Application of a Handheld Infrared Sensor for Monitoring the Distribution of Vitamins and Minerals in Fortified Corn-based Snacks

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

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2009

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ABSTRACT

The food processing industry is the largest contributor to Ohio’s manufacturing economy accumulating $5.9 billion per year. On-line or real-time spectroscopic methods can provide a valuable window into in-process food manufacturing in order to optimize the production rate, quality, and safety of foods. Advances in infrared spectroscopic instrumentation combined with multivariate data analysis make this technology ideal for the snack food industry. The objective was to develop a real-time methodology for monitoring fortification of whole grain cornmeal throughout the industrial mixing process using a handheld infrared sensor.

Whole grain cornmeal was fortified with a blend of zinc, iron, vitamin E, and calcium at different levels (0.5-5.5%) and mixed for 30 minutes to ensure homogenous distribution of the fortificants. Real-time infrared analysis was achieved by pressing an aliquot (0.1g) onto an ATR diamond crystal of a portable handheld spectrometer and spectra were collected. Pattern recognition analysis was used to examine the data collected for monitoring distribution uniformity. The model predicted the level of fortification in the whole grain cornmeal with correlation coefficient, \( r=0.98 \) (5 factors) and standard error of cross-validation <0.35%, and was able to cluster the samples into classes corresponding to 3 different fortification levels. A simple, handheld FT-IR instrument could provide a real-time method to evaluate the fortification of whole grain cornmeal snack foods.
DEDICATION

Dedicated to my family

Thank you for all your love, support and encouragement and for always believing in me.
ACKNOWLEDGMENTS

I would like to thank my parents and my sister for all of their support throughout my academic career. Without their love and support this would not have been possible.

Thanks to my best friend Ashley thanks for all of your guidance and encouragement throughout this program.

I would like to thank Dr. Luis Rodriguez-Saona for taking me on as his student after my first year at OSU. Thank you for your support, guidance and continuous patience.

I would also like to thank Dr. Sheryl Barringer and Dr. James Harper for supporting me after I changed advisors and for their support and direction throughout my research.

Thank you to all the members of my lab group for their willingness to help me whenever I needed it and for encouraging me along the way.

Furthermore, I would like to thank the rest of my family for their encouragement and support as well. This would not have been possible without the guidance, patience, love, and support of everyone in my family.
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CHAPTER 1: LITERATURE REVIEW

1.1 FORTIFICATION

The fortification of food is one of the most effective public health interventions used to prevent nutrient deficiencies (Bishai and others 2002). The practice of fortification has been going on for several decades in Western countries as well as in some developing countries (Fletcher and others 2004). Many terms are associated with the addition of nutrients to food, such as restoration, fortification, enrichment, and nutrification (Gregory 2008). Currently, the terms enrichment and fortification are often used interchangeably to indicate the addition of nutrients to food (Berner and others 2001; Sichert-Hellert and others 2000). Fortification is the addition of one or more nutrients to a food in amounts that are greater than those naturally occurring, or that are not present in the fortified food at all (Gregory 2008, Berner and others 2001; Sichert-Hellert and others 2000; Richardson 1990; Richardson 1997). The addition of nutrients not normally associated with the food or the addition to levels above that present in the unprocessed food are examples of fortification (Gregory 2008, Anonymous 2003). Mass fortification refers to the supplementation of nutrients to foods that are commonly consumed by the public, which is initiated, mandated, and regulated by the government sector (Anonymous 2003). The main criterion for adding selected nutrients to foods are that they are shown to be required, safe, and valuable (Richardson 1990).
1.1.2 Fortified Food Regulations

The addition of specific amounts of selected nutrients is known as enrichment and is associated with a standard of identity which is defined by the U.S. Food and Drug Administration (FDA) (Gregory 2008, Anonymous 2001). During the 1940s and 1950s, the FDA established standards of identity for staple foods such as flour, bread, rice, and cornmeal; because these cereal grain products were thought to be good vehicles for fortification since they offer the advantage of reaching most of the targeted U.S. population without requiring changes in consumers’ food selection patterns (Crane and others 1995). In the past, food fortification decisions made by the FDA were based solely on dietary data alone; however, current choices made concerning food fortification are based primarily on clinical and biochemical data (Crane and others 1995). Furthermore, there must be assurance that the addition of specified amounts of nutrients to the food supply is safe for the more than 260 million Americans who would be exposed to them throughout their life times (Crane and others 1995).

The Codex Alimentarius Commission of the Food and Agriculture Organization/World Health Organization, and the US FDA have established general principles for the addition of nutrients to foods (Richardson 1990). However, regulations concerning the addition of nutrients to foods vary from country to country, and many problems can arise due to the lack of harmonization (Richardson 1990). The main criteria for selecting the suitable nutrients to add to foods are that they must be shown to be safe, effective, and beneficial to the nutritional status of the target population (Richardson 1997). Four categories of foodstuffs have been identified where the addition of vitamins and minerals to foods is obligatory in some countries: (a) foods used for
special dietary applications, (b) foods that have lost nutrients during manufacturing, (c) foods which resemble another common food and (d) staple foods that represent standard vehicles for nutrients (Richardson 1990).

According to the FDA, foods used for special dietary reasons are defined as follows: (a) they supply particular dietary needs that exist by reason of a physical, physiologic, pathologic, or other condition; (b) they supply particular dietary needs that occur due to age; (c) they supplement or fortify the normal diet with any vitamin, mineral, or other dietary property (Committee on Nutrition 2003). A good example of this would be the development of nutritional standards for the composition of infant formulas, which are intended for use as a substitute for human milk and should meet the nutritional requirements of infants up to 4-6 months of age (Richardson 1990).

In the UK, the addition of nutrients to all types of flour, except whole meal flour, during manufacture is compulsory due to the loss of nutrients of these foodstuffs during manufacture (Richardson 1990). They must be enriched with the minerals thiamin, niacin, iron and calcium (Richardson 1990). Similarly, in the US, most flours are enriched with thiamin, riboflavin, nicotinamide, and iron, due to the loss of nutrients during the manufacture of different flours (Richardson 1990).

A good example of compulsory enrichment of foods resembling a common food is the addition of vitamins A and D to margarine in order to give margarine a nutritive value comparative to that of butter (Richardson 1990). The addition of vitamin A is required in margarines for sale in the US, and the addition of vitamin D to margarine is optional (CFR Title 21, Volume 2, 2003). The Committee on Medical Aspects of Food Policy in the UK, stated that “any substance promoted as a replacement or an alternative
to a natural food should be nutritionally equivalent in all but unimportant aspects of the natural food which it would simulate.” (Richardson 1990).

The addition of nutrients to staple foods in different countries can help to eliminate or reduce the most widespread nutritional disorders. Tea is consumed on a regular basis in India, even by small children, so this drink is used as the vehicle for enrichment (Richardson 1990). In Guatemala, staple foods used for enrichment with vitamin A are rice and table sugar (Richardson 1990). A good example of a staple food that has been fortified and decreased the occurrence of a nutritional deficiency is the addition of iodine to salt, which diminished the cases of goiter reported (Richardson 1990).

The addition of vitamins and minerals to food is beneficial; however it could also have the potential for abuse which could cause harm to the consumer (Gregory 2008). For these reasons, the U.S. FDA has established important guidelines outlined in 21 CFR Section 104.20(g) which state that the nutrient added to a food should be: (1) stable under usual conditions of storage, distribution, and use; (2) physiologically available from the food; (3) present in an amount where it is guaranteed that there will not be excessive intake, considering cumulative amounts from other sources in the diet; and (4) suitable for its intended purpose and in compliance with provisions of the act and regulations governing the safety of substances in food (Gregory 2008; Berner and others 2001; Richardson 1990). Recommendations similar to the former have also been developed and sanctioned by the Council on Foods and Nutrition of the American Medical Association (AMA), the Institute of Food Technologists (IFT), and the Food and Nutrition Board (FNB) of the National Academy of Sciences-National Research Council.
The guidelines established by the AMA, IFT, and FNB recommend that the following prerequisites be met to justify fortification: (1) the intake of the particular nutrient is inadequate for a substantial portion of the population; (2) the food (or category of food) is consumed by most individuals in the target population; (3) there is reasonable assurance that excessive intake will not occur; and (4) the cost is reasonable for the intended population (Gregory 2008).

