SENSORY ACCEPTABILITY AND NUTRIENT STABILITY OF DOUBLE-FORTIFIED WHEAT FLOUR

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ABSTRACT

Wheat flour in Thailand is produced from at least 11 large modern millers. Due to the increase in wheat flour consumption in the country, wheat flour has become an interesting vehicle for research into micronutrient fortification. Cake (low extraction) and all-purpose (high extraction) wheat flours were fortified with iron and folic acid and tested for shelf stabilities under accelerated conditions (fluorescent light, 40± 2ºC) for 3 months, after being packed in 250 g polyethylene, laminated film (OPP/PE/L-LDPE/EAA), and woven polypropylene bags. Each kind of wheat flour was fortified with 51 ppm Fe from either ferrous sulfate or ferrous fumarate, or 102 ppm Fe from elemental iron either H-reduced (H-reduced EI) or electrolytic (Electrolytic EI), and 1.4 ppm folic acid. Before the shelf stability test, the double-fortified cake and all-purpose flours were used for preparing angel cake, cookies and fresh alkaline noodles and evaluated for their differences compared to products made from unfortified flours. This was done by using the sensory evaluation method, i.e. sensory difference from control test (n=24). Ferrous fumarate affected sensory quality the most, therefore was eliminated from the study. During storage, the double-fortified wheat flours (DFW) were analyzed for color and oxidative rancidity by spectro-colorimeter and thiobarbituric acid reactive substances (TBARS) respectively; difference from control on rancidity and color by sensory evaluation (n=24); iron and folate retention by Atomic Absorption Spectrophotometer and microbiological assay (*Lactobacillus casei*), respectively; and moisture content and Aw by oven drying and water activity meter. TBARS of DFW and unfortified flours increased slightly, but significantly during the 1st months (0.41-0.71 to 1.10-1.90 mg MDA/kg), and increased substantially in the 3rd months (1.10-1.90 to 2.00-3.43 mg MDA/kg). Significant differences in rancidity intensity were found in DFW fortified with ferrous sulfate packed in PE and laminated film bags after 2-3 months storage. L*, a*, b* colors of all DFW were significantly different from unfortified flours; however they were not significantly detectable by sensory evaluation. Reductions in moisture content and Aw during storage were significant with final values of 9-10% and 0.33-0.45, respectively. After 3 months storage, there were no significant changes in iron content, while folate in DFW retained > 90%. The highest losses of folate in unfortified flours were 17-25%. Per serving, DFW fortified with ferrous sulfate and elemental iron provided Fe of 10.5 and 20.4% RDI, respectively; and 21% RDI of folic acid.

KEY WORDS: WHEAT FLOUR/ FORTIFICATION / IRON / FOLIC ACID / SHELF STABILITY
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LIST OF ABBREVIATIONS

A_w	Water activity
^oC	Temperature in degree Celsius
CV	Coefficient of variation
Fe	Iron
H	Hour
IDA	Iron deficiency anemia
ml	Millilitre
mo	Month
ng	Nanogram
ppm	Part per million
Thai RDI	Thai Recommended Daily Intakes
s	Second
SD	Standard deviation
TBHQ	Tert-butylhydroquinone
TBARS	Thiobarbituric acid reactive substances
µg	Microgram
µl	Microliter

CHAPTER I

INTRODUCTION

Iron deficiency is the most common and widespread nutritional disorder in the world. As well as affecting a large number of children and women in developing countries, it is the only nutrient deficiency which is also significantly prevalent in industrialized countries. Two billion people or over 30% of the world's population are anemic due to iron deficiency (1). In Thailand, the prevalence of iron deficiency anemia (IDA) has been found in school-aged children, pregnant women, lactating women and over 60 years old, at about 40%, 26%, 21% and 36% respectively (2). Not only the impact on health, anemia also has consequences on economics. Anemia in early pregnancy can be harmful to development of unborn baby. It can also increase the risk of a mother having a miscarriage or stillbirth, or delivering a low-birth-weight baby, which, in turn, is linked to increased risk of prenatal and infant mortality. Anemic mothers may also be at increased risk of maternal mortality, especially those that have complications at birth that result in hemorrhaging (3). The causative factors of IDA are complicated; the primary cause of IDA is inadequate dietary intake of bioavailable iron (4-5). In addition, iron also plays a role in regulating response of the body to low-oxygen conditions or hypoxia. Body also uses iron in many enzymes that are critical for metabolism. These enzymes, called cytochromes are required for the metabolism and detoxification of many natural compounds in the body as well as chemicals, drugs and environmental pollutants. DNA synthesis also requires an iron-containing enzyme, which makes iron become so important for growth, development and wound healing. Bioavailability of iron is influenced by iron intake and absorption but the diets of people in developing countries are mainly composed of cereal products that poorly available form of iron absorption (6).

Another nutrient with well-established evidence regarding its importance to fetal development is folic acid or folate. Folate derivatives act as a coenzyme substrate in many reactions associated with the metabolism of amino acid and nucleotides. Various studies and randomised trials over the last three decades have shown that adequate intake of folate reduces the risk of abnormalities in early embryonic brain development and, specifically the risk of malformations of the embryonic brain/spinal cord, collectively referred to as neural tube defects (NTDs) (7-8). Deficiency of folate can also lead to a certain type of anemia. Consuming diets which are adequate in folate can reduce risk of heart disease and colorectal cancer (9-10). In 2000 the average cardiovascular death rate per 100,000 populations was 31.9 (37.9 in male, 26.0 in female) and the neural tube defect problem can cause death in children at a rate up to 85 cases/year in Thailand (11). Natural folates found in foods are all conjugated to a polyglutamyl chain containing different numbers of glutamic acids depending on the type of food (12). The bioavailability of natural folates is affected by the removal of the polyglutamyl chain by the intestinal conjugase enzyme. This process is apparently not complete, thereby reducing the bioavailability of natural folates by as much as 25-50 %. In contrast, synthetic folic acid appears to have a bioavailability of close to 100 % (13-14). The problem of cardiovascular disease in Thailand could be partly due to folate deficiency.

Wheat flour has been used as a vehicle for iron and folate fortification since it is a staple food and a major component of energy intake in many parts of the world. Wheat consumption in Thailand kept increasing every year due to higher consumption of bakery and pasta products (3). At present, wheat flour in Thailand is produced from 11 large-scale millers. In 2000, Asian Development Bank (ADB) has proposed that wheat flour should be a suitable food vehicle for fortifying iron and folate in Southeast Asian countries, since wheat flours in these countries are produced from large-scale millers. According to ADB, wheat flour in Southeast Asia should be fortified with iron and folic acid at the levels of 60 ppm and 140 µg/ 100 g flour, respectively (11). However, it was estimated in 2000 that average Thais consumed 24.3 g of wheat flour/person/day, while 30 % of the population who had high income consumed 72.6 g/person/d. One serving size of wheat flour based on Thailand's Food and Drug

Administration is 30 g, which is much lower than the consumption amount estimated by international organizations. In order to obtain a significant impact from the fortified nutrients, fortification dosages, therefore need to be much higher than what was formerly recommended.

In order to study the feasibility of fortifying wheat flour, effects of the fortified nutrients at such high dosages on sensory qualities of the flour and the foods prepared needed to be evaluated. In addition, the evaluation should be done on changes in shelf life of the fortified flours that are packed for distribution in different markets.

CHAPTER II
OBJECTIVES

General Objective

To study the technical feasibility in double-fortification of wheat flour with iron and folic acid.

Specific objectives

1. To select appropriate forms of iron for using in double-fortified wheat flours of different extraction rate,
2. To study shelf life of the double-fortified wheat flours those are packed in packagings that are used for distribution in the markets.
3. To study stability of the fortified nutrients during storage by using folic acid as an indicator.

CHAPTER III
LITERATURE REVIEW

3.1 Iron deficiency and background

Iron is present in every cell in the human body, and plays a vital role in red blood cells by helping to carry oxygen. In severe form, iron deficiency results in anemia, a condition in which the body does not have enough red blood cells and cannot function at full capacity. This decreased capacity is manifest in lower work productivity in men and women, decreased intelligence in children, and greater risk for low birth weight babies in pregnant women (15-16). Women and children are most at risk for iron deficiency due to conditions of high blood loss and high growth, respectively (16-17). The iron requirements of a child are approximately 10 times those of a grown man. Compounding the problem, infant diets typically are poorer in dietary quality than the diets of the rest of the family. Women also have high iron requirements, especially during pregnancy. A woman is more at risk to develop iron deficiency during pregnancy if she has had a lower iron status before pregnancy. This pre-pregnancy iron status is stressed by poor dietary intake, multiple micronutrient deficiencies, and parasitic infections which increase normal blood losses (17).

The causes of iron deficiency are different in industrialized versus developing countries. In industrialized regions such as North America and Europe, iron deficiency is caused almost primarily by low consumption of absorbable iron. However, in less-developed countries the problem of iron deficiency is more one of low bioavailability of consumed iron. While normal developing world diets are high in iron, most of that iron is consumed from plant sources which have high levels of natural iron-absorption inhibitors such as phytic acid and phenolic compounds (18). Staple foods in most developing countries also contain low levels of bioavailable iron,

thus increasing these populations vulnerability to iron deficiency (19).

Iron deficiency is the most common nutritional deficiency in the world. The World Health Organization (1) estimates that approximately 30% of the world's population is anemic as the result of severe iron deficiency. Anemia has a high economic cost: a nation looses approximately US $4 per capita per year for every individual with iron deficiency. South Asia is thought to be impacted by these losses most heavily, loosing as much as $5 billion annually (20). In Thailand, the 5th Thailand National Nutrition Surveys (NNS) in 2003-2004 has been found iron deficiency anaemia (IDA) in school-aged children, pregnancy women, lactating women and over 60 years elderly, at about 40%, 26%, 21% and 36% respectively.

3.2 Folate biochemistry and background

The nutritional benefit of folate was first reported by Wills who, in 1931, demonstrated that a constituent of yeast cured the macrocytic anaemia of pregnancy. In the early 1940s, the beneficial factor was isolated from spinach and named folic acid, from the Latin *folium* (meaning "leaf"). It was subsequently found that derivatives of folic acid occur widely in nature in both plant and animal sources. Collectively they comprise a family of compounds generically known as folates that exertsimilar vitamin activities. The simplest structural form of the vitamin is pteroylglutamic acid monoglutamate (folic acid), composed of an aromatic pteridine ring joined to *p*-amino benzoate and a single glutamic acid residue. This form of the vitamin does not occur naturally, but may be formed from other folate species during the isolation process. Folic acid is chemically stable during food processing and storage and is efficiently absorbed and converted to active forms of folate *in vivo* (80 to 100% bioavailability). It can be synthesized commercially and is the form of folate commonly added to foods or manufactured in supplement form (21).

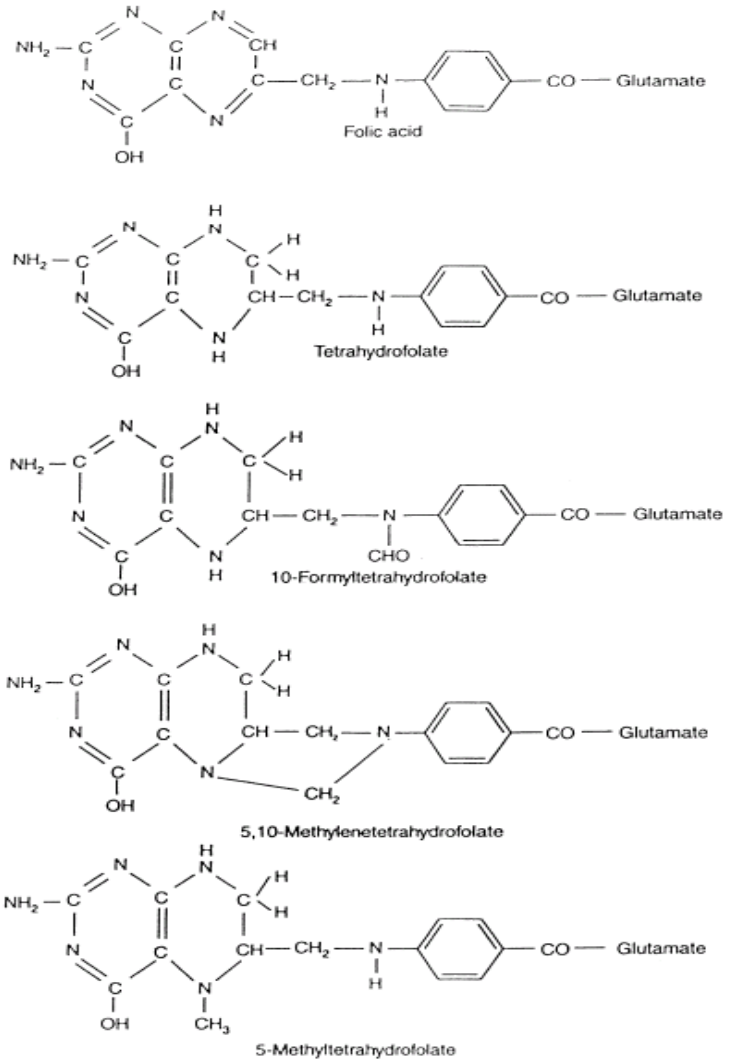


Figure 1. The chemical formulae of synthetic folic acid and some of the naturally-occurring reduced folates. (Scott, 1999)(22).

Natural food folates are structurally diverse. They differ from the basic folic acid structure in three ways: they exist in the reduced state as dihydrofolate (DHF) or tetrahydrofolate (THF); methyl or other carbon groups are inserted into the pteridine ring at the N-5 or N-10 position; and a polyglutamate side chain is attached to the benzene ring. The different combinations of these variations allow for numerous forms in which the molecule can occur (Figure 1) (22).

Functional folates have one-carbon units derived from several metabolic precursors (e.g. serine, N-formino-L-glutamate, folate, etc.). These one-carbon units (as a formyl group) are passed on to enzymes in the purine pathway that insert the C-2 and C-8 into the purine ring. A methylene group (-CH_2-) attached to tetrahydrofolate is

used to convert the uracil-type pyrimidine base found in RNA into the thymine base found in DNA. A further folate cofactor, i.e. 5-methyltetrahydrofolate, is involved in the remethylation of the homocysteine produced in the methylation cycle back to methionine. After activation to *S*-adenosylmethionine this acts as a methyl donor for the dozens of different methyltransferases present in all cells (Figure 2) (23).

The inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced. The deficiency will be most obvious in cells that are rapidly dividing, for example, in red blood cell production, producing anaemia. Secondary folate deficiency, the most obvious expression of the decrease in the methylation cycle is an elevation in plasma homocysteine that is implicated in the aetiology of cardiovascular disease (24).

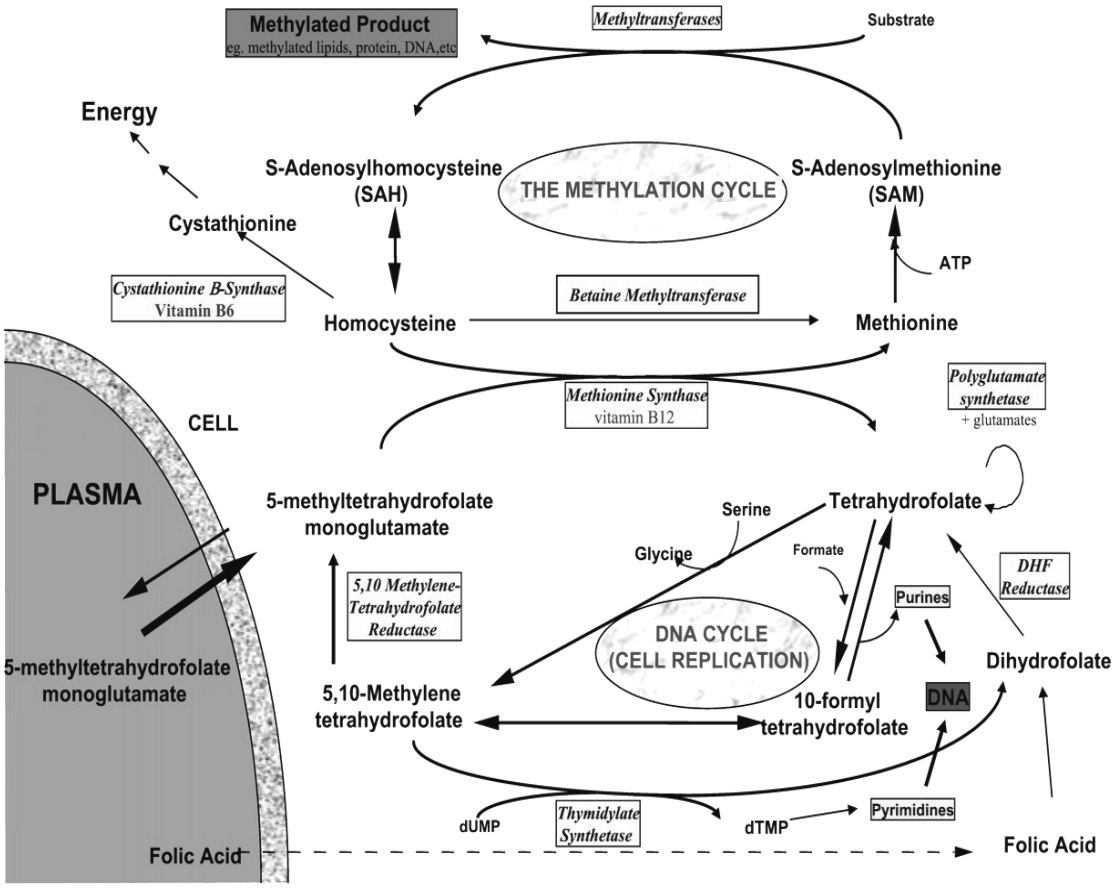


Figure 2. The biochemical pathways involving the folates and vitamin B_{12}. DOPA,3,4-dihydrocyphenylalanine.(Scott, 1999) (23)

3.3 Intervention strategy for combating micronutrients deficiency

Several strategies have been proposed to address the problem.The efficiency intervention strategies against micronutrient deficiency are direct supplementation of vulnerable populations with micronutrient supplements, dietary improvement and food fortification.

