systems. However, it was suggested that food-based strategies must be taken into account at household level food security and food habits of community in order to have a workable and practical food combination (73). Two main important points of view i.e. food to food fortification and selection of food vehicle are considered in this work.

2.10.1 Food to food fortification

Food to food fortification is a prominent food-based strategy, which has been using worldwide as a tool for solving malnutrition problems. In Thailand, the food to food fortification strategy had been introduced to Thai population at different levels including family, community and industry (73). Some food products such as cracker (Khao-Kreb-Khung) were used as the vehicle for the fortification of iron using animal blood as iron source (73). In other counties such as China, the biscuit has been used as a vehicle for iron fortification using dry blood powder as iron source (30). In Chile, the research on the hemoglobin fortified cereal (74-76) as well as the use of a bovine heme iron concentrate in the fortification of biscuits had been carried out (29). Comparing with other food fortification strategies, the food to food fortification posses

more advantageous point than that of others. This might ground on the fact that there are different fortification sources, which can be selected appropriately for use as source of iron in each location. Among the fortificants used in food to food strategy, animal blood such as chicken and porcine blood are selected more frequently than other sources. Blood as a waste can be easily taken from the slaughterhouse. Therefore, the use of chicken or porcine blood as iron sources is a value-added strategy, as well.

2.10.2 Selection of food vehicles

There are four criteria for selecting an appropriate food vehicle for fortification. First, the food vehicle should be staple food consumed throughout the year by the large proportion of the target population, especially for young children, adolescents and pregnant women. Second, the level of fortification must be such that it contributes significantly to nutritional requirement and it should be no risk of over consumption. Third, the fortificants should not affect the organoleptic properties (e.g. taste, smell, appearance, texture), physical structure or shelf life of the food vehicle. Finally, the fortified product should not cost significantly more than the unfortified food (23). Snack food is one of the eligible foods that have been selected as a vehicle for iron fortification. Successful methods of fortifying common snack foods such as cookies, biscuit, and extruded cereals with animal blood have been developed in Chile, China, Peru, Taiwan and Russia (29, 30, 76-77).

2.11 Utilization of Animal Blood in Food Products

2.11.1 Animal blood and its nutritive value

The blood is a visceral scarlet-colored fluid confined to the circulatory system of the animal body (78). The content of blood is estimated about 3-4% of animal live weight (79). In cattle and hogs, the yield of blood has been reported to be 10-12 liters and 2.5 liters/animal, respectively (80). The amount of blood in chicken ranges between 3.2-3.5% of their body weight (80). The blood recovered from slaughtered animals can be classified as inedible and edible by-products (81-83). Edible blood by-product was used as human food whereas an inedible by-product was used for different purposes such as fertilizer or feed ingredients (79-80). Customarily, the use of blood in foods is prohibited in some religions such as Muslims and Jews (78-79).

Whole blood is a liquid with dry matter content of 18-20%, of which 90-95% is protein (79). Although blood is a source of protein and essential amino acids, it is deficient in isoleucine and low in methionine (82). Fresh blood contains abundant of hemoglobin of about 125 mg/g wet weight and the iron of about 0.44 mg/g wet weight (84). Blood is the excellent source of heme iron (80, 84). Iron contents in chicken, porcine and cattle blood are 23.9, 20.4 and 44 mg/100 g, respectively (85). The iron contents in fresh ox and pig blood are about 49 and 55 mg/ 100 g, respectively (86). Level of hemoglobin in animal blood varies with age, sex, nutrition, pregnancy, lactation, disease and living altitude (87).

Iron contents of cooked chicken and porcine blood curds are 9.94 and 16.18 mg/100 g, respectively (27). Heme iron content of cooked chicken and porcine blood curd were 8.6 and 14.2 mg/100 g, respectively (27). A study in Taiwan indicated that porcine blood curd contained a higher iron content of about 22.4 mg/100 g, of which

18.2 mg/100 g is heme iron (28). Other forms of blood product are blood powder and bovine hemoglobin concentrate which usually contains a much higher iron content i.e. 230-260 and 280 mg/100g, respectively. (30, 74).

2.11.2 Processing of animal blood

(a) Preparation of porcine blood curd

Sodium chloride was added in to porcine blood and stirred throughly. A liquors of the blood were transferred into the cup. The blood were boiled until being curd and then were removed and cooled in cool water.

(b) Preparation of chicken blood curd

About five ml of sodium chloride (NaCl) solution was poured into a 300 ml cup using for chicken blood collection. The chicken was bled by cutting the jugular vein. Into each cup, about 300 ml of blood was collected through the collecting funnel. The blood in cup will clot in a short period of time after collection. The clot blood was then transferred into boiling water for 15-20 minutes, and immediately put into cold water. The chicken blood curd is used as an alternative food source for iron.

In addition to human food, animal blood can also be used as animal feed, laboratory reagent, medical preparation, industrial use, and as fertilizer (79). Commercially, animal blood is also produced in dried form as shown in (Figure 3), since it can reduce storage and transportation costs (78).

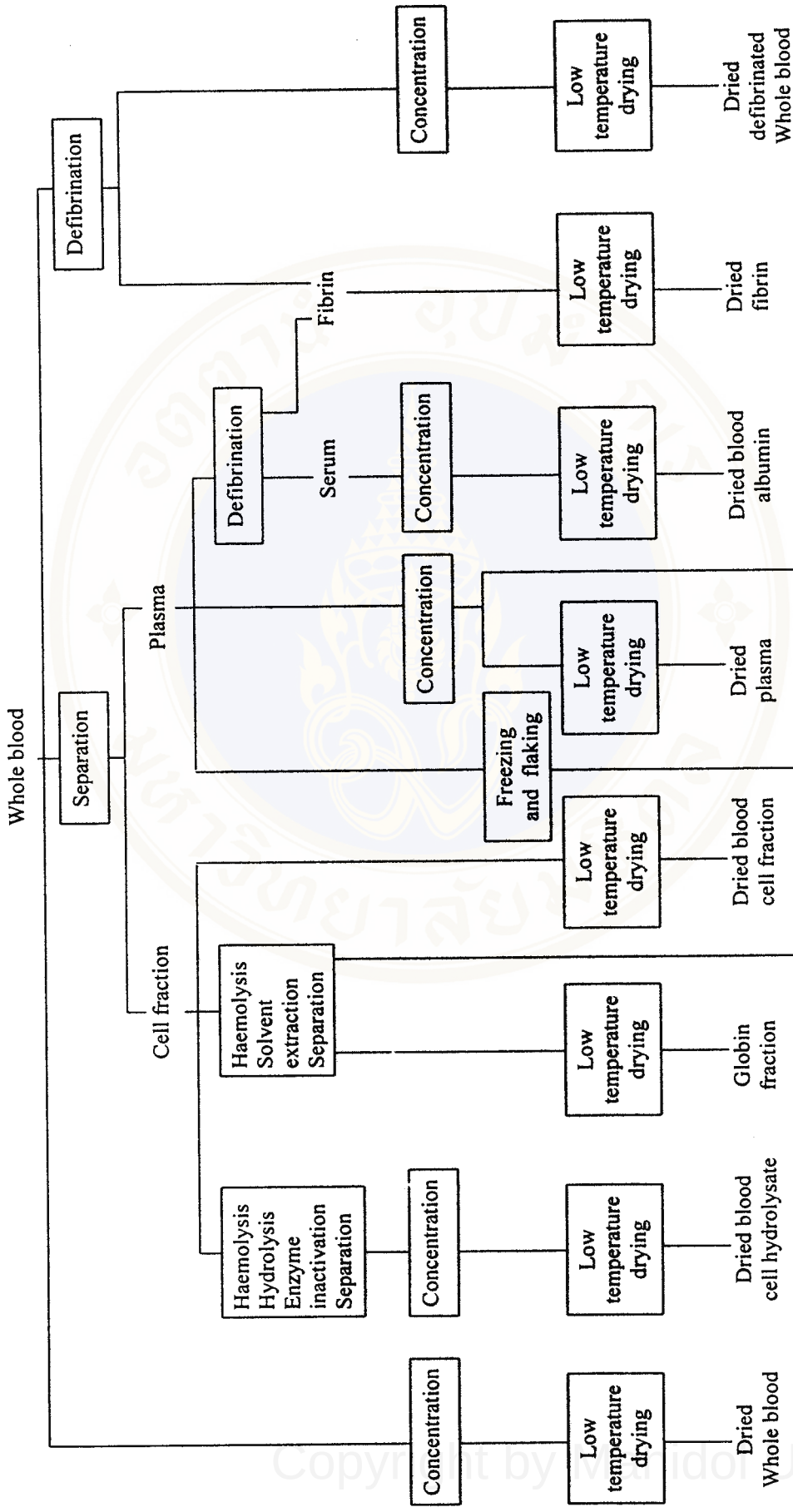

Figure 3 Outline scheme for the processing and fractionation of whole animal blood. Source Vernam and Sutherland 1995 (83).

2.11.3 Functional properties of hemoglobin in food product

Hemoglobin is a highly soluble protein and has high capacity to emulsify fat and develop stable foam (82). It has a very good capacity to bind water upon swelling and does not produce gel upon heating but becomes creamy (81). In the production of biscuit, about 50% of egg could be replaced by blood protein (82). Isolation of globin removes the color problem and appears to be beneficial with respect to the functional properties of the protein (81).

2.11.4 Animal blood containing food products

In many European countries, animal blood is utilized in sausages, black pudding, soup, bread, cookies, and pancake. The addition of 1-2.5% of whole blood to sausage improved the color, flavor and meat taste (82). In Europe, the main ingredient in black pudding is fresh blood which accounts for 48% (83). In Thailand, fresh blood is sometimes added into certain local dishes. Cooked chicken and porcine blood curd are used in certain kinds of curry (88-89). Similarly, porcine blood curd is one of the popular dishes in Taiwan (28). Chiang et al. (1995) used 10-25% fresh porcine blood as raw material in extruded product (76). Fajvisevskij et al. (1993) conducted an experiment on extrusion technology of processing and utilization of slaughterhouse blood and concluded that extrusion processing of blood and cereal mixture is a useful method for manufacturing of blood containing foods for use as a dietary iron source (90). Kocovski et al. (1985) used 15% bovine blood (2.5% of dried hemoglobin) in meat products (frankfurters, Kraljevska sausage, hamburgers and meat patties) and found that total iron content increased by a factor of 3.08-3.33 compared to conventional products. Moreover the proportion of Fe^{2+} in total iron was increased (91). Asenjo et al. (1985) used 6% bovine heme iron concentrate in the

fortification of biscuit. At this level, the product contains adequate iron and protein quantities (29). Xiuyun et al. (1987) fortified biscuit with dried blood and the product contained 15 mg Fe/100 g (30). Benyasut (1988) fortified chip with cooked porcine blood, it was found that the fried chip contains 0.83 mg Fe/serving (92).

(a) Effect of animal blood on sensory quality of food products

In order to utilize animal blood for human consumption and take advantage of its iron content, acceptable product has to be obtained. As the amount of animal blood is increased in the food product; end product become darker in color (29). Benyasut (1988) found that the chip fortified with porcine bloods of more than 30% is unacceptable due to the intense color (92). The color is therefore the main sensory quality problem of this kind of product (29, 75, 92-93). The dark color is due to oxidation of heme into hemin as hemoglobin is heated (82). Slinde and Martens (1982) illustrated that the dark color of the sausages appeared after the sausages were heated (94). The dark color problem can be decrease with the emulsification of blood in a pressure homogenizer (81). Addition of animal blood in food product also causes off-flavor (29, 75, 93,). It was found that off-flavor in sausage could be observed as animal blood had been added up to 3.5% (94). Hertrampf et al. (1990) reported that fortification extruded rice flour with 5% bovine hemoglobin concentrate (BHC) resulted in unacceptable end product due to the dark color and lack of taste (75). Asenjo et al. (1985) reported that biscuit fortified with 4% BHC could be acceptable but not for the biscuit fortified with 8% BHC (29). The unacceptable blood taste was also observed in improperly cooked blood replaced meat sausage (94).

(b) Bioavailability of iron from animal blood fortified in food products.

The capacity of iron in foods and diets to meet body requirements depends on the content of dietary iron and iron bioavailability (54). The bioavailability of iron can be defined as the capability of iron in food or diet that can be absorbed within the body (32). Factors affecting iron absorption and utilization regarding to food ingredient and composition have been studied. Food processing and preparation can also have a significant effect on both the iron bioavailability and the ratio of heme to nonheme iron in food (28, 65-66, 70,96). The use of heme iron fortified cereal with bovine hemoglobin concentrate (BHC) at 5% level provides an appropriate amount of absorbed iron and adequate energy density and a protein which could complement milk protein for infant (74). The use of an extruded rice flour fortified with a bovine hemoglobin concentrate containing 14 mg Fe/100 g of powder showed that the iron fortified cereal has a high iron bioavailability, good protein quality and amino acid score (75). The hemoglobin iron bioavailability of the biscuits fortified with 6% of a bovine hemoglobin concentrate in school-age children showed that heme-iron absorption was about 19.7% (77). In China, it was found that when about 30 g of the blood fortified wheat flour biscuit containing 15 mg Fe/100 g was offered daily for 42 days to the 65 primary school children (age 8-10), both the hemoglobin and the hematocrit of the experiment group were higher than in the control group (30). Such evidence indicated that food products fortified with animal blood sources provide high iron bioavailability.

2.12 Shelf stability test of food products

The shelf stability of food is the time that the quality of the product declines to an unacceptable level or to a minimum acceptable level. The length of the shelf life of any product depends on processing method, packaging, and storage condition. The major causes of food product deterioration during storage can be divided into biological, chemical, and physical factors. At one time many forms of deterioration may take place, depending on the food and environmental conditions. The deterioration of food product may include losses in sensory desirability, nutritive value, safety and aesthetic appeal (97).

2.12.1 Sensory evaluation

Measuring the sensory properties and determining the importance of these properties as a basis for predicting acceptance by the consumer represent major accomplishments for sensory evaluation (98). Sensory evaluation defines as a scientific discipline used to evoke, measure, analyze and interpret reactions to the characteristics of food and materials as they are perceived by the sense of sight, smell, taste, touch and hearing (99). The human senses play a ubiquitous role in food acceptance and it is used to accurately describe the sensory properties of food (80). In the sensory evaluation process, all characteristics of the tested food must be controlled to minimize or counterbalance extraneous factors that affect judgment. When the appropriate method, qualified panelists, experimental design and method for analysis were considered and applied, sensory evaluation can be the objective criteria (96). Moreover, the detail of preparation for serving the product must be considered at quiet area free from distraction, with controlled lighting, partitions between panelist to minimize visual contact and neutral colors of walls (98).

(a) Hedonic scale

The hedonic relates to pleasant and unpleasant states of an organism. It indicates the affective rate of preference or liking and disliking (100). Hedonic scale is an important tool for evaluating sensory acceptability of a product. Since, the test question is subjective, high number of qualified tester (at least 50) is usually required. This scale consists of a series of 5 and 9 labeled scale points ranging from 1 = 'dislike extremely' to 5 or 9 = 'like extremely', with a neutral category of 3 or 5 = ' neither like nor dislike' (98). The data obtained from this method were analyzed by using the parametric statistical analysis such as analysis of variance. The analytical results can provide information on product differences (98).

(b) Just-about-right scale

The just-about-right scale is most frequently encountered in larger scale consumer testing. These scales have three or five categories, usually anchored with statements of too much, too little, or about right for each product attribute. The just-about-right scales are championed as a diagnostic tool for consumer tests. These scales combine attribute intensity and preference in a single response, and are highly susceptible to interpretive and/or semantic errors because the product attribute to be measured is given a name. The analyses of data from these scales also present numerous problems. Frequently, only the percentage responding in each category is reported without any rule for determining how much of a difference between percentages is to be considered significant (98). However, just-about-right scale is the only level available for determining the suitability of the intensity of an attribute in food product. Such in product can be very useful in the product quality process.

(c) Line scale

Line scale is a consistent method with the concept of functional measurement and graphic rating-scale approach generally used for sensory evaluation of the products. Although various forms of the line scales have been used for product evaluation for many years, the philosophical approach developed by Anderson and his co-worker in the year 1970 proved to be especially useful (98). .A line with the length of 6 inch with word anchors located ½ inch from each end is generally used for the evaluation purpose. In metric term, the line length can be 15 cm to be fully effective, with anchors located at approximately 1.5 cm from each end. The specific length of the line, however, varied according to the specific research. In many case, line scale is used for measuring the intensity of an attribute in food. The scale direction always goes from left to right with increasing intensity, i.e. weak to strong, light to dark or some similar designated set of word anchors.

The subject's task is to make a vertical line across the horizontal line at the point that best reflects the relative intensity for that attribute to the tested products. This mark is later converted to a numerical value by measuring the distance from the left end of line. The responses obtained from each subject are used directly in statistical analysis, that is, no transformations are necessary and the monitoring of response patterns is directly achieved (98).

2.12.2 Water activity (Aw)

Water activity is defined by the ratio of the vapor pressure of food substrate or solution to the vapor pressure of pure water at the same temperature (101). Pure water has a water activity of 1.00, while fresh food and food products vary in water activity, which is ranged from 0.20-1.00 (102). Foods contained high water content is usually

reported deterioration due to biological and chemical changes (102). Furthermore, water activity is a major influence on growth of mold, yeast and bacteria in food (103). It is well accepted that the water activity at 0.91 is the lowest point that most of spoilage bacteria can grow, whereas spoilage molds can grow at the water activity as low as 0.80. The food poisoning bacteria has been found to grow at Aw as low as 0.86 (101). The lowest water activity value for halophilic bacteria, xerophilic mold and osmophilic yeast has been found to grow at Aw of 0.75, 0.65 and 0.61, respectively (101). The limiting value of water activity for the growth of any microorganisms in food is about 0.6 (103). Therefore the products such as cookies, crackers, bread crusts and so on , which have Aw of about 0.3 do not allow any microbial proliferation (Table 3).

