STABILITY OF MICRONUTRIENTS IN NUTRIGEMS DURING COOKING AND ACCELERATED STORAGE

BY

ELIANA ROSALES

THESIS

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Advisors:

Professor William G. Helferich, Chair
Professor Faye M. Dong
Associate Professor Nicki J. Engeseth
ABSTRACT

Micronutrient undernutrition is one of the most common and preventable nutritional problems in the world. Several strategies have been developed to prevent and control micronutrient deficiency; however, the problem still persists. We have developed Nutrigems™, a food-based micronutrient delivery vehicle which represents a simple technology to provide critical micronutrients to children using the food service logistics of an on-going school lunch program in developing countries. This thesis work was aimed at establishing the stability of micronutrients in Nutrigems after cooking and accelerated storage. Nutrigems made with corn flour and all purpose wheat flour was evaluated as a potential vehicle to deliver vitamin A (VA, as retinol palmitate), iron and the combination. Two types of iron sources were evaluated: sodium iron (III) ethylenediaminetetraacetate or iron EDTA (FEDTA) and ferrous sulfate (FS). Nutrigems and rice were mixed (1:100 w/w) and cooked in a conventional rice cooker. Samples were stored at two relative humidities (RH) 33% and 77% within two temperatures 23 °C and 40 °C. Color, VA content and malondialdehyde-thiobarbituric acid (MDA-TBA) were measured at 0, 7 and 14 days after storage. VA was quantified using reverse phase HPLC with Photodiode Array (PDA) detection. Iron and MDA-TBA were quantified using spectrophotometric methods. After cooking, percent retention respectively were 92, 99 and 68% for VA, FEDTA and the combination. Vitamin A stability after storage was more affected by temperature than RH. However, this changed in the presence of iron. The source of iron had the greatest effect on vitamin A stability, the level of oxidative rancidity and the final color of Nutrigems. FEDTA was chosen as it was the least reactive iron source. The optimal conditions for storage with iron EDTA were low RH conditions, which allowed respective retentions of VA of 87.7% and 84.37% for Nutrigems formulations containing only VA and the combination of VA with iron.
EDTA. These studies describe a promising technological alternative for its use in point of consumption, local fortification food programs for delivering critical micronutrients and improving nutritional quality of the meals provided in the School Lunch Program in Honduras or other programs with similar logistics.
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CHAPTER 1

INTRODUCTION

Micronutrient deficiency is one of the most common and preventable nutritional problems in the World. The World Health Organization (WHO) identified iron, iodine and vitamin A as the most prevalent deficiencies and estimated that they affect as many as 2 billion people in the World (1). Although people in all population groups may be affected, the most widespread and severe problems are usually found in developing countries where poverty, lack of access to a variety of foods, lack of knowledge of appropriate dietary practices and high incidence of infectious diseases are recurrent problems (2).

Several strategies have been developed to prevent and control micronutrient deficiency; however, the problem still persists (3). Food fortification has been the most efficient, cost-effective strategy to reduce micronutrient deficiency in industrialized countries. However, similar micronutrient interventions in developing countries have not been easily sustainable and have had less impact in rural areas for various reasons such as high implementation costs, distribution constraints, low coverage, non-compliance with intake of supplements and lack of economic access to processed foods (4). Therefore, there is a need for a simple and easy-to-transfer strategy that can be used to address micronutrient deficiencies among vulnerable groups like children and pregnant women in rural settings.

We have developed Nutrigems™, a food-based micronutrient delivery vehicle that provides critical micronutrients to children, using as a platform the food service logistics of an on-going school lunch program, in developing countries. Nutrigems™ technological package consists of the process of making a food-based delivery vehicle, the tools to support the process
and training. In simple terms, the product is created from manually extruded dough, which is made of local staple flours into which specific micronutrients are easily incorporated during its preparation. Flours and micronutrient pre-mixes are measured using simple and available cooking utensils. Dough formation process is very similar to making tortillas. Wet dough is shaped into noodles using a wooden hand press specially designed for this technology. Strings of noodles are dried overnight and then broken into small rice-like pieces in a common plastic bag. Uncooked fortified rice-like noodles are eventually added to local rice and cooked conventionally. Nutrigems™ is adaptable to recurrent deficiencies, easy to make, inexpensive and does not use electricity, gas or steam.

This research describes the characterization of the stability of micronutrients in Nutrigems™ and consisted of two phases: 1) determining the stability of vitamin A, iron or their combination in Nutrigems™ after cooking; and 2) determining the stability of these micronutrients after accelerated storage conditions.
CHAPTER 2

LITERATURE REVIEW

2.1 Micronutrient Deficiency (MD)

Micronutrients are required by the human body in very small amounts; these substances enable the body to produce enzymes, hormones and other substances essential for proper growth and development (5). Micronutrients include all vitamins and minerals and the best way to ensure their consumption is through a balanced diet. Unfortunately, this is far from being achieved everywhere since it requires universal access to adequate amount and quality of foods and appropriate dietary habits (2). Inadequate dietary intake of micronutrients is referred as micronutrient malnutrition or micronutrient deficiency (5).

Micronutrient deficiency (MD) is better known as hidden hunger because it is often ignored and underappreciated as there are no visible or physical symptoms of deficiency until later in life. MD alters physiological functions before the deficiencies manifest clinically (2). Subclinical deficiencies in children hinder mental performance during the critical school years and reduce resistance to disease. In adults, MDs lower work performance and endurance, and in pregnant women increase risk of maternal mortality (2).

In 2000, the World Health Organization identified iodine, iron and vitamin A as important micronutrients that affect at least one-third of the world’s population, the majority of whom are in developing countries (6). Of the three micronutrients, iron deficiency (ID) is the most prevalent. It is estimated that just over 2 billion people suffer from ID. Iodine deficiency is the greatest single cause of mental retardation and brain damage and approximately 1.9 billion people are at risk of iodine deficiency Vitamin A deficiency affects approximately 25 percent of
preschoolers in the developing world. It is associated with blindness, susceptibility to disease and higher mortality rates affecting approximately 3 million children each year (7).

2.2 Micronutrients involved in the study

2.2.1 Role of iron in human health

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell hemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (8). Key functions of the iron-containing enzymes include the synthesis of steroid hormones and bile acids, detoxification of foreign substances in the liver, and signal control of some neurotransmitters such as the dopamine and serotonin systems in the brain (9-11).

2.2.2 Iron Deficiency (ID)

ID is the most common nutritional deficiency in the world (6, 11). ID occurs when dietary iron consumption is not enough to keep adequate iron levels in the body, and thus, there is an insufficient supply of iron to various tissues. ID is the leading cause for Iron Deficiency Anemia (IDA). ID and IDA are often incorrectly used as synonyms, but both have different implications. ID is a condition resulting from too little iron in the body, whereas IDA implies that ID is so severe that oxygen delivery to tissues is impaired. ID and IDA are prevalent not only in developing but also in industrialized countries. ID and IDA lower work performance and have been linked to reduced resistance to infection (11). In young children, iron deficiency anemia can decrease their learning capability and cognitive development; it also severely increases the risk of morbidity and mortality in pregnant women. Populations most at risk for ID
and IDA are infants, children, adolescents, and women of childbearing age, especially pregnant women. The mean prevalence among specific population groups are estimated to be: pregnant women, infants and children aged 1–2 years, 50%; preschool-aged children, 25%; schoolchildren, 40%; adolescents, 30–55% and non-pregnant women, 35% (5, 12).

2.2.3 Role of vitamin A (VA) in human health

Vitamin A is an essential vitamin for normal functioning of the visual system, the maintenance of cell function for growth, integrity epithelial cells, immune function and reproduction (10).

Dietary requirements for VA are normally provided as a mixture of preformed vitamin A (retinol) present in animal source foods; and provitamin A carotenoids, derived from foods of vegetable origin, which have to be converted into retinol by tissues such as the intestinal mucosa and the liver in order to be utilized by cells (8).

2.2.4 Vitamin A Deficiency (VAD)

VA is common in many foods (8). Most individuals in the developed world readily meet their daily needs, but that is not the case in developing countries where the consumption of animal products and vitamin A fortified foods is much lower (6). In addition, diets tend to be high in fiber and low in fat, protein and other micronutrients which might affect absorption and storage of VA (13).

Vitamin A deficiency is the leading preventable cause of blindness. Mild VAD may result in changes in the conjunctiva called Bitot's spots. Severe or prolonged vitamin A deficiency causes a condition called xerophthalmia. WHO defines VAD as tissue concentrations
of VA low enough (serum retinol < 0.7 µmol/L) to have adverse health consequences even if there is no evidence of clinical xerophthalmia (8).

WHO estimates between 100 and 140 million children are VA deficient and 250,000 to 500,000 become blind every year with half of them dying within 12 months of losing their sight (1).

Besides the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, nonspecific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anemia, and slowed growth and development (1, 8).

2.3 Micronutrient deficiency in developing countries

Although people in all population groups in all regions of the world may be affected by MD, the most widespread and severe problems are usually found in developing countries where poverty, lack of access to a variety of foods, lack of knowledge of appropriate dietary practices and high incidence of infectious diseases are recurrent problems (2). Food consumption of most families in these countries, especially in rural areas, is generally monotonous with limited variability in food items and intake of animal source food and vegetables is often low. Specifically, rural populations face severe nutritional challenges associated with culture, geographical isolation, access to limited variety of food items, limited food transportation systems and other lifestyle related factors (14).