The strategies for combating nutrients deficiency include nutrients supplementation, food diversification, and food fortification. Food fortification is being recognized as a sustainable, relatively simple, and realistic way to reduce and prevent nutrients deficiency (18, 25). However, in many situations food fortification can be the most cost effective and simple way of delivering nutrients to the people.

Fortification is the way to added vitamins and/or minerals to food to increase its overall nutritional content. Fortification may not necessitate change in the consumer diet. It could often be dovetailed into existing food production and distribution systems. For these reasons, food fortification can often be implemented and yield results quickly and be sustained over a long period of time. It can thus be the most cost-effective means of overcoming micronutrient deficiency (26). A key to success of the national fortification program is the identification of a suitable food vehicle. Identifying and appropriate vehicle includes a combination of factors including: food consumption and market patterns, the capacity of the food industry to adapt to the fortification process, and interactions of the diet with the specific fortificants.

3.3.1 Supplementation

Supplementation is the most common strategy used to control micronutrients deficiency in developing countries. Increasing micronutrients intake through the use of pharmaceutical preparations by injection or in the form of capsule or tablet is an effective strategy for improving nutrition status of at risk groups such as pregnant women and young children. In critical or urgent situation, micronutrient supplementation is a relatively low cost measure against micronutrient deficiency. To succeed, programs require a reliable distribution system for delivering good-quality supplements when and where they are needed, as well as appropriate activities to sensitize both health care professionals and the public at large about the need for and appropriate use of supplements.(3, 27)

3.3.2 Dietary improvement

Dietary diversification is a food-based strategy, which involves increasing dietary quality to include higher level of micronutrients. A population's dietary quality can be improved through consumer education to change the types of foods consumed. Several factors limit the efficacy and sustainability of dietary diversification programs as a method to prevent and control micronutrient deficiencies. The first is that micronutrient malnutrition within the household often has much to do with food distribution globally and within the household (28) and the quality of diets typically fed to infants and children (20) and less to do with a paucity of micronutrient-rich

foods or a lack of food. In addition, while empirically many diets in areas of high iron deficiency and anemia are high in iron, most of this iron is consumed from plant and not animal sources. Plant-source iron is only minimally absorbed because of natural plant iron- absorption inhibitors. Some scientists have calculated that it is unfeasible for an individual to consume enough plant based foods to obtain iron sufficiency (29). Similarly in the case of folate consumption, natural folates are less bioavailable than synthetic folic acid (30). While women judged to have a reasonable diet in England consume 331 micrograms of folic acid per day from dietary sources, this level of folate consumption is not sufficient to increase red cell folate concentrations to levels high enough to prevent birth (31).

3.3.3 Food fortification

The term "food fortification" refers to the addition of one or more essential nutrients to a food, regardless of whether they occur naturally in the food. The purpose of fortification is to correct a recognized population-wide micronutrient deficiency or to add micronutrients lost in processing back to their original levels (restoration) or even higher. Government may mandate or encourage collaboration between the public or private sector and the health or agricultural sector through food fortification legislation, regulation, or a variety of incentives. Food fortification is also often used as a marketing tool by the food sector to increase sales. Thus, the types and levels of nutrients that are added are greatly influenced by which of the above purposes takes precedence (3).

3.4 Food Fortification

The World Bank has indicated that micronutrient interventions are among the most-cost effective of all health interventions (32). More specifically, fortification has been determined to be the least-cost method for reducing clinical deficiency in a population. For example, it is three times more cost- efficient at lowering iron deficiency than supplementation with iron among pregnant women (33). Food fortification is also an attractive strategy because it does not require behavioural modification. While supplementation and dietary diversification require population

compliance, the micronutrient status of a population can be improved through people continuing to consume a traditional diet of newly-fortified staples (34). That a population's micronutrient status can be improved through traditional diets is important as national agricultural policies in food-deficient nations are more oriented more toward calorie-dense dietary staples than micronutrient-rich vegetable products (28). Because of its efficacy, scientists in many countries including China, Israel, and India have advocated for a national flour fortification strategy to control micronutrient deficiency (29, 34-35).

3.4.1 Selection of food vehicle

Criteria for selecting food vehicle are depicted as follow (36).

1. Consumption

- High proportion of the population covered,
- Regular consumption in relatively constant amount,
- Minimal regional variation in consumption pattern,
- Appropriate serving size to meet a significant part of daily requirement of the micronutrient added,
- Consumption not related to socioeconomic status,
- Low potential for excessive intake (to avoid any probable toxicity),
- No change in consumer acceptability after fortification, and
- No change in quality (in a board sense) as a result of micronutrient addition.

2. Processing and storage

- Centralized processing,
- Simple, low cost technology,
- Good masking qualities (dark color and strong odor of the vehicle to mask slight changes to original color),
- High stability and bioavailability of added micronutrient in final product,
- Minimal segregation of the fortificant and vehicle,
- Good stability of the fortificant and vehicle,
- No micronutrient interaction

3. Marketing

- Appropriate packing that will ensure stability,
- Labelling according to prescribed standards, and
- Adequate turnover rate.

3.4.2 Selection of a food fortificant

General criteria for selection of a fortificant

- Good bioavailability during normal shelf life of the fortified product,
- No interaction with flavour or color system,
- Affordable cost,
- Acceptable color, solubility, and particle size,
- Free commercial availability of food grade marterial,
- Available in encapsulated form if required, and
- Feasibility of addition and dispersion through dry blending or spray coating premixes if required.

3.4.3 Fortificants

3.4.3.1 Iron

The success of iron fortification depends as much on the fortification compound as on the food vehicle. Fortification with iron is technically more difficult than with other nutrients because bioavailable forms of iron are chemically reactive and often produce undesirable effects when added to the diet. Since the population will seldom accept the fortified vehicle if the added iron can be detected, inert iron compounds are commonly used, but these are poorly absorbed and have little effect on iron status. A critical step in the design of an iron fortification program is the selection of an iron compound that is both unobtrusive and well absorbed (37). Iron source commonly used in food fortification are usually classified according to solubility, namely those that are Table 1.

a) Water-soluble fortificants

Waters soluble iron compounds have the highest relative bioavaibility (RBV,

approximately 100) of conventional iron compounds and if organoleptically accepted they should be the first choice for food fortification. Ferrous sulfate is usually the relative standard of bioavailability for other iron fortificants. This group of fortificants is unfortunately, also the most chemically change. Dried ferrous sulfate is the cheapest fortificants, mostly used to fortified infant cereal formula, bread, pasta and low acid foods. Moreover, it can also be added to wheat flour when stored for short period, but possibly trigger fat oxidation and off flavours in wheat and other cereal flours stored for long periods (25, 38-40).

b) Poorly soluble in water but soluble in dilute acids

Iron compounds in this group are secondly considered when freely soluble iron compounds causes undesirable organoleptic changes to food vehicles. These compounds cause less orgonoleptic problems than water soluble compounds, but have the slightly lower RBV. Recently , only ferrous fumarate is widely used as iron fortificants in this group. Ferrous fumarate is regularly added to commercial infant cereal and ferric sacchatrare in added to chocolate drink powder (25, 39-40).

Table 1. Characteristics of iron sources commonly used to fortified food (41).

Iron compounds	Approximate Fe (%)	Average relative bioavailability Rat	Man	potential for adverse Sensory Changes Color	Fat Oxidation	Relative cost*
Freely water soluble						
Ferrous sulphate $7H_2O$	20	100	100			1.0
Dried ferrous sulfate	33	100	100			0.7
Ferrous gluconate	12	97	89			5.1
Ferrous lactate	19	----	106	high	high	4.1
Ferric ammonium citrate	18	107	----			2.1
Ferrous ammonium sulfate	14	99	----			2.1
Ferric choline citrate	14	102	----			11.0
Poorly water soluble /soluble in dilute acid						
Ferrous fumarate	33	95	100			1.3
Ferrous succinate	35	119	92			4.1
Ferric saccharate	10	92	74			5.2
Ferric glycerophosphate	15	93	----			10.5
Ferrous citrate	24	76	74	low	low	3.9
Ferrous tarreate	22	77	62			3.9
Water insoluble/ Poorly Soluble in Dilute acid						
Ferrous pyrophosphate	25	45-58	21-74			2.3
Ferric orthophosphate	28	6-46	25-32			4.1
Elemental Fe powders:						
Electrolytic	97	16-70	75	neg	neg	— +
H-reduced	97	13-54	13-148			— +
CO-reduced	97	12-32	ND			— +
Atomized	97	ND	ND			— +
Carbonyl	99	35-66	5-20			— +

*Relative to ferrous sulphate $7H_2O$ = 1.0, for the same level of total iron

+ In general less expensive than ferrous sulphate. Cost of different powder types varies approximately seven-fold, with carbonyl iron

ND = not determined neg = negligible

Source: modified from Hurell, 1999 (41).

c) Water-insoluble and poorly soluble in dilute acids

These compounds are often used in food fortification because they did not cause any organoleptic changes in food vehicles. However, their main specific attribute is that they dissolve slowly and incompletely in the gastric juice during digestion. Their absorption is difficult to prophesy because their degree of solubility depends on their physical properties (size, shape, and surface area of particles) as well as the enhancing or inhibiting effects of meal composition. There are two different types of insoluble iron fortificants: the iron phosphate and the elemental iron powders (25).

Elemental iron compounds are wildly used to fortify cereal flours, breakfast cereal, and infant cereals. There are five different types of elemental iron powders used for food fortification. Hurrell et al (2002) addressed the usefulness of elemental iron powders for food fortification and concluded that electrolytic iron (325) was the best choice for food fortification in that time (42).

d) Protected compounds

- Ferric sodium ethylenediaminetetraacetic acid (NaFeEDTA)

The attractive advantages of NaFeEDTA over other fortification compounds are that it prevents iron binding to inhibitors of iron fortification, moreover it has high stability and fewer undesirable characteristics. Thus, NaFeEDTA is added to cereal foods or to meals consisting high amount of phytic acid, is two to three times absorption higher than ferrous sulfate (43). In cast of dietary inhibitors, Fidler et al. reported that the absorption of NaFeEDTA may be similar to the absorption of ferrous sulfate. It is a useful compound fortification of cereal foods, fish sauce, and soy sauce (44). It is slowly water soluble and thus may cause unacceptable color changes in some food vehicle, although it dose not induce fat oxidation in stored wheat flour (45).

- Iron amino acid chelates

Iron amino acid chelates, such as iron glycinate chelates, have been developed to be used as food fortificants and therapeutic agents in the prevention and treatment of iron deficiency anemia. Ferrous bis-glycine chelate (FeBC), ferric tris-glycine chelate, ferric glycinate, and ferrous bis-glycinate hydrochloride are available

commercially. FeBC is the most studied and used form. Iron absorption from FeBC is affected by enhancers and inhibitors of iron absorption, but to a lesser extent than ferrous sulfate. Its absorption is regulated by iron stores. FeBC is better absorbed from milk, wheat, whole maize flour, and precooked corn flour than is ferrous sulfate. Supplementation trials have demonstrated that FeBC is efficacious in treating iron deficiency anemia. Consumption of FeBC-fortified liquid milk, dairy products, wheat rolls, and multi-nutrient beverages is associated with an improvement of iron status. The main limitations to the widespread use of FeBC in national fortification programs are the cost and the potential for promoting organoleptic changes in some food matrices. Other amino acid chelates should also be evaluated. Finally there is an urgent need for more rigorous efficacy trials designed to define the relative merits of amino acid chelates when compared with bioavailable iron salts such as ferrous sulfate and ferrous fumarate and to determine appropriate fortification level (46).

Bioavailability of food iron is strongly influenced by enhancers and inhibitors in the diet. Presently, there is no satisfactory in vitro method for predicting the bioavailability of iron in a meal. Iron absorption can vary from 1% to 40%, depending on the mix of enhancers and inhibitors in the meal. Therefore, the adequacy - i.e. bioavailability - of iron in usual diets can be improved by altering meal patterns to favour enhancers, lower inhibitors, or both (47).

Enhancers of iron absorption include:

- Haem iron, present in meat, poultry, fish, and seafood;
- Ascorbic acid or vitamin C, present in fruits, juices, potatoes and some other tubers, and other vegetables such as green leaves, cauliflower, and cabbage; and
- Malic and tartarlic acid, present in carrot, potato, beetroot, pumpkin, broccoli, tomato, cabbage and turnip.
- Some fermented or germinated food and condiments, such as sauerkraut and soy sauce (note that cooking, fermentation, or germination of food reduces the amount of phytates).

Inhibitors of iron absorption include:

- Phytates, present in cereal bran, cereal grains, high-extraction flour, legumes, nuts, and seeds;
- Food with high inositol content;
- Iron-binding phenolic compounds (tannins); foods that contain the most potent inhibitors resistant to the influence of enhancers include tea, coffee, cocoa, herbal infusions in general, certain spices (e.g. oregano), and some vegetables; and
- Calcium, particularly from milk and milk products.

3.4.3.2 Folic acid (FA)

Folic acid, synthetic form appears a yellow-orange crystalline powder and bioavailability close to 100 percent. Folic acid (a monoglutamic acid) is the oxidized and most active form of the vitamin; found rarely in food, it is the form used in vitamin preparations and food fortification. The distinction between food folate and folic acid is important because of differing bioavailability (ie, food folate is only about half as available as folic acid consumed on an empty stomach). Folic acid can interfere with a number of drugs (anti-folate drugs, drugs used to treat epilepsy, anti-inflammatory drugs). Folate, vitamin B6 and vitamin B12 metabolism are linked *via* the enzyme methionine synthase (which requires vitamin B12 as a cofactor). Some authors have reported a negative effect of folate supplementation on zinc status. Some animal studies have suggested that iron deficiency may cause folate depletion (48).

3.5 Wheat flour fortification

Wheat is a cereal grain. Other cereal grains include corn (maize), oats, rice, and rye. Widespread consumption of cereal grains began in the Middle East about 10,000 years ago, when agriculture first began. It was then that wheat was first planted and cultivated.

Wheat is a natural source of vitamin B1 (thiamin), vitamin B2 (riboflavin), vitamin B6 (pyridoxine), niacin, vitamin E, iron, zinc, and small amounts of folic acid. Despite wheat's high empirical iron content, most of the iron in unprocessed wheat is unavailable to humans due to natural iron-absorbing inhibitors in flour such as

phytates. When flour is processed into white flour, the phytates are removed from the flour and any iron in the flour is more bioavailable. Due to this reason, iron in white flour whether from fortification or from natural sources has more potential to be bioavailable than the iron found in natural whole-grain wheat or less processed flours (49, 50). However, the milling process involves removing bran and germ from the endosperm. When this is done, vitamins and minerals, dietary fibres, and protein and fat from the bran and germ are removed. It is likely that other important unidentified nutrients are also removed.

Fortified flour is generally able to supply a person's full daily requirement of a particular micronutrient. There are a number of good reasons why wheat flour are fortified with deficient micronutrients (50).

1. They are food staples, consumed in significant quantities by all age groups and economic classes at nearly every meal. This makes them ideal vehicles for getting deficient nutrients to the general population.
2. Most of the micronutrients being added are naturally present in the whole grain but greatly reduced by the milling refinement process. Many fortification programs simply call for restoring deficit nutrient levels to that contained in the whole grain, often called enrichment or restoration
3. Fortification at the flour mill is fairly simple and easy to control and regulate.
4. The mills producing the bulk of the flour are large, modern and centrally located.
5. Some micronutrients, like folic acid and other B vitamins, are ideally suited for addition to milled cereals. There is no other food staple as well suited for B vitamin fortification.
6. Wheat flour has been fortified now for sixty years, so the concept, technology and sustainability are well established.
7. The milling equipment, design and quality control procedures for flour fortification have all been developed and are readily available.

8. There are a number of commercial concerns operating worldwide that supply fortification premix and mill equipment at reasonable prices due to heavy competition.
9. Fortification of wheat flour is an established and proven public health measure with widespread support by the medical and milling communities.
10. Cereal fortification is safe because a person cannot eat enough fortified wheat flour to exceed the upper safety levels of micronutrient intakes.
11. Fortification at the mill is relatively inexpensive and affordable. It will not noticeably impact the cost of the food to the consumer; yet the public will eventually pay for it with a small, overall price increase.
12. There are a number of groups - including the Micronutrient Initiative, UNICEF, USAID/MOST, ADB and GAIN - available to provide technical, promotional and financial support for establishing cereal fortification programs.