The interaction of water activity with temperature, hydrogen ion, oxygen, carbon dioxide and inhibitory substances can make effective in preventing the microorganisms growth. When any of those factors is sub optimal, the inhibitory effect of reduced water activity tends to be enhanced (102).

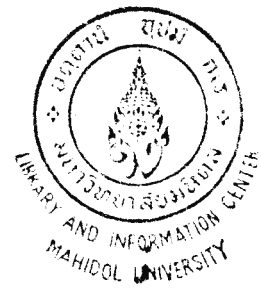

Table 3 Water activity and growth of microorganisms in food

Range A_w	Microorganisms generally Inhibited by lowest A_w in this range	Food generally with in food
1.00-0.95	Pseudomonas, Escherichia, Proteus, shigella, klebsiella, Bacillus, Clostridium perfringens, some yeasts	Highly perishable(fresh) foods and canned fruits, vegetables, meat, fish, and milk; cooked sausages and breads; foods containing up to approximately 40%(w/w) sucrose or 7% sodium chloride
0.95-0.91	Salmonella, Vibrio parahaemolyticus, C. botulinum, Serratia, Lactobacillus, Pediococcus, somemolds, yeasts (Rhodotorula,Pichia)	Some cheeses(cheddar, Swiss, Muenster, provolone), cured meat (ham), some fruit juice concentrates; foods containing 55%(w/w) sucrose or 12% sodium chloride
0.91-0.87	Many yeasts(Candida, Torulopsis, Hansenula), Micrococcus	Fermented sausage(salami), sponge cakes, dry cheese, margarine; foods containing 65%(w/w) sucrose (saturated) or 15% sodium chloride
0.87-0.80	Most molds(mycotoxigenic penicillaia), Staphylococcus aureus, most Saccharomyces(bailli) spp., Debaryomyces	Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrups; flour, rice, pulses containing 15-17% moisture; fruit cake, country-style ham, fondants, high-ratio cakes
0.80-0.75	Most halophilic bacteria, mycotoxigenic aspergilli	Jam, marmalade, marzipan, glace'ed fruits, some marshmallows
0.75-0.65	Xerophilic molds(Aspergillus chevalieri, A. Candidus, Wallemiasebi), Saccharomyces bisporus	Rolled oats containing approximately 10% moisture, grained nougats, fudge, marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts
0.65-0.60	Osmophilic yeasts(Saccharomyces rouxii), few molds(Aspergillus echinulatus, Monascus bisporus)	Dried fruits containing 15-20% moisture, some toffees and caramels; honey
0.50	No microbial proliferation	Pasta containing approximately 12% moisture; spices containing approximately 10% moisture
0.40	No microbial proliferation	Whole-egg powder containing approximately 5% moisture
0.30	No microbial proliferation	Cookies, crackers, bread crusts, and so on containing 3-5% moisture
0.20	No microbial proliferation	Whole milk powder containing 2-3% moisture, dried vegetables containing approximately 5% moisture, corn flakes containing approximately 5% moisture, country-style cookies, crackers

Source: Beauchat (1981), (104)

2.12.3 Peroxide value

The peroxide value, an indicator of primary oxidation of fat and oil, was observed to increase during processing and storage (105). The deterioration of fats and oils take place by oxidation process (106). The rate of oxidation increases with an increase in temperature, exposure to oxygen in the air, the presence of light and contact with materials that are classified as pro-oxidants (107). An excellent example of a pro-oxidant is nonheme iron (68). Lipid oxidation renders the product unacceptable because of the production of rancid off-flavors and off-odors (108). Deterioration of cookies were generally due to loss of flavor or acquisition of stale flavors due to chemical changes such as the oxidation reaction (109). The cookies have a distressing tendency to turn rancid because of their relatively high fat content (109). For the fish cracker and some other fried snacks, the deterioration in quality due to oxidation of lipids in the product since these products contain high oil content (107). The observations of peroxide value usually support the results of sensory evaluation (105).

CHAPTER III

MATERIALS AND METHODS

3.1 Selection of Iron -rich Foods

The food was primarily selected from the Food Composition Tables of Division of Nutrition, Ministry of Public Health of 1987 and 1992 (85, 110). The selection was based on the following criteria:

(i) The food must be from animal source and contain high iron content in heme form.

(ii) The food should be normally consumed, affordable and accessible.

(iii) The food must be available throughout the year.

The selected foods were bought from the nearby market and analyzed for total iron and moisture contents. Cost of iron per mg in each food was then calculated. Table 6 indicated the list of selected foods being analyzed. Blood curd was selected since it could serve the mentioned criteria.

3.2 Selection of Food Vehicle

Traditional Thai chip "Kow krieb pla" (fish tapioca chip) and chocolate cookies were chosen to be the vehicles for blood curd fortification since they were widely consumed all over the country. They also had good masking qualities (dark color and strong odor) and reasonable shelf life under normal conditions.

3.3 Development of Iron-rich Food Products

3.3.1 Preparation of pressed blood curd

Cooked chicken and porcine blood curds were used as iron-rich raw materials in this study. Blood curd was cut into block of 2x2x2 cubic centimeters, placed into a nylon bag, and later pressed in a hydraulic press machine (SAKAY 10 TURBO, M310RZ). The pressed blood curd was then minced in an electric chopper (National chopper, MK-C 300N). The minced blood curd was used as iron fortificant in blood fortified products at the levels of 10, 20, 30 and 40 percent.

3.3.2 Preparation of iron-rich food products

(a) Cookies

The ingredients used for blood-fortified cookies were listed in Table 4. Margarine and salt were mixed together in a Kitchen Aid™ mixer (Heavy Duty, Model K5SS, USA) for 5 minutes until soft. Minced blood curd was then added and beaten at medium speed for 15-20 min until fluffy. Sugar was gradually added, and mixed until the mixture became creamy. Eggs and flavors were added and beaten vigorously until again smooth and creamy. The mixture was then mixed for another 1 minute at medium speed. Other ingredients were later added slowly and mixed thoroughly. The mixture was chilled until firm enough, and then formed into a roll of 4 cm dia. on a wax paper. The roll was wrapped with wax paper, chilled until firm (2 h), cut for about 6 mm thick, and baked on greased cookies sheet at 175 °C for 15 min. Cookies were removed from the oven and cooled on a cooling rack. Cookies was packed in a sealed polypropylene bag and kept in a dark plastic-can during the shelf-life study. The preparation method of blood-fortified cookies is shown in Figure 4.

Table 4. Ingredients (as percentage) used for preparation of blood-fortified cookies

	Fortification level (%)			
Ingredient	10	20	30	40
Minced chicken blood or Minced porcine blood	10	20	30	40
Cake flour	32.73	32.73	32.73	32.73
Baking powder	0.29	0.29	0.29	0.29
Cocoa powder	0.90	0.90	0.90	0.90
Baking soda	0.22	0.22	0.22	0.22
Margarine	22.42	22.42	22.42	22.42
Salt	0.17	0.17	0.17	0.17
Sugar	17.94	17.94	17.94	17.94
Whole egg	8.97	8.97	8.97	8.97
Chocolate flavor	0.34	0.34	0.34	0.34
Butter flavor	0.34	0.34	0.34	0.34
Roasted, crusted peanuts	15.69	15.69	15.69	15.69
Total	110.01	120.01	130.01	140.01

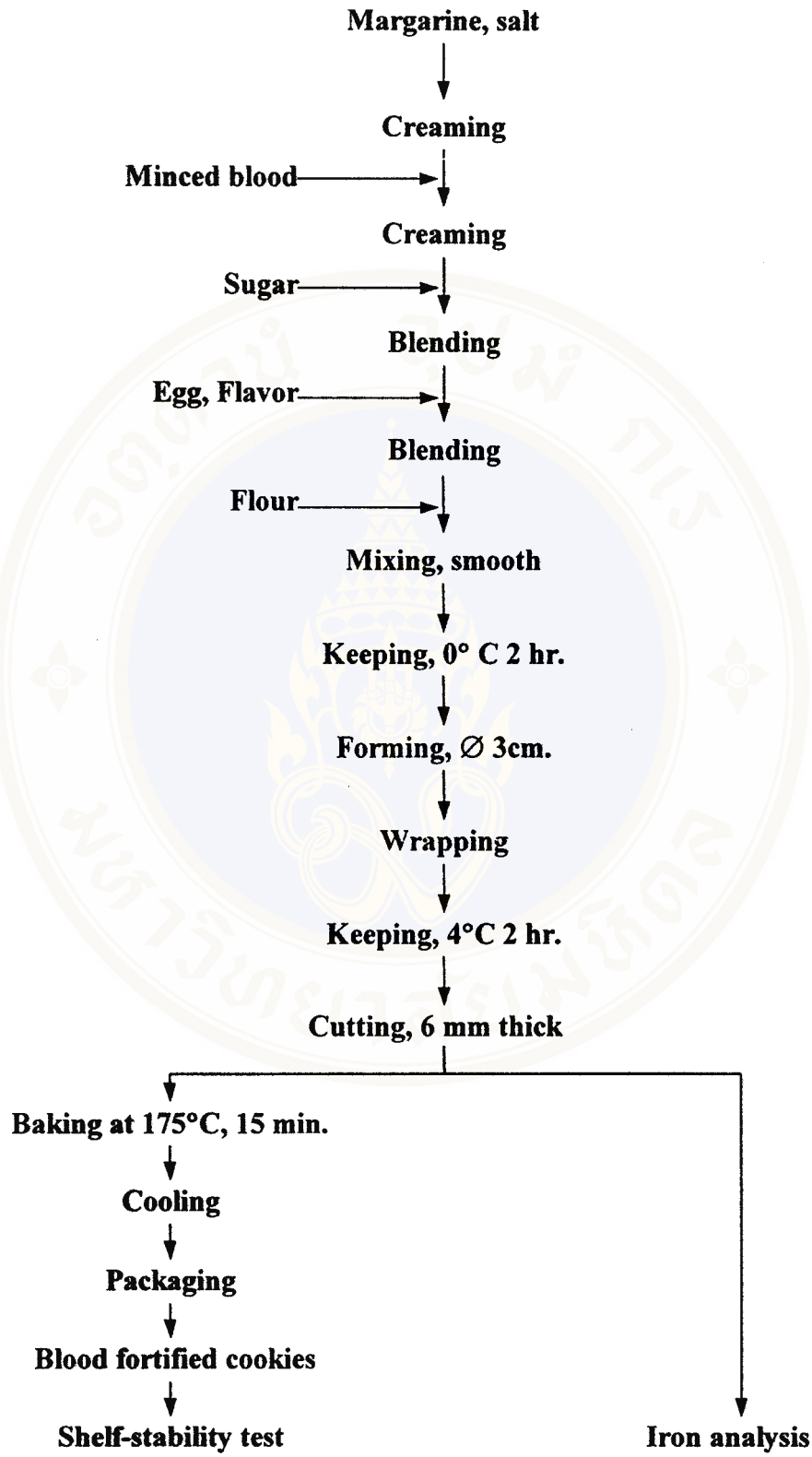

Figure 4 Flow diagram for the processing of blood fortified cookies

(b) Traditional Thai chips (Kow krieb pla)

The ingredients used for blood-fortified chips were listed in Table 5. Flour mixture (tapioca flour and all-purpose flour) and seasonings (pepper, sugar, salt and monosodium glutamate) were mixed in a Kitchen Aid™ mixer (Kitchen Aid Heavy Duty model K5SS, USA) until homogenous. Minced garlic, fish sauce, minced fish, minced blood curd and water were added and again mixed. The mixture was then removed from the mixing bowl and hand-kneaded until a homogeneous dough was obtained. The dough was formed into a roll of 25-30 cm long and 3 cm dia. The roll were steamed for 40 min., cooled on a rack at room temperature, and chilled at 5-10°C for overnight or until firm. The dough was then sliced to a thickness of 1.1-1.5 mm and dried in a hot air oven at 60 °C for 3 h. The dried chip was deep-fried with palm oil at 200°C and packed in polypropylene bag. Finished product was kept in a dark plastic-can during the shelf-life study. The preparation method is shown in Figure 5.

Table 5. Ingredients (as percentage) used for preparation of blood-fortified chips

	Fortification level (%)			
Ingredient	10	20	30	40
Minced chicken blood or Minced porcine blood	10	20	30	40
Tapioca flour	58.39	58.39	58.39	58.39
All-purpose flour	5.84	5.84	5.84	5.84
Pepper	1.17	1.17	1.17	1.17
Salt	0.88	0.88	0.88	0.88
Sugar	3.50	3.50	3.50	3.50
Monosodium glutamate (MSG)	0.44	0.44	0.44	0.44
Minced garlic	5.84	5.84	5.84	5.84
Fish sauce	3.50	3.50	3.50	3.50
Minced fish	20.44	20.44	20.44	20.44
Total	110	120	130	140

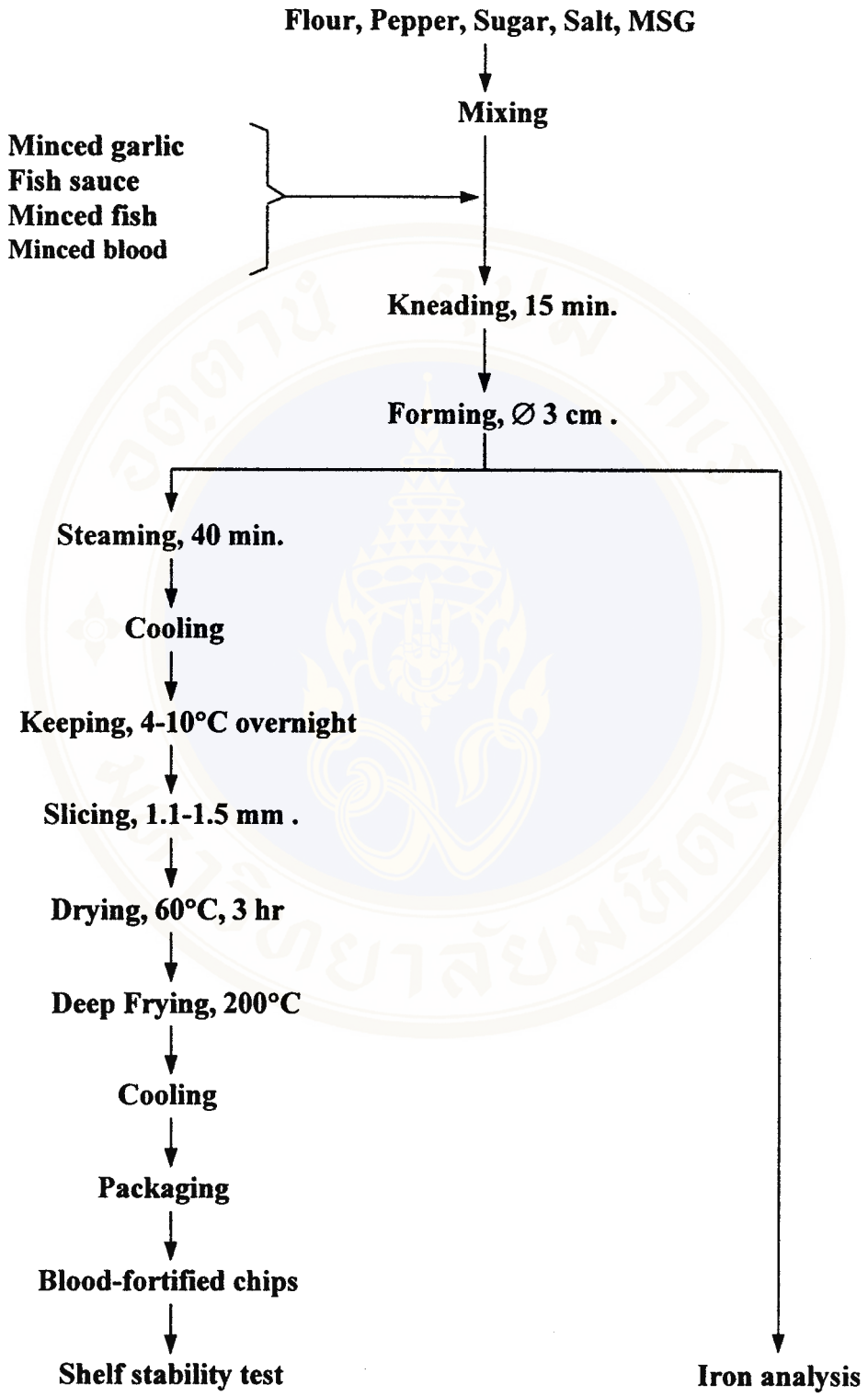

Figure 5 Flow diagram for processing of blood fortified chips

3.3.3 Selection of the developed iron-rich food products for further study

The products developed in the experiment 3.3.2 were selected for the further studies by using sensory acceptability test. One piece of product packed in a sealed 12.5x7 cm polypropylene bag was labeled with a three-digit random number. Products of four different fortification levels were randomly served to each subject. Panelists were asked to rinse their mouth with soda and distilled water in between each sample. The test was conducted at the sensory science laboratory by 45 panelists, who were staffs and graduate students at the Institute of Nutrition, Mahidol University (INMU). The sensory evaluation included (i) nine-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely) for general appearance and overall acceptability, (ii) five-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much) for odor acceptability, (iii) five-point just about right (1, too light; 3, just about right; 5, too dark) for color, taste and texture suitabilities, and (iv) fifteen centimeter line scale (1, none; 15, too much) for degree of off-flavor. In case of blood-fortified chip, the suitability of crispness was additionally evaluated on a five-point just about right scale. The questionnaires used in the sensory test are illustrated in Appendix A and B.

The difference among mean scores of each sensory acceptability characteristic was statistically analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test on SPSS/PC^{+} software. The samples that obtained mean overall acceptability score of five and above were then used for the further study.