A large multi-country study on anthropometric status of rural schoolchildren in Ghana, Tanzania, Indonesia, Vietnam and India found a high prevalence of stunting and underweight, ranging from 48 to 56% for stunting and 34 to 62% for underweight (15). Anemia is estimated to
affect 46% of 5-12 year old children in low income countries, with the highest prevalence in South Asia (50%) (16). Similarly, the prevalence of vitamin A deficiency (serum retinol < 0.7 µmol/L) in South Asian schoolchildren is estimated to be 23.4%, of whom one in ten have xerophthalmia (17). In Latin America and Caribbean region the panorama is not different. Honduras is one of the countries with highest incidences of poverty and inequality (2, 18). According to the Micronutrient Initiative Group, it is estimated that 34% of the children less than 5 years of age in Honduras suffer from iron deficiency anemia, 12 % and 15% from iodine and vitamin A deficiencies, respectively (7, 19).

2.4 Implications of MD among school age children

In general, defining MD status is often a challenge in developing countries, especially in school age children. Prevalence and diseases associated with deficiencies of most micronutrients are not well documented; however, the negative implications are well known. MD can lead to retarded growth (20), anemia (16), reduced immune function (21), and impaired motor and cognitive development (22) all of which may adversely affect academic performance through reduced learning capacity and poor school attendance (23). However, the timely provision of micronutrients through nutritional interventions, often accompanied by other health improvement measures, could reverse such deficiencies and their associated developmental impairments (24). Additionally, infections such as diarrhea, malaria, and helicobacter pylori infestation have been associated with poor micronutrient status (25, 26).
2.5 Food fortification

Micronutrient fortification is defined as the addition of one or more micronutrients to foods in order to increase their intake to correct or prevent a deficiency and provide health benefit for a population or specific subgroup(s) within the population (5). This strategy does not require compliance or change in dietary pattern of populations and is often considered as one of the most cost-effective and sustainable micronutrient interventions (27).

Until recently, the majority of fortification programs have used centrally processed foods that require a wide distribution network to reach its intended beneficiaries. In industrialized countries, where processed foods are widely consumed and distribution systems are efficient, fortification has been successful in the prevention and control of several vitamin and mineral deficiencies. In contrast, in others parts of the world, the impact of similar mass fortification efforts has been less effective because of poor distribution networks and low purchasing power, which limits physical and economic access to centrally processed foods (28).

2.5.1 Community level fortification approach

In rural areas, most foods are often grown, processed and eaten at the community level (28). Therefore, the application of food fortification strategy at the community level is needed to provide critical micronutrients for rural populations. Community level fortification approaches promote proactive community participation, empowering people to participate in the process of fortifying their own foods. This approach enables service providers and community leaders to collectively set realistic goals and develop workable plans. Studies have shown that community level nutrition programs increased the acceptability, sustainability and adherence to the program while often generating employment for local women (29). A good example of this approach is
the case of The Integrated Child Development Service (ICDS), a large nutrition program in India that successfully distributes khichdi (rice and lentil mixture), a supplement that provides macronutrients. This program studied the efficacy of adding a premix fortified with iron and vitamin A to prepared khichdi on pilot locations at the community level. Results demonstrated a reduction in the prevalence of IDA among children who received fortified khichdi for 24 weeks, but without significant changes in vitamin A status, measured as serum retinol (30). A similar approach was conducted by a fortification program in Ghana that studied the efficacy of the addition of micronutrients in the form of powder (Sprinkles), soluble or crushable tablets (Nutritabs) and spreads to foods just before consumption in infants recruited at 5 months age. The variables evaluated were intake from complementary foods, growth, and observed motor milestone acquisition at 12 months. Results showed supplements were well accepted, did not affect growth, improved iron status and had positive effects on motor milestone acquisition by 12 months compared with no the intervention group (31).

2.5.2 A new approach: Nutrigems™ in school lunch programs in Honduras

The Healthy Schools Program is the main public food program in Honduras. This program provides meals to more than 1 million children in urban and rural schools. The distribution strategy requires participation of the World Food Program, school teachers, and most importantly, the parents who prepare the lunches at their homes, transport and distribute them at the schools. The program provides raw food items such as rice, corn, beans, oil and corn soy blend to schools, and in exchange it requires organization of the mothers of children for the preparation of the meals. Meals are served at the school. The program has great success in delivering fortified foods to schools. Common foods served include white rice, beans, tortillas and corn-soy blend (CSB) that contains mineral and vitamin premixes. Even though these meals
provide micronutrients, the micronutrient requirements for most children are still not satisfied. Therefore, other technologies for the delivery of critical micronutrients using inexpensive vehicles for distribution are necessary.

To enhance the micronutrient content of the meals and eventually the micronutrient status of children in schools participating in the Healthy Schools Program in Honduras, we proposed the use of a low-cost, culture-based and point-of-consumption micronutrient fortification technology called Nutrigems™. Nutrigems™ technology is the process, the tools and training that create a micronutrient delivery vehicle (MDV). It consists of manually cold-extruded dough made of local staples into which specific micronutrients are easily incorporated during its preparation. Uncooked-fortified rice-like noodles are formed and eventually will be combined (1:100 w/w) with rice and cooked conventionally. It is designed to be easily prepared by the parents participating in the Healthy School Program. This additional step would add 10 to 15 minutes to regular food preparation process time. The goal would be to increase micronutrient status among school children who receive lunch meals fortified with Nutrigems™ delivering iron, vitamin A or their combination.

2.6 Micronutrient sources for food fortification

2.6.1 Iron fortification

Iron is the most challenging micronutrient to add to foods (13, 32, 33). Despite these challenges, the effectiveness of iron fortification has been demonstrated in several world regions. Iron fortification of infant formulas reduced prevalence of anemia in children aged under 5 years in the United States (34). Similar results were observed in Venezuelan children when wheat and maize flours were fortified with iron (35). Fortification of milk with iron and vitamin C in Chile
produced a rapid reduction in the prevalence of iron deficiency in infants and young children (3, 5). Cereal flours (wheat and maize) are currently the most common vehicles for iron fortification (32).

2.6.1.1 Challenges of iron sources in food fortification

In the case of iron pre-mixes, the two most common problems in product development are increased rancidity due oxidation of unsaturated lipids and unwanted color changes. Color changes typically include a green or bluish coloration in cereals, a graying of chocolate and cocoa, and darkening of salt to yellow or red/brown (36). Also, changes to food sensory characteristics are highly variable and not always predictable because the same pre-mix can behave differently in interaction with components of the food matrix (5).

The choice of food vehicle and the iron source should be based upon careful consideration since it is required that the fortified food should be acceptable to consumption by the target population in terms of cost and organoleptic properties. The ideal iron pre-mix should be safe, inexpensive, highly bioavailable and at the same time should not cause unacceptable changes to the sensory properties of the food vehicle (5, 33). Unfortunately, in the case of iron this rarely happens. Generally, the most absorbable and inexpensive iron compounds are usually most reactive with the food matrix causing detrimental effects in sensory characteristics and those that are not that reactive are generally poorly absorbed (5).

The most common iron compounds used to fortify foods are ferrous fumarate, ferrous sulfate and ferrous(ic) bisglycinate (33). However, ferrous sulfate is the most frequently used water-soluble iron pre-mix, principally because is the cheapest form available. It has been ubiquitously used for a wide range of foods, especially flours (5). For this reason, it is common
to compare the relative bioavailability (RBV) of any iron pre-mix to the RBV of hydrated ferrous sulfate.

2.6.1.2 Novel pre-mixes: Ferrazone™, iron sodium EDTA

Ferrazone™ is Akzo Nobel’s brand name for sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA). This compound is a chelated type of iron, soluble in water and chemically inert. It has a molecular weight of 367.05 g/mol and contains 13% of iron (Figure 1). It is tightly bound to the EDTA molecule and as a consequence, no significant free iron is generated in the solution to be available to react and form off-flavors or colors, as is often the case with other water-soluble iron forms. Ferric sodium EDTA does not interact with other cations which makes it suitable for multiple fortification in the presence of vitamins and other minerals (37).

Iron EDTA is stable, highly bioavailable and resistant to the common inhibitors of non-heme iron absorption, especially in phytate-rich diets such as found in countries of Latin America. The typical diet of the rural population in Honduras consists of corn made into tortillas, beans, plantains, rice, and coffee, with only occasional supplements of meat or fish (15).

In addition, this type of iron offers a number of other advantages: it does not promote lipid oxidation in stored cereals, or the formation of precipitates in foods that are high in free peptides, such as soy sauce and fish sauce (38), and does not change color and sensory quality of the products made from wheat flour (39).
Iron NaFeEDTA has been selected as a suitable iron source for government-led soy sauce fortification and wheat flour fortification programs in China, and fish sauce fortification in Vietnam (4).

![Structure of sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA)](image)

**Figure 1.** Structure of sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA)

### 2.6.2 Vitamin A fortification

Challenges associated to VA fortification are mainly due to low stability. VA stability is affected by the presence of oxygen, heat, moisture, trace elements and light (40). Therefore, the selection of a stable VA form is important to assure nutritional stability of a fortified product. Most manufactures of VA producers stabilize VA using the method of sealing within a physical matrix, generally gum acacia or gelatin to stabilize VA (41).