The addition of micronutrients to flour while it is being milled is a common practice among millers in North and South America. The USA began fortifying flour with thiamin, riboflavin, and niacin during World War II to prevent deficiencies. South America began to fortify flour in the 1950's to be consistent with US standards (51). Originally iron and folic acid were only added to flour to restorative levels, or to make up for nutrient losses during milling. More recently, iron and folic acid deficiency has been recognized and national policies have been developed to fortify flour with micronutrients above pre-milling levels (52).

3.5.1 Dosage of vitamins and minerals for food fortification

A guideline for food fortification developed by Thai Food and Drug Administration has set up the maximum level of nutrients including iron that are allowed to be used in the fortified product. The value (as shown in Table 2) is based on the body requirement and the safety level of consumption.

Table 2. The maximum allowance of iron for food fortification

Micronutrient	Thai RDI	Total safe Daily intake	Maximum allowance for fortification			
			Per day		Per serving	
			Amount (mg)	Thai RDI (%)	Amount (mg)	Thai RDI (%)
Iron	15	60	18	120	6	40

Source: The Thai Food and Drug Administration, 1999 (53)

The study of fortified wheat in Sri Lanka shown the information to recommend the suitable fortificant forms and upper-level for wheat flours fortification in Table 3 and 4 (50).

Table 3. Potential use of different iron forms in the fortification of wheat flour

Product	%Extraction rate	Ferrous sulfate	Ferrous fumarate	Ferric-orthophosphate	Reduced iron	Electrolytic iron	Iron EDTA
All-purpose	75-80	O	O	O	O	R	O
Bread flour	75-80	R	O	O	O	O	O
Whole wheat	97	N	N	O	O	O	R
Pastry flour	45	O	O	O	O	R	O
Cake flour	40-50	O	O	O	O	R	O
Semolina	60-65	R	O	O	O	O	O

R= Recommended; O = Optional; N = not Recommended

Source: Iron fortification of foods: stability trials in Sri Lanka ; 1985 (50)

Table 4. Upper-level sensory thresholds (ppm) for iron fortificants added to wheat flour stored up to 3 months

	High-temperature (30-40ºC), high relative humidity (70-80%)	Low-moderate temperature (20-30ºC), low relative humidity (<50%)
Ferrous sulfate	30	40
Ferrous fumarate	60	NA
Reduced iron	66	88
Electrolytic iron	66	NA
Sodium-iron-EDTA	15	NA

NA = Published data or study results not available

Source: Iron fortification of foods: stability trials in Sri Lanka; 1985 (50)

The US National Academy of science and Food and Durg Administration of the United states, after reviewing literature, has suggested an upper limit at 1000 µg folic acid / day. Thus, 400 µg/day of folic acid, in addition to dietary folate, would seem safe. There is probably no great risk of toxicity at range between 400 and 1000 µg of folic acid per day with the exception of some increased difficulty in diagnosing pernicious anemia resulting from the masking of the anemia (54, 55).

Addition, Asian Development Bank recommends for Thailand that wheat flour should be fortified with iron and folic acid at the level of 60 ppm and 140 µg per 100 g flour, respectively.

Table 5. % RDI of folic acid and Iron after fortified base on 100g wheat flour

	Thai RDI	ADB recommended		% of Thai RDI
FA	200µg	140 µg/100g flour	140 µg	70
Iron	15 mg	60 ppm(electrolytic iron)	6 mg	20*

*% Iron depend on fortificants and bioavailability (bioavailability of electrolytic iron is half of ferrous sulfate)

3.6 Fortification effects on product qand acceptability

As a guiding principle it is essential that the fortification of wheat flour and maize meal will not change consumer acceptability of the fortified food. It would be ideal for the fortification to be invisible to the consumer. That is, there would be no detectable difference in the appearance, sensory properties or even the price of the fortified product, but this is not always achievable (51).

Colour and appearance

The visual appearance of any food is the first of the organoleptic senses that a consumer experiences. Therefore if the fortified food has had any changes in colour and appearance the consumer is more likely to reject the product. At the current fortification levels normally found in wheat flour there is no adverse impact on colour or appearance. Elemental iron powders may cause a slight darkening of flour, while high levels of folic acid can cause a slight yellowing, but these changes are accepted once all flour is similarly treated. Ferrous sulphate does not cause any colour problems in the dry flour but could lead to off-colours in cooked flour products (51).

Flavour and aroma

The same criteria for colour and appearance apply for flavour and aroma. The consumer must not be able to detect a discernable difference. Any detectable change in flavour and aroma caused by fortification is unacceptable (51).

Shelf life

As a general rule of thumb the addition of micronutrients to produce a fortified flour of wheat must not reduce the normal or expected shelf life of the flour. Any reduction of shelf life will result in lost products and reduced consumer acceptance of the food. The usual cause of reduced shelf life is due to the development of rancidity in the flour caused by soluble iron and zinc salts. This is particularly true for high extraction and whole grain flours (51).

Taste and mouth-feel

There must be no change in product texture and mouth-feel. Rancidity will affect the taste and mouth-feel as well as aroma and flavour of both the flour and the finished products as consumed. Rancid products have a slightly soapy mouth-feel and a distinctive unpleasant odour.

Sensory testing

There has been extensive testing and experience to show that fortification can be accomplished without adversely affecting any of these sensory properties in standard products such as flour, maize meal, bread, cakes, instant noodles and pasta. However, there are some cereal-based foods unique to different regions of the world that have not been tested. These should be tested prior to starting a general fortification program (51).

3.7 Sensory evaluation

Sensory evaluation is a scientific discipline, which is normally used in the analyze, measure and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, taste, smell, touch and hearing, The complex sensation that result from the interaction of these senses is normally used to measure food quality in program quality control, new product development and shelf stability test of food products (56).

3.7.1 Sensory testing methods

There are three primary types of sensory tests: discriminatory testing, preference/acceptance tests and descriptive tests. Discriminatory tests are used to determine whether a difference exists between samples. The panelist does not allow his personal likes and dislikes influencing his response. Laboratory difference panels can be used to determine if there is a difference among samples. Preference/ acceptance tests are affective tests based on a measure of preference or a measure from which relative preference can be determined. The personal feeling of a panelist toward the products directs his response. Descriptive tests involve the detection and the description of both the qualitative and quantitative sensory aspects of a product by trained panels of 5 to 100 subjects (57-58).

a) Triangle test

Triangle test is the most famous method for discrimination test. It is a three sample test in which all three sample are coded and the subject’s task is to determine which two samples are the same or which sample is different from the other two. The chance probability associated with this test is only 1/3 which probably accounts for its offer orders of presentation with replicate within the session. Each subject is served

with two set of sample. The orders of presentation must be equally balanced between both samples for each subject. This method usually requires a minimum of 20 subjects per test (57-58).

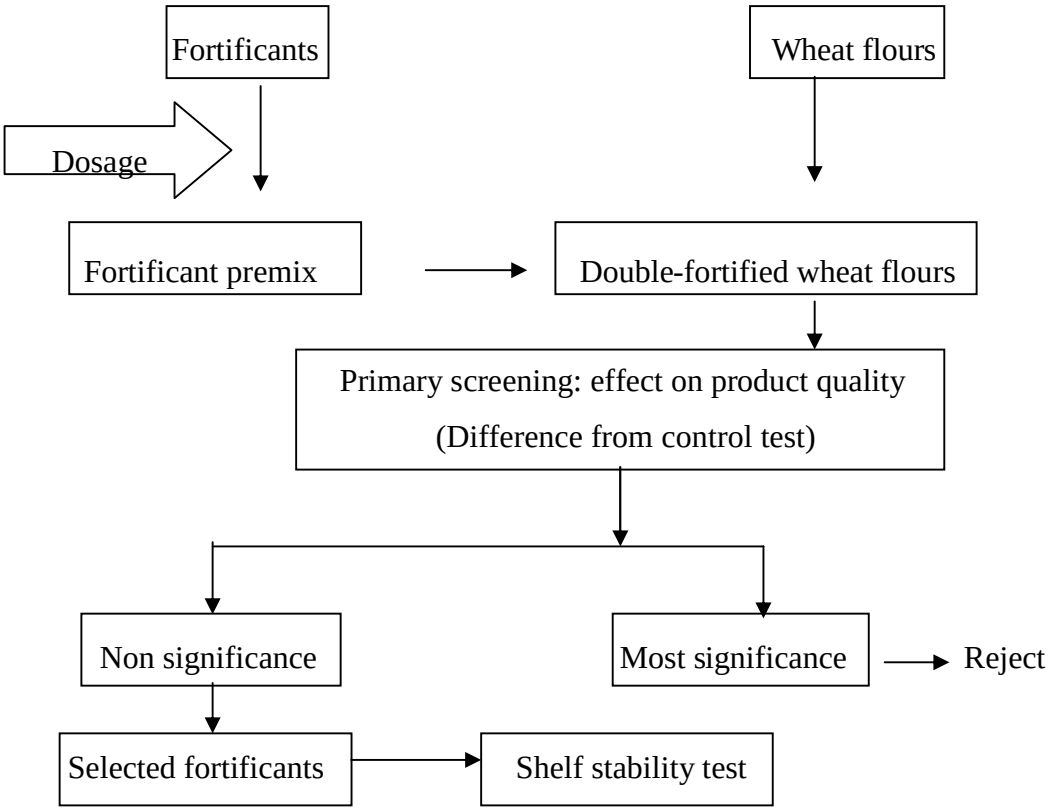
b) Hedonic scale

The hedonic scale is considered to provide a measure of the “amount of” or “degree of liking”. The hedonic scale emerged from food research at the U.S. Army Quartermaster Corporation in Chicago. It is pretty straight forward to scale liking - the panelists or dislikes the samples, and to what degree. The hedonic scale is easy to use, intuitive, and makes no pretense of measuring anything other than an individual likes of product. The most commonly used scale is the nine-point scale (59).

c) Difference from Control test

Difference from Control test normally requires at less 20 panelists. The samples are presented to each panelist as randomized Complete Block design. In the tests, the unfortified sample was labeled as “R”, and panelists are asked to rate for the degree of difference from the “R” sample. The “R” sample is also coded with randomized 3 digit number and tested as an internal control.

CHAPTER IV
MATERIALS AND METHOD



- Homogeneity test
- Packagings
  - Polyethylene bag - Laminated film bag
  - Woven polypropylene bag
- Acceleration test
  - Iron retention at 0, and 3rd month
  - Folic acid retention, Thiobabituric Acid Reactive Substances (TBARS) at at 0, 1st, 2nd and 3rd month
  - Color (L*, a*, b*), Moisture content, A_w and sensory evaluation at 0, 1st, 2nd and 3rd month

Figure 3. Shelf life stability and sensory acceptability of double fortified wheat flour diagram

4.1 Wheat flours

All-purpose wheat flour Kite™ brand which were widely used in Thailand represented high extracted wheat flour. While cake flour Royal Fan™ brand represented low extracted wheat flour. Both were purchased from United Flour Mill Co., Ltd.

4.2 Fortificants

-Iron source: ferrous sulfate ($FeSO_4$) (33% iron), ferrous fumarate (33% iron), were obtained from Dr. Paul Lohmann Co., Ltd., Lüneburg, Germany. H-reduced elemental iron (97% iron) and electrolytic elemental iron (97% iron) were obtained from North American Hoganas. Inc, 111 Hoganas Way Hollsopple, Pennsylvania, USA.

-Folic acid (FA) was obtained from DSM Nutritional Products, Kaiseraugst, Switzerland.

4.3 Fortification doses

Fortification was performed based on the serving size of wheat flour mentioned in the Ministry of public health notification no 182 on Nutrition Labeling, which is 30 g. Iron was fortified at 10% RDI, while folic acid was fortified at 21%. Fortification levels of iron used was the minimum level for nutrient claim as fortified product, while for folic acid was the level recommended by ADB. Due to their low bioavailabilities, elemental irons i.e. H-reduced and E were fortified at double level.

Details of fortification are shown in Table 6.

Table 6. Fortification levels of fortificants (iron and folic acid) and their fulfillments to the Thai RDI [1].

Fortificants	Level (ppm)	Fe	% Thai RDI per serving (30 g)
Ferrous sulfate	155	51.15	10.2
Ferrous fumarate	155	51.15	10.2
H-reduced	105	101.85	20.4
Electrolytic	105	101.85	20.4
Folic acid	1.4	NA	21

[1]Thai RDI of iron and folate were 15 mg and 200µg per day, respectively (60).

4.4 Preparation of fortificant premix

To prepare fortificant premix, 10 g of wheat flour was mixed with 0.1 g of folic acid or 2 g of FeSO4 or 2 g of Fe fumarate or 2 g of elemental iron and then the mixture enlarge volume to 100 g with wheat flour. The mixtures were well-mixed in swollen polypropylene bag. The premix that contained 2% Fe and 0.1% FA were kept in glass bottle at desiccators.

4.5 Primary screening: effects of fortificants on product quality

One kg of wheat flour was mixed with the premix in a polypropylene bag for 15 min at the appropriate ratio and then the double-fortified all-purpose and cake wheat flours were used for making alkaline fresh noodles, and angel cake and sugar cookies, respectively. Products made from fortified flours were tested for their difference from the unfortified products by using Difference from Control test. The most significantly different formula was discarded from this study.

4.6 Shelf life study

Fortificants which passed the primary screening test were then used for shelf life study in different kinds of packaging that were normally used for commercial distribution.

4.6.1 Pilot-scale production of double-fortified wheat flours

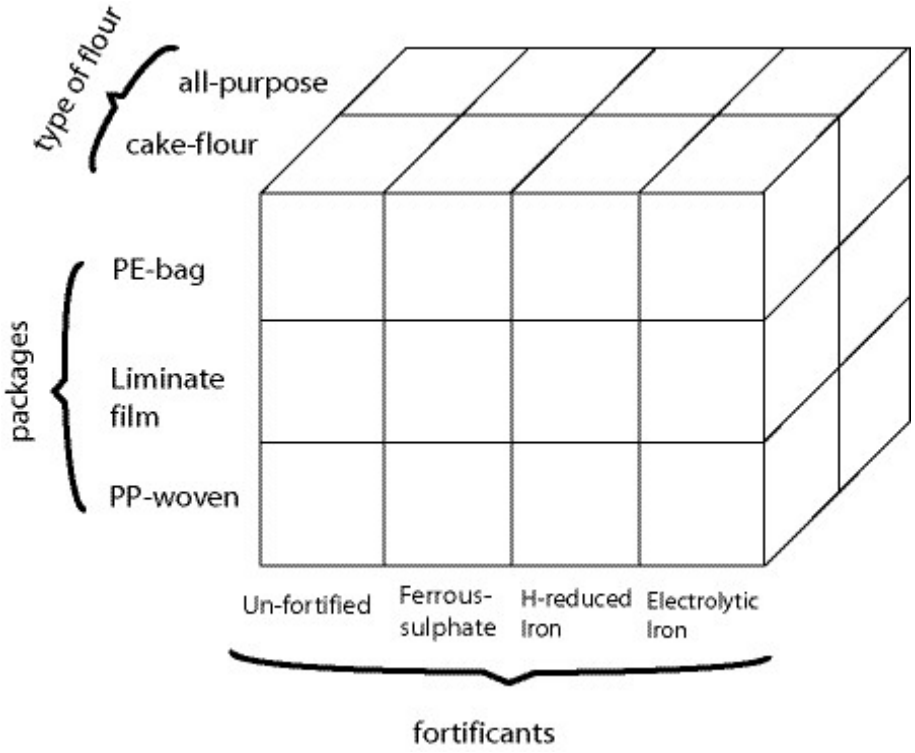
Sixteen kg of wheat flour were mixed with 0.5 kg of the wheat flour mixed with fortificant premix at the fortification level in a Vee shape mixer for 30 min. The mixed double-fortified wheat flour was sampled for 5 spots and analyzed for its homogeneity, before being packed in different packagings.

4.6.2 Homogeneity test

Iron contents from the 5 sampled wheat flour samples were determined as representative because iron is mineral that substantially stable. The acceptable degree of homogeneity should be at >10% coefficient of variation (CV).

4.6.3 Packagings

. Approximately 250 g of double-fortified wheat flours were packed and heat-sealed in 3 kinds of packaging i.e.



1) Polyethylene bag (0.09 mm thickness), which is normally used in lower income market
2) Laminated film bag (OPP 30 µ/PE 20 µ+MB8000CL-5%/L-LDPE 30 µ/EAA25 µ), which was normally used in higher income market
3) Woven polypropylene bag ($10\times10/in^2$), which was normally used for industrial distribution.

4.6.4 Acceleration test

The packed products were stored under fluorescent light at 40 ± 2 ^{o} C (relative humidity = 25-35%) for 3 mo. The products were sampled at 0 mo, 1st mo, 2nd mo, and 3rd mo to determine sensory quality, lipid oxidation as Lipid-Oxidation-Thiobarbituric Acid Reactive Substances (TBARS), water activity (A_w), moisture content, Color (L*, a*, B*), folate and iron contents. If samples could not analyze immediately, samples were stored in aluminium foil at -20 ^{o} C until analysis.