3.4 Iron analysis

Total iron, was analyzed in duplicate to select the iron-rich raw materials used for product development. Heme and non heme iron were analyzed in triplicate to evaluate iron content in blood curds and changes in heme iron content during preparation and storage of the developed products. The differences among mean scores of iron contents during processing were statistically analyzed using t-test. With the mean different of nonheme iron contents during storage was analyzed by using one-way analysis of variance (ANOVA) and Duncan multiple range test on SPSS/PC^{+} software. The methods for iron analyses were described as follows.

3.4.1 Total iron (TFe)

Dry ashing technique was used to prepare samples for the determination of total iron. Ash was dissolved in 4 N nitric acid solution and then determined for iron content using an atomic absorption spectrophotometer (Model Spectr AA-20, Varian Associated, Australia) set at a wavelength of 248.3 nm. (111)

3.4.2 Heme Iron (HFe)

Heme iron values were determined by the Hornsey method for total pigment analysis (112). The samples were extracted in acid-acetone solution. The supernatant was filtered through paper filter (Whatman no. 42). The absorbancy of the filtrate at 640 nm (Spectronic 21, BAUSCH & LOMB^{TM}) was measured. Heme iron in the sample was calculated. Detail of the analytical method is illustrated in Appendix C.

3.4.3 Nonheme Iron (NHFe)

Nonheme iron was determined according to Schricker et al. (1982) with the modifications of Rhee and Ziprin (1987) (65, 113). Heme iron was separated from the

sample by digestion with sodium nitrite ($NaNO_2$) and then precipitating the bound heme iron by using trichloroacetic acid (TCA). The supernatant was determined for free nonheme iron using absorbancy at 540 nm in spectrophotometer (Spectronic 21, BAUSCH & LOMB™). The method for nonheme iron determination is illustrated in Appendix D.

3.5 Proximate analysis

3.5.1 Moisture

The moisture content was determined by drying the sample in 80 mm diameter and 30 mm high glass pots. The drying process was carried out under mercury level of 30 mm at 70°C in the vacuum-oven until a constant weight was obtained (102).

3.5.2 Protein

The nitrogen content of the sample was analyzed using the Macro-Kjeldahl method. Crude protein was calculated by multiplying the nitrogen content by 6.25. (111)

3.5.3 Fat

The sample was hydrolyzed by 4 N hydrochloric acid and dried in a hot-air oven at 60°C. The dried sample was extracted with petroleum ether in a soxhlet apparatus (Soxhlet System HT™ 1043 Extraction unit). Samples was dried in hot-air oven at 60°C to constant weight. (111)

3.5.4 Ash

Dry ashing method was used for the determination of ash content. Sample was burnt on a hotplate, and transferred to a muffle furnace and burnt at 450°C. The residue was weighed. (111)

3.5.5 Carbohydrate

Amount of carbohydrate was calculated by subtracting the percentages of crude fat, crude protein, moisture content and ash from 100. (111)

3.5.6 Energy

Energy was determined by calculation using the following equation: Energy (kcal/100g) = (% Protein x 4) + (% Fat x 9) + (% Carbohydrate x 4). (111)

3.6 Shelf Stability Test

The selected products packed in a 12.5x7 cm heat-sealed polypropylene bag were kept in the dark plastic-can and stored at room temperature for 30 days. The product was sampled at 0, 15 and 30 days for the following evaluations.

3.6.1 Changes of iron forms

The selected products were analyzed for the heme and nonheme iron contents at the 0^{th} day. During storage, changes in nonheme iron content were analyzed at the 15^{th} and 30^{th} days.

3.6.2 Changes in sensory qualities

The sensory quality changes of the selected products were evaluated by using the method mentioned in method 3.3.4. However, the tests were conducted using 40 panelists.

3.6.3 Changes in physical property

Water activity (A_w) was the physical property used for the shelf stability test of the selected product. The sample was cut into small pieces and placed in the chamber of water activity meter at 24°C ±1°C for 30 min using Novasina^{TM} MIK 3000 instrument (NOVASINA AG, Switzerland).

3.6.4 Changes in chemical quality

Change in peroxide value (PV) during storage could be an indicator for development of rancid flavor during storage. Lipid in sample was extracted by using the mixture of methanol and chloroform, and evaporated in a rotary vacuum evaporator (Eyela Tokyo Rikakiki Co., Ltd.). The extracted oil was dissolved in glacial acetic acid and chloroform, and then potassium iodide was added. The solution was titrated with 0.01 N sodium thiosulphate solution ($Na_2S_2O_3.5H_2O$) by using starch solution as an indicator.

3.7 Cost estimation

The cost was estimated only for the raw materials not including other costs. The estimation was performed during 1996 to 1997. Prices of the food commodities were obtained from 7 markets i.e. Thevej, Bangsue, Klongtoei, Bangkapi, Salanumron, Bangkhae and Phuttamonthon.

CHAPTER IV

RESULTS

4.1 Selection of Iron-rich Foods from Animal Source

4.1.1 Iron content and cost of iron-rich food from animal sources

Total iron content of iron-rich foods and their costs are shown in Table 6. The contents were different among sources. The first three highest iron-rich foods included cooked chicken blood curd, cooked porcine blood curd and fresh porcine spleen. However, the cost per milligram of iron for cooked chicken and porcine blood curds are much lower than the costs of other sources. Both kinds of cooked blood curds were therefore selected for use as the fortificants in the study. Since blood curd contained high water content which might interfere in the further formulation process. The curd was therefore pressed in order to remove some water . Table 7 showed that total iron content in the pressed blood curd (wet basis) became higher than in the unpressed one due to water loss. However, the iron content as dry basis of the unpressed blood curd was higher than the pressed one, which affected the cost of the pressed cooked blood curd to be slightly higher (Tables 6 and 7).

Table 6 Content and cost of iron in different iron-rich foods.

Iron-rich food	Total Iron mg%(wet basis)	Total Iron mg% (dry basis)	Cost of Iron[1] Baht/mg Fe
Anchovy, dried	2.81	4.39	3.20
Anchovy, boiled, dried	6.49	9.14	1.54
Anchovy, big, boiled, dried	4.35	6.21	1.61
Anchovy, small, boiled, dried	2.46	3.51	12.20
Mussel, dried	12.85	17.60	1.56
Chicken blood, cooked	15.00	220.59	0.09
Porcine blood, cooked	15.30	246.77	0.08
Porcine spleen, fresh	35.67	187.74	0.20
Porcine lung, fresh	4.51	23.74	0.51
River shrimp, fresh	2.70	13.50	2.22
River shrimp, dried	16.00	19.05	0.63
Tuna dark flesh, cooked	6.11	21.82	0.13

[1]Baht, Thai monetary unit (US $ 1 = 25 Baht at the experimental period, 1995-1996).

Table 7 Content and cost of iron in pressed cooked chicken and porcine blood

Pressed cooked blood	Total Iron[1] mg% (wet basis)	Total Iron[1] mg% (dry basis)	Cost of Iron[2] Baht/mg Fe
Chicken blood	46.85	194.00	0.13
Porcine blood	51.45	194.89	0.10

[1] Baht, Thai monetary unit (US $ 1= 25 Baht at the experimental period, 1995-1996).

4.1.2 Variation of iron contents in cooked blood curds from different sources

Heme, nonheme and total iron contents of unpressed and pressed cooked chicken and porcine blood curds from 6 different sources are shown in Table 8 and 9, respectively. Wide ranges of the values could be observed in both kinds of blood curd. The average values also indicated that the unpressed cooked blood curd contained higher iron content than the pressed one. Heme content in the blood curds were about 2-3 times higher than the nonheme.

4.1.3 Variation of iron contents in cooked blood curds of different batches from single source

The iron contents including heme, nonheme and total iron in cooked chicken and porcine blood curds which were purchased from Phuttamonthon market for 6 times are shown in Table 10. The contents were not significantly different among the kinds of blood ($p>0.05$). The significant differences among the unpressed and pressed blood curds were found. The pressed cooked blood curds contained higher dry matter

content than the unpressed ones ($p<0.05$). While the iron contents (dry basis) in the pressed cooked blood curds were significantly lower ($p<0.05$).

Table 8 Concentration of heme, nonheme and total iron in unpressed and pressed cooked chicken blood from 6 sources[1]

Sources	Unpressed Chicken Blood			Pressed Chicken Blood		
	mg /100 g (dry basis)			mg /100 g (dry basis)		
	Heme[2]	Nonheme[2]	Total Iron[3]	Heme[2]	Nonheme[2]	Total Iron[3]
1	178.33	97.04	275.37	136.08	79.34	215.42
2	157.02	71.12	228.14	155.18	54.56	209.74
3	141.92	69.36	211.28	132.18	53.17	185.35
4	114.42	54.38	168.80	107.50	41.75	148.25
5	125.71	72.47	198.18	114.86	61.70	175.56
6	165.67	70.70	236.37	138.11	53.03	191.14
mean±SD	147.18±24.38	72.51±13.75	219.69±36.26	130.65±17.17	57.53±12.57	187.58±24.39

[1]Samples were purchased from the following markets: Thevej, Bangsue, Klongtoy, Bangkapi, Salanumron and Bangkhae.
[2]Values represents the mean of triplicate analyses and the unit is in mg per 100 g of dry basis.
[3]Total Iron content = Heme + Nonheme.

Table 9 Concentration of heme, non-heme and total iron in unpressed and pressed cooked porcine blood from 6 sources[1].

Sources	Unpressed Porcine Blood			Pressed Porcine Blood		
	mg /100 g (dry basis)			mg /100 g (dry basis)		
	Heme[2]	Nonheme[2]	Total Iron[3]	Heme[2]	Nonheme[2]	Total Iron[3]
1	177.69	77.06	254.75	162.80	62.40	225.19
2	153.79	68.14	221.93	145.23	58.26	203.49
3	165.43	74.53	239.96	94.44	66.03	160.48
4	172.19	58.70	230.90	112.99	46.71	159.70
5	181.97	70.80	252.77	142.66	63.32	205.97
6	192.04	77.38	269.43	147.79	54.80	202.60
mean±SD	173.85±13.32	71.10±7.06	244.96±17.37	134.32±25.40	58.59±7.04	192.91±26.73

[1]Samples were purchased from the following markets: Thevej, Bangsue, Klongtoy, Bangkapi, Salanumron and Bangkhae.
[2]Values represents the mean of triplicate analyses and the unit is in mg per 100 g of dry basis.
[3]Total Iron content = Heme + Nonheme.

Table 10 Dry matter (DM), heme (HFe), nonheme (NHFe) and total iron (TFe) contents in unpressed and pressed cooked chicken (CB) and cooked porcine blood (PB)[1] of 6 batches from Phuttamonthon market.

	Process	
Qualities of each Blood source	Unpressed	Pressed
DM[2](%) CB	7.66±0.46[c]	24.70±1.43[b]
	(7.11-8.41)	(22.84-27.02)
PB	7.47±0.66[c]	27.06±2.14[a]
	(6.34-8.16)	(23.96-29.44)
HFe[2] (mg/100g) CB	178.83±13.21[a]	140.71±17.94[b]
	(164.19-195.56)	(113.78-160.93)
PB	182.89±12.69[a]	145.86±19.05[b]
	(159.21-197.34)	(117.04-169.44)
NHFe[2] (mg/100g) CB	66.15±3.50[a]	55.56±7.11[bc]
	(62.13-70.16)	(49.09-66.54)
PB	62.76±6.40[ab]	50.11±6.15[c]
	(53.89-71.69)	(42.51-59.99)
TFe[2,3](mg/100g) CB	244.98±13.83[a]	196.27±21.03[b]
	(226.32-262.37)	(171.13-227.47)
PB	245.65±12.88[a]	195.97±16.37[b]
	(225.61-261.13)	(170.51-218.23)

1Mean ± SD (Range)
[2]Mean values within the same horizontal block (same quality different sources and different processes) with different superscripts differ significantly ($p < 0.05$).
[3]Total Iron content = Heme + Nonheme.

4.2 Selection of Appropriate Fortification Levels

4.2.1 Sensory acceptability of blood fortified cookies

The sensory acceptability scores of all characteristics at different fortification levels of cookies fortified with chicken and porcine blood curds are shown in Tables 11 and 12, respectively. The general appearance scores of the products with lower level of fortification were higher in both kinds of blood however insignificance in case of chicken blood. Colors of the products with higher degree of fortification were significantly darker and in the unacceptable ranges. The degree of odor acceptability tended to be lower as more blood curd was added. Taste of cookies fortified with porcine blood curd became significantly milder as more curd was added, however such evidence was not found in case of chicken blood curd. While the texture of the cookies fortified with higher chicken blood curd became significantly harder. The rating of off-flavor characteristic from 15 cm line scale was presented as mode, which most panelist rated at lower than 1 cm at all levels of fortification (except for the one that fortified with 40% chicken blood curd), however the percentage of selection became lower as the fortification level increased (Table 15). The overall acceptability scores of both kinds of cookies significantly decreased as more blood curds were added; especially in the 40% level of the porcine blood curd which was scored lower than the acceptable range of nine point hedonic scale (lower than 5). Base on the mention overall acceptability score, the cookie recipes selected for further study were 10 and 40% fortification levels for chicken blood, and 10 and 30% for porcine blood.

Table 11 Sensory acceptability[1,2] scores of cookies fortified with four levels of chicken blood curd

Fortification Level	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Texture[4]
10%	6.29±1.58	2.93±0.81^{d}	6.71±1.99^{a}	3.67±0.80^{a}	2.58±0.54	2.84±0.42^{c}
20%	6.29±1.50	3.36±0.68^{c}	6.58±1.54^{a}	3.58±0.84^{ab}	2.60±0.62	3.07±0.62^{bc}
30%	5.76±1.73	3.80±0.79^{b}	5.87±1.79^{b}	3.30±1.07^{b}	2.47±0.76	3.13±0.81^{b}
40%	5.87±1.77	4.18±0.78^{a}	5.82±1.89^{b}	3.29±0.97^{b}	2.64±0.74	3.44±0.69^{a}

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with different superscripts differ significantly ($p < 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4]Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 12 Sensory acceptability[1,2] scores of cookies fortified with four levels of porcine blood curd

Level	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Texture[4]
10 %	6.93± 1.14 [a]	3.22± 0.64 [c]	6.89 ± 1.43 [a]	3.87± 0.84 [a]	2.73 ± 0.50 [a]	3.00± 0.60
20 %	5.91 ± 1.79[b]	3.84± 0.85[b]	6.20± 1.75[b]	3.69± 0.87[a]	2.62± 0.65[ab]	3.20± 0.50
30 %	5.73± 1.70[b]	4.18± 0.78[a]	5.49± 1.66[c]	3.20± 1.08[b]	2.53±0.76[ab]	3.36± 0.83
40 %	5.67± 1.82[b]	4.22± 0.82[a]	4.98± 2.01[c]	3.13± 1.01[b]	2.40± 0.84 [b]	3.07± 1.12

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with different superscripts differ significantly ($p < 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

4.2.2 Sensory acceptability of blood fortified chips

The sensory acceptability scores of all characteristics at different fortification levels of chips fortified with chicken and porcine blood curds are shown in Tables 13 and 14, respectively. The general appearance scores of the products with lower levels of fortification were significantly higher in both kinds of blood chips. The color of the products with higher levels of fortification was darker. Odor acceptability tended to be lower but not significant as more blood curd was added. While tastes of the chips fortified with blood curd at different levels were not significantly different. The same evidence was found in cases of crispness and texture of the fortified chips. The off-flavor characteristic was also presented as mode, which were rated lower than 1 cm on 15 cm line-scale for all levels of fortification. The chips with higher levels of fortification had lower percentages of selection at $\leq$ 1 cm (Table 15). The overall acceptability scores of chips produced from both kinds of blood at different levels of fortification were not significantly different on nine point hedonic scale ($p>0.05$). As considering all characteristics concerning sensory acceptability scores especially overall acceptability scores of over 5.00, the chicken and porcine blood fortified chips at 10 and 40% of the fortification levels, were selected for further study.

Table 13 Sensory acceptability[1,2] scores of chips fortified with four levels of chicken blood curd

Fortification Level	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste [4]	Crispness[4]	Texture[4]
10%	6.27±1.76[a]	3.09±0.73[d]	6.67±1.40	3.82±0.75	2.56± 0.62	3.04±0.37	3.20± 0.46
20 %	5.93±1.84[ab]	3.69±0.76[c]	6.51±1.49	3.78±0.85	2.58±0.78	3.11±0.44	3.09±0.51
30 %	5.73±1.91[bc]	3.91±0.73[b]	6.40±1.59	3.80±0.84	2.71±0.87	3.11±0.32	3.20±0.59
40 %	5.36±2.02[c]	4.31±0.70[a]	6.22±1.74	3.58±1.06	2.60±0.81	3.22±0.60	3.20± 0.63

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with different superscripts differ significantly ($p < 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5]Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 14 Sensory acceptability score [1,2] of chips fortified with four levels of porcine blood curd

Fortification Level	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor [5]	Taste [4]	Crispness[4]	Texture[4]
10%	6.16±1.66[a]	3.29±0.66[c]	6.89±1.42	3.80±0.73	2.71±0.55	3.09±0.29	3.09±0.47
20%	5.87±1.55[ab]	3.78±0.67[b]	6.60±1.42	3.56±0.84	2.73±0.75	3.11±0.38	3.07±0.54
30%	5.51±1.65[bc]	4.33±0.56[a]	6.47±1.18	3.67±0.67	2.82±0.65	3.18±0.49	3.09±0.63
40%	5.20±1.77[c]	4.53±0.59[a]	6.64±1.17	3.73±0.72	2.76±0.77	3.13±0.34	3.07±0.62

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with different superscripts differ significantly ($p < 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4]Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 15 Frequency of rating (as %) for off-flavor intensity of the blood fortified products

off-flavor Intensity[1] (cm)	Cookie Products								Chip Products							
	Chicken blood curd				Porcine blood curd				Chicken blood curd				Porcine blood curd			
	10%	20%	30%	40%	10%	20%	30%	40%	10%	20%	30%	40%	10%	20%	30%	40%
≤1	67	60	53	36	71	64	62	56	51	49	49	44	64	58	58	41
>1-7	24	24	38	44	20	15	16	27	33	33	40	42	29	31	29	33
>7	9	16	9	20	9	20	22	18	16	18	11	13	7	11	13	16

[1]Intensity was rated on 15 cm line scale which 1 cm refered to none and 14 cm refered to very strong

4.3 Changes of Forms of Iron in Iron-rich Food Products

4.3.1 Cookie products

Heme and nonheme iron contents of cookies at different blood fortification levels selected from the previous study which were analyzed before and after baking the products at 175°C in an oven are shown in Figures 6 and 7, respectively. The iron contents especially heme iron in the formula with a higher level of fortification were significantly higher ($p<0.05$) both in unbaked or baked cookies. After baking, heme iron in all formulas decreased significantly ($p<0.05$), while Figure 7 indicated that nonheme iron also increased at the same proportion. Decreases in heme iron of about 20% were observed in the baked 10%CkC and CkP, and about 30% for the baked 40%CkC and 30%CkP.