The fortification of widely consumed staples with VA has been a successful strategy to reduce vitamin A deficiency. Solon and colleagues (2000) evaluated the efficacy of daily consumption of a vitamin A fortified wheat-flour bun (30 µg retinyl palmitate) for 30 weeks in improving the vitamin A status measured as serum retinol concentrations and modified relative dose response (MRDR) in school-age Filipino children (6-10 years). They found improvement in initial serum retinol concentrations, and differences in the MRDR showed higher liver stores of
the VA as high as 50% change (42). Similarly, sugar fortification with VA is a strategy that has been used extensively throughout Central America and is extending to other countries in the region. Sugar delivers vitamin A at a concentration average of 9 mg of VA per kilogram (43). Arroyave and colleagues (1981) reported that an evaluation of serum retinol levels of preschool-aged children (1 to 5 years) in Guatemala revealed that after a year of consumption of fortified sugar, 76% of the children experienced an elevation of serum retinol concentration (44). This program is one of the most successful mass VA fortification programs and is a still ongoing program in Guatemala.

2.6.2.1 Vitamin A sources for food fortification

Retinol by itself is an unstable compound; therefore, commercial preparations require its esterification, usually with palmitic or acetic acids. Retinyl acetate, retinyl palmitate, and provitamin A (β-carotene) are the main commercial forms of VA available for use as food pre-mixes. However, the intense orange-yellow color of β-carotene limits its use as a pre-mix in many foods (5). Several VA sources are available in the market for food fortification. The choice of VA pre-mix is determined mainly by the characteristics of the food vehicle and technological and regulatory limitations (5).
Figure 2. Vitamin A sources for fortification
2.6.2.2 Factors affecting vitamin A oxidation

The biological activity of VA depends on its stability. The presence of five conjugated double bonds (Figure 2) in the configuration of vitamin A (retinol) makes it an easy target for oxygen attack. Consequently, oxidized VA has lower biological activity (45). Factors that reduce vitamin A activity are atmospheric oxygen, light, heat and other oxidizing agents (40, 46).

Paquette and colleagues (1985) studied the kinetics of retinyl acetate and retinol in the presence of three potential oxidizing agents: light, air and temperature. They concluded that the major factor that cause oxidation was light (40). Manan and colleagues (1991) looked at the stability of retinol in the presence of iron, copper, zinc and calcium. The results showed that mineral fortification reduced the retinol stability by 36% (45). These authors also reported that in the presence of 10% to 25% of mineral supplementation this degradation can be reduced if ascorbic acid is added.

2.6.2.3 Lipid oxidation

Lipid oxidation detrimentally affects vitamin A content in foods. It is an autocatalytic, free-radical mediated reaction that degrades polyunsaturated fatty acids. It proceeds as a sequence of three stages: initiation, propagation and termination (47).

Initiation of lipid oxidation involves the formation of an initial lipid free radical in the presence of metals, ionizing radiation, singlet oxygen, heat or other free radicals in the food system. In the propagation stage, this initial free radical sets off an autocatalytic chain reaction, generating many more free radicals. Finally, the termination step occurs by two mechanisms: radical recombination, in which two radicals react to form a non-radical product, and radical
scission, in which alkoxyl radicals undergo scission and form end products by reacting with water. These non-radical molecules do not propagate the chain reaction, but can generate undesirable end products such as polymers, ketones, and aldehydes, which are responsible for the characteristic off-odors and flavors in oxidized food products (48).
CHAPTER 3

STABILITY OF MICRONUTRIENTS AFTER COOKING

This experiment was conducted to determine the level of protection provided by the Nutrigems matrix in comparison to the addition of the pre-mix alone after cooking process. Our hypothesis was that Nutrigems reduces degradation of vitamin A with and without iron when cooked with rice. After cooking, characteristics evaluated in rice were: 1) vitamin A (VA) and iron content per serving size, 2) vitamin A percent retention, 3) iron percent retention when rice was washed before cooking and 4) color changes. Two sources of iron were used: Ferrous Sulfate (FS) and iron NaEDTA (FEDTA).

3.1 Materials

Ferrazone™ XP (NaFeEDTA) was donated from Akzo Nobel Fuctional Chemicals (Deventer, Netherlands). Retinyl palmitate premix was obtained from AgD Nutrition (Chicago, IL, USA). Ferrous sulfate was purchased from Spectrum Chemical Corporation (Gardena, CA, USA). All purpose flour Gold Medal, corn flour MASECA and soybean oil were obtained from Wal-Mart stores.

Standards of retinyl palmitate, retinyl acetate and iron atomic absorption standard were obtained from Sigma (St. Louis, MO, USA). Bathophenanthroline disulfonic acid were obtained from Sigma Aldrich Inc. (St Louis, MO, USA). 1-propanol, ethanol, and methanol HPLC grade and sodium EDTA were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Acetonitrile was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA).
3.2 Methods

3.2.1 Nutrigems™ preparation

Flours and chosen micronutrients (Table 1) were accurately weigh and manually mixed in a stainless steel bowl with a spoon for 2 minutes. Then, oil was added and mixed for additional 2 minutes. Finally, deionized water was slowly added to the mixture. Ingredients were first mixed with a spoon and then by hand for 5 minutes until pliable dough was formed. Dough was allowed to let sit for 5 minutes, then cold extruded using a stand mixer Kitchen Aid® with pasta maker accessory (Model FGA-2). The dough was then extruded at a rate of approximately 4 kg per hour. Occasional interruptions in the flow were necessary to avoid temperature increase in the extrusion process. Samples were dried in a dark cabinet at 47 ± 3 °C for 10 hours. Immediately, after samples were made (initial time $t=1$), they were tested for VA and iron concentrations. The formulations of Nutrigems™ used in this experiment are shown in Table 1.

3.2.2 Rice preparation

Nutrigems™ formulations (Table 1) were combined with long grain rice in a ratio of 1:100 (w/w) and cooked in conventional rice cooker for 15 min. For treatments fortified with micronutrient solutions, the procedure varied slightly. Pre-mixes were accurately weighed and dissolved in water, then combined with rice and cooked. For determination of iron losses after rinsing, the mixture of rice and Nutrigems™ was soaked in 200 mL of deionized water (25 °C) and agitated for 30 seconds. Then, the excess water was discarded carefully without losing rice. For the 60 sec treatment, this procedure was completed twice, and for 90 seconds, three times. Then, fortified rice was cooked in conventional rice cooker for 15 min. At the end of preparation, rice was mixed with a spoon to assure even distribution.
### Table 1. Nutrigems™ formulations

<table>
<thead>
<tr>
<th>Nutrigems™ Formulations</th>
<th>APF</th>
<th>Corn flour</th>
<th>Oil</th>
<th>VA</th>
<th>VA&lt;sup&gt;a&lt;/sup&gt; premix</th>
<th>Iron</th>
<th>Iron&lt;sup&gt;b&lt;/sup&gt; Premix</th>
<th>Final fortification level&lt;sup&gt;c&lt;/sup&gt; VA&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Iron mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrigems</td>
<td>55.5</td>
<td>41.7</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nutrigems-VA</td>
<td>55.5</td>
<td>41.7</td>
<td>2.7</td>
<td>616</td>
<td>4.7</td>
<td>-</td>
<td>200</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nutrigems-VA-FEDTA</td>
<td>55.5</td>
<td>41.7</td>
<td>2.7</td>
<td>616</td>
<td>4.7</td>
<td>5</td>
<td>38</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Nutrigems-FEDTA</td>
<td>55.5</td>
<td>41.7</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>38</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nutrigems-VA-FS</td>
<td>55.5</td>
<td>41.7</td>
<td>2.7</td>
<td>-</td>
<td>4.7</td>
<td>5</td>
<td>13</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> VA premix: Retinyl palmitate.

<sup>b</sup> Iron premixes. FEDTA: FeNaEDTA premix (Ferrazone™). FS: Ferrous sulfate.

<sup>c</sup> Final fortification level refers to the amount of micronutrients per serving size after combined with rice in a ratio of 1:100 w/w. Rice serving size was set at 60 g of raw rice. A total of 60 g of each Nutrigems formulation is enough to fortify 100 children meals.

<sup>d</sup> VA: Retinol equivalents.

The target fortification level for vitamin A was 50% RDA of children (4-8 years) which represents 200 µg of retinol per serving size. For iron, the fortification level was 30% RDA which is approximately 3 mg of Fe per serving. Calculations were based on a typical rice serving size. In this study, a serving size of rice was set at 60 g of raw rice or 120 g of cooked rice.

Treatments for analysis of stability after cooking are shown in Table 2.

#### 3.2.2.1 Direct solvent extraction of vitamin A

Samples of 120 g of cooked rice were accurately weighed and placed in conventional blender. Internal standard, 1000 µL (concentration of 300 µg of retinyl acetate per mL of 1-propanol and BHT (0.01%, w/v) solution was added immediately. Then, 200 mL of deionized
water (25 °C) were added, mixture was blended at medium velocity to form slurry. Aliquots of 40 g samples were placed in erlenmeyer and wrapped in foil to prevent light oxidation during extraction procedure. 100 mL of 1-propanol and BHT (0.01%, w/v) solution was added to the mixture. Samples were sonicated (Fisher Scientific Sonicator, Model FS20H) for 15 min and transferred to 50 mL centrifuge tubes (Falcon centrifuge tubes, BD Biosciences). Finally, samples were centrifuged (Sorvall R17 Plus®) for 15 min at 10 °C at 2012 xg. Supernatant was filtered using 0.45 μm PTFE membrane filter (Gelman Laboratories, USA) directly into HPLC amber drone vials (4 mL) with clear spring inserts (250 uL). All steps were carried out in the dark and all solvents were HPLC grade.