4.7 Quality analysis

4.7.1 Physical analysis

4.7.1.1 Color measurement

Changes in colors of the double-fortified and unfortified wheat flour during storage were measured by Spectro-colorimeter Model JS 555 (Color Techno System Corporation, Tokyo, Japan). A Tungsten halogen lamp was used as alight source. L*value represents darkness (0) and lightness (100), a* value represents green (-) and red (+) color tones, and b* value represents blue (-) and yellow (+) color tones.

4.7.1.2 Water activity (A_w)

Water activity was determined by using water activity meter (NOVASINA IC-500 A_w-Lab, Axair Ltd., Pfaffikon, Switzerland) under controlled temperature at 25 ± 1 ºC. Sample of wheat flour were placed in equilibrium chamber of the meter until the constant water activity was obtained.

4.7.1.2 Moisture content

Moisture content was analyzed by drying the sample in a hot air oven at 100 ± 5 ^{o} C until obtaining constant weight (61).

4.7.2 Chemical analysis

4.7.2.1 Lipid oxidation thiobarbituric acid reactive substances (TBARS)

TBARS was used for measuring lipid oxidation in the product. After 0.2 g of sample was added with tertiary butyl hydroxyl quinine (TBHQ) solution and trichloroacetic acid – hydrochloric acid reagent, it was flushed with nitrogen gas and mixed. The mixture was added with thiobarbituric acid solution and again flushed with nitrogen gas. After mixing, the mixture was incubated in 100 ^{o} C water bath for 30 min and placed in ice bath for 5 min to stop the reaction. The mixture was then separated by mixing with chloroform and centrifuging at 3,000 rpm, 4 ^{o} C for 10 min.

The top layer was transferred into cuvette and measured for absorbency at 535 nm using Spectrophotometer model SHIMADZU Pharmaspec UV.-1700. (62-63).

4.7.2.2 Folic acid content

Folic acid was analyzed by using microbial assay, based on the growth of *Lactobacillus casei* ATCC7469. The product was added with 0.2 M phosphate buffer, autoclaved at 120 ^{o} C for 10 min, and cooled down to room temperature. The mixture was then added with amylase enzyme at 0.2 g/ml and shaken at 37 ^{o} C for 3 h. After being boiled in water bath at 100 ^{o} C for 5 min, the mixture was diluted with 100 ml de-ionized water, filtered through Whatman no.42, adjusted pH to 6.2 and added with phosphate buffer. The filtered mixture was inoculated with *Lactobacillus casei* ATCC7469 inoculum. After 17 h, growth of the bacteria was determined from the mixture turbidity by using Spectrophotometer (MILTON SPECTRONIC 1001PLUS) at wavelength of 630 nm (64-65).

4.7.2.3 Iron content

Iron content was determined by wet digestion method. After the product had been digested in nitric and perchloric acids for overnight, the predigested product was diluted with de-ionized distilled water and measured iron contents by using Atomic Absorption Spectrophotometer model Varian; Spectr AA-20 (lamp 5 mA and spectral band pass 0.5 nm) at wavelength of 248.3 nm (66).

4.7.3 Sensory evaluation

Sensory evaluation was performed in individual air-conditioned booth under daylight at the sensory science laboratory of the Institute of Nutrition, Mahidol University at Salaya, Nakhonpathom. Samples were coded with 3-digit random number and randomly served. Complete block design was used in the study. Twenty-four staffs and graduate students are joining the panel.

4.7.3.1 Primary screening test: Difference from Control Test which panelists rated the differences of products quality for appearance and taste on 5-point scale rated 0 as no difference and 4 as very difference was used.

4.7.3.2 Shelf stability test: Difference from Control Test which panelists rated the differences of products quality on 9-point scale rated 0 as no difference and 4, -4 as very difference was used. Samples for color evaluation were placed in transparent plastic bag while the ones for odor and rancidity evaluation were placed in concealed color bottle, and closed with plastic lid.

4.8 Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) for Windows, version 13.0. Differences between means of the analytical results were tested at a significance level of $p = 0.05$ by using one-way analysis of variance (ANOVA) and Duncan multiple comparisons, except for iron contents that used independent t-test. Differences in means of sensory quality were analyzed with ANOVA and Tukey multiple comparisons.

4.9 Cost estimation

Additional costs due to fortification were estimated based on the costs of iron and folic acid fortificants.

CHAPTER V
RESULTS

5.1 Fortificant screening: effect on sensory quality

The iron sources that significantly affected general appearance, especially color ($p \leq 0.05$) of fresh noodles were ferrous sulfate and ferrous fumarate (Table 7). Numerically, the more effects were observed after the products were kept refrigerated for 4 days. As the noodles were cooked, differences in sensory quality (appearance and taste) were still significant in both kinds of iron. In case of cake flour, angel cake's general appearance (table 7, appearance), was only significantly affected by ferrous fumarate ($p \leq 0.05$). As the cookies prepared from different kinds of iron were tasted (table8), subjects could not identify the difference from the unfortified one ($p > 0.05$). Therefore, ferrous fumarate was eliminated from the next study.

Table 7 Scores of the Difference from Control test for general appearances of the double-fortified wheat flour products as compared to the unfortified ones (n = 25)

Fresh noodles		**Test score[1,2,3]**	
Formula[4]	**Fortificant**	**1st day**	**4th day**
AU	Unfortified	0.21±0.41[a]	0.54±0.88[a]
AF	Ferrous sulfate iron, folic acid	0.83±0.82[b]	1.38±1.06[bc]
AFF	Ferrous fumarate iron, folic acid	0.79±0.78[b]	1.83±1.09[c]
AH	H-reduced iron, folic acid	0.12±0.34[a]	0.96±1.08[ab]
AE	Electrolytic iron, folic acid	0.42±0.50[ab]	0.75±0.79[ab]
Angel cake		**Test score[1,2,3]**	
Formula[4]	**Fortificant**	**1st day**	
CU	Unfortified	0.54±0.59[a]	
CF	Ferrous sulfate iron, folic acid	1.04±0.69[ab]	
CFF	Ferrous fumarate iron, folic acid	1.17±0.96[b]	
CH	H-reduced iron, folic acid	0.5±0.88[a]	
CE	Electrolytic iron, folic acid	0.5±0.59[a]	

[1] Mean ± standard deviation

[2] Mean scores within the same column of the same product of the same day having the same superscript are not significantly different ($p>0.05$).

[3] Score of "Difference from Control" ranged from 0, no difference from reference sample; 1, slight difference; 2, moderate difference; 3, much difference; 4, very much difference.

[4] A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; FF: Ferrous fumarate H: H-reduced iron; E: Electrolytic iron.

Tables 8 Scores of the Difference from Control test for flavor of the double-fortified wheat flour products as compared to the unfortified ones (n = 25)

Cooked noodles		**Test score[1,2,3]**	
Formula[4]	**Fortificant**	**1st day**	**4th day**
AU	Unfortified	0.12±0.34[a]	0.46±1.06[a]
AF	Ferrous sulfate iron, folic acid	0.67±0.76[b]	1.12±0.85[ab]
AFF	Ferrous fumarate iron, folic acid	0.42±0.50[ab]	1.21±0.93[b]
AH	H-reduced iron, folic acid	0.12±0.71[ab]	0.71±0.86[ab]
AE	Electrolytic iron, folic acid	0.29±0.62[ab]	0.79±0.83[ab]
Cookies		**Test score[1,2,3]**	
Formula[4]	**Fortificant**	**1st day**	**4th day**
UCF	Unfortified	0.54±0.98[a]	0.79±0.93[a]
CF	Ferrous sulfate iron, folic acid	0.42±0.78[a]	0.79±0.93[a]
CFF	Ferrous fumarate iron, folic acid	0.42±0.58[a]	0.88±0.74[a]
CH	H-reduced iron, folic acid	0.46±0.66[a]	1.08±1.06[a]
CE	Electrolytic iron, folic acid	0.50±0.66[a]	0.92±0.97[a]

[1] Mean ± standard deviation

[2] Mean scores within the same column having the same superscript letter are not significantly different ($p>0.05$).

[3] Score of "Difference from Control" ranged from 0, no difference from reference sample; 1, slight difference; 2, moderate difference; 3, much difference; 4, very much difference.

[4] A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; FF: Ferrous fumarate; H: H-reduced iron; E: Electrolytic iron.

5.2 Homogeneity of the nutrients in products

The 3 iron sources (ferrous sulfate iron, H-reduced iron and Electrolytic iron) were selected for the shelf life study of double fortified wheat flour (DFW). Homogeneity of fortified flour prepared for the next the study was measured to ensure that it is homogeneous.

Table 9 indicates that percents coefficient of variation (CV) of the fortified flours from 5 different positions of each formula were less than 10%, which mean that nutrients were homogenously mixed under the process used in laboratory.

Table 9 Degrees of homogeneity of iron content in each formula of double-fortified all-purpose and cake wheat flours from different sampling points.

Position	Iron content (mg/100 g)							
	Formula[1]							
	AU	AF	AH	AE	CU	CF	CH	CE
1	0.88	5.21	8.51	8.92	0.46	4.65	9.3	8.77
2	0.88	5.12	8.65	8.97	0.46	4.29	8.71	8.75
3	0.87	4.72	8.84	9.06	0.48	4.58	8.54	8.47
4	0.87	4.68	9.38	8.57	0.47	5.18	9.71	9.79
5	0.9	4.51	9.26	8.58	0.47	5.12	9.26	8.7
Mean±SD	0.88±0.01	4.85±0.30	8.92±0.38	8.82±0.23	0.47±0.01	4.76±0.38	9.10±0.48	8.90±0.51
%CV	1.36	6.26	4.23	2.61	1.79	7.92	5.22	5.77

[1]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

5.3 Iron and folate contents in double-fortified wheat flours (DFW).

Table 10 indicates the contents of iron and folate in different kinds of wheat flour that had been fortified as compared to the unfortified ones. The mentioned contents in fact did not include the nutrients that were found naturally in the wheat flours (unfortified wheat flour). The iron contents were about 80-90% of the desirable values, while the folate contents were about 95% of the desirable values.

Table 10 Iron and folate contents in each kind of wheat flour[1]

Formula[2]	Initial value (wet basis)	
	Iron (mg/100g)	Total Folate (µg/100g)
AU	0.88±0.012	10.68±0.46
AF	4.85±0.30	143.63±7.28
AH	8.92±0.38	140.12±1.19
AE	8.82±0.23	139.11±2.17
CU	0.47±0.01	9.01±0.29
CF	4.76±0.38	140.10±4.88
CH	9.10±0.48	140.99±12.26
CE	8.90±0.51	139.57±10.01

[1] Mean ± standard deviation

[2]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

5.4 Shelf life study

5.4.1 Color measurement

Tables 11, 12 and 13 show changes in colors of DFW which were stored in different kinds of packaging. Slight difference but significance in L* value of the DFW of different iron sources. The difference could be detectable since the beginning of fortification especially for elemental iron. Slight fluctuation in the L* value was observed during storage in different kinds of packaging, however not in the same direction. Iron fortification affected in red (a*) and yellow (b*) color tones of the fortified wheat flours (Tables 12 and 13). Changes in the red and yellow tones were based on the changes in initial color tones of the unfortified flours, as well. Overall, the changes in L*, a*, b* values after fortification and storage were slightly but instrumentally were significant.

5.4.2 Sensory evaluation

Even color differences were detectable from instrumental measurement. However, they were not significant in the sensory evaluation. **Table 14** shows that scores of difference from control tests of color were not significant between unfortified and double-fortified products which were packed in different packagings during 3 mo storage. The same evidence was also found in case of differences in odor (**Table 15**), except in case of the flours that were fortified with ferrous sulfate packed in PE and laminated bags.

Table 11 L* color changes of double fortified wheat flours that were stored in different packaging during 3-month storage under accelerated condition**.**

Packaging	Time(mo)	L* value[1,2,3]			
		AU	AF	AH	AE
PE	0	94.29±0.06^{a}	94.15±0.03^{a}	93.77±0.14^{b}	93.62±0.05^{b}
	1	94.4±0.15^{a}	94.24±0.05^{a}	93.87±0.06^{b}	93.84±0.10^{b}
	2	94.51±0.05^{a}	94.17±0.07^{b}	93.84±0.01^{c}	93.78±0.02^{c}
	3	94.59±0.05^{a}	94.2±0.08^{b}	94.22±0.04^{b}	94.27±0.10^{b}
Laminated	0	94.29±0.06^{a}	94.15±0.03^{a}	93.77±0.14^{b}	93.62±0.05^{b}
	1	94.48±0.04^{a}	94.01±0.13^{b}	93.84±0.03^{c}	93.90±0.03^{bc}
	2	94.43±0.05^{a}	93.98±0.06^{b}	93.74±0.02^{c}	93.74±0.02^{c}
	3	94.57±0.01^{a}	94.11±0.06^{c}	94.17±0.04^{bc}	94.27±0.11^{b}
Woven PP	0	94.29±0.06^{a}	94.15±0.03^{a}	93.77±0.14^{b}	93.62±0.05^{b}
	1	94.42±0.09^{a}	93.94±0.03^{b}	93.84±0.01^{b}	93.86±0.03^{b}
	2	94.24±0.06^{a}	93.89±0.06^{b}	93.76±0.01^{c}	93.76±0.04^{c}
	3	94.36±0.05^{a}	94.17±0.02^{b}	94.10±0.06^{b}c	94.07±0.04^{c}
Packaging	Time(mo)	CU	CF	CH	CE
PE	0	94.79±0.10^{a}	94.59±0.12^{b}	94.31±0.07^{c}	94.13±0.08^{d}
	1	95.63±0.08^{ab}	95.68±0.05^{a}	95.49±0.15^{b}	95.58±0.06^{ab}
	2	95.53±0.03^{a}	95.51±0.01^{a}	95.60±0.01^{b}	95.62±0.03^{b}
	3	96.14±0.10^{a}	96.02±0.04^{b}	95.98±0.02^{b}	96.01±0.02^{b}
Laminated	0	94.79±0.10^{a}	94.59±0.12^{b}	94.31±0.07^{c}	94.13±0.08^{d}
	1	95.64±0.00^{a}	95.65±0.02^{a}	95.53±0.09^{b}	95.62±0.00^{ab}
	2	95.61±0.06^{a}	95.61±0.01^{a}	95.59±0.02^{a}	95.61±0.03^{a}
	3	95.96±0.05^{b}	96.03±0.03^{a}	96.01±0.02^{ab}	95.92±0.04^{c}
Woven PP	0	94.79±0.10^{a}	94.59±0.12^{b}	94.31±0.07^{c}	94.13±0.08^{d}
	1	95.62±0.06^{a}	95.65±0.04^{a}	95.48±0.02^{b}	95.46±0.12^{b}
	2	95.62±0.11^{a}	95.63±0.00^{a}	95.54±0.06^{a}	95.56±0.01^{a}
	3	95.62±0.07^{ab}	96.01±0.04^{a}	95.96±0.05^{ab}	95.82±0.12^{b}

[1]Mean ± SD (n = 3)

[2]Means with the same superscript of the same color value within the same row are not significantly different ($p > 0.05$)

[3]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron

[4] "L*" represented white (100) → dark (0)

Table 12 a* color changes of double fortified wheat flours that were stored in different packaging during 3-month storage under accelerated condition**.**

Packaging	Time(mo)	a* value[1,2,3]			
		AU	AF	AH	AE
PE	0	0.07±0.04^{a}	0.28±0.06^{b}	0.47±0.04^{c}	0.58±0.02^{d}
	1	0.41±0.11^{a}	1.27±0.04^{b}	1.39±0.01^{c}	1.39±0.01^{c}
	2	0.49±0.12^{a}	1.14±0.13^{b}	1.31±0.01^{c}	1.34±0.00^{c}
	3	0.13±0.13^{a}	0.22±-0.02^{ab}	0.27±0.01^{b}	0.32±0.00^{b}
Laminated	0	0.07±0.04^{a}	0.28±0.06^{b}	0.47±0.04^{c}	0.58±0.02^{d}
	1	0.83±0.12^{a}	1.31±0.01^{b}	1.38±0.01^{b}	1.39±0.01^{b}
	2	0.86±0.05^{a}	1.28±0.02^{b}	1.21±-0.19^{b}	1.35±0.00^{b}
	3	0.15±0.03^{a}	0.24±0.02^{b}	0.30±0.01^{c}	0.33±0.01^{c}
Woven PP	0	0.07±0.04^{a}	0.28±0.06^{b}	0.47±0.04^{c}	0.58±0.02^{d}
	1	1.14±0.13^{a}	1.39±0.01^{b}	1.40±0.00^{b}	1.42±0.02^{b}
	2	1.26±0.05^{a}	1.38±0.05^{b}	1.38±0.02^{b}	1.44±0.08^{b}
	3	0.37±0.10^{a}	0.47±0.01^{ab}	0.51±0.06^{b}	0.55±0.05^{b}
Packaging	Time(mo)	CU	CF	CH	CE
PE	0	0.49±0.02^{a}	0.55±0.02^{b}	0.59±0.02^{c}	0.64±0.02^{d}
	1	1.18±0.00^{a}	1.21±0.00^{b}	1.21±0.01^{b}	1.24±0.00^{c}
	2	1.15±0.02^{a}	1.20±0.01^{c}	1.18±0.01^{b}	1.15±0.00^{a}
	3	0.07±0.01^{a}	0.10±0.02^{a}	0.08±0.01^{a}	0.10±0.00^{a}
Laminated	0	0.49±0.02^{a}	0.55±0.02^{b}	0.59±0.02^{c}	0.64±0.02^{d}
	1	1.18±0.02^{a}	1.20±0.01^{ab}	1.22±0.01^{c}	1.25±0.01^{d}
	2	1.12±0.00^{a}	1.20±0.01^{d}	1.17±0.00^{c}	1.15±0.01^{b}
	3	0.09±0.01^{a}	0.08±0.01^{a}	0.08±0.01^{a}	0.10±0.00^{b}
Woven PP	0	0.64±0.02^{a}	0.59±0.02^{b}	0.55±0.02^{c}	0.49±0.02^{d}
	1	1.28±0.01^{a}	1.23±0.01^{a}	1.20±0.01^{b}	1.21±0.01^{c}
	2	1.13±0.02^{b}	1.20±0.00^{c}	1.15±0.00^{ab}	1.16±0.01^{a}
	3	0.09±0.01^{b}	0.08±0.00^{c}	0.07±0.00^{d}	0.14±0.00^{a}