4.3.2 Chip products

Heme and nonheme iron contents of chips fortified with different kinds of blood at different levels are shown in Figure 8 and 9, respectively. Total and heme iron contents of deep-fried chips of higher levels of fortification were significantly higher ($p<0.05$). After deep-frying, heme iron content decreased significantly ($p<0.05$). Losses of heme iron due to deep-frying process were 35-40% in deep-fried 10% ChP and ChC, and 50% in deep-fried 40% ChC and ChP. Meanwhile the amounts of nonheme iron were found to increase proportionally upon deep-frying (Figure 9).

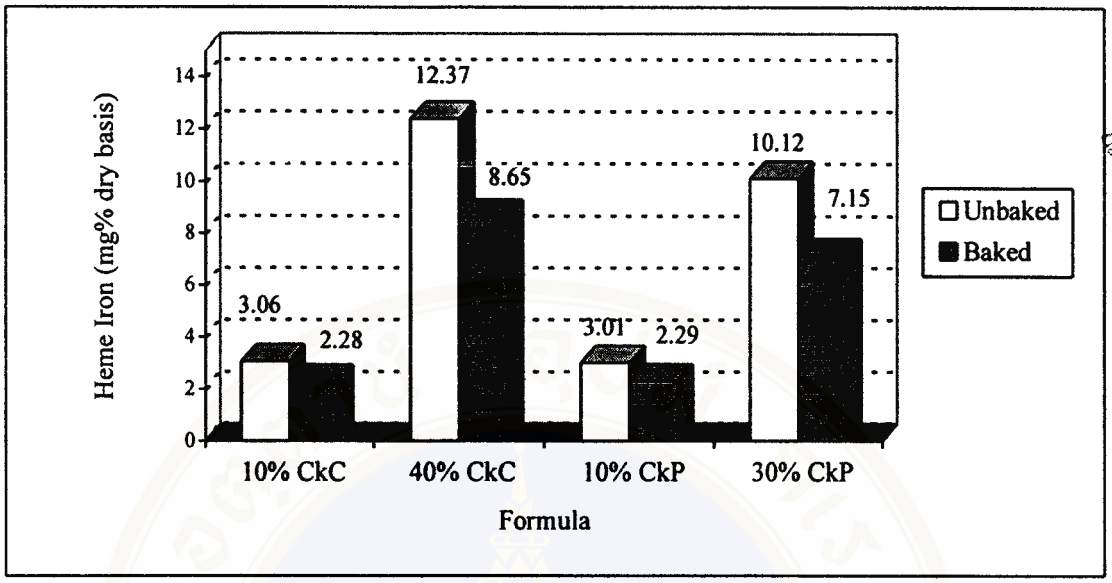

Figure 6 Heme iron contents of selected cookies fortified with different kinds of animal blood at different levels (10% and 40% CkC refer to 10 and 40 percent chicken blood fortification levels, 10% and 30% CkP refer to 10 and 30 percent porcine blood fortification levels).

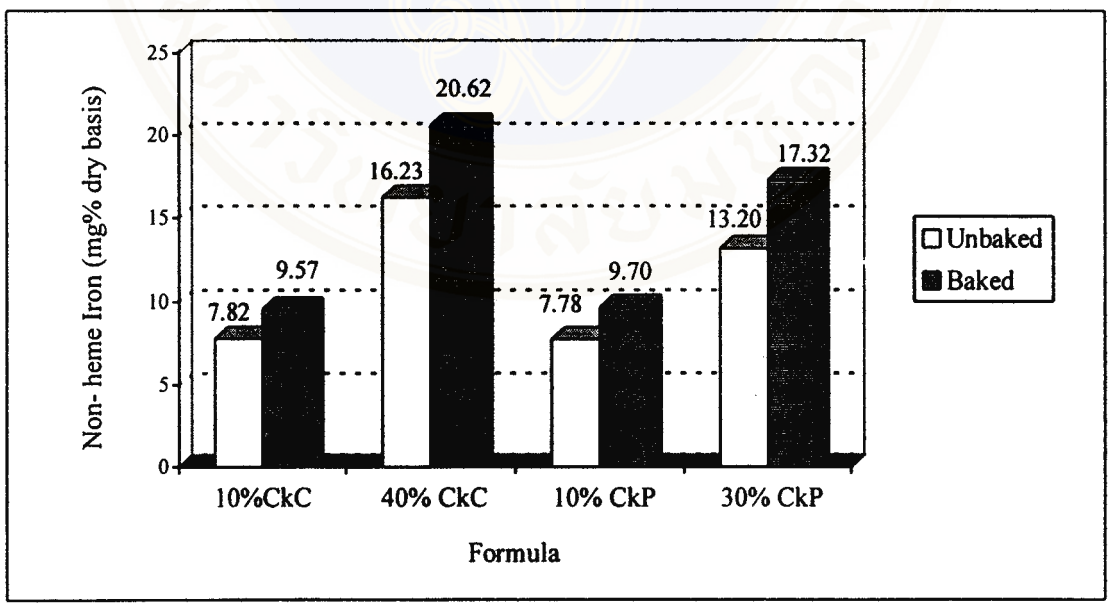

Figure 7 Non-heme iron contents of selected cookies fortified with different kinds of animal blood at different levels (10% and 40% CkC refer to 10 and 40 percent chicken blood fortification levels, 10% and 30% CkP refer to 10 and 30 percent porcine blood fortification levels).

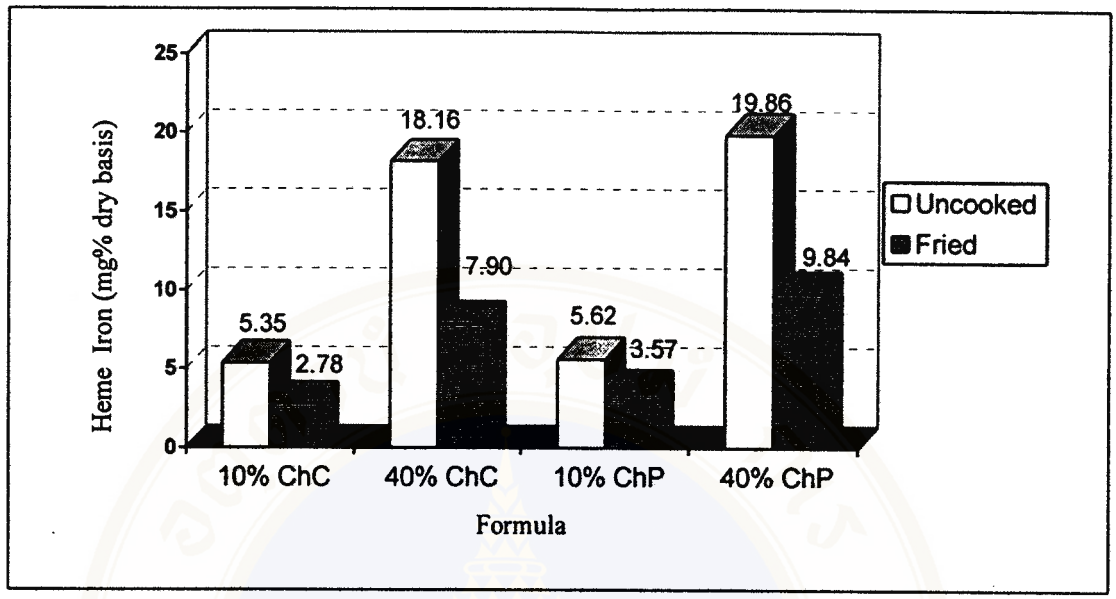

Figure 8 Heme iron contents of chips fortified with different kinds of animal blood at different levels (10% and 40% ChC refer to 10 and 40 percents chicken blood fortification levels, 10% and 40% ChP refer to 10 and 40 percents porcine blood fortification levels)

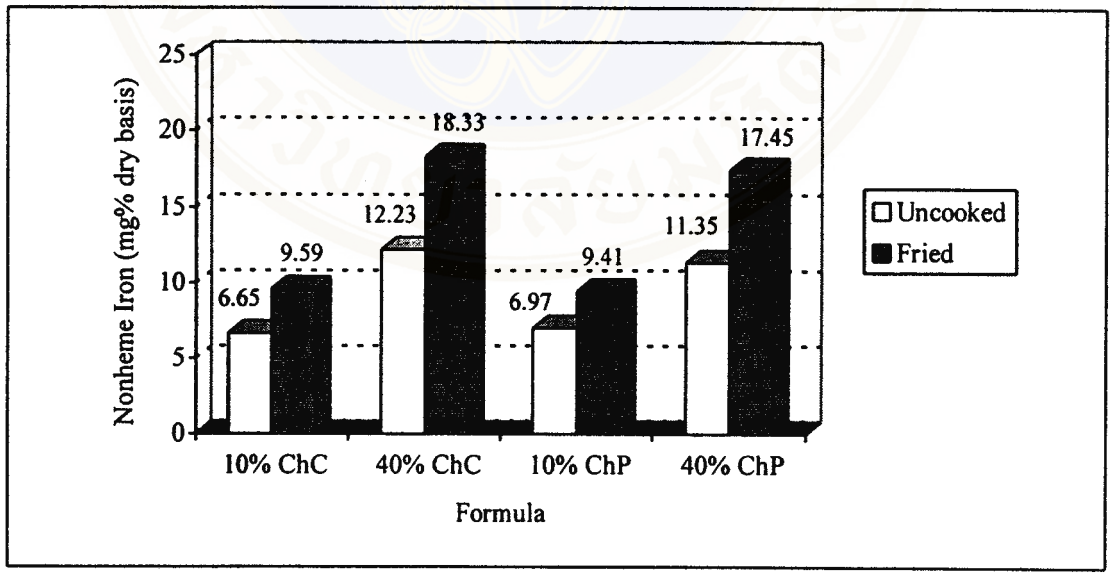

Figure 9 Nonheme iron contents of chips fortified with different kinds of animal blood at different levels (10% and 40% ChC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% ChP refer to 10 and 40 percent porcine blood fortification levels).

4.4 Shelf stability test of Iron-rich Food Products

4.4.1 Sensory acceptability evaluation

(a) Sensory acceptability of blood fortified cookies

Sensory acceptability scores of the selected cookies fortified with chicken and porcine bloods during 30-day storage are shown in Tables 16, 17, 18 and 19. The sensory acceptability scores did not significantly change during storage ($p>0.05$). However, Table 20 shows that off-flavor intensity rated on 15 cm line scale became higher in the stored cookies which were fortified with a higher level of blood.

(b) Sensory acceptability of blood fortified chips

The sensory acceptability scores of the blood fortified chips during storage are shown in Tables 21-24. The general appearance scores of 40%ChC, 10%Chp, and 40%Chp formulas during each storage period were significantly different ($p<0.05$). The differences in general appearance were found significantly only at the 15 day storage of the mentioned formulas. The same evidence could be observed in the odor scores of the 10%ChC. Meanwhile, the scores for other sensory characteristics were not significantly different ($p>0.05$) during the storage. Table 25 showed that the changes in frequency of off-flavor intensity rating on 15 cm line of the fortified chips was found not related to the storage period. Such evidences could be observed at all levels of fortification.

Table 16 Sensory acceptability scores[1,2] of cookies fortified with 10 % chicken blood curd during 30 day storage.

Day	General Appearance[3]	Color[4]	Overall-Acceptability[3]	Odor[5]	Taste	Texture
0	6.53±1.34	3.08±0.73	6.68±1.65	3.88±0.99	2.78±0.53	2.80±0.52
15	6.55±1.40	3.03±0.80	6.25±1.39	3.60±0.90	2.73±0.55	3.00±0.82
30	6.93±1.33	3.03±0.66	6.53±1.60	3.70±0.97	2.75±0.63	2.95 ± 0.50

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4]Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5]Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 17 Sensory acceptability scores[1,2] of cookies fortified with 40% chicken blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall-Acceptability[3]	Odor[5]	Taste[4]	Texture[4]
0	5.24±1.78	4.50±0.69	5.00±2.03	3.05±0.95	2.32±0.66	3.97±0.79
15	4.81±1.92	4.50±0.65	5.00±1.82	2.89±1.04	2.61±0.87	3.94±0.75
30	5.08±1.68	4.47±0.61	4.72±1.68	2.83±1.13	2.53±0.77	3.94±0.79

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 18 Sensory acceptability scores[1,2] of cookies fortified with 10% porcine blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall-Acceptability[3]	Odor [5]	Taste[4]	Texture[4]
0	6.63 ± 1.44	3.15 ± 0.58	7.00 ± 1.24	3.98 ± 0.73	2.85 ± 0.43	2.80 ± 0.52
15	6.60±1.28	3.18± 0.68	7.00 ± 1.30	3.98 ± 0.73	2.73 ± 0.55	2.93 ± 0.57
30	6.78±1.23	3.05 ± 0.68	7.13 ± 1.07	3.90 ± 0.81	2.65 ± 0.53	2.85 ± 0.53

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 19 Sensory acceptability score[1,2] of cookies fortified with 30% porcine blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall-Acceptability[3]	Odor[5]	Taste [4]	Texture[4]
0	5.30 ± 1.91	4.30 ± 0.69	5.64 ± 1.81	3.33 ± 0.94	2.48 ± 0.72	3.10 ± 0.74
15	5.33 ± 1.99	4.30 ± 0.65	5.41 ± 1.97	3.15 ± 1.08	2.65 ± 0.89	3.41 ± 0.79
30	5.45 ± 1.74	4.33 ± 0.66	5.41 ± 1.63	2.83 ± 0.93	2.50 ± 0.88	3.38 ± 0.67

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 20 Frequency of rating (as%) for off-flavor intensity[1] of the blood fortified cookie products during 30 day storage

Off-flavor Intensity (cm)	Cookie Products											
	Chicken blood						Porcine blood					
	10% Fortification Level			40% Fortification Level			10% Fortification Level			30% Fortification Level		
	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day
≤1	60	58	63	55	39	36	72	65	70	63	48	30
>1-7	20	20	30	19	17	22	15	20	23	22	27	30
>7	20	22	7	26	44	42	13	15	7	15	25	40

[1]Intensity was rated on 15 cm line scale which 1 cm refered to none and 14 cm refered to very strong

Table 21 Sensory acceptability scores[1,2] of chips fortified with 10% chicken blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Crispness[4]	Texture[4]
0	6.31±1.44	3.13±0.82	6.28±1.75	3.68±0.86[a]	2.78±0.73	3.25±0.49	3.00±0.55
15	5.48±1.75	3.13±0.94	6.18±1.24	3.08±0.97[b]	2.90±0.63	3.18±0.50	3.18±0.64
30	5.98±1.86	3.13±0.79	6.43±1.28	3.38±0.98[ab]	2.83±0.71	3.15±0.43	3.15±0.58

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$)
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 22 Sensory acceptability scores [1,2] of chips fortified with 40% chicken blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Crispness [4]	Texture[4]
0	4.97±1.96[ab]	4.43±0.65	5.40±1.75	3.20±1.08	2.80±0.90	3.29±0.52	3.03±0.57
15	4.64±1.64 [b]	4.42±0.55	5.42±1.90	2.86±1.07	2.53±0.97	3.28±0.57	3.03±0.74
30	5.82±1.83 [a]	4.18±0.73	5.95±1.72	3.39±0.79	2.82±0.93	3.24±0.49	2.97±0.64

[1]From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 23 Sensory acceptability scores [1,2] of chips fortified with 10 % porcine blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Crispness[4]	Texture[4]
0	6.43±1.48[a]	3.35±0.92	6.70±1.26	3.73±0.82	2.90±0.55	3.10±0.38	3.05±0.45
15	5.48±1.63[b]	3.43±0.87	6.30±1.30	3.30±0.97	2.88±0.61	3.15±0.43	3.05±0.50
30	6.28±1.60[a]	3.28±0.75	6.48±1.35	3.63±0.70	2.74±0.68	3.20±0.46	3.08±0.42

[1] From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 24 Sensory acceptability scores [1,2] of chips fortified with 40% porcine blood curd during 30 day storage

Day	General Appearance[3]	Color[4]	Overall Acceptability[3]	Odor[5]	Taste[4]	Crispness[4]	Texture[4]
0	5.34±1.89 [a]	4.50±0.69	6.03±1.70	3.39±0.98	2.74±0.83	3.11±0.31	3.11±0.56
15	4.08±1.98 [b]	4.66±0.63	5.63±1.44	3.13±0.96	2.79±0.93	3.16±0.49	3.00±0.62
30	5.23±1.95 [a]	4.60±0.59	5.80±1.68	3.08±0.99	2.60±0.81	3.25±0.63	3.03±0.58

[1] From 45 subjects.
[2] Mean ± SD and means within the same column with the same or no superscripts were not significantly different ($p > 0.05$).
[3] Using a 9-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely).
[4] Using a 5-point just about right scale (1, too light/mild/soft; 3, just about right; 5, too dark/strong/hard).
[5] Using a 5-point hedonic scale (1, dislike very much; 3, neither like nor dislike; 5, like very much).