### 1.1.3 History of Food Fortification

The recommendation for the fortification of some foods began as early as 1831, when the French physician Boussingault suggested adding iodine to salt to prevent goiter (Fletcher and others 2004). The introduction of the fortification of salt occurred in Switzerland in 1923 and later in the U.S., particularly Michigan, in 1924 (Subar and others 1988; Berner and others 2001; Bishai and others 2002; Fletcher and others 2004). Vitamin A was first added to margarine voluntarily in the United Kingdom in 1927, and eventually it became mandatory, along with the addition of vitamin D to attain the nutritional equivalence to butter (Fletcher and others 2004). The voluntary enrichment of grain products, including bread, with niacin occurred in 1938, and the addition of thiamin, riboflavin, iron, and niacin became mandatory in 1940 (Fletcher and others 2004). The FDA made the addition of folic acid to enriched grain products such as bread, rolls, crackers, tortillas, pasta and rice, mandatory in 1998 in order to reduce neural-tube defects in newborns, cardiovascular disease, and certain cancers (Fletcher and others 2004).
Certain nutritional deficiencies were prevalent in the population of the U.S. during the 1930’s and newly developed synthetic vitamins were being used to fortify foods with little or no scientific guidance (Anonymous 1954). The addition of vitamins and minerals to food was done to prevent and treat deficiency diseases such as goiter, anemia, and rickets (Mertz 1997). The Council on Foods and Nutrition of the AMA adopted policies in 1939 and again in 1945, on the proper addition of vitamins and minerals to foods. Currently, in the U.S., goiter, rickets, beriberi, and pellagra have been virtually eliminated due to the fortification of food and the implementation of fortification programs at the turn of the century (Bishai and others 2002; Crane and others 1995).

1.1.4 Strategies to Tackle Malnutrition

Micronutrient malnutrition is a widespread problem throughout the world in both industrialized and developing countries, which can be caused by socioeconomic conditions or by the local geochemical environment, having serious health and economic implications (Mertz 1997; Fletcher and others 2004). Socioeconomic conditions affect only certain parts of a population including poverty ridden areas, while the environment can provide deficient amounts of nutrients in specific geochemical regions (Mertz 1997). In the U.S. today, socioeconomic problems include poverty, but are caused for the most part by the lack of knowledge, the partiality for refined foods, and the current trend that associates slimness with health (Mertz 1997).

One of the main strategies employed to tackle these serious global issues continues to be fortification (Fletcher and others 2004). Many populations suffer from vitamin and mineral deficiencies, and the fortification of common processed foods can
protect large populations using sustainable market channels (Governments 2004). Initially, the addition of nutrients to foods was to address the issue of micronutrient deficiencies, but now many of the formerly widespread nutrient deficiencies are infrequent in the United States due to the successful fortification programs that were implemented at the turn of the century (Berner and others 2001; Crane and others 1995). Fundamental factors that proved successful for the elimination of many micronutrient deficiencies were: the increased understanding in preventing diseases that occurred during the 20th century, the aptitude to synthesize specific vitamins and their large scale production which enabled the fortification of foods with the suitable vitamins and minerals (Fletcher and others 2004).

1.1.5 Future Focus of Fortification

The focus of fortification has shifted from the prevention of the deficiency diseases to the optimization of nutrient intakes in order to prevent chronic diseases and for overall health and well being, as well as preventing over-fortification of the food supply with certain nutrients (Berner and others 2001; Crane and others 1995). It is necessary to ensure that consumers are not at placed at risk for over-consumption due to the relatively low margin between the recommended intake and the measure of safe intake in certain nutrients (Fletcher and others 2004). The risk of excessive intakes of some nutrients is potentially high, whereas for others it is negligible, but it depends on the margin between the daily requirement and the respective UL for each case (Fletcher and others 2004). Nutrients that show a slender margin of safety are: vitamins A and D, as well as the minerals iron, phosphorus, zinc and selenium (Fletcher and others 2004). The persons who have an increased risk of surpassing the UL are those that consume
large amounts of food, or those who choose proportionally more fortified foods or take dietary supplements (Fletcher and others 2004).

Using tools such as the USDA Food Guide Pyramid is the best way to select a variety of foods which can promote optimal health and reduce the risk of chronic diseases while providing a desirable balance of macronutrients as well as micronutrients, but for certain nutrients or individuals, the addition of fortified foods into the diet may also be desirable (Anonymous 2001). Vitamins and minerals consumed from fortified foods can help people meet their nutritional requirements as specified by science-based nutrition standards like DRIs (Anonymous 2001). The fortification of frequently consumed foods may be an effective and consistent way to achieve health benefits (Anonymous 2001).

Recommended Dietary Allowances define the desirable levels of nutrients in foods and are still used as the foundation of the U.S. fortification policy (Mertz 1997). Nutritional reference standards have been established to evaluate the impact of food composition and intake patterns on the nutritional status of individuals and populations. They also determine the nutritional effects of particular food processing and handling practices (Gregory 2008). Dietary Reference Intakes (DRIs) are made up of a set of four nutrient-based reference values, which include the Recommended Dietary Allowance (RDA), Adequate Intake (AI), Tolerable Upper Intake Level (UL), and Estimated Average Requirement (EAR), each having its own special use (Institute of Medicine 1998). Recommended Dietary Allowances were developed in the United States and have been published since 1941 by the National Academy of Sciences (Institute of Medicine 1998).
Figure 1.1 Dietary reference intakes. This figure shows that the Estimated Average Requirement (EAR) is the intake at which the risk of inadequacy is 0.5 (50%) to an individual. The Recommended Dietary Allowance (RDA) is the intake at which the risk of inadequacy is very small—only 0.02 to 0.03 (2% to 3%). The Adequate Intake (AI) does not bear a consistent relationship to the EAR or the RDA because it is set without being able to estimate the average requirement. It is assumed that the AI is at or above the RDA if one could be calculated. At intakes between the RDA and the Tolerable Upper Intake Level (UL), the risks of inadequacy and of excess are both close to 0. At intakes above the UL, the risk of adverse effects may increase.

Source: IOM 1998

According to the Committee on Dietary Allowances of the Institute of Medicine’s FNB, RDA values are defined as “The average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97-98%) healthy individuals in a particular stage of life and gender group” (2008). The number of micronutrients with RDAs has increased from eight to 26 in the past 40 years (Mertz 1997). In 1994, the FDA revised the labeling regulations and the term RDA has been replaced by Reference Daily Intake RDI, however, the current RDI is equivalent to the previous RDA (Gregory 2008). The vitamin content listed on the nutrition label is currently expressed as a percentage of the RDI and is listed on the label as “% Daily Value” (Gregory 2008). Tolerable upper
intake levels (UL) are the reference standard used to evaluate the risk of excessive intake of individual micronutrients, and they are an estimation of the highest level of long-term intake that bears no risk to health in the general population (Fletcher and others 2004).

1.1.6 Stability and Bioavailability of Fortified Food

Historically, the fortification of foods has been used to improve the nutritional quality of the U.S. food supply (Crane and others 1995). However, during the manufacture, storage, and preparation of foodstuffs, nutrients are exposed to a wide range of physical and chemical factors which can affect the stability as well as the bioavailability of the nutrients (Richardson 1990). Deterioration of nutrients in foods is gradual and depends on the severity of the processing method which can either accelerate or retard the changes in nutrient levels (Karmas 1975). Processing technologies and food preparation techniques such as heat treatment(s), drying, chilling, freezing, fermenting, milling, and irradiation can all affect the levels of micronutrients in food (Richardson 1997).