Table 2. List of Nutrigems™ formulations for analysis of stability after cooking

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>Long-grain, white rice cooked alone.</td>
</tr>
<tr>
<td>Rice-VA solution</td>
<td>VA premix solution was added to rice.</td>
</tr>
<tr>
<td>Rice-VA-FEDTA solution</td>
<td>VA and FEDTA solutions were added to rice.</td>
</tr>
<tr>
<td>Rice-Nt-VA</td>
<td>Nutrigems-VA was added to rice (1:100 w/w ratio).</td>
</tr>
<tr>
<td>Rice-Nt-VA-FEDTA</td>
<td>Nutrigems-VA-FEDTA was added to rice (1:100 w/w ratio).</td>
</tr>
<tr>
<td>Rice-Nt-FEDTA</td>
<td>Nutrigems-FEDTA was added to rice (1:100 w/w ratio).</td>
</tr>
<tr>
<td>Rice-Nt-FS</td>
<td>Nutrigems-FS was added to rice (1:100 w/w ratio).</td>
</tr>
</tbody>
</table>

3.2.3 Vitamin A quantification

3.2.3.1 Standards preparation and concentration measurements

In order to measure the concentration of VA in the extracted samples, a calibration curve was developed using retinyl palmitate and retinyl acetate standards. Initial concentrations of standards were calculated using the Beer-Lambert law, where molar absorptivity coefficients were 950 and 1550 L·mol⁻¹·cm⁻¹ for retinyl palmitate and acetate, respectively (49). Concentrations were measured using a spectrophotometer (Spectronic Instruments, Inc, NY, USA) at 326 nm and units were expressed in mol/L. Appropriate dilutions were made to adjust the standards to 0.5, 1, 5, 10 and 20 µg/mL in 1-propanol containing BHT (0.01%, w/v) solution. Concentrations of retinyl palmitate and retinyl acetate were calculated from peak area determined by Empower® software integrator (Waters, Milford, MA). Standard curves were constructed using a linear fit function.

3.2.3.2 High performance liquid chromatography (HPLC) procedure

HPLC analysis was carried out on Waters™ system consisting of Waters™ 600S pump, Waters™ 717 plus autosampler, Waters™ PDA 996 – UV-VIS detector and Zorbax®, Rx C8 column (250 x 4.6 mm). The conditions used were: 20 µL injection volume at 10 °C and 35 °C autosampler and column detector temperatures, respectively. The mobile phase used consisted of acetonitrile, methanol and propanol (98:1:1, v/v). Separation was carried in isocratic conditions and a flow rate of 1.0 mL/min. Photodiode detection was recorded at a wavelength of 326 nm. Peak evaluation was based on peak area. Controlled data acquisition and processing were performed on chromatograms (326 nm) using Empower® software integrator (Waters, Milford, MA). Retinols were identified comparing their retention time and on-line UV-spectra with those
of authentic standards calibrated using know extinction coefficients, 950 and 1550 L mol\(^{-1}\) cm\(^{-1}\) for retinyl palmitate and acetate, respectively (49). Quantification was performed using calibration curves covering concentrations close to those found in the products.

### 3.2.4 Percent retention of vitamin A

VA percent retention after cooking was calculated using the formula:

\[
\% \text{ Retention of VA} = \frac{\text{VA measured by HPLC after cooking}}{\text{VA added before cooking}} \times 100 \quad \text{Eq [1]}
\]

### 3.2.5 Total iron determination

Iron analysis was performed using the method for fortified foods developed by Kosse, et al. (2001). This method involved the reaction from iron dissolved in the iron-extracting solution with bathophenanthroline disulfonic acid in the presence of reducing agent producing a red color complex (50) that was measured using a spectrophotometer (Spectronic Instruments, Inc, NY, USA) at 535 nm. These values were correlated to a calibrated atomic iron standard curve to determine the total iron content in the samples. Iron standards were prepared by diluting a stock iron solution.

#### 3.2.5.1 Rice samples preparation

Samples of 120 g of cooked rice were accurately weighed and placed in a conventional blender. Then, 200 mL of deionized water was added and blended to form slurry. The slurry was transferred to a 500 mL Erlenmeyer and 300 mL of iron extraction solution was added. Mixture was sonicated (Fisher Scientific Sonicator, Model FS20H) for 15 min. Aliquots of 40 g were transfer to a 50 mL centrifuge tubes (Falcon centrifuge tubes, BD Biosciences). Finally, samples
were centrifuged (Sorvall R17 Plus®) for 15 min at 10 °C, 2012 xg. Supernatant was filtered using 0.45 μm PTFE membrane filter (Gelman Laboratories, USA). Supernatant was combined with bathophenanthroline disulfonic acid as described by Kosse and colleagues (2001).

3.2.6 Iron percent retention

Iron percent retention in rice after cooking was calculated using the formula:

\[
% \text{Retention of iron} = \frac{\text{iron measured after cooking}}{\text{iron added before cooking}} \times 100 \quad \text{Eq [2]}
\]

3.2.7 Color

Color of cooked samples was measured by Hunter L*a*b* spectrophotometric system using the Minolta ChromaMeter Cr-400/410 (Konica Minolta; Mahwah, NJ), where L indicates lightness (100=white, 0=blank), a indicates red to green (100=red, -100=green), and b indicates yellow to blue (100=yellow, -100=blue). L*a*b* samples values were obtained in triplicate using 5 x 15 mm Petri dishes. Samples were measured at approximately 25 °C.

These values were then used to calculate the color change ∆E (Eq 3), an overall measurement to measure color change in comparison to a standard, in this case, white rice. ∆E was calculated based on the following formula:

\[
\Delta E = \sqrt{[(L)^2+(a)^2+(b)^2]} \quad \text{Eq. [3]}
\]
3.2.8 Statistical analysis

A minimum of three replicates were analyzed of each sample. Data are shown as the means ± Standard Deviation (SD). All statistical analyses were conducted using the SAS software v. 9.2 (SAS Institute Inc., Cary, NC) to determine Analysis of Variance (ANOVA). If significant differences were found, means were compared by the Tukey’s test and deemed significant at $P<0.05$.

3.3 Results

3.3.1 Vitamin A percent retention

Figure 3 shows VA percent retention when VA and FEDTA premixes were added and cooked with long grain rice in two different forms: within Nutrigems™ (Nutrigems-VA and Nutrigems-VA-FEDTA) and as separate solutions of equivalent concentrations (VA and VA-FEDTA solutions). Overall, the cooking process decreased VA content ($P<0.05$). However, VA retention was higher in the presence of Nutrigems after cooking, even when iron was present.

Rice samples fortified only with VA with and without the presence of Nutrigems had cooking retentions of 95% and 83%, respectively. Presence of Nutrigems reduced significantly VA losses ($P<0.05$). The addition of iron into the Nutrigems matrix increased VA degradation ($P<0.001$) resulting in 20% reduction of the original VA present. Nevertheless, fortified rice with Nutrigems-VA-FEDTA had 12% higher recovery of VA than rice fortified with the combination of both solutions of FEDTA and VA. Overall, the distribution of VA in the cooked rice was constant per children serving size.
Figure 3. Percent retention of vitamin A (VA) of rice fortified with Nutrigems™ after cooking. Bars represent mean percent retention + SD. White bars represent rice fortified with micronutrient premix solutions of VA and VA plus iron EDTA (FEDTA). Black bars represent rice fortified with micronutrients within Nutrigems (NT). Different letters represent significant differences after Tukey’s test ($P<0.05$).

### 3.3.2 Iron Content

Results for iron retention after cooking are shown in Table 3. A minimum of four replicates were analyzed of each sample. Results show that iron content after cooking was retained at same level with or without the presence of Nutrigems. However, rinsing rice before cooking reduced iron content in 15, 34 and 47% after rice was rinsed with an excess of double deionized water after 30, 60 and 90 seconds, respectively. The distribution of iron in cooked rice was relatively constant per children serving size (Table 4). Variability (Coefficient of Variation,
CV%) among different treatments was reduced as the size of the sample used for extraction was increased (Table 4).

**Table 3. Changes in iron content after cooking process**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iron concentration (µg Fe/ g rice)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(^a)</td>
<td>Expected</td>
</tr>
<tr>
<td>Without fortification</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Rice fortified with Nutrigems-FEDTA</td>
<td>8</td>
<td>32.1</td>
</tr>
<tr>
<td>Rice fortified with Nutrigems-FEDTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinced for 30 seconds before cooking</td>
<td>4</td>
<td>32.2</td>
</tr>
<tr>
<td>Rinced for 60 seconds before cooking</td>
<td>4</td>
<td>32.1</td>
</tr>
<tr>
<td>Rinced for 90 seconds before cooking</td>
<td>4</td>
<td>31.9</td>
</tr>
<tr>
<td>Rice fortified with FEDTA solution</td>
<td>4</td>
<td>43.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. n\(^a\): Sample size. Values in the same column followed by different letters are significantly different after Tukey’s test (P<0.05).