Table 25 Frequency of rating (as%) for off-flavor intensity[1] of the blood fortified products during 30 day storage

Off-flavor Intensity (cm)	Chip Products											
	Chicken blood						Porcine blood					
	10% Fortification Level			40% Fortification Level			10% Fortification Level			40% Fortification Level		
	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day
≤1	60	43	55	50	36	58	58	45	60	66	32	50
>1-7	28	42	25	28	33	24	27	30	30	13	44	35
>7	12	15	20	22	31	18	15	25	10	21	24	15

[1]Intensity was rated on 15 cm line scale which 1 cm refered to none and 14 cm refered to very strong

4.4.2 Changes in forms of iron during storage

Figure 10 shows that the nonheme iron contents of all cookie formulas increased significantly during storage ($p \leq 0.05$). More changes could be observed in case of higher fortification levels. The nonheme iron contents in chips fortified with 10% blood curds did not show any significant changes ($p > 0.05$) during the storage (Figure 11). The evidence in the chips with higher fortification levels (40%ChC and 40%Chp) was the same as in cookies; the nonheme iron increased significantly during the storage ($p \leq 0.05$).

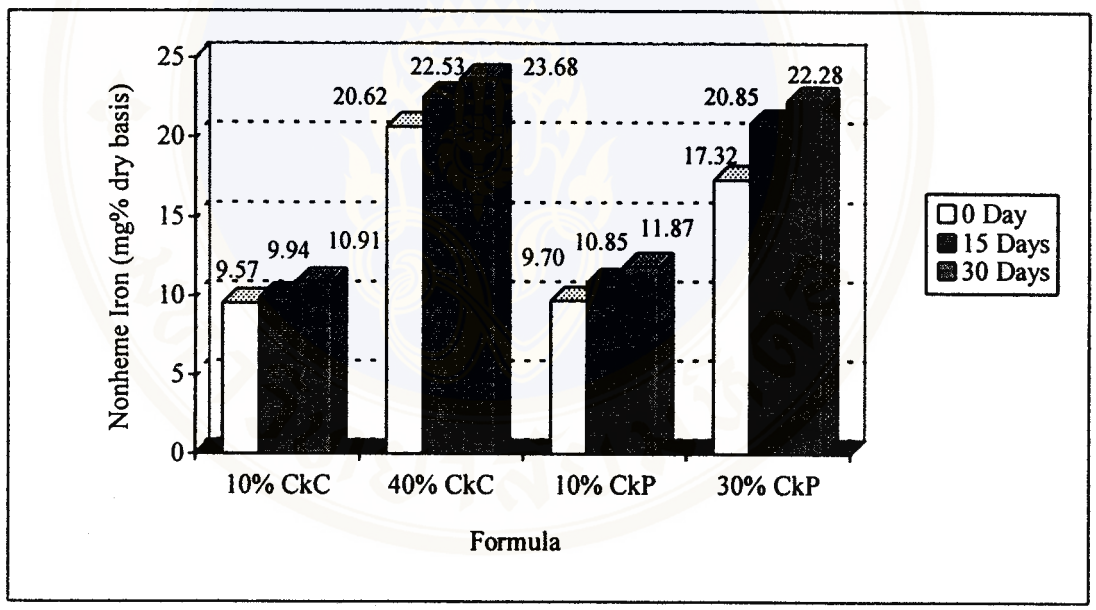

Figure 10 Nonheme iron contents of selected cookies fortified with different kinds of animal blood at different levels during storage at ambient temperature in sealed polypropylene bags (10% and 40% CkC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% CkP refer to 10 and 30 percent porcine blood fortification levels).

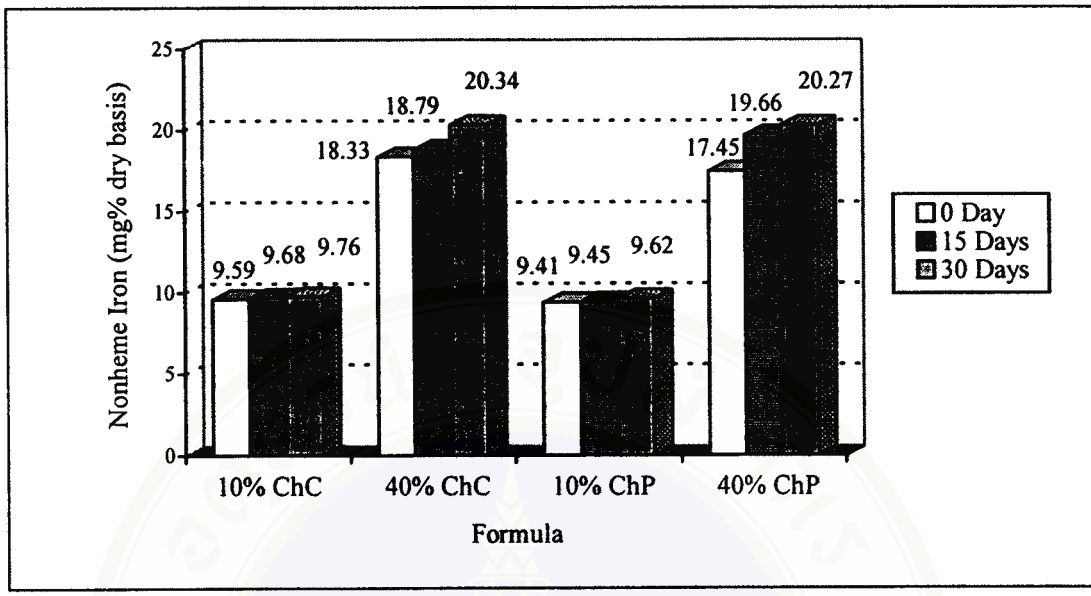

Figure 11 Nonheme iron contents of selected chips fortified with different kinds of animal blood at different levels during storage at ambient temperature in sealed polypropylene bags (10% and 40% ChC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% ChP refer to 10 and 40 percent porcine blood fortification levels).

4.4.3 Nutritional Quality in Iron-rich Food Products

(a) Cookie products

The nutrient composition of blood fortified cookies from different animal sources were quite similar in terms of proximate analysis and kinds of iron (Tables 25 and 26). As the fortification level was increased, the product contained more protein, iron and moisture. Amounts of fat and carbohydrate became less when more blood curd was used in the fortification. The nutrient composition and iron contents of both forms of blood fortified cookies expressed as per 100 g edible portions are shown in Appendix E, respectively. A photograph of the blood-fortified cookies per one serving is shown in Appendix G.

Table 26 Nutrient composition of blood fortified cookies (per serving size of 30 g or about 5 pieces)

Formula	Energy (Kcal)	Moisture (%)	Protein (g)	Fat (g)	CHO (g)	Ash (g)	Iron[2] (mg)
10% CkC[1]	164.12	0.72	3.36	9.39	16.53	0.50	3.07
40% CkC	158.25	1.72	5.94	8.46	14.60	0.49	8.62
10% CkP[1]	163.37	0.83	3.56	9.33	16.28	0.58	3.68
30% CkP	159.38	1.57	5.13	8.72	15.11	0.58	7.24

[1]CkC = chicken blood fortified cookies; CkP = porcine blood fortified cookies.
[4]Total Iron = Heme + Nonheme.

Table 27 Heme, nonheme and total iron contents in blood fortified cookies (per serving size of 30 g or about 5 pieces)

Formula	Heme	Nonheme	Total Iron[3]
	mg per serving (wet basis)		
10% CkC[1]	0.83	2.24	3.07
40% CkC	2.57	6.05	8.62
10% CkP[1]	0.75	2.90	3.68
30% CkP	2.13	5.11	7.24

[1]CkC = chicken blood fortified cookies; CkP = porcine blood fortified cookies.
[4]Total Iron = Heme + Nonheme.

(b) Chip products

The nutrient composition of blood fortified chips from different animal sources were also very similar (Tables 28 and 29). As comparing to the blood fortified cookies, the chips contained less iron contents. The chips with higher level of fortification also contained higher protein. The nutrient composition and iron contents of both forms expressed as per 100 g edible portions are shown in Appendix F. A photograph of blood fortified chips per serving size is shown in Appendix G.

Table 28 Nutrient composition of deep-fried fortified blood chips (per serving size of 30 g or about 22 pieces).

Formula	Energy (Kcal)	Moisture (%)	Protein (g)	Fat (g)	CHO (g)	Ash (g)	Iron (mg)
10%ChC	162.02	2.32	1.92	9.63	17.01	0.84	2.39
40%ChC	162.80	1.63	3.53	9.51	15.84	0.72	5.15
10%ChP	165.71	1.44	2.06	10.25	16.63	0.83	2.57
40%ChP	166.44	1.61	3.52	10.33	14.92	0.81	5.06

[1]ChC = chicken blood fortified chips; ChP = porcine blood fortified chips.
[4]Total Iron = Heme + Nonheme.

Table 29. Heme, nonheme and total Iron content in deep-fried blood fortified chips (per serving size of 30 g or about 22 pieces)

Formula	Heme	Nonheme	Total Iron[2]
	mg /serving (wet basis)		
10% ChC[1]	0.54	1.85	2.39
40% ChC	1.54	3.61	5.15
10% ChP	0.70	1.87	2.57
40% ChP	1.82	3.24	5.06

[1]ChC = chicken blood fortified chips; ChP = porcine blood fortified chips.
[2]Total Iron = Heme + Nonheme.

4.4.4 Physical property evaluation

Water activity (A_w) of blood fortified cookies increased and became stable at about 0.3 (Figure 12). The cookies with higher levels of fortification, which already contained higher moisture content started with a higher water activity and remained the same during storage. Figure 13 shows that water activity of blood fortified chips increased and remained after 15 day storage, except for 10%ChC. The water activities of most developed products were in the range of 0.3-0.4 after 30 day storage.

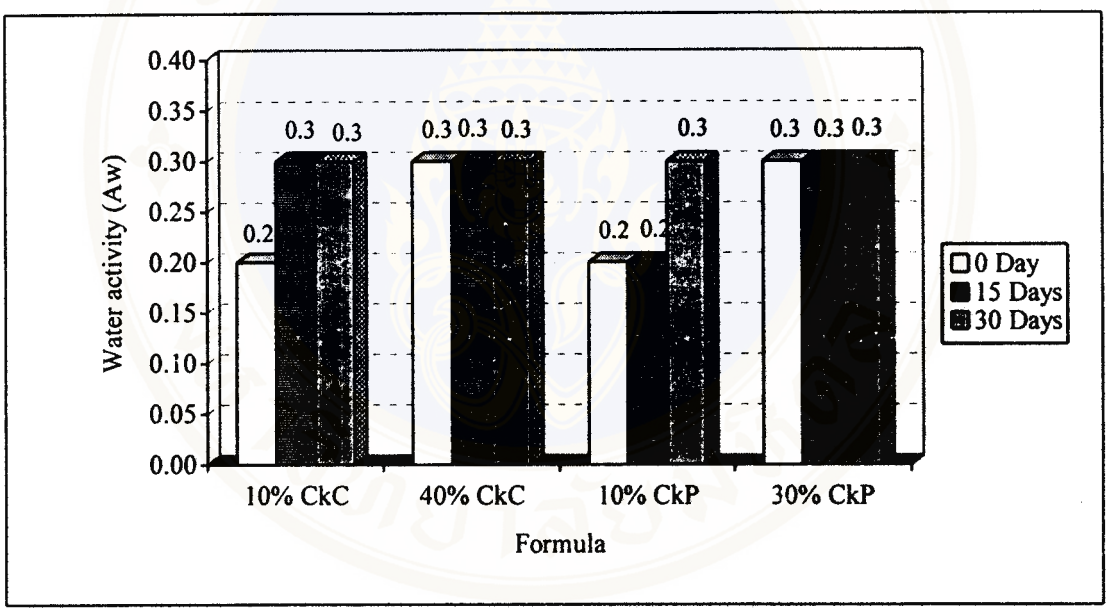

Figure 12 Water activity of selected cookies fortified with different kinds of animal blood at different levels during storage at ambient temperature in sealed polypropylene bags. (10% and 40% CkC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% CkP refer to 10 and 40 percent porcine blood fortification levels).

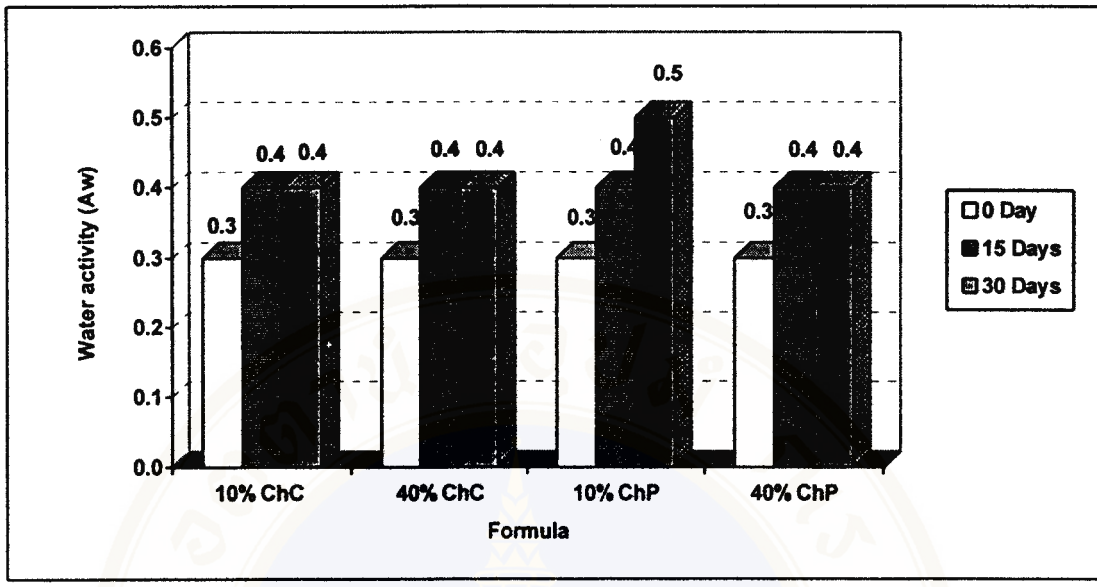

Figure 13 Water activity of selected chips fortified with different kinds of animal blood at different levels during storage at ambient temperature in sealed polypropylene bags. (10% and 40% ChC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% ChP refer to 10 and 40 percent porcine blood fortification levels).

4.4.5 Chemical property evaluation

Peroxide values of the 10%CkC and 10%CkP formulas were lower than those of the 30%CkP and 40%CkC formulas (Figure 14). Peroxide values of all formulas of deep-fried chips were quite similar regardless of the fortification levels (Figure 15).

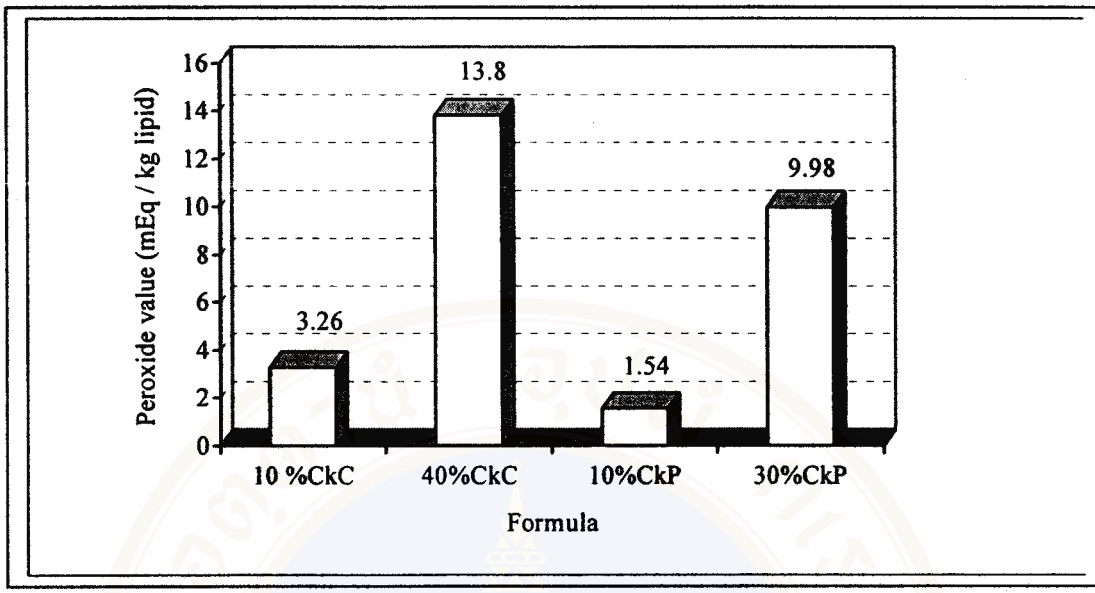

Figure 14 Peroxide value of selected cookies fortified with different kinds of animal blood at different levels at Day 0 (10% and 40% CkC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% CkP refer to 10 and 40 percent porcine blood fortification levels).