[1] Mean ± SD (n = 3)

[2] Means with the same superscript of the same color value within the same row are not significantly different ($p > 0.05$)

[3] A means All purpose wheat flour with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron

[4] "a*" represented red (+) and green (-)

Table 13 b* color changes of double fortified wheat flours that were stored in different packaging during 3-month under accelerated condition**.**

Packaging	Time(mo)	b* value[1,2,3]			
		AU	AF	AH	AE
PE	0	8.32±0.09^{a}	8.47±0.03^{b}	8.49±0.05^{b}	8.51±0.02^{b}
	1	8.08±0.07^{a}	8.70±0.09^{b}	8.69±0.08^{b}	8.76±0.04^{b}
	2	8.17±0.07^{a}	8.51±0.08^{b}	8.55±0.01^{b}	8.54±0.01^{b}
	3	7.99±0.14^{a}	8.33±0.12^{c}	8.17±0.02^{bc}	8.14±0.05^{ab}
Laminated	0	8.32±0.09^{a}	8.47±0.03^{b}	8.49±0.05^{b}	8.51±0.02^{b}
	1	8.29±0.10^{a}	8.90±0.06^{b}	8.76±0.04^{b}	8.80±0.01^{b}
	2	8.33±0.02^{a}	8.79±0.25^{b}	8.59±0.08^{b}	8.61±0.06^{b}
	3	7.90±0.02^{a}	8.52±0.17^{c}	8.19±0.05^{b}	8.16±0.01^{b}
Woven PP	0	8.32±0.09^{a}	8.47±0.03^{b}	8.49±0.05^{b}	8.51±0.02^{b}
	1	8.50±0.10^{a}	8.81±0.02^{b}	8.76±0.03^{b}	8.83±0.03^{b}
	2	8.51±0.08^{a}	8.72±0.03^{b}	8.66±0.02^{b}	8.64±0.04^{b}
	3	8.02±0.11^{a}	8.30±0.01^{b}	8.29±0.04^{b}	8.30±0.03^{b}
Packaging	Time(mo)	UC	CF	CH	CE
PE	0	7.10±0.09^{a}	7.17±0.08^{a}	7.17±0.04^{a}	7.24±0.07^{a}
	1	7.00±0.02^{a}	7.12±0.04^{b}	6.99±0.10^{a}	7.17±0.05^{b}
	2	6.82±0.02^{a}	6.84±0.06^{a}	6.87±0.02^{a}	6.99±0.02^{b}
	3	6.08±0.30^{a}	6.14±0.10^{a}	6.26±0.06^{a}	6.33±0.03^{a}
Laminated	0	7.10±0.09^{a}	7.17±0.08^{a}	7.17±0.04^{a}	7.24±0.07^{a}
	1	7.11±0.04^{ab}	7.18±0.01^{b}	7.04±0.08^{a}	7.16±0.05^{b}
	2	6.79±0.01^{a}	7.01±0.00^{d}	6.87±0.04^{b}	6.94±0.01^{c}
	3	6.27±0.03^{a}	6.33±0.04^{a}	6.24±0.08^{a}	6.35±0.08^{a}
Woven PP	0	7.10±0.09^{a}	7.17±0.08^{a}	7.17±0.04^{a}	7.24±0.07^{a}
	1	7.01±0.03^{a}	7.00±0.01^{a}	6.87±0.16^{a}	7.02±0.02^{a}
	2	6.72±0.10^{a}	6.89±0.11^{b}	6.84±0.02^{ab}	6.84±0.07^{ab}
	3	6.14±0.08^{ab}	6.17±0.04^{b}	6.06±0.02^{a}	6.33±0.03^{c}

[1] Mean ± SD (n = 3)

[2] Means with the same superscript of the same color value within the same row are not significantly different ($p > 0.05$)

[3] A means All purpose wheat flour with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron

[4] "b*" represented yellow (+) and blue (-)

Table 14 Scores of color difference of double fortified wheat flour that were packed in different kinds of packaging during 3-month storage under accelerated condition as compared to the unfortified one.

All-purpose flour	Formula[1,2,3]											
	PE				Laminated				Woven PP			
Time (mo)	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic
0	0.12±0.74[a]	0.04±0.46[a]	0.04±0.81[a]	-0.08±0.50[a]	0.12±0.74[a]	0.04±0.46[a]	0.04±0.81[a]	-0.08±0.50[a]	0.12±0.74[a]	0.04±0.46[a]	0.04±0.81[a]	-0.08±0.50[a]
1	-0.17±1.00[a]	0.21±0.93[a]	-0.12±1.12[a]	0.04±1.04[a]	0.08±1.06[a]	0.21±1.14[a]	-0.04±0.91[a]	-0.17±1.13[a]	-0.08±0.65[a]	-0.08±0.72[a]	-0.21±0.51[a]	-0.17±1.09[a]
2	0.38±1.01[a]	-0.04±0.99[a]	0.08±0.78[a]	0.38±1.24[a]	0.04±0.99[a]	0.67±0.96[b]	0.25±0.90[ab]	-0.04±0.75[a]	0.08±0.78[a]	0.04±0.46[a]	0.08±0.83[a]	0.08±0.83[a]
3	0.37±0.88[a]	0.75±1.26[a]	0.5±0.98[a]	0.25±1.15[a]	0.17±0.92[a]	0.38±0.92[a]	-0.08±0.65[a]	0.04±0.91[a]	0.04±0.86[a]	0.00±0.78[a]	0.04±0.62[a]	0.00±1.02[a]
Cake flour	Formula[1,2,3]											
	PE				Laminated				Woven PP			
Time (mo)	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic
0	0.04±0.46[a]	0.00±0.51[a]	-0.08±0.41[a]	0.04±0.62[a]	0.04±0.46[a]	0.00±0.51[a]	-0.08±0.41[a]	0.04±0.62[a]	0.04±0.46[a]	0.00±0.51[a]	-0.08±0.41[a]	0.04±0.62[a]
1	-0.04±0.69[a]	0.42±0.83[a]	-0.08±1.14[a]	0.08±0.93[a]	0.21±0.98[a]	0.58±1.25[a]	0.29±0.81[a]	0.33±1.13[a]	0.12±0.95[a]	0.54±0.83[a]	0.21±0.66[a]	0.33±1.05[a]
2	0.04±1.12[a]	-0.08±0.65[a]	0.16±0.82[a]	0.29±0.95[a]	0.12±0.61[a]	0.21±0.58[a]	0.08±0.58[a]	0.38±0.62[a]	0.00±0.98[a]	0.08±0.78[a]	-0.12±0.85[a]	0.08±0.78[a]
3	0.42±0.86[a]	0.42±0.46[a]	0.42±1.04[a]	0.38±1.06[a]	0.00±0.66[a]	-0.04±0.81[a]	0.25±0.53[a]	0.17±0.76[a]	0.08±0.50[a]	0.17±0.56[a]	-0.12±0.80[a]	0.17±0.76[a]

[1]Means ± SD (n=24).

[2]Means within the same row of the same packaging followed by the same superscript (represents effect of each fortificant) are not significantly different ($p > 0.05$).

[3]Score of "Difference from Control" ranged from 0, no difference from reference sample; 1, slighly darker; 2, moderatly darker; 3, much darker; 4, very much darker; -1, slightly lighter; -2, moderately lighter; -3, much lighter; -4, very much lighter.

Table 15 Scores of rancidity intensity difference of double fortified wheat flour that were packed in different kinds of packaging during 3-month storage under Accelerated condition as compared to the unfortified one.

All-purpose flour	Formula[1,2,3]											
	PE				Laminated				Woven PP			
Time (mo)	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic
0	0.29±0.95[a]	0.83±0.96[a]	0.17±0.82[a]	0.29±1.16[a]	0.29±0.95[a]	0.83±0.96[a]	0.17±0.82[a]	0.29±1.16[a]	0.29±0.95[a]	0.83±0.96[a]	0.17±0.82[a]	0.29±1.16[a]
1	-0.12±1.62[a]	0.71±1.30[a]	0.21±1.64[a]	0.00±1.25[a]	0.29±1.12[ab]	1.21±1.69[b]	0.00±1.86[a]	-0.12±1.26[a]	0.08±1.44[a]	0.29±1.37[a]	0.29±1.37[a]	-0.38±1.31[a]
2	0.67±1.13[a]	1.50±1.38[b]	0.79±0.88[a]	0.67±1.13[a]	0.25±0.68[a]	0.87±0.99[b]	0.71±0.95[ab]	0.29±0.86[a]	0.17±0.56[ab]	0.62±0.71[b]	0.17±1.09[ab]	-0.12±0.80[a]
3	-0.12±1.11[a]	0.96±1.12[b]	0.46±0.88[a]	0.04±1.23[a]	0.08±0.65[a]	1.54±1.18b	0.54±1.18[ab]	0.29±0.86[a]	0.21±0.93[a]	0.17±0.87[a]	-0.12±1.11[a]	0.08±0.83[a]
Cake flour	Formula[1,2,3]											
	PE				Laminated				Woven PP			
Time (mo)	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic	Reference	Ferrous sulfate	H-reduced	Electrolytic
0	0.46±0.98[a]	0.08±0.93[a]	0.25±1.07[a]	0.29±0.86[a]	0.46±0.98[a]	0.08±0.93[a]	0.25±1.07[a]	0.29±0.86[a]	0.46±0.98[a]	0.08±0.93[a]	0.25±1.07[a]	0.29±0.86[a]
1	-0.04±0.69[a]	0.62±0.65[a]	0.00±1.18[a]	0.17±0.0[a]	0.25±0.99[a]	0.58±1.25[a]	0.33±0.82[a]	0.38±1.13[a]	0.21±0.98[a]	0.54±0.83[a]	0.33±0.70[a]	0.42±1.06[a]
2	0.04±1.23[a]	0.38±1.47[a]	0.29±1.08[a]	0.08±1.06[a]	0.04±0.95[a]	0.38±1.28[a]	0.08±0.88[a]	0.17±0.87[a]	0.12±0.95[a]	0.25±0.85[a]	0.17±0.92[a]	0.21±0.72[a]
3	-0.21±1.22[a]	0.54±0.83[b]	-0.08±1.02[a]	-0.21±1.25[a]	-0.08±0.83[a]	0.79±1.02[b]	0.25±0.99[ab]	0.17±0.92[ab]	-0.12±1.15[a]	-0.21±1.14[a]	0.17±0.92[a]	0.08±0.83[a]

[1] Means ± SD (n=24).

[2] Means within the same row of the same packaging followed by the same superscript (represents effect of each fortificant) are not significantly different ($p > 0.05$).

[3] Score of "Difference from Control" ranged from 0, no difference from reference sample; 1, slightly stronger; 2, moderately stronger; 3, much stronger; 4, very much stronger; -1, slightly milder; -2, moderately milder; -3, much milder; -4, very much milder.

5.4.3 Thiobarbituric acid reactive substances (TBARS)

Table 16 indicates that TBARS in unfortified wheat flour were low at the beginning, significantly increased until the 1st mo and remained unchanged in 2nd mo, however the TBARS were significantly increased again in the 3rd mo. The same trends of changes were also observed in case of the fortified wheat flours however at higher TBARS values especially in the wheat flours that were fortified with ferrous sulfate. Types of packaging did not significantly affect TBARS**.**

5.4.4 Folate and iron retention

Types of packaging did not significantly affect folate loss in both kinds of fortified wheat flours (**Tables 17 and 18**). Rate of folate loss in unfortified wheat flour was found to be significantly higher. However, folate losses in the fortified wheat flours were not significant during storage. **Table 19** indicates that there were no significant losses of iron in both kinds of wheat flour in all packagings during storage.

Table 16 Thiobarbituric acid reactive substances (TBARS) of double-fortified all-purpose and cake wheat flours from different packages

Formula[3]	Time(mo)	TBARS (mg MDA/kg)[1],[2]		
		PE	Laminated	Woven
AU	0	0.49±0.05^{Aa}	0.49±0.05^{Aa}	0.49±0.05^{Aa}
	1	1.10±0.23^{Ba}	1.16±0.11^{Bab}	1.48±0.14^{Bb}
	2	1.06±0.15^{Ba}	1.04±0.19^{Ba}	1.28±0.10^{Ba}
	3	2.00±0.35^{Ca}	2.16±0.39^{Ca}	2.34±0.32^{Ca}
AF	0	0.41±0.07^{Aa}	0.41±0.07^{Aa}	0.41±0.07^{Aa}
	1	1.36±0.25^{Ba}	1.25±0.12^{Bab}	0.95±0.14^{ABb}
	2	1.26±0.08^{Ba}	1.36±0.22^{Ba}	1.50±0.35^{Ba}
	3	2.34±0.22^{Ca}	2.47±0.12^{Ca}	2.40±0.38^{Ca}
AH	0	0.62±0.20^{Aa}	0.62±0.20^{Aa}	0.62±0.20^{Aa}
	1	1.20±0.15^{Ba}	1.24±0.38^{Ba}	1.37±0.19^{Ba}
	2	1.21±0.17^{Ba}	1.29±0.17^{Ba}	1.45±0.17^{Ba}
	3	2.30±0.15^{Ca}	2.08±0.09^{Ca}	2.19±0.18^{Ca}
AE	0	0.54±0.04^{Aa}	0.54±0.04^{Aa}	0.54±0.04^{Aa}
	1	1.10±0.28^{Ba}	1.45±0.05^{Ba}	1.14±0.11^{Ba}
	2	1.52±0.12^{Ca}	1.29±0.17^{Ba}	1.31±0.10^{Ba}
	3	2.24±0.09^{Da}	2.28±0.06^{Cb}	2.33±0.10^{Cb}
CU	0	0.45±0.10^{Aa}	0.45±0.10^{Aa}	0.45±0.10^{Aa}
	1	1.70±0.22^{Ba}	1.73±0.20^{Ba}	1.82±0.20^{Ba}
	2	1.60±0.13^{Ba}	1.63±0.20^{Ba}	1.68±0.04^{Ba}
	3	2.47±0.21^{Ca}	2.55±0.40^{Ca}	2.92±0.20^{Ca}
CF	0	0.71±0.08^{Aa}	0.71±0.08^{Aa}	0.71±0.08^{Aa}
	1	1.66±0.05^{Ba}	1.88±0.14^{Ba}	1.87±0.18^{Ba}
	2	1.59±0.17^{Ba}	1.63±0.13^{Ba}	1.62±0.09^{Ba}
	3	3.43±0.11^{Ca}	3.42±0.25^{Ca}	3.82±0.42^{Ca}
CH	0	0.71±.011^{Aa}	0.71±0.11^{Aa}	0.71±0.11^{Aa}
	1	1.90±0.09^{Ba}	1.33±0.25^{Bb}	1.31±0.13^{ABb}
	2	1.96±0.17^{Ba}	1.84±0.21^{Ca}	1.41±0.11^{Bb}
	3	3.15±0.40^{Ca}	2.81±0.30^{Da}	3.36±0.64^{Ca}
CE	0	0.71±0.10^{Aa}	0.71±0.10^{Aa}	0.71±0.10^{Aa}
	1	1.61±0.17^{Ba}	1.82±0.17^{Ba}	1.89±0.06^{Ba}
	2	1.58±0.16^{Ba}	1.88±0.14^{Ba}	1.78±0.18^{Ba}
	3	3.22±0.22^{Cb}	2.50±0.44^{Ca}	3.34±0.36^{Cb}

[1]Means of mg malonaldehyde (MDA/kg product) ± SD(n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same row followed by the same small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

[3]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

Table 17 Total folate retention of double fortified all purpose flours that were packed in different kinds of packaging during 3-month storage under accelerated condition.