**Table 4. Iron distribution in different sample sizes.**

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Iron concentration (µg Fe/ g rice)</th>
<th>CV %</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Measured</td>
<td></td>
</tr>
<tr>
<td>4 g</td>
<td>50.0</td>
<td>52.0 ± 6.3</td>
<td>12.2</td>
</tr>
<tr>
<td>60 g</td>
<td>50.0</td>
<td>45.2 ± 3.9</td>
<td>8.6</td>
</tr>
<tr>
<td>120 g</td>
<td>50.0</td>
<td>47.3 ± 1.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. % CV: coefficient of variation in sampling. No significant differences in % recovery were observed.
Table 5 shows the color profile of rice fortified with different formulations of Nutrigems. Among all Nutrigems formulations, only Nutrigems-VA-FS caused a significant increase in whiteness (L values) in the color of cooked rice ($P<0.05$). However, in all formulations the addition of Nutrigems caused a slight increase in whiteness (L values) and trend to yellowness (b values), but without reaching statistical significance.

When analyzing change of color $\Delta E$ values, we found that Nutrigems alone and fortified only with VA had differences in color compared to white rice. However, Nutrigems fortified with iron had even higher values. Between Nutrigems-FEDTA ($\Delta E = 1.78$) and Nutrigems-FS ($\Delta E = 2.81$), FS indicated the highest color change value among cooked rice samples.

**Table 5. Color of rice fortified with Nutrigems$^{\text{TM}}$**

<table>
<thead>
<tr>
<th>Rice treatments</th>
<th>Color values</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>Rice alone</td>
<td>68.46$^a$ ± 0.40</td>
<td>-1.62 ± 0.10</td>
</tr>
<tr>
<td>Rice + Nutrigems</td>
<td>68.58$^a$ ± 1.27</td>
<td>-1.91 ± 0.03</td>
</tr>
<tr>
<td>Rice + Nutrigems-VA</td>
<td>69.70$^a$ ± 0.36</td>
<td>-1.71 ± 0.09</td>
</tr>
<tr>
<td>Rice + Nutrigems-VA-FEDTA</td>
<td>68.77$^a$ ± 0.25</td>
<td>-1.55 ± 0.32</td>
</tr>
<tr>
<td>Rice + Nutrigems-FEDTA</td>
<td>67.57$^a$ ± 0.95</td>
<td>-1.69 ± 0.47</td>
</tr>
<tr>
<td>Rice + Nutrigems-VA-FS</td>
<td>71.04$^b$ ± 2.06</td>
<td>-1.9 ± 0.30</td>
</tr>
</tbody>
</table>

Values in the same column followed by different letters are significantly different according to Tukey’s test ($P<0.05$). L: 100 = white, 0 = black; a: 100 = red, -100 = green; b: 100 = yellow, -100 = blue. $\Delta E$: change in color of fortified rice with Nutrigems after cooking. $\Delta E$ was calculated using Equation 3.
3.4 Discussion

Determination of VA stability before and after cooking is necessary to assess the final amount of actual vitamin that will reach the end user and to calculate what amount should be added to any delivery vehicle.

*Nutrigems protected VA from degradation after cooking, even in the presence of iron.* In this study, retention of VA in rice fortified with Nutrigems-VA was 95%. This retention was 18% higher than the retention reported in a similar product Ultra Rice™ (UR), which was 77.31% (51). In their studies, Lee and colleagues used similar cooking conditions, long grain rice and the delivery vehicle at the ratio of 1:100 (w/w) in a conventional rice cooker. Additionally, they tested two different cooking methods, boiling rice in excess and without excess water. VA retention was 75% and 87%, respectively, which is still 8% lower than Nutrigems-VA retention observed in this study. Murphy and colleagues (1992), also evaluated cooking stability of VA in formulations of synthetic rice premixes containing different types and combinations of oils and antioxidants. The cooking method consisted in the addition of synthetic rice premix to rice in a proportion of 1:200 (w/w) in boiling water until all cooking water was absorbed by rice. Retention of VA ranged from 55% to 96% where the formulation containing BHT as antioxidant and no oil had the highest, and the formulation containing tocopherols and coconut oil had the lowest VA retention. This group concluded that the type of lipids and antioxidants used in the formulation of fortified synthetic rice play a crucial role on VA stability (52). UR and fortified synthetic rice contained a complex antioxidant mixture and additional components within the matrix to increase stability of labile vitamins such as VA (51, 52). In contrast, Nutrigems formulations contained 3% of soybean oil and there was no addition of antioxidants other than the ones originally present in flours used for its preparation. This is an important characteristic to
differentiate among delivery vehicles as additional components to the formulation will raise the cost of the final product and could be an undesirable characteristic for their use in rural environments.

Changes in the final amount of VA after cooking depends on one or several well-known parameters that negatively affects VA stability such as heat (40), light (40, 53), presence of metals (40), among others. One important factor is the type of VA premix used. Besides brands, the type of VA premixes used in the investigations of Lee and colleagues (2001) and Murphy et al. (1992), had different encapsulation components. Commercial stabilization techniques greatly influence stability of VA in specific food systems and under specific conditions (54).

In this study, Nutrigems contributed to the reduction in VA losses. There are two significant factors that might contribute to achieve this positive characteristic. First, Nutrigems provides a physical barrier to reduce surface contact with pro-oxidant agents such as heat or light and second, the presence of antioxidants in ingredients used for the preparation of Nutrigems such as wheat, corn flours and oil. Absence of Nutrigems resulted in additional 12% degradation. Higher losses occurred when iron was added (17% loss). Although iron used is chemically chelated with EDTA, a small proportion is still free to react. Indeed, FEDTA added as a solution to rice promoted more degradation of VA than added within Nutrigems. Presence of Nutrigems still provided protection by almost 10%. This effect can be explained by the minimization of the contact between the iron and the VA. Thus the oxidation potential of the iron is reduced because it is trapped in the matrix.

The delivery of iron in Nutrigems containing VA increased VA degradation (P<0.001) in more than 20%. This was expected as it is well recognized that the presence of iron can speed up
vitamin A degradation (40, 45). As reported by Paquette and colleagues (1985), VA was less stable in the presence of iron, copper, zinc and calcium, where mineral fortification reduced the retinol stability by 36% (45). However, this degradation can be reduced if ascorbic acid is added (45, 55).

Negative effects between the interaction of VA and iron is a constant challenge in food fortification (5, 10). However, not all the interactions of VA and iron are negative. Laramisse et al. (1997) suggested that VA interaction with non-heme iron in precooked maize flour VA could increase iron absorption. This is possible because VA binds iron during the digestive process forming a complex that acts as a chelating agent, thus blocking the effect of hydroxyl radicals present in phytates and polyphenols, therefore, reducing the inhibitory effect of phytates on iron absorption (56).

In many developing countries, children are in high risk of VA and iron deficiencies (57). Finding a suitable vehicle for fortification with iron and VA is difficult but might cost less than separate fortification programs. Nutrigems™ technology is characterized to be a flexible, low cost and point-of-use fortification and might be a suitable option to deliver both VA and iron to isolated rural areas in developing countries.

*Due to extreme solubility in water, Nutrigems containing iron EDTA should be added after rice is washed.* Rinsing rice with Nutrigems containing iron EDTA resulted in iron losses of 15, 34 and 47% after 30, 60 and 90 seconds of washings. This is an important consideration because in developing countries, rice is often washed before cooking usually to: 1) remove impurities and stones; 2) remove extra amylose; and 3) increase thermal conductivity. Estimates of nutrient losses due to rinsing/washing, including those of iron in unenriched, milled rice, have
been estimated at about 60% (58). The use of Nutrigems to deliver iron could reduce the washing effect as it protects iron in the matrix and it is directed at delivering iron after rice has been washed. Data also imply that FEDTA is extremely water soluble as had been reported by Le and colleagues (2007) who showed that 70% of the iron EDTA present in fortified noodle dissolves within 5 min into the soup (32).

Data on Table 3 shows that iron content after cooking was retained at same level with or without Nutrigems. This was expected because minerals are much more stable than vitamins, and negative effects on their stabilities after processing and storage are rarely seen. If there are some losses of minerals, they are more likely affected by the processing or environmental parameters, such moisture or due to the reactions with other food components such as proteins and carbohydrates (36).

Addition of Nutrigems causes slight color changes in cooked rice. The color of formulations of Nutrigems alone and cooked with rice varied. Even the addition of 1% of Nutrigems or Nutrigems-VA caused slightly changes in color of cooked rice. When iron was present in formulations these changes increased. The formulation of Nutrigems™ with ferrous sulfate caused the most significant changes in color, leading to a final cooked product different (whiter, more yellow and red) than white rice alone. Similar findings were reported by Murphy (1996), when he attempted to fortify artificial rice grains with VA and iron (ferrous sulfate), his results showed that besides VA oxidation by iron, rice grains also resulted in a discolored product. Therefore, they produced the combination of two separately simulated rices one with VA and one with iron. In this way the VA oxidation was reduced, however discoloration due to added iron was still an unresolved issue (59).
CHAPTER 4

STABILITY OF MICRONUTRIENTS AFTER STORAGE

This set of studies was conducted to determine the degree of degradation of VA in different formulations of Nutrigems containing VA alone and in the presence of iron under different accelerated storage conditions. Our hypothesis was that the stability of Vitamin A in Nutrigems after storage will be the same with and without iron. Two sources of iron were used: Ferrous Sulfate (FS) and iron NaEDTA (FEDTA). Analyses conducted in these stored samples included moisture, color, vitamin A (VA), and thiobarbituric acid reactive substances (TBARS).