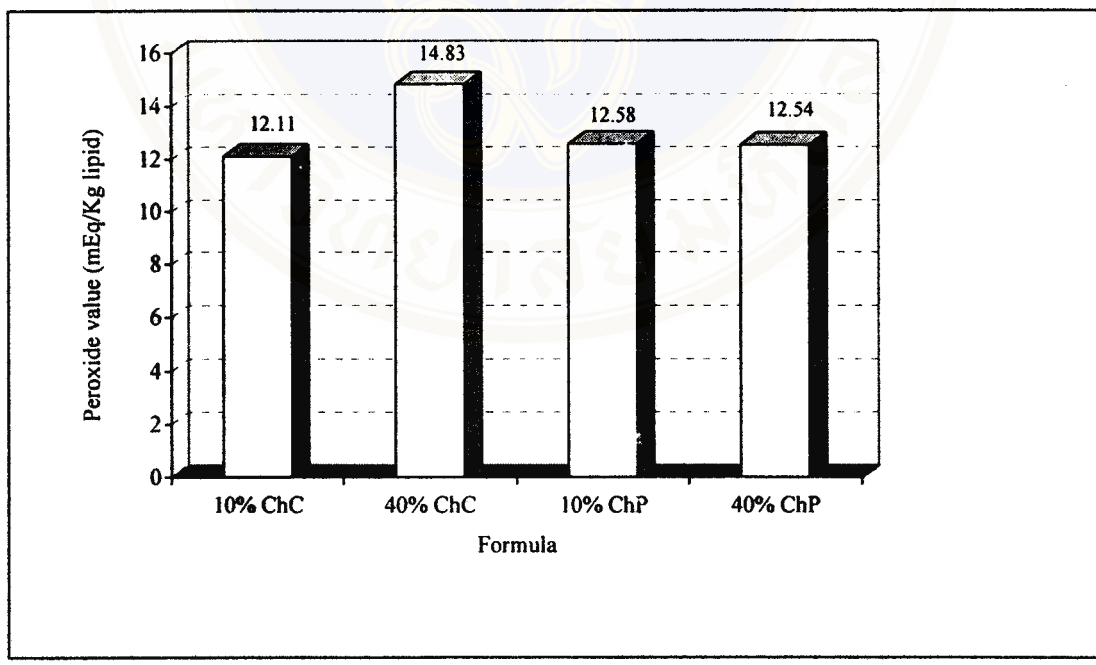

Figure 15 Peroxide value of selected chips fortified with different kinds of animal blood at different levels at Day 0 (10% and 40% ChC refer to 10 and 40 percent chicken blood fortification levels, 10% and 40% ChP refer to 10 and 40 percent porcine blood fortification levels).

4.4.6 Cost of blood fortified products

Cost per kilogram of blood fortified products were calculated from raw materials used and shown in Tables 30 and 31. Costs of the fortified products became higher due to the blood curd ingredient.

Table 30 Costs of blood fortified cookies

Formula[1]	Cost/kg (Baht)[2]
10%CkC	55.86
40%CkC	68.15
10%CkP	54.59
30%CkP	60.87

[1]CkC = chicken blood fortified cookies; CkP = porcine blood fortified cookies.
[2]Baht, Thai monetary unit(US $1 = 25 Baht at the experimental period :1996-1997).

Table 31 Costs of blood fortified chips

Formula[1]	Cost/kg (Baht)[2]
10%ChC	46.93
40%ChC	61.31
10%ChP	50.11
40%ChP	58.21

[1]ChC = chicken blood fortified chips; ChP = porcine blood fortified chips.
[2]Baht, Thai monetary unit (US $1 = 25 Baht at the experimental period :1996-1997).

CHAPTER V

DISCUSSION

By considering the cost of iron per mg (Table 6), the cooked porcine and chicken bloods the most suitable iron sources which are eligible for using as natural source for fortification. However, the variation of iron content in blood curds obtained from different markets could be observed. The variation might be from the differences in production process as well as age, sex, nutrition status, state of health and species of animal (87). Such variation needed to be considered if animal blood was used as an iron source. Heme iron, which was known for better absorption, contributed more than 70% of the total iron contents in both chicken or porcine bloods. This property made animal blood an interesting source of iron. The disadvantage in using blood curd as an ingredient was the too high water content. An expression technique was therefore introduced in order to remove certain amount of water (115). Iron content in the pressed blood curd increased. However, certain amount of iron also lost in the drained liquid, which could be observed in the dry-basis iron content in blood curds (Table 8-10). The lowering in iron content due to the expression also affected the cost of iron per mg to be slightly higher.

Cookies and traditional Thai fish/shrimp chips were used as the model for iron-fortification of food products. Both were the popular snacks among Thais, which had reasonable shelf lives and easily consumed. The chip, which was starch-based, could be easily fortified with blood curd, and was normally consumed among people both in the rural and urban areas. While, many kinds of cookies, which were widely

consumed, could be acceptable even with the dark color such as chocolate and chocolate-chip cookies. The recipes of both kinds of snack usually contained high fat content, which was an important source of energy especially for pregnant woman. Cooking conditions of the products were different but severe. The impact of heat processing on changes in contents of heme and nonheme iron in food was clearly indicated in the processes of making blood fortified cookies and chips (Figure 6-9). Furthermore, oxidation reached during air-drying of chips and storage of the product could also affecting loss of heme iron. Heme pigments could be destroyed by lipid hydroperoxides with the formation of various oxidation products containing Fe^{3+}iron decreased as the product was heated while the nonheme iron proportionally increased (Chen et al, 1984 (67); Hamdaoui et al, (1993) (116) and Garcia et al, (1996) (64). Such change was probably due to the release of iron from destroyed porphyrin ring (Chen et al, 1984 (67); King et al 1990 (70); Wang and Lin, 1994 (28), which might decrease the bioavailability of iron in the products. The loss of heme iron in chips was higher than in cookies, due to passing more severe processes. An increase in nonheme iron content has been found to be highly correlated with size and shape of food, and cooking time and temperature (Hamdaoui et al, 1993 (116); Wang and Lin, 1994 (28). However, certain studies indicated that only slight loss of total iron was observed during the heat processing such as baking, frying (Fillion and Henry, 1998) (72). Upon serving, the chip was also needed to be deep-fried, which contributed certain amounts of cooking oil into the product and lowered the percentage of iron content. Per serving, cookies therefore provided more iron content with higher ratio of heme to nonheme iron than chips.

The studies on sensory acceptability of cookie (Tables 11 and 12) and chip products (Tables 13 and 14) indicated that product colors could have strong influence on the acceptability of general appearances of both products. Product with a higher blood content contained more hemoglobin, which provided brownish color shade. However, the difference in color did not worsen the overall acceptability of the chips. The overall acceptability of cookies lowered, as the fortification level was higher. Such differences were not contributed solely from color, however other factors especially odor and off-flavor could also play significant roles (Tables 15, off-flavor).

The sensory overall acceptability scores indicated that the chips fortified with chicken or porcine blood curd at the level of 40% could still be acceptable (about 6 from 9-point hedonic scale). Such level of fortification could contribute iron up to 33% of Thai RDI per serving (10% of RDI was from heme iron). In case of cookies, the fortification level of chicken blood curd, which was still sensory acceptable was 40%, and could contribute iron up to 57% of RDI per serving (17% of RDI was from heme iron). While the fortification level of porcine blood curd in cookies could be only at 30%, which could contribute iron up to 48% of RDI per serving (14% of RDI was from heme iron).

The shelf life of the product either chips or cookies could be at least 30 days without any significant changes in the sensory qualities ($p > 0.05$). Kinds of blood used did not have any significant effect on the shelf life. It had been expected that iron could be a catalyst for the oxidation reaction of the high fat products like deep-fried chip and cookies. However, during the test period, the panelists did not significantly change their acceptability to the stored products. A study performed by Asenjo et al. (1985) showed that biscuits fortified with heme-iron concentrate could be stored for

about 7 months at room temperature in hermetically sealed plastic laminated aluminum foil bag (29). Benyasut (92) found that the shelf life of dried (non-fried) traditional Thai shrimp chips fortified with swine blood curd at 10 to 30 % fortification levels could be up to 10 weeks under room temperature. During the one month storage, changes in the form of iron were observed especially in the ones with higher fortification levels. Certain amount of heme iron changed into nonheme, which could affect iron bioavailability. Gomez-Basauri and Regenstein (1992) found that an increase of nonheme iron during storage was due to the breaking down of the heme structure (62). However the rate of increase usually depends on other factors during processing i.e. processing methods, storage time and temperature (62). The water activities of both products during storage increased slightly, however they were still lower than 0.6. It is well established that the low moisture food ($a_w < 0.6$) do not support microbial growth.

Peroxide value of the fortified cookies increased, as the fortification level was higher. This indicated the potential that iron in the fortified blood curds could be a catalyst for lipid oxidation reaction in cookies. However, such evidence was not found in the deep-fried chips, since the peroxide values of the fortified chips were similar at all fortification levels. During processing of chips, there were many severe steps that could cause oxidation to the ingredients i.e.steaming, sundrying, deep-frying (117). The reaction became faster in the presence of a catalyst such as iron. At the 0^{th} day, peroxide value of the fortified chips was already highest, and the peroxide value of blood-fortified chips (12 mEq/kg lipid) was also much higher than the commercial unfortified chips (5 mEq/kg lipid) (unreported data). Lipid auto-oxidation occurred much at the faster rate in the presence of iron from hemepigment(116).

Costs of the fortified products were increased due to the addition of blood curds. The recipe with higher fortification level was more expensive. The costs of fortified cookies and chips were about 5-7 Baht/kg higher than the normal recipe, respectively. Since there were not so much differences in costs, the fortified products therefore had potential for commercially produced.

In this study, the products were in the ready to eat form, packed only in normal propylene plastic bag, and stored at room temperature. The shelf life of at least 30 days should be reasonable for such economical packing condition and could make the product accessible and affordable to the target population with a lower income.

CHAPTER VI

CONCLUSION

1. Chicken and porcine blood curds were suitable iron sources from animal due to the high iron content and low cost.
2. There was the potential in using blood curds for fortifications of cookies and chips at community level up to 30- 40% fortification levels, which could contribute iron up to 50% of RDI per serving.
3. The blood-fortified products could be stored in a sealed polypropylene bag for at least 30 days at room temperature without changing the sensory quality.
4. Costs of the fortified products were about 5-7 Baht/kg higher than the normal ones.

REFERENCES

1. Nutrition Division, Ministry of Public Health. Report on the prevalence of iron deficiency anemia Thailand in 1997

2. ไม่ปรากฏชื่อผู้แต่ง คนใต้ยังครองแชมป์ขาดธาตุเหล็กเผยเหตุใหญ่กินอาหารไม่ครบหมู่. มติชนรายวัน 2538 ธันวาคม 11;หน้า 21

3. De Maeyer EM. Preventing and controlling iron deficiency anemia through primary health care. WHO, Geneva, Switzerland. 1989.

4. Freire WB. Strategies of Pan American Health Organization/World Health Organization for the control of Iron Deficiency in Latin America. Nutr Rev 1997, 55(6):183-8.

5. กองโภชนาการ กรมอนามัย กระทรวงสาธารณสุข รายงานการสำรวจภาวะอาหารและโภชนาการของประเทศไทยครั้งที่ 3 พ.ศ. 2538

6. คณะกรรมการพิจรณาการแสดงคุณค่าทางโภชนาการบนฉลากของอาหาร หลักเกณฑ์และวิธีปฏิบัติเกี่ยวกับการเติมสารอาหารในผลิตภัณฑ์อาหาร สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข กรุงเทพ 2538

7. คณะกรรมการจัดทำข้อกำหนดสารอาหารประจำวันที่ร่างกายควรได้รับของประชาชนชาวไทย ข้อกำหนดสารอาหารที่ควรได้รับประจำวันและแนวทางบริโภคอาหารสำหรับคนไทย พิมพ์ครั้งที่ 1 กรุงเทพมหานคร โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก 2532:161

8. Anonymous. Food processing and Thailand's economic prosperity. Asia Pacific Food Industry 1996, 8(7/8):76-84.

9. Yip R and Dallman PR. Iron. In: Ziegler EE and Filer LJ, editor. Present knowledge in nutrition. 7th ed. Washington, DC: ILSI Press, 1996:227-92.

10. Beard JL, Dawson H, Pinero DJ. Iron metabolism: A comprehensive review. Nutr Rev. 1996;54(10):295-317

11. Lee K, Clydesdale FM. Iron sources used in food fortification and their changes due to food processing. In: Furia TE, editor. CRC Critical reviews in food science and nutrition. Volume 11, Issue 2. Florida: CRC Press, 1978: 117-54.

12. Carpenter CE, Mahoney W. Contributions of heme and nonheme iron to human utrition. C Rev Fd Sci Nutr 1992;31(4):333-67

13. Spallholz JE, Boylan, LM, Driskell J A. 1999. Nutrition Chemistry and Biology, second edition. CRC Press, USA, 345 pp.

14. Silver J. Chemistry of Iron. Glasgrow, Blackie Academic &Professional, 1993: 306pp

15. World Health Organization. Report of WHO/UNICEF/ Joint Committee on Health Policy, 30th Session. Strategic approach to operationalizing selected end-

decade goals: reduction of iron deficiency anemia by one-third of the 1990 levels. JCHP 30/95/4.5. Geneva: WHO, 1994

16. Hallberg L. Iron. Present Knowledge in nutrition. 5 th, ed. Washington, DC: The Nutrition, Inc, 1984: 459-78.

17. Kreutler PA. Nutrition in perspective. New Jersey: Prentice-Hall Inc, 1980:337-63.

18. Gulthrie HA, Picciano MF. Human Nutrition. Mosby Year Book, Inc, 1995: 334-51.

19. Reed PB. Nutrition: an applied science. Minnesota: West Publishing Company, 1980:351-9

20. Kuchel PW, Ralston GB. Theory and problems of biochemistry. Singapore:McGraw-Hill,1988. 555p

21. Fairbanks VF. Iron in medicine and nutrition. In: Maurice ES, Olson JA Shike M, editors. Modern nutrition in health and disease. 8 th ed. Philadelphia: Lea & Febiger, 1994: 185-213

22. Bothwell T H, Charlton RW, Cook J. D. Finch CA. Iron metabolism in man. Blackwell Scientific Publications, London, 1979. 576 pp.

23. Anonymous. a quarterly update on micronutrients, Nutrition and Health. Iron fortification. Nutriview. 3:1996

24. Mahan LK, Escott-stump S, Krause S. Food, Nutrition, Diet Therapy. 9 th ed. W. B. Saunders Company, 1996: 717-36.

25. Alais C, Linden G. Food Biochemistry. England. 1991. Ellis Horwood limited. 219 pp.

26. King FS, Burgess A. Nutrition to developing countries. 2nd ed. Oxford: Oxford Medical Publications, 1993. 37-41p.

27. Kangsadalampai O, Wattanapenpaiboon N. Total and heme content in animal food.J of Nutr Assoc of Thailand 1987;21:162-72.

28. Wang FS, Lin CW. The effects of heating and chemical treatment on the haem and non-haem iron content of heat-induced porcine blood curd. J Sci Food Agric 1994;65:209-3.

29. Asenjo J, Amar M, Cartagena N, King J, Hiche E, Stekel A. Use of a bovine heme iron concentrate in the fortification of biscuits. J Fd Sci 1985;50:795-9.

30. Xiuyun L, Leyi Y, Xiaoming Y, Furu L, Yanli S. Effect of dried blood powder fortified biscuit on prevention of iron deficiency anemia. Research Institute of child and adolescent Health. Beijing Med. Univ. September:1987;1-8.

31. Johnson CD, Berry MF, Weaver CM. Soybean hull as an iron source for bread enrichment. J Fd Sci 1985;50:1275-7,1305.

32. The Micronutrient Initiative (MI) and International Agriculture Center (IAC). Micronutrient fortification of food: current practices, research, and opportunities. 1996:106.

33. Brittin MC, Nossaman CE. Iron content of food cooked in iron utensils. J Am Diet Assoc 1986;86(7):897-901.

34. Liu DY, Chen ZG, Lei HQ, Lu MQ, Li R, Li LX. Investigation of the amount of dissolved iron in food cooked in Chinese iron pots and astimation of daily iron intake. Biomed Enviroon Sci. 1990,3(3):276-80.

35. Borigato EVM, Martinez FE. Iron nutritional status is improved in Brazilian preterm infants fed food cooked in iron pots. J Nutr 1998;128(5):855-9.

36. Hamdaoui M, Doghri T, Tritar B. Effect of different levels of an ascorbic acid and tea mixture on nonheme iron absorption from a typical Tunisian meal fed to healthy rats. Ann Nutr Metab 1990;39:310-6.

37. Hallberg L, Rasmussen EB, Garby L, Pleehachina, Suwanik R. Iron absorption from Southeast Asian diets and the effect of iron fortification. Am J Clin Nutr 1978;31:1403-8.

38. Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulthen L, Pleehachinda R, Suwanik R, Hallberg L. Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. Am J Clin Nutr 1991;53:554-7.

39. Siegenberg D, Baynes RD, Bothwell TH et al., . Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 1991;53:537-41.

40. Cook JD, Dassenko SA, Whittaker P. Calcium supplementtation: effect on iron absorption. Am J Clin Nutr 1991;53:106-11.

41. Hallberg L, Brune M, Erlandsson M, Sandberg A-S, Hulten LA. Calcium: effect of different amounts on nonheme and heme iron absorption in humans. Am J Clin Nutr 1991;53:11219.

42. Bothwell TH, Baynes RD, Macfarlane BJ, Macphail AP. Nutritional iron requirements and food iron absorption. J Internal Med 1989;226:357-65.

42. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 1976;29:859-67.

43. Hallberg L, Rossander L. Improvement of nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid and ferrous sulfate on iron absorption from a simple Latin American-Type of meal. Am J Clin Nutr 1984;39:557-83.

44. Rossander-Hulthen L, Gramatkovski E, Gleerup A, Hallberg L. Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. Eur J Clin Nutr 1995;49:794-808.

45. Davisson L, Walczyk T, Morris A, Hurrell RF. Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. Am J Clin Nutr 1998;67:873-7.

46. Frølich W. Chelating properties of dietary fiber and phytate. The role for mineral availability. In: Furda I, Brine CJ, editors. New developments in dietary fiber. New York: Plenum Press, 1990:83-93.

47. Reddy MB, Hurrell RF, Juillerat MA, Cook JD. The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. Am J Clin Nutr 1996;63:203-7.

48. Rossander L, Sandberg AS, Sandstrom B. The influence of dietary fiber on mineral absorption and utilization. In: Schweizer TF, Edwards CA, editors. Dietary fiber a component of food. London: Springer-Verlag London Limited, 1992:197-216.

49. Cook JD, Reddy MB, Hurrell RF. The effect of red and white wines on nonheme iron absorption in humans. Am J Clin Nutr 1995;61:800-4.