Changes in the nutritional content of products are copious and they can occur before, during, and after processing (Karmas 1975). Many nutrients can be lost by leaching out with water used during manufacturing, therefore in order to maximize vitamin retention, aqueous extraction (leaching) must be minimized (Gregory 2008; Richardson 1997). The loss of nutrients may be due to the temperature at which the product is exposed, the length of the manufacturing process, processing conditions such as the presence or absence of water, air, light, and/or acidity, nutrient-nutrient interactions, and nutrient-food matrix interactions (Richardson 1997). Since vitamins are variable in
nature, the possibility of each combination of added nutrients interacting in a different way with the food matrix, and the infinite number of ways to prepare, cook, and store food, it is practically impossible to simplify on the effect of individual factors on stability; however much information has been collected on the behavior of individual vitamins (Richardson 1990).

**Table 1.1** Factors Influencing Vitamin Stability

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Temperature</td>
</tr>
<tr>
<td>2.</td>
<td>Moisture</td>
</tr>
<tr>
<td>3.</td>
<td>Oxygen</td>
</tr>
<tr>
<td>4.</td>
<td>Light</td>
</tr>
<tr>
<td>5.</td>
<td>pH</td>
</tr>
<tr>
<td>6.</td>
<td>Oxidizing and reducing agents</td>
</tr>
<tr>
<td>7.</td>
<td>Presence of metallic ions (e.g. Fe, Cu)</td>
</tr>
<tr>
<td>8.</td>
<td>Presence of other vitamins</td>
</tr>
<tr>
<td>9.</td>
<td>Other components of food such as SO₂</td>
</tr>
<tr>
<td>10.</td>
<td>Combination of the above</td>
</tr>
</tbody>
</table>

*Source:* Berry Ottaway, 1993

Vitamins encompass a diverse group of organic compounds that are nutritionally essential micronutrients; however, quantitatively they are minor constituents of foods (Gregory 2008). Vitamins can function as reducing agents, radical scavengers, reactants in browning reactions, and as flavor precursors; all of which can influence the chemical nature of the food (Gregory 2008). Today, there is a plethora of information regarding the stability and properties of vitamins; conversely, our knowledge about their behavior in the complex milieu of food is limited (Gregory 2008). Many studies have been
published using well defined model systems, or even buffer solutions, to simplify the investigation of vitamin stability, but the results of these studies should be interpreted with caution since the degree to which these model systems imitate complex food systems is unknown (Gregory 2008). Furthermore, these studies have provided valuable insight into the chemical variables affecting vitamin retention, but the results are limited for predicting the behavior of vitamins in complex food systems (Gregory 2008).

The nutrient retention in the food is influenced by any structural changes that occur in the food as well as any mechanical processes to which the product is subjected (Richardson 1997). There are many factors that affect the retention of vitamins in complex food systems such as (a) processing or storage time and temperature; (b) the concentration and temperature dependence of the degradation reaction; (c) environmental variables; (d) the rate of competing or sequential reactions; (e) the relative stability of the various forms of the vitamin present; (f) the chemical nature of other food constituents; (g) the mechanism(s) of chemical loss of vitamin activity in the food; and (h) the food system’s water activity (Gregory 1985). The loss of nutritional quality with respect to certain vitamins is often inevitable during food processing and storage due to the complex interactions of the aforementioned variables (Gregory 1985).
Table 1.2 General Stability\textsuperscript{a} of Vitamins

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Neutral</th>
<th>Acid</th>
<th>Alkaline</th>
<th>Air or Oxygen</th>
<th>Light</th>
<th>Heat</th>
<th>Maximum Cooking Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>S</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>40</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>100</td>
</tr>
<tr>
<td>Biotin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>60</td>
</tr>
<tr>
<td>Carotenes</td>
<td>S</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>30</td>
</tr>
<tr>
<td>Choline</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>S</td>
<td>S</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin B_{12}</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>S</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>40</td>
</tr>
<tr>
<td>Folate</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>5</td>
</tr>
<tr>
<td>Niacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>75</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin B_{6}</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>40</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>75</td>
</tr>
<tr>
<td>Thiamin</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>S</td>
<td>U</td>
<td>80</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>55</td>
</tr>
</tbody>
</table>

Note: Caution: these conclusions are oversimplifications and may not accurately represent stability under all circumstances.
\textsuperscript{a} S, stable (no important destruction); U, unstable (significant destruction).

Source: Adapted from Harris, R. S. (1971).

The stability of vitamins can be improved by producing a coated or encapsulated vitamin (Richardson 1997). Nutrient premixes are prepared when a number of vitamins and/or minerals are being added, and the nutrients are dispersed in a compatible base (Richardson 1997). The use of this technique provides many advantages such as complete premix dispersion throughout the product, greater accuracy with the level of addition, and the premix can be used to manufacture a number of defined batches or days’ productions and ‘tracer’ nutrients can be used to check each batch and reduce analytical tests (Richardson 1997).
Biological activity is a term that refers to the inherent potency of a compound in meeting a specific nutritional requirement; however bioavailability refers to the extent of intestinal absorption and metabolic utilization of a nutrient; bioavailability involves both utilization and absorption of a nutrient as consumed (Gregory 1985, Gregory 2008). To fully understand the nutritional adequacy of a food, three important factors must be known: (1) the vitamin concentration at the time of consumption; (2) the various chemical species of the vitamin present must be known; and (3) the bioavailability of these forms of the vitamin as they exist in the meal consumed (Gregory 2008).

The bioavailability of a nutrient can be affected by its physical state or chemical form, as well as other dietary components that could affect its absorption and metabolism (Gregory 1985). The bioavailability of some nutrients may change due to food processing parameters; therefore when foods have added nutrients, attention must be paid to their bioavailability (Richardson 1990; Richardson 1997). Also, there are three main factors that influence the bioavailability of vitamins, including (1) the composition of the diet (can influence transit time in the intestine, viscosity, emulsion characteristics, and pH); (2) the form of the vitamin since different forms may vary in rate or extent of absorption and stability in the stomach and intestine prior to digestion; and (3) the interactions that occur between a vitamin and the components of the diet, like proteins, starches, dietary fiber, etc. which can interfere with the absorption of the vitamin in the intestine (Gregory 2008).

The understanding of the relative bioavailability of the various species of each vitamin is rapidly improving; however, the complex influences of food composition on vitamin bioavailability remain poorly understood (Gregory 2008). Also, processing and
storage effects on vitamin bioavailability have only been partially determined, due to the complexity of food matrices, as well as the interactive effects of individual foods and the sources of variation among individual people (Gregory 2008).

Bioavailability, in the case of mineral nutrients, is principally determined by the efficiency of absorption from the intestinal lumen into the blood; however, absorbed nutrients may be in a form that cannot be utilized (Gregory 2008). The bioavailability of mineral nutrients can range from 1% to greater than 90%, and this wide range can be attributed to the many factors that interact to determine the bioavailability of a nutrient (Gregory 2008).
Table 1.3 Factors that may influence mineral bioavailability from foods.