Iron Fortificant	Time(mo)	Total Folate (µg/100g)[1,2] dry basis		
		PE (% retention)	Laminated (% retention)	PP (% retention)
Unfortified	0	11.75±0.75^{Aa} (100)	11.75±0.75^{Aa} (100)	11.75±0.75^{Aa} (100)
	1	10.82±0.52^{ABa} (90.18)	11.24±0.32^{Aa} (93.67)	11.45±0.59^{ABa} (95.41)
	2	10.42±0.39^{Cb} (86.87)	11.52±0.34^{Aa} (95.97)	10.63±0.50^{ABb} (88.61)
	3	11.07±0.25^{ABab} (92.23)	11.36±0.63^{Aa} (94.64)	10.04±0.75^{Bb} (83.68)
Ferrous sulfate	0	161.31±8.17^{Aa} (100)	161.31±8.17^{Aa} (100)	161.31±8.17^{Aa} (100)
	1	157.70±3.75^{Aa} (97.76)	159.11±1.03^{Aa} (98.63)	154.94±2.74^{ABa} (96.05)
	2	158.18±2.57^{Aa} (98.06)	158.29±4.67^{Aa} (98.13)	146.32±9.80^{Ba} (90.71)
	3	151.49±4.31^{Aa} (93.91)	151.22±5.99^{Aa} (93.74)	146.98±4.16^{Ba} (91.12)
H-reduced	0	157.33±1.34^{Aa} (100)	157.33±1.34^{Aa} (100)	157.33±1.34^{Aa} (100)
	1	158.17±1.02^{Aa} (100.53)	158.39±3.58^{Aa} (100.67)	152.57±2.04^{ABb} (96.97)
	2	157.23±5.02^{Aa} (99.93)	158.83±5.48^{Aa} (100.96)	143.63±2.75^{Cb} (91.29)
	3	146.58±10.41^{Aa} (93.17)	148.08±5.47^{Ba} (94.12)	149.10±5.03^{BCa} (94.77)
Electrolytic	0	156.29±2.44^{Aa} (100)	156.29±2.44^{Aa} (100)	156.29±2.44^{Aa} (100)
	1	160.37±3.21^{Aa} (102.61)	154.52±2.66^{Aa} (98.87)	153.79±5.37^{Aa} (98.40)
	2	150.12±3.11^{Aa} (96.05)	155.26±4.84^{Aa} (99.34)	154.44±3.78^{Aa} (98.81)
	3	152.64±4.04^{Aa} (97.66)	150.77±1.54^{Aa} (96.47)	149.87±8.94^{Aa} (95.89)

[1]Results are means of folate retention (µg/100g) ± SD (n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same row followed by the same small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

Table 18 Total folate retention of double fortified cake flours that were packed in different kinds of packaging during 3-month storage under accelerated condition.

Iron Fortificant	Time(mo)	Total Folate (µg/100g)[1,2] dry basis		
		PE (% retention)	Laminated (% retention)	PP (% retention)
Unfortified	0	10.14±0.32^{Aa} (100)	10.14±0.32^{Aa} (100)	10.14±0.32^{Aa} (100)
	1	9.15±0.07^{Bb} (90.23)	9.75±0.42^{Aa} (96.14)	9.81±0.20^{Aa} (96.81)
	2	6.98±0.25^{Da} (68.85)	6.84±0.21^{Ca} (67.45)	6.98±0.32^{Ba} (68.90)
	3	7.63±0.22^{Ca} (75.27)	7.47±0.32^{Ba} (73.70)	7.41±0.09^{Ba} (73.14)
Ferrous sulfate	0	157.47±5.49^{Aa} (100)	157.47±5.49^{Aa} (100)	157.47±5.49^{Aa} (100)
	1	158.54±4.31^{Aa} (100.67)	158.56±3.34^{Aa} (100.69)	147.51±6.71^{Ab} (93.67)
	2	143.48±5.62^{Bb} (91.11)	157.93±4.16^{Aa} (100.29)	150.72±6.61^{Aab} (95.71)
	3	138.88±1.02^{Bb} (88.19)	153.44±2.30^{Aa} (97.43)	150.65±3.87^{Aa} (95.67)
H-reduced	0	158.41±13.66^{Aa} (100)	158.41±13.66^{Aa} (100)	158.41±13.66^{Aa} (100)
	1	148.30±1.47^{Aa} (93.62)	153.72±0.89^{Aa} (97.04)	150.48±9.91^{Aa} (94.99)
	2	154.33±4.68^{Aa} (97.42)	154.61±5.96^{Aa} (97.60)	155.64±4.45^{Aa} (98.26)
	3	150.01±11.79^{Aa} (94.69)	148.61±6.63^{Aa} (93.81)	144.99±8.73^{Aa} (91.53)
Electrolytic	0	156.84±11.25^{Aa} (100)	156.84±11.25^{Aa} (100)	156.84±11.25^{Aa} (100)
	1	151.82±8.09^{Aa} (96.79)	150.20±5.03^{Aa} (95.76)	147.15±3.58^{Aa} (93.82)
	2	157.36±2.20^{Aa} (100.33)	157.96±5.12^{Aa} (100.71)	147.35±12.10^{Aa} (93.95)
	3	145.42±6.08^{Aa} (92.72)	146.44±1.45^{Aa} (93.37)	150.55±1.85^{Aa} (95.99)

[1]Results are means of folate retention (µg/100g) ± SD (n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same row followed by the same small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

Table 19 Iron retention of double fortified wheat flour that were packed in different kinds of packaging during 3-month storage under Accelerated condition.

Formula[3]	Time(mo)	Iron (mg/100g)[1,2] dry basis		
		PE	Laminated	PP
AU	0	0.98±0.02 ^{Aa}	0.98±0.02 ^{Aa}	0.98±0.02 ^{Aa}
	3	0.96±0.09 ^{Aa}	0.99±0.05 ^{Aa}	1.06±0.04 ^{Aa}
AF	0	5.45±0.34 ^{Aa}	5.45±0.34 ^{Aa}	5.45±0.34 ^{Aa}
	3	5.50±0.34 ^{Aa}	5.31±0.10 ^{Aa}	5.46±0.32 ^{Aa}
AH	0	10.02±0.42 ^{Aa}	10.02±0.42 ^{Aa}	10.02±0.42 ^{Aa}
	3	10.01±0.78 ^{Aa}	9.94±0.60 ^{Aa}	9.48±0.30 ^{Aa}
AE	0	9.91±0.26 ^{Aa}	9.91±0.26 ^{Aa}	9.91±0.26 ^{Aa}
	3	9.66±0.49 ^{Aa}	9.58±0.29 ^{Aa}	9.34±0.37 ^{Aa}
CU	0	0.53±0.01 ^{Aa}	0.53±0.01 ^{Aa}	0.53±0.01 ^{Aa}
	3	0.54±0.01 ^{Aa}	0.53±0.01 ^{Aa}	0.54±0.01 ^{Aa}
CF	0	5.35±0.42 ^{Aa}	5.35±0.42 ^{Aa}	5.35±0.42 ^{Aa}
	3	5.48±0.16 ^{Aa}	5.31±0.42 ^{Aa}	5.07±0.30 ^{Aa}
CH	0	10.23±0.53 ^{Aa}	10.23±0.53 ^{Aa}	10.23±0.53 ^{Aa}
	3	10.22±0.61 ^{Aa}	10.15±0.52 ^{Aa}	9.76±0.38 ^{Aa}
CE	0	10.00±0.58 ^{Aa}	10.00±0.58 ^{Aa}	10.00±0.58 ^{Aa}
	3	9.75±0.30 ^{Aa}	9.76±0.36 ^{Aa}	9.90±0.84 ^{Aa}

[1]Means ± SD (n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same row followed by the same small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

[3]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

5.4.5 A_w and moisture content changes

Upon storage, both A_w and moisture contents of all products decreased at faster rate in the 1st mo, especially for the products that were packed in PP woven **(Tables 20 and 21)**. The changing rates were not so high in the products packed in PE and laminated bags.

Table 20 Changes in water activities (A_w) of double fortified wheat flours that were packed in different kinds of packaging during 3-month under accelerated condition.

Formula[3]	Time(mo)	A_w[1,2] PE	Laminated	Woven
AU	0	0.54 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}
	1	0.48 ± 0.02^{Ba}	0.50 ± 0.00^{Ba}	0.39 ± 0.02^{Bb}
	2	0.46 ± 0.02^{Bb}	0.51 ± 0.01^{Ba}	0.43 ± 0.02^{Bb}
	3	0.45 ± 0.02^{Bb}	0.50 ± 0.01^{Ba}	0.44 ± 0.03^{Cb}
AF	0	0.54 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}	0.54 ± 0.01^{Aa}
	1	0.46 ± 0.03^{Ba}	0.50 ± 0.01^{Ba}	0.38 ± 0.02^{Bb}
	2	0.46 ± 0.02^{Bb}	0.50 ± 0.01^{Ba}	0.39 ± 0.01^{Bc}
	3	0.43 ± 0.01^{Bb}	0.48 ± 0.03^{Ba}	0.40 ± 0.01^{Bc}
AH	0	0.55 ± 0.01^{Aa}	0.55 ± 0.01^{Aa}	0.55 ± 0.01^{Aa}
	1	0.45 ± 0.01^{Bb}	0.50 ± 0.00^{Ba}	0.34 ± 0.00^{Cc}
	2	0.44 ± 0.02^{Bb}	0.50 ± 0.01^{Ba}	0.37 ± 0.01^{Bc}
	3	0.44 ± 0.01^{Bb}	0.48 ± 0.01^{Ca}	0.39 ± 0.01^{Bc}
AE	0	0.55 ± 0.00^{Aa}	0.55 ± 0.00^{Aa}	0.55 ± 0.00^{Aa}
	1	0.47 ± 0.02^{Bb}	0.52 ± 0.03^{ABa}	0.38 ± 0.02^{Cc}
	2	0.49 ± 0.01^{Ba}	0.51 ± 0.01^{Ba}	0.42 ± 0.02^{Bb}
	3	0.45 ± 0.02^{Cb}	0.50 ± 0.01^{Ba}	0.43 ± 0.03^{Bb}
CU	0	0.53 ± 0.01^{Aa}	0.53 ± 0.01^{Aa}	0.53 ± 0.01^{Aa}
	1	0.44 ± 0.01^{Cb}	0.48 ± 0.01^{Ba}	0.42 ± 0.00^{Cb}
	2	0.47 ± 0.01^{Ba}	0.48 ± 0.00^{Ba}	0.44 ± 0.01^{Bb}
	3	0.48 ± 0.01^{Ba}	0.49 ± 0.00^{Ba}	0.45 ± 0.01^{Bb}
CF	0	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}
	1	0.44 ± 0.00^{Bb}	0.48 ± 0.00^{Ba}	0.38 ± 0.02^{Bc}
	2	0.41 ± 0.01^{Cb}	0.49 ± 0.01^{Ba}	0.40 ± 0.04^{Bb}
	3	0.41 ± 0.01^{Cb}	0.46 ± 0.01^{Cb}	0.40 ± 0.03^{Bb}
CH	0	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}
	1	0.46 ± 0.01^{BCb}	0.49 ± 0.01^{ABa}	0.45 ± 0.00^{Bb}
	2	0.47 ± 0.00^{Bb}	0.48 ± 0.00^{BCa}	0.42 ± 0.01^{Cc}
	3	0.45 ± 0.01^{Ca}	0.47 ± 0.02^{Ca}	0.41 ± 0.01^{Db}
CE	0	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}	0.51 ± 0.00^{Aa}
	1	0.47 ± 0.01^{Cab}	0.49 ± 0.01^{ABa}	0.47 ± 0.01^{ABb}
	2	0.50 ± 0.01^{ABa}	0.50 ± 0.02^{Aa}	0.41 ± 0.04^{BCb}
	3	0.48 ± 0.01^{BCa}	0.48 ± 0.02^{Ba}	0.43 ± 0.02^{Cb}

[1] Means ± SD (n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same rows followed by the small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

[3]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

Table 21 Change in **%** moisture content of double fortified wheat flour that were packed in different kinds of packaging during 3-month storage under accelerated condition.

Formula[3]	Time(mo)	%Moisture content[1],[2]		
		PE	Laminated	Woven
AU	0	10.97±0.11^{Aa}	10.97±0.11^{Aa}	10.97±0.11^{Aa}
	1	10.45±0.18^{ABa}	10.59±0.05^{Ba}	9.18±0.15^{Cb}
	2	10.32±0.32^{Ba}	10.63±0.24^{Ba}	9.78±0.23^{Bb}
	3	9.77±0.43^{Ca}	10.10±0.14^{Ca}	9.61±0.30^{Ba}
AF	0	10.96±0.04^{Aa}	10.96±0.04^{Aa}	10.96±0.04^{Aa}
	1	10.22±0.44^{Ba}	10.64±0.30^{Aa}	9.06±0.41^{Bb}
	2	9.91±0.27^{Ba}	10.56±0.08^{ABa}	8.89±0.58^{Bb}
	3	9.75±0.17^{Ba}	10.11±0.39^{Ba}	9.11±0.07^{Bb}
AH	0	10.94±0.06^{Aa}	10.94±0.06^{Aa}	10.94±0.06^{Aa}
	1	10.32±0.09^{Bb}	10.58±0.08^{Ba}	8.35±0.16^{Cc}
	2	10.05±0.28^{BCb}	10.74±0.20^{ABa}	9.03±0.37^{Bc}
	3	9.90±0.20^{Ca}	10.13±0.13^{Ca}	8.74±0.49^{BCb}
AE	0	10.99±0.11^{Aa}	10.99±0.11^{Aa}	10.99±0.11^{Aa}
	1	10.29±0.31^{BCa}	10.44±0.15^{ABa}	9.20±0.22^{Cb}
	2	10.64±0.09^{ABa}	10.90±0.14^{ABa}	9.77±0.21^{Bb}
	3	9.89±0.46^{Cab}	10.64±0.47^{Ba}	9.14±0.38^{Cb}
CU	0	11.15±0.10^{Aa}	11.15±0.10^{Aa}	11.15±0.10^{Aa}
	1	10.02±0.10^{Ca}	10.41±0.10^{Ba}	9.96±0.82^{Ba}
	2	10.34±0.12^{Ba}	10.37±0.08B^{Ca}	9.46±0.78^{Ba}
	3	10.25±0.12^{Ba}	10.22±0.10^{Ca}	9.60±0.19^{Bb}
CF	0	11.02±0.12^{Aa}	11.02±0.12^{Aa}	11.02±0.12^{Aa}
	1	10.13±0.16^{Bab}	10.58±0.11^{ABa}	9.30±0.92^{Bb}
	2	9.88±0.75^{Bab}	10.76±0.29^{ABa}	9.10±0.87^{Bb}
	3	9.71±0.27^{Bab}	10.47±0.44^{Ba}	9.26±0.50^{Bb}
CH	0	10.99±0.10^{Aa}	10.99±0.10^{Aa}	10.99±0.10^{Aa}
	1	10.11±0.10^{Bab}	10.91±0.27^{ABa}	10.00±0.65^{Bb}
	2	10.39±0.50^{Ba}	10.57±0.17BCa	10.05±0.21^{Ba}
	3	10.37±0.06^{Ba}	10.40±0.17Ca	9.97±0.17^{Bb}
CE	0	11.01±0.10^{Aa}	11.01±0.10^{Aa}	11.01±0.10^{Aa}
	1	10.48±0.23^{BCa}	10.65±0.17^{Aa}	9.94±0.84^{ABa}
	2	10.64±0.10^{Bab}	10.73±0.36^{Aa}	9.57±0.89^{Bb}
	3	10.33±0.08^{Ca}	10.48±0.58^{Aa}	9.42±0.74^{Bb}

[1]Means ± SD(n = 3).

[2]Means within the same column followed by the same capital letter (represents effect of storage time of each fortificant) and the same rows followed by the same small letter (represents effect of packaging) are not significantly different ($p > 0.05$).

[3]A and C means all-purpose and cake wheat flours, respectively with U: Unfortified; F: ferrous sulfate; H: H-reduced iron; E: Electrolytic iron.

5.6 Cost estimation

Additional costs due to fortification based on the prices of iron and folic acid are shown in **Table 22**. Costs of fortificants for double fortified wheat flours were 11.2 to 27 baht per MT wheat flour (excluded feeder for the fortification process and analytical cost for approximately). While, hypothetical costs of wheat flour fortification per year at 1 mill using a continuous fortification system shown in **table. 23.**

Table 22 Cost of fortificants used for double fortified of wheat flour[1]

Fortificant	cost/kg (US $)	cost/MT flour (US $)	cost/MT flour (baht)
Ferrous sulfate	2.5	0.39	12.4
H-reduced	2.0	0.21	6.72
Electrolytic	6.7	0.70	22.51
Folic acid	100	0.14	4.48

[1]Exchange rate: 32 Baht = 1 US$

Table 23 Estimated hypothetical costs of wheat flour fortification (30,000 metric tons (MT) per year (one production line) at 1 mill using a continuous fortification system**)**

	Annual (US$)
1. Capital investment	
Depreciation of dosifier[1]	2,500
5% annual maintenance on equipment	1,000
Equipment of laboratory and quality control[2]	100
2. Fortificants cost elemental iron and folic acid = 0.35-0.84 $ per MT)	10,500-25,200
3. Quality control cost[3]	6000
Total annual factory costs	20,100-34,800
Cost per metric ton of fortified wheat flour	0.67-1.16 **(21.44-37.12 baht)**

[1]Amortization 10 years.