50. Hallberg L, Rossander L. Effect of different drinks on the absorption of nonheme iron from composite meals. Human Nutr Appl Nutr 1982;36:116-23.

51. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. Am J Clin Nutr 1983, 37:416-20.

52. Barton JC, Conrad ME, Parmley RT. Calcium inhibition of inorganic iron absorption in rats. Gastroenterology 1983;84:90-101.

53. Gleerup A, Rossander-Hulthen L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr 1995;61:97-104.

54. U.S. Agency for international Development. Micronutrient interactions. Impact on child health and nutrition. Program/Abstracts. Food and Agriculture Organization. World Health Organization. 1996.

55. Bender AE. Food Processing and Nutrition. Food Science and Technology A Series of monographs. Academic Press. London. 1978. 243pp.

56. Hein RE, Hutchings IJ. Influence of Processing on vitamin-minerals content and biological availability in processed foods. Nutrients in processed foods. Vitamins Minerals. USA: Publishing Sciences Group. Inc, 1974:59-70p. (193pp)

57. Kimura M, Itokawa Y. Cooking losses of minerals in foods and its nutritional significance. J Nutr Sci Vitaminol 1990; 36:s25-s33.

58. Ensminger AH, Ensminger ME, Kouland JE, Robson JRK. Iron. Food and Nutrition Encyclopedia. 2nd, ed. USA: CRC Press, Inc, 1994, 2 (I-Z): 1246-51.

59. Torelm I, Danielsson R, Appelqvist L-A, Bruce A. Variations in major nutrients and minerals due to interindividual preparation of dishes from recipes. J Food Comp Anal 1997; 10:14-27.

60. Jansuittivechakul O, Mahoney AW, Cornforth DP, Hendricks DG, Kangsadalampai K. Effect of heat treatment on bioavailability of meat and hemoglobin iron fed to anemic rats. J Fd Sci 1985;50:407-28.

61. Han D, Mcmillin KW, Godber JS, et al. Iron distribution in heated beef and chicken muscles. J Fd Sci 1993;58:697-700.

62. Gomez-Basauri JV, Regenstein JM. Processing and Frozen storage effects on the iron content of cod and mackerel. J Fd Sci 1992;57:1332-6.

63. Mahoney AW, Carpenter CE, Cornforth DP. Heating and the distribution of total and heme iron between meat and broth. J Fd Sci 1988;53:43-5.

64. Garcia MN, Martinez-Torres C, Leets I, Tropper E, Ramirez J, Layrisse M. Heat treatment on heme iron and iron-containing proteins in meat: iron absorption in humans from diets containing cooked meat fractions. 1996;7:49-54.

65. Schricker BR, Miller DD, Stouffer JR. Measurement and content of nonheme and total iron in muscle. J Fd Sci 1982;47:740-3.

66. Schricker BR, Miller DD. Effects of cooking and chemical treatment on heme and nonheme iron in meat. J Fd Sci 1983;48:1340-9.

67. Chen CC, Pearson AM, Gray JI, Foodladi MH, Ku PK. Some factors influencing the nonheme iron content of meat and its implications in oxidation. J Fd Sci 1984;49:581-4.

68. Igene JO, King JA, Pearson AM, Gray JI. Influence of heme pigments, nitrite, and nonheme iron on development of warmed-over flavor (WOF) in cooked meat. J Agric Food Chem 1979;27:838-2.

70. King J, Pablo SDE, Montes de Oca F. Nonheme iron evaluation of hemoglobin iron concentrate. J Fd Sci 1990;55:593-4.

71. Gall KL, Otwell WS, Appledorf H. Effect of four cooking methods on the proximate, mineral and fatty acid composition of fish fillets. J Fd Sci 1983;48(4):1068-74.

72. Fillion L, Henry CJK. Nutrient losses and gains during frying: a review. International Journal of food Sciences and Nutrition 1998; 49: 157-68.

73. Chavasit V, Tontisirin K. Thailand's experiences with fortified weaning foods. Micronutrient interactions impact on child health and nutrition. 1996 July 29-30. Washington,Dc. IlSI Press,1998:69-74.

74. Calvo E, Hertrampf E, Pablo S, Amar M, Stekel A. Hemoglobin-fortified cereal: An alternative weaning food with high iron bioavailability. Eur J Clin Nutr 1989;43:237-43.

75. Hertrampf E, Olivares M, Pizarro F, et al. Hemoglobin fortified cereal: A source of available iron to breast-fed infants. Eur J Clin Nutr 1990; 44: 793-98.

76. Chiang W, Tsai LJ. Application of extrusion technology for processing of porcine blood-containing products. Food Science, Taiwan 1995,22(3):325-34.

77. Salinas-Pielago JE, Vega-Dienstmaier JM, Rojas-Oblitas M. Effect of biscuits fortified with heam iron on the intellectual status of pre-school children. Rev Neurol 1998; 27(157):400-4.

78. Divakaran S. Animal blood processing and utilization. Room. Food and Agriculture organization of the United Nations, 1982: p97.

79. Anonymous. Animal by-product processing. In: Hui YH, editor. Encyclopedia of Food Science and Technology. USA: Wiley Inter Science Publication, 1992, 1:42-8.

80. Kotula AW. By products. In: Putnam PA, editor. Animal Science. San Dieago: Academic Press, 1991:295-317.

81. Pedersen JW. Utilization of animal blood in meat products. Food Tech 1979; 76-80.

82. Pedersen JW. Use of hemoglobin in foods. Meat Sci 1988;24:31-45.

83. Vernam AH, Sutherland JP. Conversion of muscle into meat. Meat and meat products, Technology, Chemistry and microbiology. London: Chapman & Hall, 1995:46-98.

84. Oellingrath IM, Slinde E. Color, pigment and iron content of meat loaves with blood, blood emulsion, or mechanically deboned meat added. J. Food Sci. 1985;50:1551-5.

85. กองโภชนาการ กรมอนามัย ตารางแสดงคุณค่าอาหารไทยในส่วนที่กินได้ 100 กรัม โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก 2530

86. Souci SW, Fachman W, Kraut H. Food composition and Nutrition tables. In Deutsche Forschungsanstalt fuer Leblesmittlechemie. 5th, ed. Stuttgart: Medpharm GmbH Scientific Publishers, 1994, 253-83.

87. Underwood EJ. Iron. Trace elements in human and animal nutrition. 4th, ed. USA: Academic Press, Inc. 1977:533pp.

88. เสริมพร สาตรพันธุ์. อาหารและขนม. กรุงเทพฯ: โรงพิมพ์พรานนกการพิมพ์; 2528.

89. จันทร ทศานนท์ อาหารไทย. กรุงเทพฯ :ศิริวัฒนาการพิมพ์; 2531.

90. Fajvisevskij ML, Lisina TN. Extrusion technology of processing and utilization of slaughterhouse blood. Tehnologija Mesa 1994,34(2/3):58-60.

91. Kocovski T, Matekalo-Sverak V, Petrovic L, Mrdanov J, Starcevic G. Possibilities of blood and hemoglobin use in production of dietetic and products enriched with iron. Tehnologija Mesa 1994,35(3):95-100.

92. Benyasut M. Use of pork blood curdin the fortification of Thai snack (Kaow Kreb Ghung) [M.S. Thesis in Public Health] Bangkok: Faculty of Graduate Studies, Mahidol University; 1988.

93. Mielnik J, Slinde E. Sausage color measured by integrating sphere reflectance spectrophotometry when whole blood or blood cured by nitrite is added to sausages. J. Food Sci. 1983;48:1723-54.

94. Slinde E, Martens M. Changes in sensory properties of sausages when small amounts of blood replace meat. J Sci Food Agric. 1982;33:760-62.

95. U.S. Agency for international Development. Micronutrient interactions. Impact on child health and nutrition. Program/Abstracts. Food and Agriculture Organization. World Health Organization. 1996.

96. Carpenter CE, Clark E. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J Agric Food Chem 1995;43:1824-7.

97. Potter NN, Hotchkiss JH. Food deterioration and its control. In: Heldman DR, editor. Food Science. 5th ed. New York: Chapman & Hall, 1995:113-36.

98. Stone H, Sidel JL. Sensory evaluation practices. In: Taylor SL, editor. 2nd ed. San Diego: Academic Press, 1993. p337.

99. Blanshard JMV, Mitchell JR. Food structure-its creation and evaluation. London: Butterworths, 1988. p489.

100. Powers JJ. Current practices and application of descriptive methods. In: Piggott JR, editor. Sensory analysis of food. 2nd ed. London: Elsevier Science Publishers, 1988:187-266.

101. Jay JM. Intrinsic and extrinsic parameters of foods that affect microbial growth. In: Heldman DR, editor. Modern food microbiology. 5th ed. New York: Chapman & Hall,1996: 38-61.

102. Troller JA, Christian JHB. Water activity-basic concepts. Food science and technology a series of monographs. New York: Academic Press,Inc,1978:1-11.217pp.

103. Adams MR, Moss MO. Food microbiology. Cambridge: The Royal Society of Chemistry, 1995:5-17.

104. Beauchat LR. Microbial stability as affected by water activity. Cereal Foods World. 1981, 26(7):345-9.

105. Ahmad S, Augustin MA. Effect of tertiarybutylhydroquinone on lipid oxidation in fish crackers. J Sci Food Agric 1985, 36:393-401.

106. Bender DA, Bender AE. Food processing. Nutrition a reference handbook. Oxford: Oxford University Press, 1997:437-50.573pp.

107. Lawson H. Food oils and fats. Technology, Utilization, and Nutrition. New York: Chapman & Hall, 1995:329pp.

108. Sherwin ER. Oxidation and antioxidants in fats and oil processing. J Am Oil Chem Soc 1978, 55:809-14.

109. Anonymous. Biscuit and Cracker Technology. In: Hui YH, editor. Encyclopedia of Food Science and Technology. USA: Wiley Inter science Publication, 1992, 1:205-10.

110. กองโภชนาการ กรมอนามัย ตารางแสดงคุณค่าทางโภชนาการของอาหารไทย โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก 2535

111. Kangsadalampai K, Sungpuag P. Laboratory manual for food analysis. Bangkok: Prayurawong, 1984.

112. Hornsey HC. The color of cooked cured pork. I-Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 1956;7 (8):534-40.

113. Rhee KS, Ziprin YA. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J Fd Sci 1987;52:1174-6.

114. Heldman DR, Hartel RW. Principles of food processing. London: Chapman & Hall. 1997. 285 pp.

115. Hamdaoui M, Esseghaier C, Hedhili A, Doghri T, Tritar B. Effect of cooking and freezing on heme and nonheme iron concentration in beef. Nutrition Abstracts and Reviews (Series A). 1993; 63 (10):900.

116. Davídek J, Vellíšek J, Pokorný J. Development in food science 21: chemical changes during food processing, Amsterdam. Elsevier. 1990. 223.pp.

APPENDIX A

แบบสอบถามการประเมินผลทางประสาทสัมผัส

ผลิตภัณฑ์ คุกกี้ชอคโคแลต

สูตรหมายเลข.....
วันที่.....
เวลา.....

ผู้ประเมิน เพศ (.....) ชาย (.....) หญิง อายุ.....ปี

คำชี้แจง : แบบสอบถามทั้งหมดมี 2 ตอน กรุณาตอบแบบสอบถามทั้งหมด

ตอนที่ 1 ก่อนชิมผลิตภัณฑ์ เมื่อท่านได้รับผลิตภัณฑ์ กรุณาให้คะแนนความชอบของท่าน โดยการมอง แล้วขีดเครื่องหมาย / ลงในช่องที่ตรงกับความคิดเห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยทั่วไปของผลิตภัณฑ์ เมื่อท่านได้เห็นผลิตภัณฑ์
   - .....ชอบมากที่สุด
   - .....ชอบมาก
   - .....ชอบปานกลาง
   - .....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)
   - .....ไม่ชอบเล็กน้อย
   - .....ไม่ชอบปานกลาง
   - .....ไม่ชอบมาก
   - .....ไม่ชอบมากที่สุด

ข้อเสนอแนะ .....

2. ความเหมาะสมของสี
   - .....สีเข้มมาก
   - .....สีเข้ม
   - .....สีเข้มกำลังดี
   - .....สีอ่อน
   - .....สีอ่อนมาก

ข้อเสนอแนะ .....

ตอนที่ 2 หลังชิมผลิตภัณฑ์ เมื่อท่านได้ชิมผลิตภัณฑ์แล้วกรุณาให้คะแนนโดยขีดเครื่องหมาย / ลงในช่องที่ตรงกับความรู้สึก และความชอบของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยทั่วไปของผลิตภัณฑ์เมื่อท่านได้ชิมผลิตภัณฑ์แล้ว

.....ชอบมากที่สุด

.....ชอบมาก

.....ชอบปานกลาง

.....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)

.....ไม่ชอบเล็กน้อย

.....ไม่ชอบปานกลาง

.....ไม่ชอบมาก

.....ไม่ชอบมากที่สุด

ข้อเสนอแนะ .....

.....

ตอนที่ 2 หลังชิมผลิตภัณฑ์ เมื่อท่านได้ชิมผลิตภัณฑ์แล้วกรุณาให้คะแนนโดยขีดเครื่องหมาย / ลงในช่องที่ตรงกับความรู้สึก และความชอบของท่านมากที่สุด

2. ความเหมาะสมของรส

.....รสจัดมาก

.....รสจัด

.....รสกำลังดี

.....รสอ่อน

.....รสอ่อนมาก

ข้อเสนอแนะ .....

.....

3. ความเหมาะสมของลักษณะเนื้อสัมผัส

.....กรอบแข็งมาก

.....กรอบแข็ง

.....กรอบกำลังดี

.....กรอบเล็กน้อยค่อนข้างนิ่ม

.....ไม่กรอบ

ข้อเสนอแนะ .....

4. ความชอบในกลิ่น

.....ชอบมาก

.....ชอบปานกลาง

.....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)

.....ไม่ชอบ

.....ไม่ชอบมาก

ข้อเสนอแนะ .....

5. ในกรณีที่ท่านได้รับกลิ่นผิดปรกติ กรุณาทำเครื่องหมาย / ลงบนเส้นตรงจุดที่ตรงกับความรู้สึกของท่านมากที่สุด

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ไม่มีกลิ่น มีกลิ่นมาก

ข้อเสนอแนะ .....

APPENDIX B

แบบสอบถามการประเมินผลทางประสาทสัมผัส

ผลิตภัณฑ์ ข้าวเกรียบ

สูตรหมายเลข.....
วันที่.....
เวลา.....

ผู้ประเมิน เพศ (.....) ชาย (.....) หญิง อายุ.....ปี

คำชี้แจง : แบบสอบถามทั้งหมดมี 2 ตอน กรุณาตอบแบบสอบถามทั้งหมด
ตอนที่ 1 ก่อนชิมผลิตภัณฑ์ เมื่อท่านได้รับผลิตภัณฑ์ กรุณาให้คะแนนความชอบของท่าน
โดยการมอง แล้วขีดเครื่องหมาย / ลงในช่องที่ตรงกับความคิด
เห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยทั่วไปของผลิตภัณฑ์ เมื่อท่านได้เห็นผลิตภัณฑ์
   .....ชอบมากที่สุด
   .....ชอบมาก
   .....ชอบปานกลาง
   .....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)
   .....ไม่ชอบเล็กน้อย
   .....ไม่ชอบปานกลาง
   .....ไม่ชอบมาก
   .....ไม่ชอบมากที่สุด

ข้อเสนอแนะ .....

2. ความเหมาะสมของสี
   .....สีเข้มมาก
   .....สีเข้ม
   .....สีเข้มกำลังดี
   .....สีอ่อน
   .....สีอ่อนมาก

ข้อเสนอแนะ .....

ตอนที่ 2 หลังชิมผลิตภัณฑ์ เมื่อท่านได้ชิมผลิตภัณฑ์แล้วกรุณาให้คะแนนโดยขีดเครื่องหมาย / ลงในช่องที่ตรงกับความรู้สึก และความชอบของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยทั่วไปของผลิตภัณฑ์เมื่อท่านได้ชิมผลิตภัณฑ์แล้ว

.....ชอบมากที่สุด

.....ชอบมาก

.....ชอบปานกลาง

.....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)

.....ไม่ชอบเล็กน้อย

.....ไม่ชอบปานกลาง

.....ไม่ชอบมาก

.....ไม่ชอบมากที่สุด

ข้อเสนอแนะ .....

.....

ตอนที่ 2 หลังชิมผลิตภัณฑ์ เมื่อท่านได้ชิมผลิตภัณฑ์แล้วกรุณาให้คะแนนโดยขีดเครื่องหมาย / ลงในช่องที่ตรงกับความรู้สึก และความชอบของท่านมากที่สุด

2. ความเหมาะสมของรส

.....รสจัดมาก

.....รสจัด

.....รสกำลังดี

.....รสอ่อน

.....รสอ่อนมาก

ข้อเสนอแนะ .....

.....

3. ความเหมาะสมของลักษณะความพองฟู

.....พองฟูมากเกินไป

.....พองฟูมาก

.....กำลังดี

.....แข็งแน่น

.....แข็งแน่นมากไป

ข้อเสนอแนะ

.....

4. ความเหมาะสมของลักษณะความกรอบ

.....กรอบแข็งเกินไป

.....กรอบแข็ง

.....กรอบกำลังดี

.....นิ่ม

.....นิ่มมาก

ข้อเสนอแนะ .....

.....

5. ความชอบในกลิ่น

.....ชอบมาก

.....ชอบปานกลาง

.....เฉยๆ(อยู่ระหว่างชอบและไม่ชอบ)

.....ไม่ชอบปานกลาง

.....ไม่ชอบมาก

ข้อเสนอแนะ .....

.....

6. ในกรณีที่ท่านได้รับกลิ่นผิดปรกติ กรุณาทำเครื่องหมาย / ลงบนเส้นตรงจุดที่ตรงกับความรู้สึกของท่านมากที่สุด

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ไม่มีกลิ่น มีกลิ่นมาก

ข้อเสนอแนะ .....