4.1 Materials

Ferrazone™ XP (NaFeEDTA) was donated from Akzo Nobel Fuctional Chemicals (Deventer, Netherlands). Retinyl palmitate premix was obtained from AgD Nutrition (Chicago, IL, USA). Ferrous sulfate was purchased from Spectrum Chemical Corporation (Gardena, CA, USA). All purpose flour Gold Medal, corn flour MASECA and vegetable oil were obtained from Wal-Mart stores.

Standards of retinyl palmitate, retinyl acetate and iron atomic absorption standard were obtained from Sigma Chem.(St. Louis, MO, USA). Thiobarbituric acid, propyl gallate, and 1,1,3,3 tetramethoxypropane and, bathophenanthroline disulfonic acid were obtained from Sigma Aldrich Inc. (St Louis, MO, USA). 1-propanol, ethanol, and methanol HPLC grade, magnesium chloride hexahydrate, sodium chloride, sodium EDTA, and hydrochloric acid were obtained from Fisher Scientific (Fair Lawn, New Jersey, USA). Acetonitrile was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA).
4.2 Methods

4.2.1 Nutrigems\textsuperscript{TM} preparation

Different formulations of Nutrigems with and without micronutrients were prepared as described in 3.2.1 and Table 1. Vitamin A, iron and TBARS content were determined in fresh samples to assess initial conditions (t=1).

4.2.2 Storage conditions: humidity chambers

Samples (Table 1) were stored under four conditions, the combination of two temperatures and two relative humidities (RH). The conditions were: 23 °C, 33% RH; 23 °C; 77% RH; 40 °C, 33% RH; and 40 °C, 77% RH. Rectangular sealed plastic chambers (0.30 x 0.15 x 0.25 m) were used to create relative humidity conditions. Each chamber was divided in two sections separated by a grid. The bottom section contained saturated salt slurries and the upper part contained the samples. Samples were in direct contact with humidity but no with saturated salts. Saturated salts including magnesium sulfate and magnesium chloride hexahydrate were used to generate both humidity conditions 33% and 77% RH, respectively (60). Chambers were placed in two different incubators at temperatures of 23 °C and 40 °C. Saturated salts slurries were prepared in small plastic weighing plates with deionized water. The equilibration time was one day. Samples were inserted in the chamber, closed and sealed tightly to maintain humidity and place in incubators. The humidity and temperature were monitored by Temperature Humidity Meter (Fluke, Model 971) placed inside each chamber.
4.2.3 Sampling procedures

All formulations were weight in aluminum plates. A total of 20 samples were inserted in each chamber and sealed tightly with high-vacuum grease (Dow Corning, MI, USA). Chambers were opened and samples were removed for analysis at two time intervals, the 7th and 14th day of storage. Analyses conducted in these stored samples included moisture, color, vitamin A, and thiobarbituric acid reactive substances (TBARS). Double deionized water (~1 mL) was added to salt slurries every time chambers were opened (45).

4.2.4 Moisture content

The moisture content of Nutrigems was determined gravimetrically according to procedure 925.09 of the AOAC International (61). Samples were weighed into aluminum trays and dried at 105 °C for 24 hours. Moisture content was measured at day 1, 7 and 14. The difference in weight was the loss in moisture of the samples and the % moisture determined as follows.

\[
\text{% Moisture} = \frac{\text{mass of dry sample}}{\text{mass of original sample}} \times 100 \quad \text{Eq [4]}
\]

4.2.5 Color determination

Color of uncooked Nutrigems samples was measured at day 1, 7 and 14 using hunter L*a*b spectrophotometric system using the Minolta ChromaMeter Cr-400/410 (Konica Minolta; Mahwah, NJ), where L indicates lightness (100=white, 0=blank), a indicates red to green (100=red, -100=green), and b indicates yellow to blue (100=yellow, -100=blue). L*a*b* samples
values were obtained in triplicate using 5 x 15 mm Petri dishes. Samples were measured at approximately 25 °C. These values were then used to calculate the color change \( \Delta E \) using Eq [3]. Color change of Nutrigems containing micronutrients were compared to color of Nutrigems alone at day 1.

4.2.6 Vitamin A quantification

4.2.6.1 Direct solvent extraction of vitamin A

Nutrigems samples were ground (5 g) using a mortar and pestle. Sub-samples (100 mg) were taken and accurately weighed in centrifuge tubes (15 mL Falcon centrifuge tubes, BD Biosciences). Immediately, 50 \( \mu \)L of internal standard (retinyl acetate 300 \( \mu \)g/mL in 1-propanol) was added to all samples. Tubes were wrapped in aluminum foil to prevent light oxidation during the extraction procedure. Then, they were dispersed with 1 mL of water containing EDTA (0.01%) following addition of 5 mL of 1-propanol and BHT (0.01%) solution. Next, samples were homogenized using a Polytron\textsuperscript{®} (Model, PT 10/35) for 45 seconds (medium speed) and sonicated (Fisher Scientific Sonicator, Model FS20H) for 15 min. Finally, samples were centrifuged (Sorvall R17 Plus\textsuperscript{®}) for 15 min at 10 °C, 2012 xg. Supernatant was filtered using 0.45 \( \mu \)m PTFE membrane filter directly into HPLC amber drone vials (4 mL) with clear spring inserts (250 uL). All steps were carried out in the dark and all solvents were HPLC grade.

4.2.6.2 Standards preparation and concentration measurements

In order to measure the concentration of VA in the extracted samples, a calibration curve was developed using retinyl palmitate and retinyl acetate standards as described in 3.2.3.1.
4.2.6.3 High performance liquid chromatography (HPLC) procedure

HPLC analysis was carried out on a Waters™ system consisting of Waters™ 600S pump, Waters™ 717 plus autosampler, Waters™ PDA 996 – UV-VIS detector and Zorbax® Rx C8 column (250 x 4.6 mm). The conditions and quantification used in this study were the same as described in 3.2.3.2.

4.2.7 Thiobarbituric acid reactive substances (TBARs)

The 2-thiobarbituric acid reactive substances (TBARs) analysis measures secondary oxidation products. Samples were analyzed using TBARs assay described by Vyncke et al. (1975) and modified by Sørensen et al. (1996) (62, 63). Briefly, Nutrigems samples (5 g) were ground using mortar and pestle. Sub-samples (1 g) were weighed and placed in testing tubes (15 mL Falcon centrifuge tubes, BD Biosciences). Then, 1 mL of 0.1% propyl gallate and 0.1% EDTA solution was immediately added to avoid further oxidation. Then, 10 mL of water was added and samples were homogenized using Polytron® (Model, PT 10/35) at medium speed for 45 seconds. Immediately, 5 mL of solution of 10% trichloroacetic acid was added and vortex for 1 minute. The mixture was centrifuged (Sorvall R17 Plus®) for 15 min at 10 ˚C, 2012 xg. Supernatant was filtered with 0.45 μm PTFE filter. Later, 0.5 mL of filtrated supernatant was combined (1:1 v/v) with 0.02 M thiobarbituric acid in 1.5 mL microcentrifuge tubes (Fisher Scientific, Fair Lawn, NJ, USA) and incubated at 94 ˚C in a water bath for 50 minutes. Next, samples were cooled in an ice bed, and the absorbance was measured using a spectrophotometer (Spectronic Instruments, Inc. Rochester NY) at 535 nm.
TBARs value was calculated by the following formula:

\[
\left( \frac{nM \text{ malondialdehyde}}{g \text{ (d.b.)}} \right) = \text{Dilution} \ast \left( \frac{\text{absorbance of test solution-blank}}{g \text{ (d.b.)}} \right)
\]

TBARs absorbances were transformed to MDA equivalent adducts using a standard curve prepared from 1,1,3,3-Tetraethoxypropane (TEP). Results were expressed as nanomoles of MDA equivalents/g dry Nutrigems with and without micronutrients. All values were corrected with the moisture content of each sample.

4.2.8 Statistical analysis

A minimum of three replicates were analyzed of each sample. A factorial design (blocked randomized design) was used for statistical data analysis to study the VA stability in each Nutrigems formulation. Data are shown as the means ± SD. All statistical analyses were conducted using the SAS software v. 9.2 (SAS Institute Inc., Cary, NC) to determine Analysis of Variance (ANOVA). If significant differences were found, means were compared by the Tukey’s test and deemed significant at \( P<0.05 \).

4.3 Results

4.3.1 Vitamin A stability under accelerated storage

Nutrigems formulations were stored under four different conditions, low and high temperature and relative humidity, for 14 days. Stability of VA was evaluated in three Nutrigems formulations: 1) vitamin A (VA); 2) vitamin A and iron EDTA (VA-FEDTA); and 3) vitamin A and ferrous sulfate (VA-FS). Vitamin A stability was more affected by temperature than relative
humidity ($P<0.0001$). However, when iron was added this changed ($P<0.0001$). VA losses were significantly lower after 7 days than 14 days of storage ($P <0.001$).