[2]The semi-quantitative assay for in-line quality control.
[3] Iron and folic acid analysis.

Source: modified from Manual for wheat flour fortification with iron: Part1-part2 (3, 50)
Exchange rate: 32 Baht = 1 US$

CHAPTER VI
DISCUSSION

6.1 Selection of iron fortificants

In this study all-purpose and cake wheat flours were selected not only because they represented high and low extraction rate products, respectively. All-purpose wheat flour is widely used for making various kinds of food in Thailand including alkaline noodles which was produced with egg under high pH condition. Alkaline noodles was therefore a food model of the worst case that could be highly affected by the fortified iron since oxidation could be catalyzed under alkaline condition and iron could react with sulfur in egg to form black color. Cake flour is normally bleached with strong oxidizing agent which the residual oxidizing agent might cause change in color of the fortified iron. Such change might be easily observed in white color cake such as angel cake. In order to observe for the change in flavor due to iron oxidation, angel cake was not a good model since it contained only 15% flour. In this study, cookies made with cake flour that contained up to 50% flour and 30% fat were used instead. As considering from sensory quality, elemental irons both H-reduced and electrolytic were the most appropriate iron fortificant for both kinds of wheat flour. In fact, ferrous sulfate and ferrous fumarate were found to be more reactive in the tested food products, however ferrous fumarate tended to cause more changes in color of fresh noodles during storage. In addition to the mentioned reason, ferrous sulfate also had higher relative bioavailability and lower cost than ferrous fumarate iron, it was therefore other choice for further study (49). Annie and Peter (2004) mentioned that ferrous fumarate and ferrous sulfate caused dark red color which could be noticeable in white flour if used at high levels (51).

6.2 Homogeneity of the nutrient in products

Since preparations of the double-fortified wheat flours were performed in batch not continuous, homogeneity of the fortified nutrients must be tested. Percents

coefficient of variation (CV) were less than 10%, which indicated that nutrients were homogenously mixed in the flours. The prepared double fortified wheat flours could be used in the further study.

6.3 Shelf stability

Slight changes in colors of the double-fortified wheat flours upon fortification and during storage could be detectable on the instrument; however they were not significant in the sensory difference from control tests. Double-fortification did not affect the colors of wheat flours that were packed in all kinds of packagings which normally used for commercial distribution. The acceleration condition used should represent shelf life's of the wheat flours in the market which were 6 mo for PE and laminated bags and 4 mo for woven PP. Nestel and Nalubola (2000) mentioned that the fortified ferrous sulfate or elemental irons at 40 and 88 ppm, respectively did not produce any noticeable change in the color of flour or dough (53). Changes due to fortification however were found in rancidity intensity of the wheat flours (Table 15). Differences in rancidity intensity were found to be significant in wheat flours that were fortified with ferrous sulfate, packed and stored in PE and laminated bags. The problem of rancidity intensity was better if the ferrous sulfate-fortified wheat flour was packed in the woven PP bag, which in fact allowed more oxygen exposure. Since the woven PP allowed better ventilation, the developed rancidity odor could be removed from the PP woven bag. While the better protected packagings i.e. PE and laminated bags did not allow the formed rancidity odors (from the reaction of residual oxygen in bag with natural fat) to be volatile from packaging. The developed off-flavors became insignificant for only 1 month, which was too short and might not be practical in term of commercial distribution. Ferrous sulfate is a pro-oxidant, which catalyzes oxidation reaction which consequently leads to rancidity of natural fat in wheat flours, approx. 1.5% (53). Ferrous sulfate might not be the choice of iron for double fortification of wheat flours in Thailand.

TBARS which is an indicator for lipid oxidation increased during storages of both unfortified and fortified wheat flours, however numerically the fortified wheat flours tended to have higher values especially in cake wheat flours that were fortified with ferrous sulfate. The TBARS values somehow did not relate to the sensory

evaluation results on rancidity intensity since the TBARS values were found to be higher in PP woven bag which in fact had lower scores for rancidity intensity (Table 15). Without the sensory evaluation, TBARS value itself might not be able to explain the change in degree of rancidity in wheat flour.

Decreases in A_w and moisture content might due to acceleration condition during the shelf stability test, which used temperature up to 40 ºC. Vapor pressure becomes higher at higher temperature, which allowed moisture to move from products into atmosphere. Such evidence could be clearly observed in less-protected packaging i.e. PP woven bag.

Changes during storage in fact only affected water activities slightly, the final A_w of all products were only 0.38-0.55, which is the range that can retard lipid oxidation by reducing metal catalysis, quenching free radicals, promoting nonenzymic browning, and impeding oxygen accessibility (67).

Losses of folate in the fortified products were much lower than the losses found in the unfortified ones. Folic acid in chemical form was found to be more stable to heat and atmospheric oxygen than folates found in natural food which are in the forms of tetrahydrofolate (THF), 5-methyl-tetrahydrofolate (5-MeTHF), and 10-formyl-tetrahydrofolate (68, 69). Natural folates rapidly lose activity in foods over periods of days or weeks, folic acid as the synthetic form used in fortified foods is mostly completely stable for months or even years (68). Under the accelerated condition, folate loss after 3 months of unfortified wheat flour were about 6-17% in all-purpose flour and 22-25% in cake flour. Losses in both unfortified flours might be at the rate but % loss in cake flour was higher due to the calculation based on less the initial content of folate. Folate retention in the fortified wheat flours in different packagings were quite high (only 3-12% folate loss). Similar result was found by Cort *et al.* (1975) that folate in fortified cereal products lost only 8.5% after 3 mo storage at 45 ^{o}C. Folate is quite stable in dry products in the absence of light and oxygen. Food fortification of breakfast cereals, flour, etc. can add significant amounts of folic acid to the diet (70). Vinodkumar et al. (2007) found that folic acid in multiple fortified salt that was stored at 30ºC, 45 % RH was stable during storage for 6 mo (71). Keagy et al. (2005) suggested that folic acid can be added to cereal products because it is stable in flour during storage, and baking causes only small loss (72). Folic acid is

chemically stable during food processing and storage and is efficiently absorbed and converted to active forms of folate *in vivo* (80 to 100% bioavailability). It can be synthesized commercially and is the form of folate commonly added to foods or manufactured in supplement form (23).

In addition, iron was found to be stable during 3 mo storage regardless of kinds of iron and packagings, which was similar to other studies. Jorge et al. reported that the elemental reduced iron in fortified corn flour retained up to 90% after 3 month storage (73), and also found that natural iron in infant milk powder was stable during 18 mo storage (74). Vinodkumar et al. (2007) found that iron in double fortified salt that was stored at 30ºC, 45 % RH was stable during storage for 2 y (75). Although, all iron fortificants did not loss during the shelf stability test, however difference in sensory characteristics of the ferrous sulfate-fortified wheat flour form the unfortified flour was significantly detectable. Therefore ferrous sulfate might not be suitable for wheat flour fortification. While both kinds of elemental iron and folic acid were more appropriate due to their stability and no significant effect on sensory quality.

6.4 Cost estimation

Costs of fortificants based on the prices of iron and folic acid were 11.2 to 27 baht per metric ton of wheat flour. According to the current price of wheat flour (2008), cost of fortification was only 0.035-0.084 % of the product price, which was within acceptable range for food fortification as recommended by Hurrell R. Guidelines on food fortification with micronutrients (76). Additional cost on feeding machine however needed to be considered.

CHAPTER VII
CONCLUSION

It was feasible to double-fortify cake and all-purpose wheat flours with iron and folic acid, which were distributed in commercially-used packagings i.e. polyethylene bag, laminated film bag, and woven polypropylene bag. The fortification dosages were 51 and 102 ppm Fe for ferrous sulfate and elemental irons, and 1.4 ppm for folic acid. As compared to ferrous sulfate, elemental irons i.e. H-reduced and electrolytic were more appropriate due to insignificant effects on sensory quality. Folic acid did not affect sensory quality and was also stable during storage. Moisture content and A_w of the stored double-fortified wheat flours decreased significantly, especially in the woven polypropylene bag. Per serving, double-fortified wheat flours with elemental iron and folic acid provided 20.4% and 21% of Thai RDI, respectively. Bioavailability of the iron fortificants needed to be further studied the wheat flour consumption patterns of the Thais.

APPENDIX

APPENDIX A

แบบทดสอบเทียบความแตกต่างจากตัวอย่างควบคุม

ผลิตภัณฑ์_____

ชื่อผู้ทดสอบ_____ อายุ_____ เพศ (___) ชาย (___) หญิง

วันที่_____ เวลา_____

คำแนะนำ

กรุณาทดสอบชิมตัวอย่างควบคุม "R" ก่อน แล้วทดสอบตัวอย่างที่มีเลขรหัส 3 ตัว แล้ว บอกขนาดของความแตกต่างเมื่อเปรียบเทียบกับตัวอย่าง "R" ตามสเกลข้างล่าง ก่อนชิมสังเกตลักษณะปรากฏของผลิตภัณฑ์ก่อน

0 = ไม่แตกต่าง	_____	_____	_____
1 = แตกต่างเล็กน้อย	_____	_____	_____
2 = แตกต่างปานกลาง	_____	_____	_____
3 = แตกต่างมาก	_____	_____	_____
4 = แตกต่างมากที่สุด	_____	_____	_____

ข้อเสนอแนะ

_____

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_____

แบบทดสอบเทียบความแตกต่างจากตัวอย่างควบคุม

ผลิตภัณฑ์_____ อายุ_____ เพศ (___) ชาย (___) หญิง

วันที่_____ เวลา_____

คำแนะนำ

โปรดพิจารณาผลิตภัณฑ์ที่นำเสนอแล้วเปรียบเทียบ**กลิ่นหืนตัวอย่าง กับตัวอย่าง "R"** โดยระบุว่าตัวอย่าง "R" กลิ่นหืนหรือไม่ แล้วทำเครื่องหมาย X ลงในช่องที่ตรงกับความคิดเห็นของท่าน หลังจากนั้นจึงทดสอบดมกลิ่นตัวอย่างที่นำเสนอเปรียบเทียบกัน "R" ว่ามีกลิ่นหืนหรือไม่ ถ้ามีแตกต่างจาก "R" แตกต่างกันเพียงใด (ก่อนเปิดขวดตัวอย่างดม ควรเขย่าก่อน)

ตัวอย่าง "R" ☐ ไม่มีกลิ่นหืน ☐ มีกลิ่นหืน

ตัวอย่าง	_____	_____	_____	_____
<u>ระดับความแตกต่าง</u>				
กลิ่นแรงกว่า "R" มากที่สุด	_____	_____	_____	_____
กลิ่นแรงกว่า "R" มาก	_____	_____	_____	_____
กลิ่นแรงกว่า "R" ปานกลาง	_____	_____	_____	_____
กลิ่นแรงกว่า "R" เล็กน้อย	_____	_____	_____	_____
กลิ่นไม่ต่างจาก "R"	_____	_____	_____	_____
กลิ่นอ่อนกว่า "R" เล็กน้อย	_____	_____	_____	_____
กลิ่นอ่อนกว่า "R" ปานกลาง	_____	_____	_____	_____
กลิ่นอ่อนกว่า "R" มาก	_____	_____	_____	_____
กลิ่นอ่อนกว่า "R" มากที่สุด	_____	_____	_____	_____

ข้อเสนอแนะ

_____

_____

_____

แบบทดสอบเทียบความแตกต่างจากตัวอย่างควบคุม

ผลิตภัณฑ์_____ อายุ_____ เพศ (___) ชาย (___) หญิง

วันที่_____ เวลา_____

คำแนะนำ

โปรดพิจารณาผลิตภัณฑ์ที่นำเสนอแล้วเปรียบเทียบ**สี และ จุดดำ ของตัวอย่าง กับตัวอย่าง "R"**แล้วทำเครื่องหมาย X ลงในช่องที่ตรงกับความคิดเห็นของท่าน โดยระบุว่าแตกต่างกันหรือไม่ และถ้าหากมีความแตกต่าง แตกต่างกันเพียงใด

ตัวอย่าง	_____	_____	_____	_____
ระดับความแตกต่าง				
สีเข้มกว่า "R" มากที่สุด	_____	_____	_____	_____
สีเข้มกว่า "R" มาก	_____	_____	_____	_____
สีเข้มกว่า "R" ปานกลาง	_____	_____	_____	_____
สีเข้มกว่า "R" เล็กน้อย	_____	_____	_____	_____
สีไม่ต่างจาก "R"	_____	_____	_____	_____
สีอ่อนกว่า "R" เล็กน้อย	_____	_____	_____	_____
สีอ่อนกว่า "R" ปานกลาง	_____	_____	_____	_____
สีอ่อนกว่า "R" มาก	_____	_____	_____	_____
สีอ่อนกว่า "R" มากที่สุด	_____	_____	_____	_____

ข้อเสนอแนะ

_____

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APPENDIX B

Storage of the MFQCR under accelerated condition (40±2 °C, RH=25-35%, fluorescent light)

Figure 4 Cabinet for storage product samples under accelerated condition

APPENDIX C

Figure 5 The samples for color difference of DFW of difference from control test.

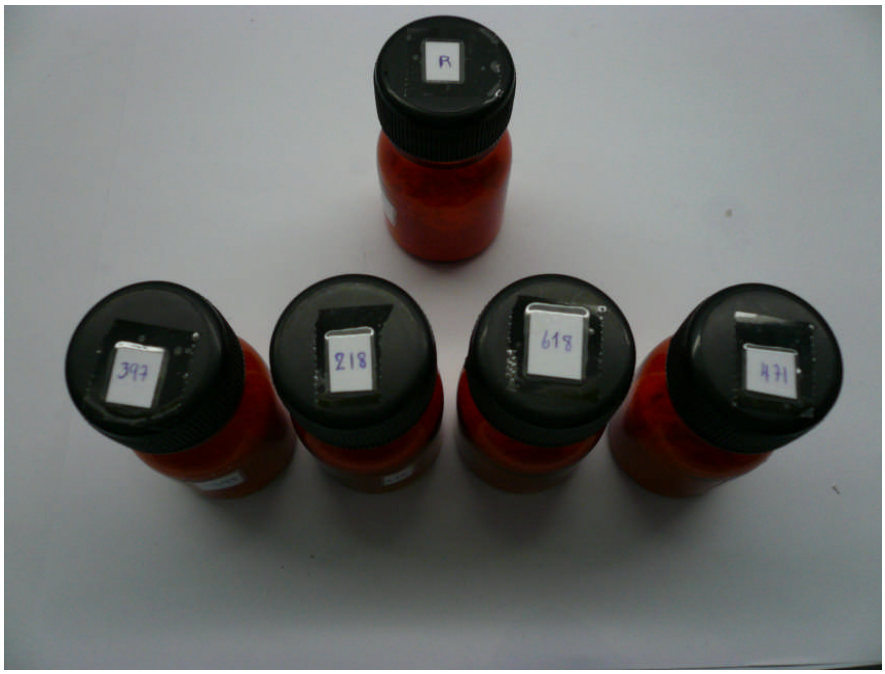

Figure 6 The samples for rancidity intensity of DFW of difference from control test.

APPENDIX D

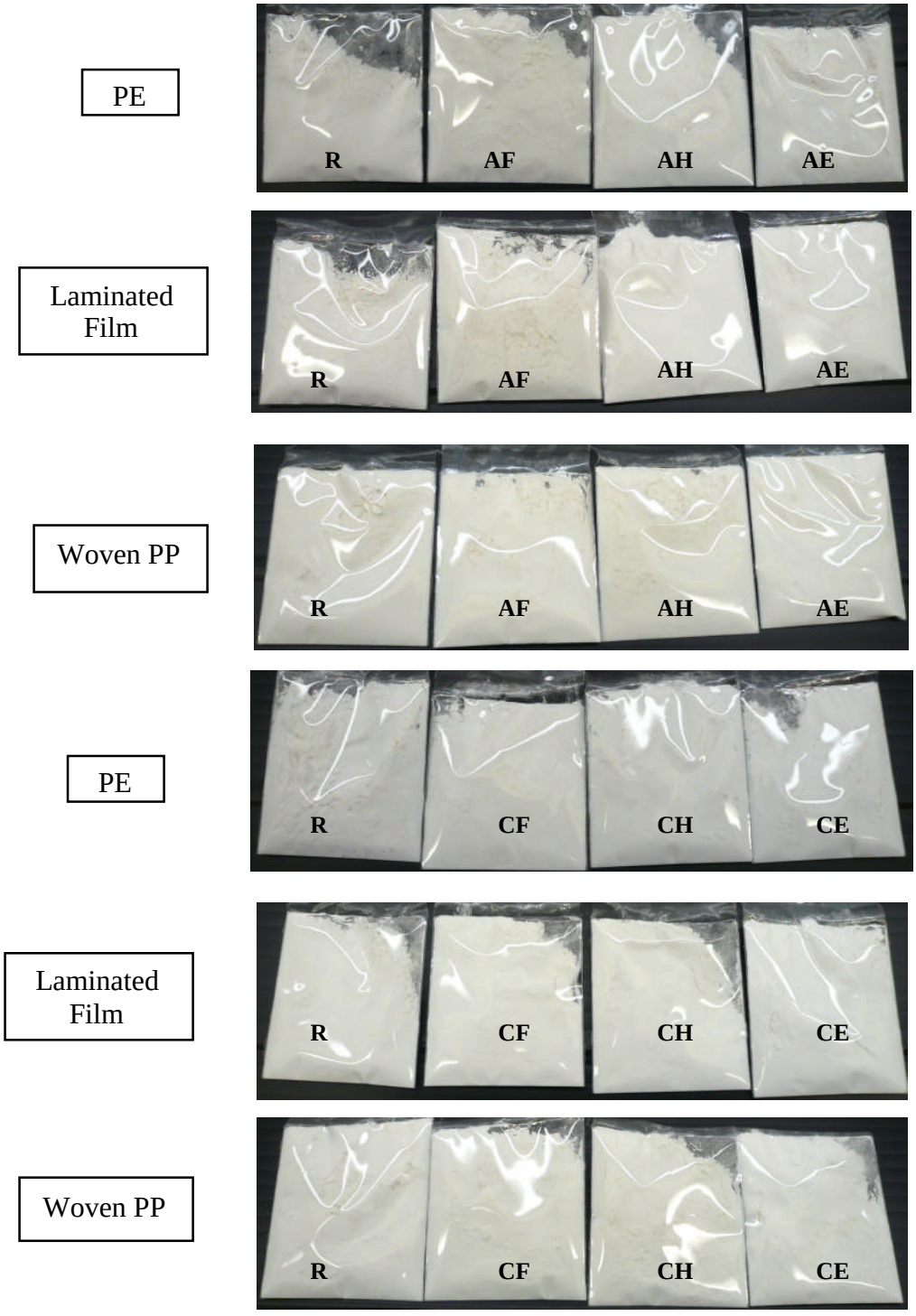

Figure 7 Color of DFW in different packaging after 3 mo storage.