APPENDIX C

Determination of heme iron

Heme iron was determined in acid-acetone extracts of the sample. The supernatant was measured at 640 nm by spectrophotometer and heme iron in the sample was calculated.

Reagents

1. Acetone (J. T. Baker)
2. Concentrated hydrochloric acid (Merck)
3. Deionized distilled water (INMU)

Apparatus

45 ml Polypropylene centrifuge tubes with covers

1,5 ml Sero pipette

10 ml Test tubes

∅ 125 mm Filter papers, No 42 Whatman

∅ 50 mm Funnels

Centrifuge (Model size-2k, USA)

Spectrophotometer (Spectronic 21, Bausch & Lomb, USA)

Vortex Genie 2 (Model G-560 E, USA)

Procedure

1. Weigh 1-5 g sample (depending on expected heme iron content) and place into polypropylene centrifuge tubes.
2. Add acetone, Conc. HCL and deionized distilled water.
3. Stoppered the tube, centrifuge by vortexing (1-2 minutes required).
4. Allow standing for 1 hour in refrigerator, then centrifuging at 2200g for 10 minutes.
5. Filter the solution through filter paper into a test-tube.
6. Prepare spectrophotometer (with 1 cm cell) at 640 nm wavelength at zero with blank sample containing 80% acetone, 2% Conc. HCL, and 18% deionized distilled water.
7. The absorbance was measured against reagent blank.

Calculation

Absorbance (OD) x (1 mole hematin / 4.8) x the final volume of extract solution / L x 55.847 / weight of sample = g Fe/g.

APPENDIX D

Determination of nonheme iron

Nonheme iron was separated from heme iron first by chelating with 0.39% (w/v) $NaNO_2$ and then precipitating the bound heme iron using an acid mixture (6N HCL, 40% TCA). After centrifugation, the supernatant was removed for the determination of free nonheme iron. The absorbance of supernatant was read at 540 nm against the reagent blank.

Reagents

1. Sodium nitrite ($NaNO_2$) reagent, (RPE CARLO ERBA). $NaNO_2$ solution, 39% (w/v) was freshly prepared with deionized distilled water daily.
2. Acid mixture : 6N Hydrochloric acid (Merck) and 40% trichloroacetic acid (Merck) were mixed in equal volumes.
3. Bathophenanthroline disulfonate reagent. Bathophenanthroline disulfonic acid (sodium salt, Sigma); 0.162 g was dissolved in 100 ml deionized distilled water and 1 ml thioglycolic acid (98%, Sigma) added. The reagents were stored in amber bottle for not more than 2 weeks in refrigerator.
4. Saturated sodium acetate solution. Sodium acetate (GR, Merck) 400 g was stirred with 500 ml deionized distilled water until sodium acetate remained undissolved.
5. Color reagent. Deionized distilled water : saturated sodium acetate solution : bathophenanthroline disulfonate reagent (20:20:1) was freshly prepared daily.

6. Iron standard. Using the iron stock standard, which was diluted with the acid-mixture from 0.5-5.0 μg Fe/ml.

Apparatus

50 ml Glass test-tube with stopper

10 ml Glass test-tube with stopper

10 ml Glass test-tube

0.5-5 ml Micro pipette

Vortex Genie 2 (Model G.560 E, USA)

Gyrotory water bath shaker (Model G 76, New Brunswick Scientific, USA)

Centrifuge (Model SIZE-2K, USA)

Spectrophotometer (Model Spectronic 21, Bausch & Lomb, USA)

Procedure

1. Weight 2-5 g samples into 50 ml test-tube together with the stopper.
2. Add 0.2 ml $NaNO_2$ reagent, then mix it thoroughly by vortexing.
3. Add fifteen ml of acid-mixture to each tube and then tightly stoppered.
4. The test-tube was incubated in a water-bath shaker at 65°C for 20 hr.
5. The test-tube was cooled to room temperature and kept in cold-room for 24 hr.
6. Centrifuge at 3500 rpm for 10 minutes.
7. One ml of supernatant was transferred to test-tube and 5 ml color reagent was added and mixed well.
8. Leave to stand for 10 minutes and the absorbance was read at 540 nm.

9. Reagent blank : 1 ml acid-mixture plus 5 ml color reagent.

Calculation

Nonheme iron = x (15+0.2 + moisture content of sample/gram) /wt. of sample = μg/g.

APPENDIX E

Table 32 Nutrient composition of blood fortified cookies (per 100 g edible portion)

Treatment	Energy (Kcal)	Moisture (%)	Protein (g)	Fat (g)	CHO (g)	Ash (g)	Iron[2] (mg)
10% CkC[1]	547.07	0.72	11.21	31.31	55.11	1.65	10.25
40% CkC	527.51	1.72	19.79	28.19	48.66	1.64	28.72
10% CkP[1]	544.55	0.83	11.88	31.11	54.26	1.92	11.92
30% CkP	531.25	1.57	17.10	29.05	50.35	1.93	24.86

Table 33 Heme, nonheme and total iron content of blood fortified cookies (per 100 g edible portion)

Treatment	mg/100 g (wet basis)		
	Heme	Nonheme	Total Iron[2]
10% CkC[1]	2.77	7.48	10.25
40% CkC	8.57	20.15	28.72
10% CkP[1]	2.49	9.66	11.92
30% CkP	7.09	17.04	24.86

[1]CkC = chicken blood fortified cookies; CkP = porcine blood fortified cookies.

[4]Total Iron = Heme + Nonheme.

APPENDIX F

Table 34 Nutrient composition of deep-fried fortified blood chips (per 100 g edible portion).

Formula	Energy (Kcal)	Moisture (%)	Protein (g)	Fat (g)	CHO (g)	Ash (g)	Iron[2] (mg)
10% ChC[1]	540.06	2.32	6.40	32.10	56.70	2.80	7.98
40% ChC	542.67	1.63	11.77	31.70	52.80	2.40	17.18
10% ChP[1]	552.37	1.44	6.87	34.17	55.43	2.77	8.58
40% ChP	554.80	1.61	11.73	34.43	49.73	2.70	16.87

Table 35 Heme, nonheme and total iron content in fried fortified blood chips (per 100 g edible portion)[1]

	mg/100 g (wet basis)		
Formula[1]	Heme	Nonheme	Total Iron[2]
10% ChC	1.81	6.17	7.98
40% ChC	5.16	12.02	17.18
10% ChP	2.34	6.24	8.58
40% ChP	6.06	10.81	16.87

[1]ChC = chicken blood fortified chips; ChP = porcine blood fortified chips.

[2]Total Iron = Heme + Nonheme.

APPENDIX G

Figure 16 Cooked chicken blood curd and grinned chicken blood curd

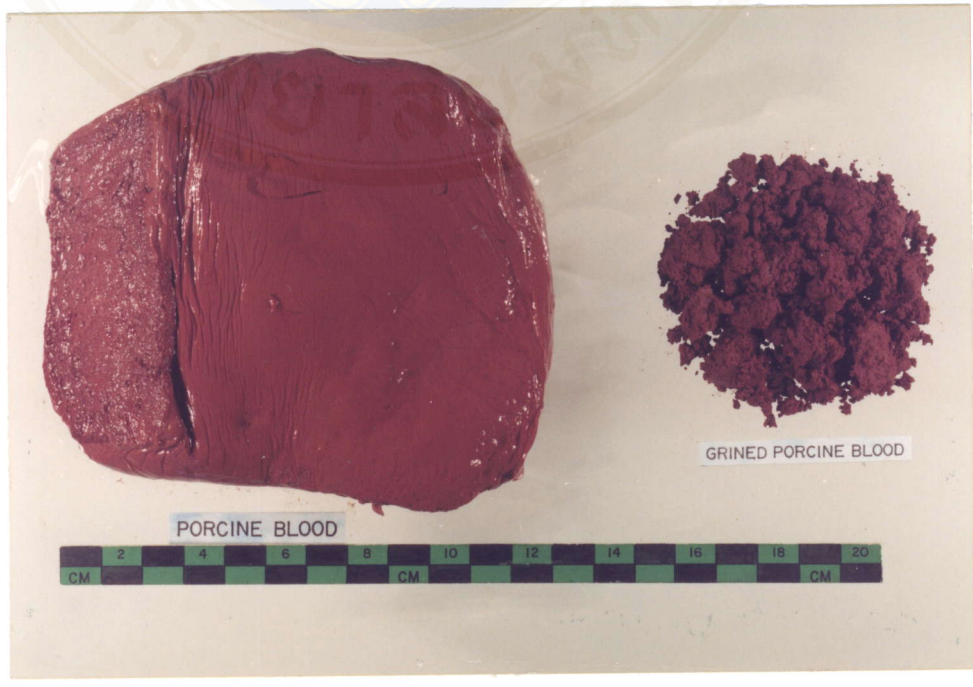

Figure 17 Cooked porcine blood curd and grinned porcine blood curd

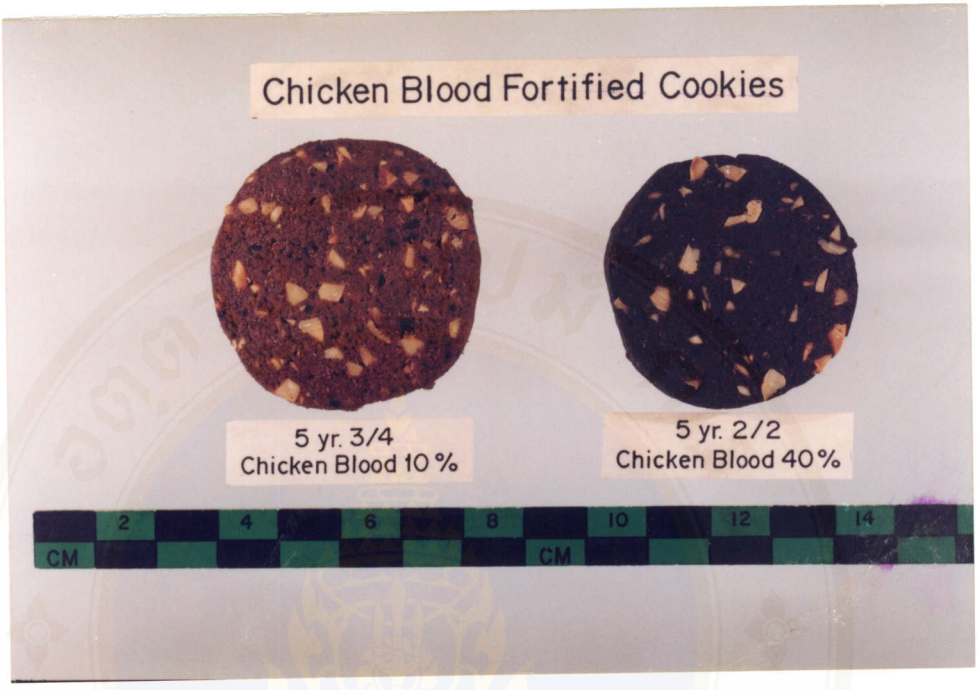

Figure 18 Chicken blood fortified cookie at 10 and 40% fortification level

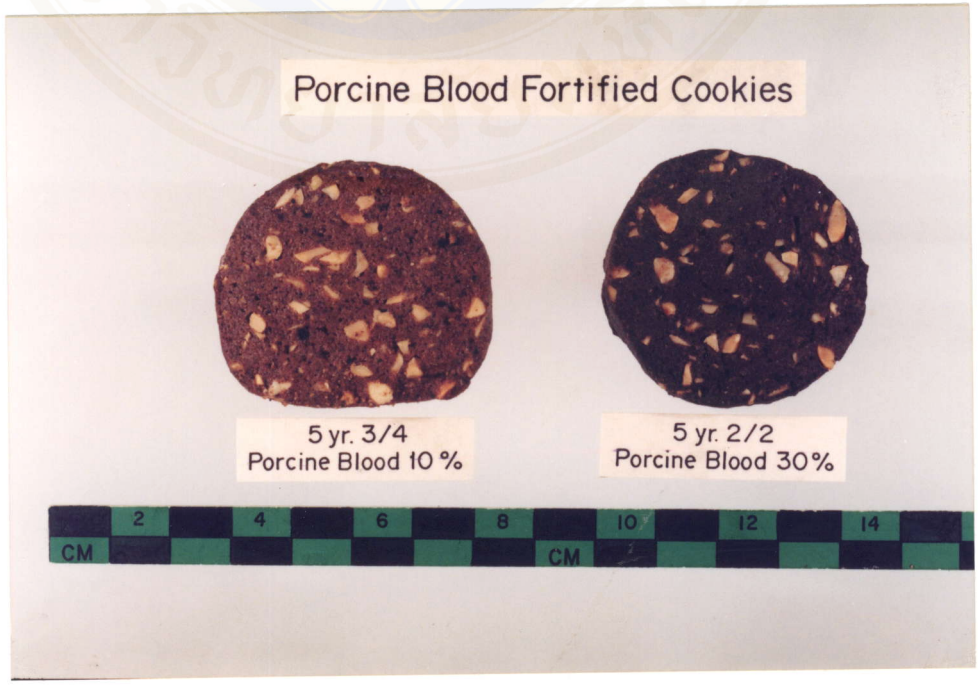

Figure 19 Porcine blood fortified cookie at 10 and 30% fortification level

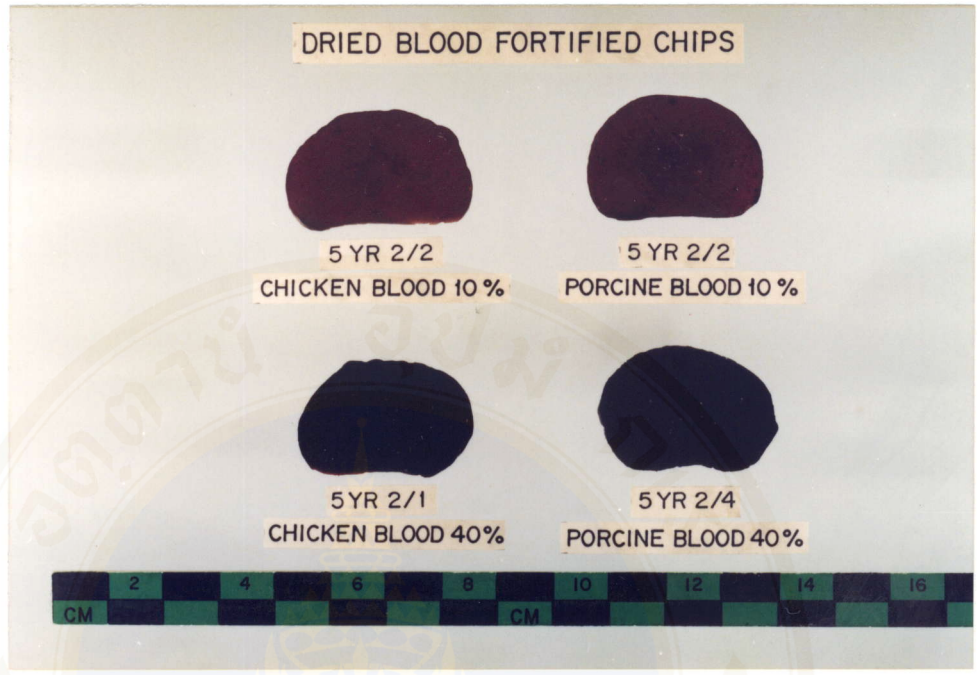

Figure 20 Dried chicken and porcine blood fortified chip at 10, 40%

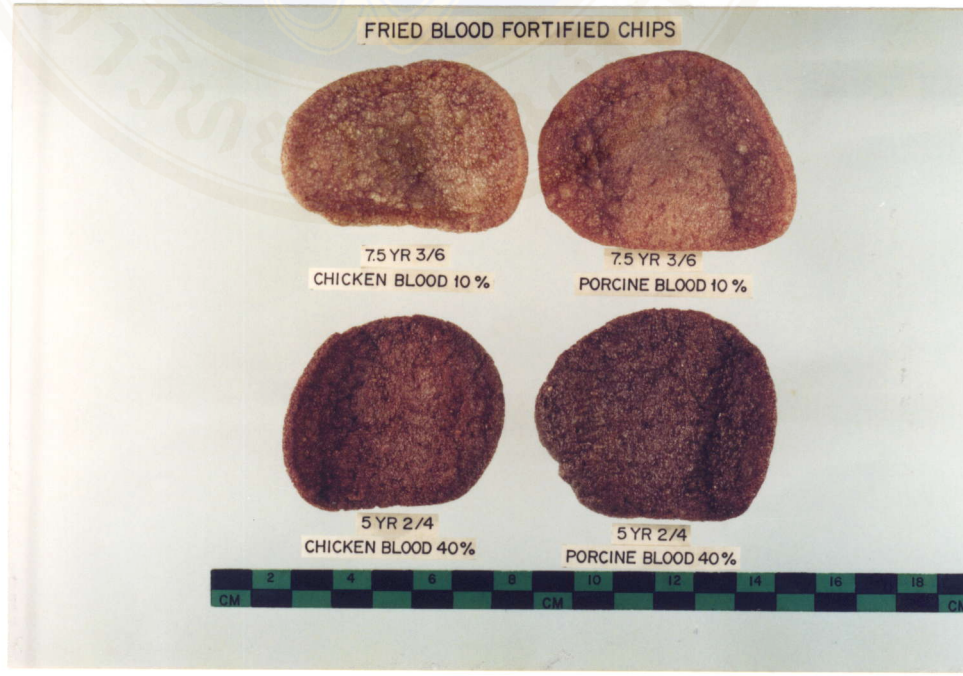

Figure 21 Fried chicken and porcine blood fortified chip at 10, 40%

Figure 22 Amount of blood fortified cookie products per serving size

(30 gram = 5-6 pieces)

Figure 23 Amount of fried blood fortified chip products per serving size

(30 gram = 17-23 pieces)

BIOGRAPHY

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DATE OF BIRTH	12 November 1960
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