1. Chemical form of the mineral in food
   a. Highly insoluble forms are poorly absorbed
   b. Soluble chelated forms may be poorly absorbed if chelate has high stability
   c. Heme iron is absorbed more efficiently than nonheme iron in most diets

2. Food ligands
   a. Ligands that form soluble chelates with metals may enhance absorptions from some foods (e.g., EDTA enhances Fe absorption from some diets)
   b. High-molecular-weight ligands that are poorly digestible may reduce absorptions (e.g., dietary fiber, some proteins)
   c. Ligands that form insoluble chelates with minerals may reduce absorption (e.g., oxalate inhibits CA absorption, phytic acid inhibits CA, Fe, and Zn absorption)

3. Redox activity of food components
   a. Reductants (e.g., ascorbic acid) enhance absorption of iron but have little effect on other minerals
   b. Oxidants inhibit the absorption of iron

4. Mineral-mineral interactions
   a. High concentration of one mineral in the diet may inhibit the absorption of another (e.g., Ca inhibits Fe absorptions, Fe inhibits Zn absorption, Pb inhibits Fe absorption)

5. Physiological state of consumer
   a. Homeostatic regulation of minerals in the body may operate at the site of absorption, resulting in up-regulated absorption in deficiency and down-regulated absorption in adequacy or overload. This is the case for Fe, Zn, and Ca
   b. Malabsorption disorders (e.g., Crohn’s disease, celiac disease) may reduce absorption of minerals and other nutrients
   c. Achromydia (reduced acid secretion in the stomach) may impair Fe and Ca absorption
   d. Age may affect mineral absorption: absorption efficiencies often decline with age
   e. Pregnancy: iron absorption increases during pregnancy

1.2 ANALYTICAL TECHNIQUES FOR FORTIFIED WHOLE GRAIN CORNMEAL

1.2.1 High Performance Liquid Chromatography

Currently, high performance liquid chromatography (HPLC) is used to determine vitamin E content in food products (Heudi and others 2004). HPLC can be applied to the analysis of any compound with solubility in a liquid (Rounds and others 2003). It is a form of chromatography where a separation occurs among the stationary phase and the mobile phase. The use of HPLC has gained approval for application in food analysis because it can be used on a variety of compounds and it is highly automated.

Five key components make up the core of HPLC (Figure 1.3) including: a pump, an injector, a column, a detector, and a recorder system (Rounds and others 2003; Bélanger and others 1997). Electronic controls regulate the pressure, flow rate and delivery rate of the mobile phase in the pump (Bélanger and others 1997). The pump is used to carry the mobile phase through the system; however, the sample must be soluble in the mobile phase, therefore it is important to keep the polarity of the sample in mind when choosing a mobile phase (Rounds and others 2003). The sole purpose of the injector is to introduce the sample into the mobile phase so it can be carried onto the column. The column is where the actual separation of the compounds occurs (Bélanger and others 1997). The most widely used type of chromatography is adsorption chromatography and it can be classified into normal phase and reverse phase (Bélanger and others 1997). During normal phase chromatography, the mobile phase uses a non-polar solvent while the stationary phase is polar (Rounds and others 2003). Reverse
phase chromatography is the exact opposite of normal phase chromatography; using a non-polar stationary phase and a polar mobile phase (Rounds and others 2003). Partition, ion exchange, and size exclusion are other types of chromatography (Bélanger and others 1997). The retention time of the solutes can be controlled by changing the pH and/or ionic strength of the mobile phase (Rounds and others 2003). The flow rate of the mobile phase is generally kept low in order to maximize separation (Bélanger and others 1997). Determining which chromatographic technique to utilize is of extreme importance seeing as the final results can be significantly affected if the wrong mobile phase or stationary phase is used. The use of an HPLC system requires a great deal of trials and tribulations due to its sensitivity.

After the analyte passes through the column, it goes through the detector. It is important to use the correct detector for the analyte since there is no universal detector for HPLC (Bélanger and others 1997). However, there are five common detectors that are used with HPLC and they include: refractive index, UV-absorption, as well as fluorescence, electrochemical, and mass detectors (Pomeranz and others 1987). The most common detector used is UV/Vis, because of its high sensitivity for the majority of compounds, its excellent selectivity, and it is easy to operate and non-destructive (Bélanger and others 1997). Detectors should be chosen depending on the compounds that are being resolved and the type of analysis being completed. Lastly, the results are presented to the analyst for further investigation. High performance liquid chromatography has been used in several studies to quantify vitamin E in many different food matrices. Examples are as follows: Lee et al. (1999) measured the vitamin E content in extruded weaning foods; Suknark et al. (2001) determined the stability of
vitamin E in snack extrudates; Park et al. (2005) measured the amount of fortification of vitamin E in fresh-cut apples; and Heudi et al. (2004) quantified vitamin E in fortified infant formula.

High performance liquid chromatography is known for being a very reliable and accurate analytical instrument; on the other hand, it does have limitations. It is a time consuming technique and requires lengthy methodology development and sample preparation. The success or failure of the method is dependent on sample preparation; furthermore, the end results are dependent on extraction efficiency, analyte stability, and the consistency of chemical or enzymatic pre-treatment (Rounds and others 2003). In addition, the stability of the compounds as well as the ability of the analyst to properly extract the compounds of interest and prepare the sample for analysis properly can all affect the end results. Moreover, HPLC is an expensive instrument that necessitates the utilization and disposal of hazardous organic chemical solvents (Halim and others 2006).

![HPLC Schematic](image)

**Figure 1.2 HPLC Schematic**
1.2.2 Brief History of Infrared Spectroscopy

The history of spectroscopic techniques dates back to 1800, when the famous scientist Frederick William Herschel discovered that the sun’s energy was not limited to what we can see; which is demonstrated by projecting a rainbow onto a bench using a prism (Baeten and others 2002). Herschel’s work was the first step in the discovery of the electromagnetic spectrum and is the root of infrared spectroscopy (Baeten and others 2002).

Fourier-transform infrared (FTIR) spectroscopy has been known for more than a century and it began with the invention of the interferometer by Michelson in the 1880s (Michelson 1891; Michelson 1892). A major breakthrough in the field of Fourier-transform infrared spectroscopy occurred in 1965, and caused a significant increase in the resolution of the spectra as well as reducing the time required for analysis (Cooley and others 1965). FTIR spectroscopy is considered one of the most powerful techniques for chemical analysis due to its simplicity, sensitivity, versatility and speed of analysis.

1.2.3 Infrared Spectroscopy

In recent years, spectroscopic techniques have become increasingly popular, attractive, and promising analytical tools for applications in the food industry due to the speed of analysis that spectrophotometric methods can provide (Baeten and others 2002). Traditional methods used for the quantification of compounds in food matrices are time consuming and expensive, as well as requiring the use of a several perilous organic solvents. Alternatively, infrared spectroscopy (IR) is an emerging diverse and versatile analytical method with which the industry is becoming familiar and which has shown to
be a very quick, sensitive, simple, and cost-effective tool (Halim and others 2006; Coates 2000).

Infrared radiation is commonly defined as electromagnetic radiation with frequencies between 14,300 and 20 cm⁻¹ (McKelvy and others 1996). The infrared spectrum is formed as an outcome from the absorption of electromagnetic radiation at frequencies which show a relationship to the vibrations that occur due to particular chemical bonds from a molecule (Coates 2000). The infrared spectrum is divided into three regions (Figure 1.2): near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). Characterization and quantification of foods frequently uses the near and mid-IR regions for analysis due to organic molecules being absorbed in these regions (Wehling 2003). Molecular motions such as rotations, vibrations, or overtones cause the dipole moment in a molecule to change, thus absorbing infrared radiation in the region of the electromagnetic spectrum (McKelvy and others 1996). Additionally, the frequencies and intensities which correspond to these infrared bands can be used to characterize the material and the spectral information gathered may be used to identify the amount or presence of a particular compound in a mixture (McKelvy and others 1996).
Infrared spectroscopy is an analytical technique used to study the interaction of infrared light with matter; it measures the absorption of different infrared radiation frequencies of a material (Smith 1996). The vibrational spectrum of a molecule is considered to be a unique physical property and the frequency of the vibration is directly proportional to the bond strength of the functional group (Wehling 2003; Coates 2000). Stretching and bending deformations occur when the molecule absorbs infrared radiation and begins to vibrate. This energy created from the vibration of the molecule produces structural features of the molecule, whether they are the backbone of the molecule or the functional groups attached to the molecule, and a spectrum rich in information that can be used to identify an unknown molecule (Coates 2000; Smith 1996).
Mid infrared spectroscopy is extremely useful in chemical analysis due to the vast majority of molecules in the universe that absorb mid-infrared light (Smith, 1999). Many functional groups show absorption in the MIR region, producing much more defined absorption bands than in the NIR and FIR regions. In addition, the region from 1200-900 cm\(^{-1}\) is known as the “fingerprint region”, which produces distinct and reproducible biochemical fingerprints that reflect the total composition of the sample. The “fingerprint region” shows major bands that represent important lipids, proteins, nucleic acids, polysaccharides and phosphate-carrying compounds (Coates 2000).