APPENDIX E

Lipid oxidation-Thiobarbituric acid reactive substances (TBARS)

Principle:

MDA (Malondialdehyde) is the principle TBARS in oxidized methyl linolenate-fatty acid ester and squalene. TBA test is useful as a measure of lipid oxidation only during the initial stages of oxidation.

Instruments:

1. 35-ml screw capped centrifuge
2. N_2 gas
3. Vortex
4. Centrifuge
5. Spectrophotometer
6. Water bath
7. Ice bath

Reagents:

1. 0.1 N NaOH: 0.4 g of NaOH are diluted to 100 ml distilled water.
2. 0.6 N HCl: 5 ml of conc. HCl (12.1 N) are diluted to 100 ml distilled water.
3. 50 ml of 25% TCA: Mix 15.6 ml of 80% TCA and 34.4 ml of distilled water.
4. TBA solution (69.4 mM): 1 g of TBA is dissolved in 75 ml of 0.1 N NaOH and diluted to 100 ml with distilled water. TBA should be stored in the regrgerator.
5. TCA-HCl reagent: 50 ml of a 25% thiobarbituric acid (TCA) solution and 20 ml 0f 0.6 N HCl are mixed with 430 ml of distilled water. Total volume is 500 ml. TCA should be freshly prepared.
6. Antioxidant mixture: 200 mg (0.2 g) of tertiary butyl hydroxy quinone (TBHQ) are dissolved in 1.6 g (1.6 ml) of propylene glycol.
7. Chloroform

Procedures:

1. Weigh 200 mg (0.2000 g) of sample in a screw-capped centrifuge tube. Blank tube is prepared without sample.
2. Extraction: Add 3 drops of antioxidant mixture followed by 17 ml of TCA-HCl reagent. Flush tube with N_2 gas then cap tube. Vortex for 30 sec.
3. Reaction: Add 3 ml of TBA solution. Flush tube with N_2 gas then cap tube. Gentle vortex. Incubate in 100 ^{o}C water bath for 30 min.

   (For high sucrose sample, incubate in 40 ^{o}C water bath for 90 min)
4. Stop reaction: Cool tube in ice bath for 5 min.
5. Extract reaction product: Add 5 ml of chloroform (use glass pipette) and vortex for 15 sec. Centrifuge at 3,000 rpm (1000 x g), 4 ^{o}C for 10 min.
6. Detection: Transfer the top layer into plastic cuvette and read absorbance at 535 nm (the bottom layer is chloroform).

Calculation:

Mg MDA/ kg sample

$$= \frac{\text{Abs}}{\text{Molar abs}} \times \text{MW of MDA} \times 100 \text{ mg} \times \frac{\text{total volume of sample}}{1000 \text{ ml}} \times \frac{1000 \text{ g}}{\text{g of sample}}$$

$$= \frac{\text{Abs} \times 72 \times 1000 \times 20 \times 1000}{156{,}000 \times 1000 \times \text{gram of sample}}$$

Conversion: µmole MDA/ kg x 72/ 1000 = mg MDA/ kg

APPENDIX E

Determination of Moisture

(Hot air oven method; AOAC 1990, 952.45)

Principle:

A well homogeneous sample is dried in an oven (usually at 100 ± 5 ^{o}C) until constant weight is obtained. The loss of weight is taken as a measure of moisture content in the sample. Acid washed sand is used to mix with the wet sample prior to dry in order to increase area for rapid and complete evaporation of water from the wet sample.

Procedure:

1. Weigh approximately 1.0 g of acid washed sand into a porcelain dish containing a small glass stirring rod and dry in hot air oven at 100 ± 5 ^{o}C for 30 min.
2. Remove the sand dish and cool in the desicator.
3. Weigh sand dish (= a) and then approximately 5 g sample. Reweigh (= b g).
4. Add small amount of distilled water to disperse the sample evenly and evaporate the water as much possible on the boiling water bath. The sample dish should be frequently mixed until dry.
5. Transfer the sample dish to hot air oven and dry the sample at 100 ± 5 ^{o}C for 2 h.
6. Remove the sample dish and cool in a desicator and weigh (= c g)
7. Return the sample dish to the hot air oven and dry until a constant weight is obtained. Reweigh every 30 min.
8. The different weight between each interval time should not be more than 1-3 mg.

Calculation:

$$\% \text{ Moisture} = (b-c)/ (b-a) \times 100 \text{ (w/ w)}$$

APPENDIX F
Determination of Total Iron
(Wet digestion)

Principle:

Wet ashing technique was used to prepare the sample for the determination total iron. The samples were digested by nitric acid and perchloric acid at ratio 5:1 and then determined the iron content using an atomic absorption spectrophotometer at a wavelength of 248.3 nm.

Reagents:

1. Conc. Nitric acid (Merk # 1.00456.2500)
2. Conc. Perchloric acid (JT Baker 3 9625-04)
3. Ferric standard solution (Merk # 1.09972): Stock standard solution (1000 ppm)
4. Intermediate standard: Dilute 10 ml of stock standard to 100 ml with de-ionized water to make 0.1 mg Fe/ml standard solution
5. Working standard: 0.5-2.0 mg Fe/100ml. Prepared by an appropriate dilution of the intermediate standard with de-ionized water, in the presence of 10% 4 N nitric acid

Instruments:

1. Teflon
2. Volumetric flask
3. Filter paper # 42 and funnels
4. Atomic absorption spectrophotometer (Varian; Spectr AA-20)

Procedure:

1. Weigh 1-5 g of the homogeneous sample (depending on expected iron content) into Teflon
2. Add 5 ml conc. Nitric acid and 1 ml perchloric acid to each of Teflon sample and tightly covered with lids.
3. Keep the Teflon sample under fume hood at room temperature for predigestion overnight.
4. Place the Teflon sample in hot air oven for 16-20 h or until the solution clear.
5. Transfer the digested sample to an appropriate volume of volumetric flask and dilute with de-ionized distilled water.
6. Measure the diluted sample, working standard iron and reagent blank by atomic absorption spectrophotometer.

The normal working condition of atomic absorption spectrophotometer (AAS) for analyzing iron as follow:

Flame	air/ acetylene
Lamp	5 mA
Spectral ban pass	0.5 nm
Wavelength	248.3 nm
Flame stoichiometry	oxidizing

Calculation:

$$\text{Total iron (mg/ 100 g)} = \frac{\text{Absorbance x Standard curve x 100}}{\text{Weight of sample (g)}}$$

APPENDIX G
Determination of Folic Acid

Principle:

The method is based on the observation that certain organisms require specific vitamins for growth, using the basal medium containing all nutrients except that to be assayed. Growth responses of the organism are then compared quantitatively with standard of known concentration.

Reagents:

- 0.2 M Stock phosphate buffer:

(A) 31.20 g NaH_2PO_4 (MW 156.01) dilute to 1 L with de-ionized water

(B) 28.39 g Na_2HPO_4 (MW 141.96) dilute to 1 L with de-ionized water

- Form of available salts must be checked carefully. Weight of reagent used varies to the form of salts.

Working buffer: Prepare freshly before use.

212.5 ml (A) + 35.5 ml (B) dilute to nearly 1000 ml with de-ionized water, add ascorbic acid to the buffer in the concentration of 0.1% (w/v), adjust pH to 6.1 with (B) or (A) and dilute to 1000 ml with de-ionized water.

- Microorganism: *Lactobacillus casei* ATCC 7469
- Stock medium: Bacto-Micro Assay Culture Agar (MACA)
- Stock culture: Stab culture (3 tubes) in MACA. Incubate 35-37 ^{o}C, 24-48 h. Store 3 tubes of the stock culture in a refrigerator. Subculture monthly in triplicate.
- Culture medium: Micro-inoculum broth
- Inoculum: Subculture *L. casei* from a stock culture to Micro-inoculum broth. Incubated at 35-37 ^{o}C, 18-24 h. Under aseptic condition, wash cells with 3 x 5 ml portions of steriled 0.9% NaCl solution (NSS). Decanted the last supernate. Diluted the inoculum to an appropriate concentration with steriled NSS (McFaland No. 0.5)

- Assay medium: Bacto-Folic acid assay medium
- Alfa amylase from aspergillus (Difco)
- Folic acid standard: Folic acid

Stock folic acid standard (100 µg/ml): dissolve 25 mg dried folic acid in 0.1 N NaOH by adding NaOH little by little until the solution is clear. Then adjust the pH of solution to 7 with 0.05 N HCl. Make up to 250 ml with 20% ethyalcohol.

Intermediate standard I: 2 ml (100 µg) 200 ml (1000 ng/ml)

Intermediate standard II : 2 ml (1000 µg) 200 ml (10 ng/ml)

Working standard: 20 ml (10 ng/ml) 250 ml (0.8 ng/ml)

Standard curve preparation: Pipette in triplicate

Folic acid concentration (ng/ tube)	0	0.2	0.4	0.8	1.2	1.6
Working standard (ml)	0	0.25	0.5	1.0	1.5	2.0
De-ionized water (ml)	2	1.75	1.5	1.0	0.5	0
Folic acid assay medium (ml)	2	2	2	2	2	2

Blank: 2 sets of un-inoculated blank for zero setting

Procedure:

Weigh 2.00 g of sample + 100 ml buffer, autoclave 120 ^{o}C for20 min. After cooling, add 1 ml Alfa amylase (0.2%) per g of sample. Incubate at 37 ^{o}C for 2 h then inactivate enzyme in in a water bath at 100 for 5 min. After cooling, dilute to 200 ml and filtered. Adjust pH of a portion of the clear filtrate to pH 6.2 and dilute to appropriate concentration of about 0.25-0.3 ng folic acid/ml.

Sample test: pipette in duplicate

ml extract	0.5	1	2
ml de-ionized water	1.5	1	0
ml medium	2	2	2

After mixing, the standard and sample tubes are steriled by boiling at 100 ^{o}C for 15 min (or autoclaving at 110 ^{o}C for 10 min). Aseptically inoculate each tube with 1 drop of appropriate inoculum, using a steriled Pasteur pipette. Mix thoroughly and incubate the set at 35-37 ^{o}C for 18-24 h. Stop growth by boiling at 100 ^{o}C for 15 min. Cool and measure growth of the tested organism by the turbidimetric method using spectrophotometer at the wavelength of 620 nm.

APPENDIX H

Protocol for fortifying wheat flour with iron or folic acid (FA)

1. Weigh approx 10 g of wheat flour to be used as filler for preparing fortificant premixes.
2. Mix 0.1 g folic acid with the weighed wheat flour to prepare folic acid premix, and 2 g iron to prepare iron premix. The preparation process must be under condition that minimizes exposure to light and oxygen.
3. The prepared premix is mixed with more wheat flour in order to obtain 100 g premix. Make sure that there is no clump of fortificant. Transfer each fortificant premix into a polypropylene bag, fill the bag with air, close tightly and shake vigorously about 10-15 min until homogeneous.
4. Fe premix contains 2 % iron (20,000 ppm), and folic acid premix contains 0.1 % folic acid (1,000 ppm).
5. Fortificant premixes are stored in desiccator cabinet at ≤20-25°C and relative humidity of ≤30%. The premix is used within 2 wk..
6. To perform larger batch fortification (16.5 kg), electrolytic iron premix 86.62 g and folic acid premix 23.10 g are mixed with 0.5 kg of wheat flour in a closed and air-filled plastic bag for approx. 15 min.
7. Add the iron-folic flour premix into 16 kg of wheat flour and mix in V-shaped mixer for approx. 30 min. Double-fortified wheat flour (DFW) contains 105 ppm elemental iron and 1.4 ppm folic acid.

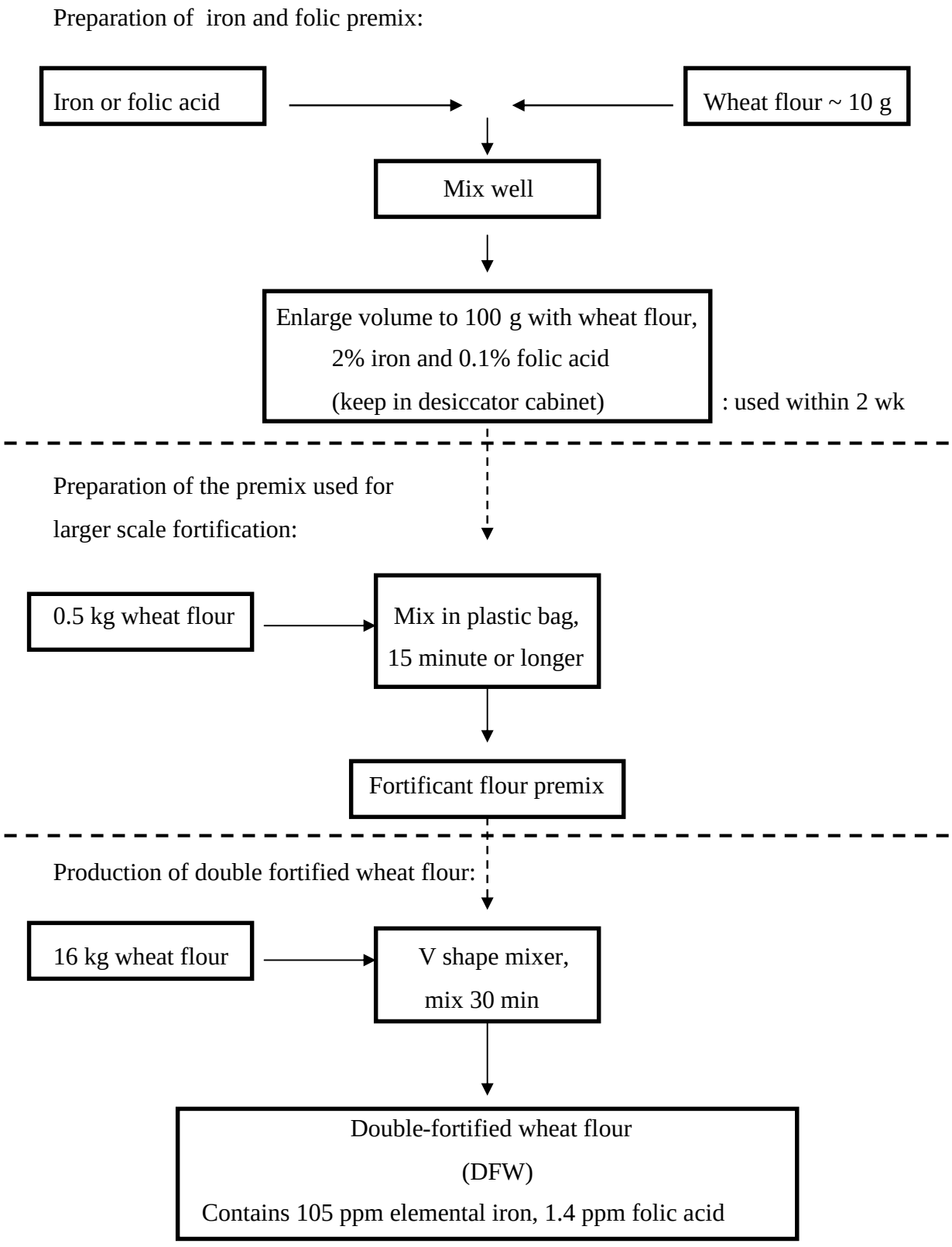

Figure 8: Protocol of double-fortification of wheat flour (DFW)