Nutrigems fortified with VA alone retained more than 80% of VA initial amount after 7 days of accelerated storage for all storage conditions (Figure 4). After 14 days, only the low temperature conditions had higher retention of VA. The highest retention was $84 \pm 6.7\%$ after 7 days at $23 ^\circ C$, 33% RH and the lowest was $41.94 \pm 7.55$ at $40 ^\circ C$, 77% RH.

![Figure 4](image)

**Figure 4.** Changes in vitamin A after 7 and 14 days of exposure to different accelerated storage conditions. Bars represent mean percent retention + SD. Bar color represent storage conditions: $23 ^\circ C$ and 33 %RH (white); $23 ^\circ C$ and 77% RH (light grey); $40 ^\circ C$ and 33% RH (darker grey); $40 ^\circ C$ and 77% RH (black). Different letters (a,b) on bars represent significant differences according to Tukey’s test at $P<0.05$ among treatments. *Figure Insert:* Factor and interaction analysis. **B:** Temperature ($23 ^\circ C$, $40 ^\circ C$). **C:** Relative Humidity (33, 77 % RH). **D:** Sampling time after storage (day 7, day 14).
**Figure 5.** Changes in vitamin A after 7 and 14 days of exposure to different accelerated storage conditions and in the presence of two different iron sources. Bars represent mean percent retention + SD. Bar color represent storage conditions: 23 °C and 33 %RH (white); 23 °C and 77% RH (light grey); 40 °C and 33% RH (darker grey); 40 °C and 77% RH (black). Different letters (a,b,c,d) on bars represent significant differences according to Tukey’s test at $P<0.05$ among treatments. *Figure Insert:* Factor and interaction analysis. 

**A:** Iron source (FS, FEDTA). **B:** Temperature (23 °C, 40 °C). **C:** Relative Humidity (33, 77% RH). **D:** Storage time (day 7, day 14).
4.3.2 Vitamin A stability in presence of iron under accelerated storage

Stability of VA was affected by the addition of iron, with significant differences observed between the two tested iron sources. Overall, addition of FEDTA resulted in lower ($P<0.0001$) losses of VA than addition of FS (Figure 5). At day 7, VA in Nutrigems-VA-FEDTA had similar stability than in Nutrigems-VA, except when stored at high temperature and high RH conditions ($40 \degree C, 33\% RH$). Addition of FS to Nutrigems-VA promoted severe VA losses. At day 7, the amounts of VA in Nutrigems-VA-FS were only 30% of initial values. Correlating VA retention and TBA-MDA equivalents in all samples showed that the addition of FS to Nutrigems increased the degradation of VA in comparison with the addition of FEDTA (Figure 6).

4.3.3 Color changes after accelerated storage conditions.

The appearance of all Nutrigems formulations varied over time as can be observed in Figure 7 and 8. The addition of different iron caused color changes in Nutrigems formulations (day 1, $P<0.0001$). Color analyses showed that Nutrigems-FS had the highest color change $\Delta E$, followed by samples that contained Nutrigems-FEDTA > Nutrigems alone > Nutrigems-VA. Different storage temperatures promoted changes in color, especially in b values. Humidity promoted color changes only at higher temperatures especially in the case of FEDTA. Samples stored at high humidity increased their $a$ and $b$ values, which represented that samples became more red and more yellow, respectively.
Figure 6. Correlation between vitamin A content and TBA-MDA equivalents in Nutrigems fortified with either iron EDTA (FEDTA) or FeSO₄ (FS) under different accelerated storage conditions. Pearson correlation coefficient for Nutrigems-VA-FEDTA was $\rho = -0.49 \ (P < 0.01)$ and for Nutrigems-VA-FS was $\rho = -0.82 \ (P < 0.0001)$. 
Figure 7. Changes in color (ΔE) over time for Nutrigems fortified with FEDTA and FS under accelerated conditions. Individual points are means ± SD. Symbols represent Nutrigems formulations: ■ Nutrigems-VA-FS; ▲ Nutrigems-VA-FEDTA.
**Figure 8.** Changes in visual appearance of Nutrigems formulations after extreme storage conditions (40 °C, 77 %RH). NT: Nutrigems. Nt-VA: Nutrigems containing vitamin A. Nt-VA-FEDTA: Nutrigems containing vitamin A and iron NaEDTA. Nt-FEDTA: Nutrigems containing iron NaEDTA. Nt-VA-FS: Nutrigems containing vitamin A and ferrous sulfate.
4.4 Discussion

Nutrigems™ is a simple technology that consists of the preparation of fortified rice-like noodles to provide critical nutrients to children in developing countries. The locations where Nutrigems™ can be prepared vary from a well-established kitchen, which has all safety requirements for food preparation, to any rural environment, where there is no access to basic services such as electricity and/or gas. Nutrigems™ is designed to be prepared within a few minutes and eaten the next day. Therefore, no additional storage procedures are involved such as the use of bags to protect it from environment or the addition of antioxidants to avoid oxidation and nutrient losses throughout time. However, we are aware that this might not be the case for all final consumers. In this study, we evaluated the stability of VA in Nutrigems when stored in accelerated conditions with and without presence of iron. Conditions tested simulated those often found in developing countries within the tropics.

In general, stability of VA is affected by factors that promote oxidation such as exposure to light, nature of the food matrix, humidity, temperature, and presence of pro-oxidant agents (40). Our stability studies were carried out in the dark; therefore, light was not considered a factor for degradation. When samples were stored under high RH, the moisture level in the samples increased, especially after 14 days of storage, this could have led to degradation due to hydrolytic rancidity reactions (64). Furthermore, hydrolytic rancidity is catalyzed by heat (65).

*Nutrigems fortified only with vitamin A was the most stable in all conditions.* Among all formulations, Nutrigems-VA was the most stable under the four different storage conditions (Figure 6). After 7 days, VA losses in all conditions were the same, about 15% on average. However, after 14 days, VA losses increased only during high temperature and high RH
conditions. As expected, VA losses were lower at low relative humidity conditions. This can be explained by the increase in concentration of oxidative reactants during such conditions. Lipids and moisture in the matrix can lead to kitol formation reactions, which promote oxidation in the matrix as described by Runge and colleagues (2000).

Another factor to consider is the presence of pro-oxidant agents in the matrix such as iron. Even though results shown in Figure 6 are for formulations that are not iron fortified, it does not mean that iron was not present in these formulations. Nutrigems preparation involves the addition of commercial all purpose wheat and corn flours that usually are fortified. The standard fortification level in wheat fortified flour in the US is 0.036 mg/g (5). Therefore, iron is present in the system in small amounts, but that can still promote oxidation.

Lee and colleagues (2001) evaluated the storage stability of VA of a similar fortification technology, Ultra Rice™ (UR). The final commercial product consists in the combination of fortified UR with rice in a ratio of 1:100 (w/w), usually packed in polyethylene bags. In their study they stored these bags under the combination of four conditions 23 °C, 55% RH; 23 °C, 88% RH; 35 °C, 55% RH; and 35 °C, 88% RH (51). Their results suggested that VA was more affected by temperature than by RH, which is consistent with our findings. Our results revealed that there was a negative interaction between RH and temperature, which became more noticeable when Nutrigems was stored for 14 days. Lee and colleagues (2001) did not describe interactions. Even though UR and Nutrigems™ technologies have similar concepts, there are major differences between them which are reflected in the storage conditions used in this study. First, Nutrigems™ fortification technology intends to be consumed fresh, therefore, there is no package associated with their use. Thus, during the storage stability study Nutrigems was exposed to direct contact with RH, air and oxygen. Second, if Nutrigems is stored it would be
kept alone, without rice. Finally, other aspect where Nutrigems differs from UR fortification technology is the nature of the matrix components. Ultra Rice™ is a complex mixture containing antioxidants and binders within this synthetic rice matrix that is reconstituted using sophisticated equipment and highly trained personnel. Nutrigems is made from local flours, and can be prepared by locals within any food preparation setting. In addition, adoption of this technology is not widely spread mostly because is still expensive as it includes a great use of technological resources, trained personnel and its distribution specially to those isolated populations is still not logistically feasible.

Addition of iron to Nutrigems affected vitamin A stability, with different iron sources resulting in different effects. Despite equivalent iron concentration of both formulations (5 mg/g of Nutrigems), VA losses were different between FS and FEDTA. There is a strong correlation between losses of VA and formation of TBAR-MDA equivalents. The correlation value for Nutrigems-VA-FEDTA was $\rho = -0.82$ and for Nutrigems-VA-FS was $\rho = -0.49$. TBARs analysis indicated that after storage all Nutrigems-VA-FS formulations had the highest rates of oxidation; followed by Nutrigems-VA-FEDTA only when stored under high RH conditions.

Ferrous sulfate was very reactive even during ingredient mixing and promoted oxidation in spite of the storage condition. After 7 days, Nutrigems-RP-FS had VA losses higher than 75%. By day 14, losses were even higher, which is consistent with the findings of Rutkowski et al. (2007) (54). These authors determined the stability of premixes of VA in the presence of different iron sources. They found that the losses of VA were 80 and 20% when either FS or FEDTA were stored for 2 months at 40 °C and 60% RH. They also reported that the introduction of three micronutrients, iodine, iron and VA, into one premix particle resulted in the reduction of
VA content to 10%. Authors discourage the combination of reactive iron and other labile vitamins such as VA.