1.2.4 Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy monitors the fundamental vibrational and rotational stretching and bending of molecules, which produces a chemical profile of the sample. MIR uses the light between 4,000-400 cm\(^{-1}\) and is a very robust and reproducible region of the electromagnetic spectrum, where very small differences in the composition of samples can be reliably measured (Subramanian and others 2008). The rich plethora of information provided from a MIR spectra helps in analyzing the composition and determining the structure of chemical molecules.

An interferometer is the heart of the Fourier-transform infrared spectrometer. The major components of an FTIR spectrometer are the IR source, beamsplitter, detector, many mirrors to direct light, and a time-reference laser. An optical diagram of an FTIR spectrometer is shown in Figure 1.3. The interferometer consists of the beam splitter and stationary and moving mirrors (Figure 1.4). The interferometer was first constructed by Albert Abraham Michelson (Michelson 1891). The beamsplitter, as the name suggests,
splits the IR light into two beams, where one part of the radiation beam goes to the moving mirror, which is capable of moving along the axis, away from and towards the beamsplitter, while the other part of the radiation beam goes to the fixed mirror (Wehling 1994; Ismail and others 1997; Jaggi and others 2006). The beams will recombine once they are reflected back from the mirrors at the beamsplitter. They will pass through the beamsplitter once again, and the recombined light from the interferometer is directed by the mirrors through the sample, and the signal produced (called an interferogram) is detected by the detector (Smith 1996, Jaggi and others 2006). A series of peaks in the spectrum will be produced when infrared energy is applied and the radiation is absorbed by the functional groups of the sample. A mathematical treatment, known as Fourier transform (FT), is applied and will transform a time domain into a frequency domain and convert the results into a typical IR spectrum (Wehling 2003).

Figure 1.4 Optical layout of a typical FTIR spectrometer.
Figure 1.5 Michelson interferometer.


Figure 1.6 FTIR spectral acquisition.

Reflectance or transmittance measurements can be taken in the MIR region, depending on the type of sample (Wehling 1994). Measuring reflectance is more popular for solid samples because it measures the light that bounces off of the sample (Smith 1996). Detection of the re-radiated light is done at a 45° angle to reduce specular reflectance (Wehling 1994). Conversely, transmittance measurements are used for liquid samples which are placed in a quartz cuvette where the light can pass through the entire sample and measure the absorbance at the wavelength of interest (Wehling 1994; Smith 1996). Furthermore, transmittance measurements are more advantageous because they use inexpensive tools for sampling as well as reducing the sample preparation time since the sample is not required to be homogenous because the IR radiation will pass through the entire sample (Wehling 1994; Smith 1996).

Attenuated total reflectance (ATR) is a widely used technique in MIR spectroscopy for both liquid and solid samples because it is one of the simplest and most convenient ways of handling samples for IR spectroscopy (Ismail and others 1997; Fabian and others 2002). Traditional IR sampling techniques using transmission are based on the samples absorption of IR radiation as the beam propagates through the sample; therefore measurements taken using this technique have severe limitations on thickness (Ismail and others 1999). A solution to this problem was the development of ATR. The total amount of energy reflected from the portion of the sample that is in direct contact with the crystal is measured by ATR (Ismail and others 1999). ATR is a reflection technique where the IR beam is directed through an internal reflection element (IRE) with a high index of refraction. The infrared light is completely reflected internally off the back surface which is in contact with the sample and the IR light penetrates into
the sample to a small degree and the IR data from the sample is obtained (Ismail and others 1999; Downey 1998). ATR is becoming more and more popular and is emerging as the technique of choice by the food industry because of its ease of use and its ability to analyze large amounts of data in a very short time.

Limitations of the FTIR are that infrared spectroscopy is not able to detect atoms or monatomic ions because they do not contain chemical bonds, such as noble gases for example. Also, homonuclear diatomic molecules, such as N$_2$ and O$_2$, do not absorb infrared light because of their symmetry (Smith 1996). In addition, complex mixtures are difficult to analyze due to the number of peaks present in the spectra. Therefore, it is advantageous to use FTIR in combination with other molecular spectroscopy techniques such as nuclear magnetic resonance (NMR), and mass spectroscopy (MS) (Smith 1996). However, the advantages of FTIR overcome its limitations. Advantages of FTIR over other methods are the high signal-to-noise ratio, simplicity, speed, sensitivity, accuracy, and the non-destructive ability of this technique (Smith 1996; Halim and others 2006).

This analytical technique offers several advantages that have attracted many researchers to adopt this practice. A summary of advantages and disadvantages obtained from various sources are listed below (Stuart 2004; Chalmers and others 2002; Mantsch and others 1996; Smith 1996; Johnston 1991). Advantages include:

1. Simplicity, sensitivity, and speed of detection.
2. High-throughput (ability to analyze several samples in a short time).
3. Non-destructive analysis depending on the application.
4. Requirement of relatively low sample volumes.
5. Less use of hazardous solvents that pose environmental and health hazards.

6. Relatively low operational cost.

Despite these advantages, the application of FTIR has some limitations that may hinder its application to certain types of analyses:

1. FTIR cannot detect atoms and monatomic ions, elements, and inert gases such as helium and argon.

2. FTIR cannot detect diatomic molecules such as N\textsubscript{2} and O\textsubscript{2}. However, in certain cases this can be seen as an advantage since it eliminates the need for vacuum analysis.

3. Biological samples including food are complex mixtures and hence their FTIR spectra are complicated with overlapping peaks and signal masking.

4. Most biological samples contain water, which has a strong absorption band that can mask important signals. Sample preparation procedures are required to reduce the effect of water.

5. Since most FTIRs are single beam instruments change in environments (carbon dioxide and water vapor) can occur during the experiment, causing uncertainties in the spectra.

1.2.5 Handheld Infrared Spectrometer

Recently, a cutting edge sensor technology directed at improving efficiency, throughput and reliability of critical processes was introduced to the food industry. The TruDefender\textsuperscript{TM} FT (Ahura Scientific) is a handheld infrared spectrometer which
incorporates the analytical precision of FTIR spectroscopy to field applications. The spectral resolution of 4 cm$^{-1}$ is equivalent to that of the benchtop instruments. Weighing less than three pounds, this handheld IR spectrometer is a portable, rugged and easy-to-use system designed for in the field analysis. This new technology can provide a valuable window into in-process food manufacturing which will permit the optimization of quality and safety as well as the production rate of many food products. This handheld infrared sensor, equipped with an ATR diamond crystal, will enable the food manufacturer to rapidly assess the quality of their food, which will allow for timely correction measure during manufacture.


Figure 1.7 Handheld infrared spectrometer.

Figure 1.8 FTIR diamond ATR components.
1.3 REFERENCES


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CHAPTER 2: APPLICATION OF A HANDHELD INFRARED SENSOR FOR MONITORING THE DISTRIBUTION OF VITAMINS AND MINERALS IN FORTIFIED WHOLE GRAIN CORNMEAL

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2.1 ABSTRACT

Fortification of snack foods is growing due to increasing consumer demand for healthier products. According to the Snack Food Association, total sales of snack foods in the U.S. grossed 26 billion dollars a year and corn based snacks accounted for 21.8% of total sales. Many snack foods produced in the U.S. are fortified with vitamins and minerals to improve their nutritional quality.

The objective was to develop a real-time methodology for monitoring the uniformity of vitamins and minerals during fortification using a handheld infrared sensor.

A fortification blend of zinc, iron, vitamin E, and calcium was added to whole grain cornmeal at different levels (0.5% to 5.5%) and mixed for 30 minutes to obtain a homogeneous matrix. For real-time infrared analysis, aliquots (0.1g) were placed onto an ATR diamond crystal of a handheld spectrometer with pressure being applied to obtain good contact between the sample and the crystal. Spectra were collected in the 4000-700 cm\(^{-1}\) range. Distribution uniformity of the supplements was verified by high pressure liquid chromatography (HPLC). Partial least squares regression (PLSR) analysis was used to correlate the concentration of supplemented vitamins and minerals in the whole grain cornmeal with the infrared spectra. The model predicted the level of fortification in cornmeal using the infrared region between 900 to 1225 cm\(^{-1}\) (r=0.98, 5 factors). The standard error of cross-validation was <0.35% which gives potential to be used as a new tool in the industry. Soft Independent Modeling of Class Analogy (SIMCA) analysis was used to examine the data collected for monitoring distribution uniformity. The model was able to cluster the samples into classes corresponding to 3 different fortification levels.
By using a simple, handheld FT-IR instrument, a methodology for real-time monitoring of whole grain cornmeal fortification was developed for the snack food industry to monitor corn-based extruded snack products. This could provide the snack food industry with a simple, real-time method to ensure the homogenous fortification of snack foods.

2.2 INTRODUCTION

Fortification is the supplementation of nutrients such as vitamin E, calcium, iron, and zinc, at levels beyond those that are naturally occurring in food (Berner and others 2001). Many populations suffer from vitamin and mineral deficiencies, and the fortification of common processed foods can protect large populations using sustainable market channels (Governments 2004). Sales of the total fortified foods market in the US reached almost $18 billion in 2001, tripling the sales achieved by the market in 1997. The requirements for labeling and safe control of dosage during the production of fortified products in complex matrices require reliable, precise and accurate concentration analysis. The industry currently relies on the use of high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) analysis to measure levels of vitamins and minerals in fortified foods (Haughey and others 2005; Chen and others 2002). These methods are often time-consuming, expensive, the precision is strongly dependent on the operation of skilled personnel, and they are not easily adapted into a quality assurance setting (Caselunghe and others 2000). In addition, unwanted interference from the sample matrix often requires the use of extensive sample preparation and clean-up (Halim and others 2006). Cutting edge sensor technologies are directed at improving efficiency, throughput and reliability of critical processes (Koca
and others 2007). On-line or real-time spectroscopic methods can provide a valuable window into in-process food manufacturing to permit optimization of production rate, quality and safety of many food products (McKelvy and others 1996). Field-based devices for rapid determination of fortification levels in foods will streamline quality assurance, protecting consumers against the risk of purchasing and consuming nutritionally inadequate, deceptively mislabeled or misbranded, impure, or unsafe foods.

Advances in Fourier transform infrared (FT-IR) spectroscopic instrumentation combined with multivariate data analysis have made this technology ideal for large volume, rapid screening and identification of various analytes (Halim and others 2006). Infrared spectroscopy provides valuable information of the biochemical composition of the samples, especially in the fingerprint region, which can be used to monitor the fortification of food samples (Koca and others 2007; Halim and others 2006).

The purpose of the study was to develop a real-time methodology for monitoring the uniformity of vitamins and minerals during fortification in corn-based extruded snack products using a handheld infrared sensor. This could provide the food industry with a simple, real-time method to ensure the homogenous fortification of snack foods.

2.3 MATERIALS AND METHODS

2.3.1 Mixing Uniformity in a Manufacturing Facility

Distribution uniformity of natural d-alpha-Tocopherol acetate (vitamin E) was determined by an HP1050 reverse-phase HPLC system equipped with a photodiode array detector (Agilent Tech, Palo Alto, CA). Samples were collected from a typical run in the industry to monitor the distribution of vitamin E in different sections (right, middle and left of the tumbler) and at different times (1 to 5 min) during mixing. Duplicate samples
were analyzed for each section/time combination. Six grams of fortification mix was extracted with a mixture of 1:1:2 acetone/ethanol/hexane solution (20 mL) and continuously shaken for 15 min (Rotamix model RK DYNAL, Dynal Biotech Inc., Lake Success, NY) at 28 rpm. De-ionized distilled water (3mL) was later added and shaken continuously for 5 min at 28 rpm to induce phase separation. The sample was centrifuged for 15 minutes and an aliquot (6 mL) of the hexane-top layer was completely dried under nitrogen flow. Dried vitamin E-containing samples were re-dissolved in 400 µL of methanol, filtered using a nylon membrane filter (Fisherbrand®, 13 mm diameter, 0.45 µm pore size, Fisher Scientific Co., Fairlawn, NJ) and analyzed by high performance liquid chromatography and infrared spectroscopy.

2.3.2 High Performance Liquid Chromatography

Samples (50 µL) were injected into the HPLC system for vitamin E analysis. A Prevail™ Organic Acid column (5µ particle size, 150 x 4.6mm) (Alltech, Deerfield, IL) and a Prevail™ All-Guard™ Organic Acid guard column (5µ particle size, 7.5 x 4.6 mm) (Alltech, Deerfield, IL) were used in-line for all separations. The elution solvent used in this experiment was 100% methanol. The column was then washed with 100% methanol for five minutes before the next analysis. The flow-rate was 1.0 ml/min with detection at 290 nm for natural d-alpha-Tocopherol acetate. The identification of natural vitamin E was carried out by comparing the retention times and absorption spectra with the reference standard, with typical retention time for natural vitamin E at ~7 minutes. Duplicate analyses were performed and the mean value was determined.
2.3.3 Fortification Samples

A total of six lots of whole grain cornmeal and two lots of fortification premix containing zinc, iron, vitamin E, and calcium were obtained from Wyandot, Inc., Marion, Ohio. The fortification premix was added to whole grain cornmeal at different levels (0.5-5.5%) and mixed for 15 minutes (Rotamix, Dynal Biotech Inc., Lake Success, NY) at 35 rpm to obtain a homogeneous matrix. An aliquot (~0.1g) of the homogenized mix was used for testing on both the benchtop and handheld ATR-IR systems. Fortified samples were analyzed in triplicate with repetitions from different batches made on different dates.

2.3.4 Benchtop Fourier Transform Infrared Spectroscopy

A Varian Excalibur 3100 spectrometer with a potassium bromide beam splitter and Deuterated Triglycine Sulfate (DTGS) detector was used for all infrared readings, operating at 4 cm\(^{-1}\) resolution. Samples were pressed onto a three-reflection diamond crystal plate accessory using a high pressure clamp (PIKE Tech, Madison, WI). Spectra were collected over the frequency region from 4000-700 cm\(^{-1}\) at 4cm\(^{-1}\) interval and interferograms of 64 scans were co-added according to Beer-Norton apodization. Spectra were displayed in terms of absorbance and viewed using Win-IR Pro Software (Varian, Palo Alto, California). Each sample was analyzed in triplicate. The instrument was continuously purged with CO\(_2\) – free dry air from CO\(_2\)RP140 dryer (Dominick Hunter, Charlotte, NC, USA).
2.3.5 Handheld Fourier Transform Infrared Spectroscopy

A TruDefender FT handheld spectrometer with an ATR diamond crystal and a pyroelectric DTGS detector was used for all readings, with a spring-loaded clamp to apply pressure and obtain good contact between the sample and the crystal (Ahura Scientific Inc., Wilmington, MA). Spectra were collected over the frequency from 4000-700 cm\(^{-1}\) and interferograms of 8 scans were co-added. Infrared spectra were collected for samples and analyzed using multivariate data analysis.