On the other hand, Nutrigems formulations that contained FEDTA were not as reactive as those that contained FS, except under high temperature and RH conditions. This increased stability of iron NaEDTA is due to the chelated iron form in EDTA, forming a tight configuration that makes the iron less reactive. In addition, iron in FEDTA is in the ferric form, which is not susceptible to oxidation (66).

Additionally, the color of Nutrigems formulations that contain both Nutrigems-VA-FS and Nutrigems-VA-FEDTA strongly varied with time, respect to other formulations. While FS was light green in color at day 1, as time passed it was converted to ferric sulfate which has a more red color. The color of FEDTA changed dramatically in high RH and high temperature condition turning in more red and more yellow. These findings are consistent with Li and colleagues (2008), who evaluated the effect of iron compounds on color after storage stability at 40 °C, 60% RH of multiple micronutrients fortified Ultra Rice™ which contained zinc and B vitamins. In their studies, they concluded that different iron sources contributed to different color shades to samples. Samples containing FEDTA showed a tendency to develop deeper red colors (positive a values), whereas FS contributed a yellowish color to the samples (positive b values). A similar study reported by Moretti et al. (2005) evaluated color of different iron-fortified reconstituted rice grains after 2 weeks of storage at room temperature (25 °C, 40% RH). They reported a brown-reddish color in the rice samples containing FEDTA after 2 weeks of storage.

Fortification of foods combining vitamin A and iron is a challenge. Addition of only vitamin A into foods is not without limitations. This challenge is even harder when combining a
transition metal such as iron and VA in the same food matrix. Degradation of VA may occur during both stages, when VA is incorporated into foods and also during the preparation of the premix. Parish and colleagues (1977) found that VA losses in mineral supplements in pill form stored at room temperature (25 °C) after one month was 8.1%, while losses in supplements in powder form was 11.7% within same conditions (67). In dry fortified products such as flours VA losses have been estimated at 30–50% (43). Masood and colleagues (2007) reported VA losses in fortified cookies after 15 days of 5.9%, and by day 30 losses were 11% when stored at room temperature inside plastic bags. They reported The sugar fortification programs in Guatemala and El Salvador experienced VA losses from 30-60% after 9 months at common storage conditions within homes (8). Ultra Rice™ technology developers have reported very low VA losses (<15%) in the final product within a month of storage, however this technology separates transition metals such as iron from the formulation that contains VA, thus creating two types of Ultra Rice™ products. Other types of products that are used in complementary feeding programs for infants or school children deliver micronutrients as final bakery products (43). Wheat-flour bread buns with multiple micronutrients, including VA, are used in school feeding programs in Philippines. This Program also reports VA losses of about 30% after baking.

In our studies, formulations of Nutrigems contained 5 mg of iron and/or 400 µg of VA palmitate. These concentrations levels are 140 times higher than any other fortified food product available in the market and are only observed in dietary supplements and supplementation pills. At these levels, negative interactions between iron and VA are expected and are generally detrimental to the food matrix. Few commercial products have similar characteristics to Nutrigems such as the triple fortified salt (TFS) that contain 30 µg iodine, 2 mg Fe (ferric pyrophosphate), and 60 µg VA per gram of salt (54). Storage stability analysis on this product
revealed that losses of iodine and VA were very low, 12–15%, after 6 months (66). Nonetheless, authors reported that the process of producing the microcapsules causes 35% losses of VA as a result of oxidation during heating and spraying. Therefore, even when using encapsulation techniques in salt, the overage of VA is approximately 50%.

The other similar product to Nutrigems, Ultra Rice™, uses a different approach to avoid negative interactions between VA and other transition metals. Developers formulate two different products, Ultra Rice™ fortified only with VA and Ultra Rice™ fortified only with iron, zinc and B vitamins, both that are ready to mix with regular rice.

In summary, there is not an easy way to fortify suitable staple foods with both VA and iron without observing negative interactions between these two micronutrients. Nutrigems™ technology proposes to mix these two micronutrients only when they are needed and prepared into the matrix, reducing the time of interaction between VA and iron. Also, this technology proposes the use of a stable source of iron, such as FEDTA, which reduces even more the probability of interaction within the window of use. In this study, the author have demonstrated that under optimal and sub-optimal storage conditions Nutrigems™ could be a good alternative to deliver VA or iron, and their combination in very high concentrations without compromising VA stability.
CHAPTER 5

CONCLUSIONS

Nutrigems™ is a local and community-based food fortification technology that consists in the preparation of fortified rice-like noodle to provide critical micronutrients to children in developing countries. This technology is simple, cheap, and flexible and can be prepared in the homes, is made with staple ingredients, and does not require expensive equipment to prepare, store or cook neither the use of electricity nor gas. For these reasons, it is an alternative to complement successful school lunch programs to reduce micronutrient deficiencies and improve children health. Besides, it empowers individuals participating in these programs, mostly women, to take ultimate responsibility over the quality of diet of their children.

The studies presented in this dissertation illustrated the impact of cooking and accelerated storage on VA content when Nutrigems fortified only with VA and in the presence of iron. Results in Chapter 3 demonstrated the impact of cooking on VA retention when Nutrigems containing 1) VA and 2) the combination of VA and iron (iron NaEDTA) was added and cooked with rice in a ratio of 1:100 (w/w) in comparison of adding separated solutions of VA and VA and iron at equivalent concentrations. Percent retention of VA was significantly \( P<0.05 \) different when rice was cooked in presence of Nutrigems. In both cases Nutrigems matrix provided on average a 12% protection to VA because it creates a physical barrier that reduces exposure to pro-oxidant agents such as heat or light, even in the presence of iron. Additionally, the Nutrigems matrix is made of flours and oil that contained antioxidants. The level of protection of having VA within a rice-like food matrix was also reported by Lee et al. (2001) and Murphy et al. (1996) that establish a range of VA retention from 75% and 96% among different
synthetic rice grains formulations that contained different types of antioxidants and oils. However, all these formulations involved the use of sophisticated equipment and ingredients to form these protective matrixes, which may be unsuitable for some isolated rural environments where dwellers cannot afford the technological cost of equipment and training. Additionally, cooking stability studies described in Chapter 3 showed the level of retention of iron in Nutrigems rinsed before cooking. This is an important consideration as many people in developing countries washed the rice principally to remove impurities and stones and to make the cooked non sticky product. Iron content was reduced in 15, 34, and 47% after rice was rinsed in excess of water for 30, 60 and 90 seconds, respectively. Therefore, Nutrigems that contains FEDTA should be added after rice is washed. The addition of 1% of Nutrigems caused slight color changes in cooked rice. The most significant color changes were caused by Nutrigems containing ferrous sulfate. In our studies, the results showed that the overage of VA alone due to losses after cooking should be only 5% and 18% when iron NaEDTA is added into the Nutrigems matrix.

Results in Chapter 4 demonstrated the losses of VA when formulations of Nutrigems containing VA and the combination of VA and iron were stored under four different accelerated storage conditions: 23 °C, 33% RH; 23 °C; 77% RH; 40 °C, 33% RH; and 40 °C, 77% RH. Nutrigems that contained only VA were the most stable under all storage conditions. Temperature affected VA stability more than relative humidity (P<0.001). However, this changed with the addition of iron. Higher RH promoted higher VA losses. VA losses were significantly lower after 7 days than 14 days of storage. After 7 days, Nutrigems containing VA alone retained more than 80% of initial VA. Also, VA retention between Nutrigems containing VA and Nutrigems containing VA and FEDTA had similar stability except at 40 °C, 77% RH.
Moreover, results showed that the source of iron 1) iron NaEDTA and 2) ferrous sulfate had significant differences on VA retention. FEDTA caused lower ($P<0.0001$) VA degradation than FS. Correlation between VA retention and TBA-MDA equivalents showed that FS increased the degradation of VA in comparison with the addition of FEDTA. Different storing temperatures promoted changes in color in Nutrigems formulations. Nutrigems-FS had the highest color changes, followed by samples that contained Nutrigems-FEDTA > Nutrigems alone > Nutrigems-VA. FEDTA color in higher RH conditions had more dramatic color changes in comparison of other FEDTA formulations.

The results of this study clearly indicate that the stability of VA in Nutrigems is satisfactory after cooking and under optimal and sub-optimal storage conditions. Therefore, Nutrigems™ could be a good alternative for delivering micronutrients and improving nutritional quality of the meals provided in the School Lunch Program in Honduras or other programs with similar logistics. In many developing countries, children are in high risk of both VA and iron deficiencies. Therefore, a flexible delivery vehicle that can carry both iron and VA is necessary. Our results demonstrated that the combination of these two micronutrients have negative effect on VA retention, color and oxidation catalysis. Nevertheless, Nutrigems™ still can be a suitable option because proposes to mix these two micronutrients only when they are needed and its uses a more stable iron form (i.e. FeNaEDTA). In this way, the time of interaction between VA and iron is reduced. However, Nutrigems™ performance may vary according to the geographical location and climate. There are potential limitations when fortifying with both VA and iron in places where there is high relative humidity and high temperature conditions. Nevertheless, these effects can be mitigated by the implementation of better practices such as consumption of Nutrigems™ within 24 hours of preparation.


61. AOAC, Method 925.10: Solids (Total) and Moisture in Flour In *Official Methods of Analysis of AOAC International*. Ed. Gaithersburg, MD, 2000, (2), p 32.1.03.