2.3.6 Multivariate Analysis

The spectra were exported as GRAMS.spc files format and imported into Pirouette®, for Windows Comprehensive Chemometrics Modeling Software, version 3.11 (Infometrix, Inc. Bothell, WA). The spectra were derivatized (5-point polynomial-fit Savitzky-Golay function) and normalized. Partial least squares regression (PLSR) models were generated to estimate the fortification level from the spectra using cross-validation (leave-one-out approach). PLSR is a bi-linear regression model which can reduce a large number of variables into a smaller number of latent variables that are linear combinations of the spectral variables; the smaller number of latent variables is used to determine the analyte’s concentration (Wold, Sjostrom and Eriksson 2001). The latent variables explain a large amount of the co-variance of X and Y (Martens and others 2001). Partial least squares regression is a versatile analytical approach because it is able to analyze large, complex and noisy data sets (Wold, Sjostrom and Eriksson 2001; Wold and others 2001). This technique is widely used in spectroscopy because it uses the concentration information (Y) to determine how regression factors are computed from the data set (X); this condenses the impact of unrelated variations in the calibration model.
(Martens and others 2001). The standard error of calibration (SEC), standard error of cross-validation (SECV), and the coefficient of correlation (r-values) are used to evaluate the validity of PLSR models.

Spectral data were also analyzed using soft independent modeling of class analogy (SIMCA) to generate clustering groups. SIMCA is a multivariate technique based on principal component analysis (PCA) which was used to evaluate the spectral data from the handheld FTIR to discriminate between whole grain cornmeal and whole grain cornmeal with different levels of fortification. Training sets are assigned to classes and a principle component model is generated for each class with distinct regions within them (De Maesschalck and others 1999). The performance of this method depends strongly on the training set for each class as well as the difference between classes (Candolfi and others 1999). Probability clouds (95%) are built around the clusters based on PCA scores, allowing SIMCA to be used as a predictive modeling system. SIMCA’s discriminating power algorithm allows identification of the wavenumbers (cm⁻¹) of bands of interest and removal of spectral bands which do not aid in the separation and differentiation of the classes (Dunn and others 1995). A sample is considered an outlier if it falls outside of the class border. Class-modeling techniques can be regarded as outlier detection methods for this reason (Candolfi and others 1999). Interclass distances, values which represent Mahalanobis distances between the classes, were used for determination of class (fortification level) separations based on factor loadings. SIMCA analysis evaluates itself by predicting each sample included in the training set and comparing that prediction to its assigned class. This assessment is referred to as
misclassifications, and zero misclassifications characterize a model in which all samples were correctly predicted to the pre-assigned class.

2.3.7 Validation Set

Six independent sets of fortified whole grain cornmeal samples with randomly selected premix fortification concentrations (0.5-5.5%) were analyzed to estimate fortification levels. Infrared spectra were collected for samples using the laboratory benchtop and handheld spectrometers as described previously, and were used to test the predictive ability of the PLSR calibration models. Predictions were performed using the Pirouette® software (Infometrix, Inc. Woodville, WA).

2.4 RESULTS AND DISCUSSION

2.4.1 Mixing Uniformity in a Manufacturing Facility

The distribution of vitamin E (Table 2.1) during the industrial production of fortified whole grain cornmeal products was quantified by using high performance liquid chromatography (HPLC) analysis (Heudi 2004) to evaluate the effect of tumbler location (left, right and middle) and mixing times (1-5 min) throughout the fortification process.
Table 2.1 Amount of vitamin E in fortified whole grain cornmeal samples taken from different locations and at different times during the mixing process.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area</th>
<th>Vitamin E (IU/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1R</td>
<td>686.10</td>
<td>11.91</td>
</tr>
<tr>
<td>T1M</td>
<td>1498.74</td>
<td>28.68</td>
</tr>
<tr>
<td>T1L</td>
<td>821.85</td>
<td>14.71</td>
</tr>
<tr>
<td>T2R</td>
<td>1458.89</td>
<td>27.86</td>
</tr>
<tr>
<td>T2M</td>
<td>1745.76</td>
<td>33.78</td>
</tr>
<tr>
<td>T2L</td>
<td>1053.23</td>
<td>19.49</td>
</tr>
<tr>
<td>T3R</td>
<td>1160.87</td>
<td>21.71</td>
</tr>
<tr>
<td>T3M</td>
<td>1053.28</td>
<td>19.49</td>
</tr>
<tr>
<td>T3L</td>
<td>884.75</td>
<td>16.01</td>
</tr>
<tr>
<td>T4R</td>
<td>1254.36</td>
<td>23.64</td>
</tr>
<tr>
<td>T4M</td>
<td>1204.15</td>
<td>22.60</td>
</tr>
<tr>
<td>T4L</td>
<td>1049.80</td>
<td>19.42</td>
</tr>
<tr>
<td>T5R</td>
<td>1265.11</td>
<td>23.86</td>
</tr>
<tr>
<td>T5M</td>
<td>1188.62</td>
<td>22.28</td>
</tr>
<tr>
<td>T5L</td>
<td>1138.33</td>
<td>21.24</td>
</tr>
</tbody>
</table>

Based on HPLC analysis, the levels of vitamin E in fortified whole grain cornmeal (elution time of ~ 7.7 min) ranged from 11.91 – 33.78 IU/100g. Time and location had a strong effect (p-value < 0.001) on the distribution of vitamin E (Table 2.1). Higher variability in the level of vitamin E was observed in the different sections of the tumbler for the first 2 minutes of mixing, while after five minutes the dispersal of vitamin E (x = 22.5 IU/100g) was consistent among the sections (Figure 2.1). Interestingly, our data suggests that the left side of the tumbler consistently showed lower levels of vitamin E, therefore it should be the target for monitoring dispersal uniformity of the fortification mix during production.
Comparison of the vitamin E levels in the finished fortified whole grain cornmeal product (~5 min mixing) was consistent with the data provided by an analytical testing company reporting levels of natural vitamin E of 24.1 IU/100g by HPLC analysis.

![HPLC chromatogram](image)

**Figure 2.1** HPLC chromatogram of fortified whole grain cornmeal after mixing for two minutes (A) and five minutes (B) ($\lambda_{\text{max}} @ 290\text{nm}$) identifying the elution of the added natural vitamin E acetate.

Multivariate regression models developed from infrared spectra obtained from fortified cornmeal samples, by using a portable handheld or an analytical benchtop spectrometer, were compared against the vitamin E levels determined by HPLC analysis and showed poor correlation statistics ($r = 0.13$). The relatively complex matrix of fortified whole grain cornmeal and low levels of vitamin E may have masked the unique spectral differences in the samples limiting the ability of PLSR to extract relevant information explaining the covariance between the spectra and analyte variables (Martens and others 2001). The development of a rapid and simple protocol for monitoring fortification of snacks is desirable but vitamin E may not be a good infrared marker for a
direct measurement method unless the effect of the matrix is minimized through a sample preparation procedure, such as lipid phase fractionation.

2.4.2 Evaluation of Lab-Scale Whole Grain Cornmeal Fortification

A fortification blend of zinc, iron, vitamin E, and calcium was added to whole grain cornmeal at different levels (0.5% to 5.5%) and mixed for 30 minutes to obtain a homogeneous matrix. In order to obtain a spectra (with both benchtop and handheld methods), the sample, in the form of a powder (~0.1g), was moistened with a drop of water (10μL) to improve contact with the ATR diamond crystal, thus enhancing spectral features. Although water has a strong absorbance in the mid-infrared region, it did not interfere with the analyte spectral signal. Unique spectral characteristics between whole grain cornmeal and the fortification premix can be seen in Figure 2.2. The premix spectra was dominated by bands centered at 1032 cm⁻¹ derived from P-O stretching vibration modes (Shirkhanzadeh 1995) of calcium phosphate ingredients (Figure 2.3), a major component of the premix formulation (~92%).
Figure 2.2 Comparison of ATR-IR spectra of whole grain cornmeal and fortification premix.

Figure 2.3 Spectral comparisons of the fortification premix and the tricalcium phosphate standard.
Comparison of the ATR-IR spectrum of fortified whole grain cornmeal samples by using the benchtop and handheld spectrometers (Figure 2.4) showed similarities with respect to spectral profile and response for both instruments generating high-quality and reproducible spectra. Second derivative (5 pt window) transformation of the spectral measurements improved the quantitative analysis by resolving overlapped bands and limiting variations in spectral baselines (Hruschka 2001). The cross-validated leave one out regression (PLSR) models based on infrared spectral information ranging from 1800 to 900 cm\(^{-1}\) showed strong correlation (\(r >0.98\)) between the IR predicted and actual levels of fortification for both infrared methods (Figure 2.5). Most of the variance was explained by the first four latent variables for the handheld methods and the first two latent variables for the benchtop instrument. Furthermore, the PLSR model based on mid-infrared spectra collected by the handheld technique (\(r_{\text{Val}}=0.98\) and \(\text{SECV}=0.34\%\)) gave similar performance statistics as models generated from spectra collected by the benchtop system (\(r_{\text{Val}}=0.99\) and \(\text{SECV}=0.21\%) (Table 2.2).
Figure 2.4 Benchtop and handheld ATR-IR spectra of fortified whole grain cornmeal.

Table 2.2 Comparison of techniques used for quantifying whole grain cornmeal fortification.

<table>
<thead>
<tr>
<th>Technique</th>
<th># of samples</th>
<th># of factors</th>
<th>SECV(^a)</th>
<th>rVal(^b)</th>
<th>SEC(^c)</th>
<th>rCal(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benchtop ATR-IR</td>
<td>36</td>
<td>2</td>
<td>0.2074</td>
<td>0.9922</td>
<td>0.1885</td>
<td>0.9942</td>
</tr>
<tr>
<td>Handheld ATR-IR</td>
<td>36</td>
<td>4</td>
<td>0.3402</td>
<td>0.9806</td>
<td>0.2755</td>
<td>0.9893</td>
</tr>
</tbody>
</table>

\(^a\)standard error of cross validation. \(^b\)coefficient of correlation for validation model.
\(^c\)standard error of calibration. \(^d\)coefficient of correlation for calibration model.
Figure 2.5 Cross-validated (leave one out) PLSR models for handheld IR spectra (A) and benchtop IR spectra (B) for whole grain cornmeal fortification from 0.5-5.5%.

Examination of the PLSR loading spectra (Figure 2.6) indicated that the 850-1300 cm\(^{-1}\) infrared region was associated with explaining most of the sample variation, showing similar absorption features for explaining the level of fortification in whole grain cornmeal samples by using either the handheld or benchtop infrared techniques. The spectral features exhibited by LV1 (Figure 2.6), explaining 65% and 83% of the total variance for the handheld and benchtop instruments, respectively, showed a major band at \(\sim 1028\) cm\(^{-1}\) typical of \((PO_4)^{3-}\) stretching vibrational mode observed in tricalcium phosphate (Figure 2.7). LV2 and LV3 (Figure 2.6) explained 24% (handheld) and 10% (benchtop) of the remaining variance and were associated with bands under 1000 cm\(^{-1}\), characteristic to deformation vibrations of the phosphate ion (Figure 2.7). Our results showed that PLSR models based on spectra of fortified whole grain cornmeal were able
to correlate the level of fortification due to the unique infrared absorption features of the inorganic phosphate components.

**Figure 2.6** Partial least squared loadings plots for cross-validated handheld (A) and benchtop (B) models for fortified whole grain cornmeal.

**Figure 2.7** Typical attenuated total reflectance infrared (ATR-IR) spectra (A), and its respective second derivative (B), for ingredients in the fortification premix.
The PLSR models for the handheld and benchtop techniques were used to predict the concentration of fortification in whole grain cornmeal samples by using an independent validation set of samples (Table 2.3). The handheld FTIR predictions gave superior standard error of prediction (SEP= 0.28%) as compared to the benchtop FTIR system (SEP= 0.44%). Given the greater simplicity, speed, versatility, ruggedness, and portability of the handheld system over the laboratory bench-top instrument, it can provide the food industry with real-time sensor tools for the reliable assessment of quality, enabling the food manufacturer to rapidly determine the quality of their food and for timely correction measures during manufacture.

Table 2.3 Handheld FTIR prediction levels in fortified whole grain cornmeal using five independent samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target Level Added</th>
<th>Predicted Level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Predicted Level&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEP&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Latent Variables&lt;sup&gt;d&lt;/sup&gt;</th>
<th>r&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Slope&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.19</td>
<td>5.11</td>
<td>6.10</td>
<td>0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>3.27</td>
<td>3.66</td>
<td>3.16</td>
<td>0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>2.45</td>
<td>2.35</td>
<td>2.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.54</td>
<td>1.34</td>
<td>1.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.76</td>
<td>0.69</td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>handheld. <sup>b</sup>benchtop. <sup>c</sup>standard error of prediction. <sup>d</sup>coefficient of correlation for prediction model.

Class projections were used to illustrate the ability of SIMCA (Soft Independent Modeling of Class Analogy) to group IR data based on spectral similarities/differences by using the first three principle components (Figure 2.8). This model offered good class separation and tight clustering among levels of fortified and unfortified cornmeal with zero misclassifications. Table 2.4 showed that class 0.5 – 1.5% and 2.5 – 3.5% were
significantly (p-value < 0.05) different from the 4.5 – 5.5% class. Generally, class distances above three are considered good for discrimination with larger interclass distances indicating well separated classes (Dunn and others 1995; Dupuy and others 1996).

Figure 2.8 SIMCA classification of whole grain cornmeal and fortified whole grain cornmeal (A) and discriminating bands (B).

Table 2.4 Interclass distances for the separation of whole grain cornmeal based on the level of fortification.

<table>
<thead>
<tr>
<th></th>
<th>Fortified 0.5-1.5%</th>
<th>Fortified 2.5-3.5%</th>
<th>Fortified 4.5-5.5%</th>
<th>Whole Grain Corn Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortified 0.5-1.5%</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fortified 2.5-3.5%</td>
<td>0.9575</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fortified 4.5-5.5%</td>
<td>1.9507</td>
<td>0.8914</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Whole Grain Corn Meal</td>
<td>1.3972</td>
<td>3.4144</td>
<td>6.0878</td>
<td>0</td>
</tr>
</tbody>
</table>
2.5 CONCLUSIONS

By monitoring the levels of vitamin E in fortified whole grain cornmeal it was shown that tumbler location and mixing time had a strong effect on the distribution of the premix components throughout the matrix. Our data suggests that the sampling on the left side of the tumbler could provide a good marker for determining the even dispersal of the ingredients.

Both the handheld and benchtop ATR-IR techniques allowed the development of PLSR and SIMCA models for the quantitative and qualitative analysis of vitamin and mineral fortification in whole grain cornmeal, with excellent performance statistics. PLSR models generated from transformed (second derivative) infrared spectral data gave correlation coefficients (r-value) > 0.98 and standard error of cross-validation (SECV) of < 0.34%. An independent validation set showed better performance statistics using the handheld infrared spectrometer with a standard error of predication (SEP) 0.28%. A portable, battery operated handheld ATR-IR spectrometer allowed for the reliable determination of fortification levels in whole grain cornmeal. This technique provides for the fast analysis of food components with minimal personnel training, simple data acquisition, and immediate predictions. The handheld device provided greater versatility, ruggedness, and portability as a real-time infrared sensor for monitoring quality assurance, providing the industry with a tool for timely correction measures during manufacture.
2.6 REFERENCES


LIST OF REFERENCES


Michelson AA. 1892. On the application of interference-methods to spectroscopic measurements, -II. Philoso. Mag. 34:280-299.


