VITAMIN AND MINERAL RETENTION AND SENSORY EVALUATION OF EXTRUDED FORTIFIED RICE

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ABSTRACT

More than 2 billion people suffer from micronutrient malnutrition worldwide. Vitamin A, iron, iodine, and zinc deficiencies are among the leading public health concerns for developing countries, particularly in Sub-Saharan Africa and many parts of Asia. Strict vegetarian diets, lack of diversity in the diet, high rates of infection, and unfavorable socio-economic conditions are risk factors for these deficiency conditions. In India alone, approximately 75% of children under the age of five suffer from iron deficiency, and 57% of children under the age of six suffer from sub-clinical vitamin A deficiency. Micronutrient deficiencies bring adverse consequences to their victims and the economies of the countries in which they live.

Fortification has proven to be an effective long-term nutrition intervention strategy, and rice has emerged as a staple food with much potential as a food fortification vehicle. Rice provides 50% of calories for more than half of the world population. However, a lack of micronutrients in white rice has become a problem for countries with high consumption rates of rice. Low intakes of iron, vitamin A, and iodine are a major concern, while low zinc intake is also a problem. An experimental product, extruded rice kernels manufactured from rice flour fortified with vitamins and minerals, has been proposed as an effective vehicle for delivering a variety of micronutrients to low income populations.

The aims of this study were 1) to select and adapt analytical methods and conduct analyses for vitamin A and vitamin C in the experimental product, as well as measure mineral retention, and 2) to conduct sensory studies to evaluate the acceptability of the experimental product by consumers and compare acceptability by South Asian consumers to that of non-South Asian consumers.

Extruded rice samples fortified with vitamins A and C and iron and zinc were evaluated for their micronutrient retention following extrusion and cooking. The average vitamin A retention in the experimental product after extrusion was 48% for dried kernels and 37% for cooked rice. The average vitamin C retention in the experimental product after extrusion was 52% for dried kernels and 48% for cooked rice. Iron and zinc retentions both averaged 84% for rice kernels after extrusion (cooked and non-cooked data combined). The vitamin retentions are consistent with studies on vitamin retention in extruded products. However, mineral retention is lower than expected. Larger sample sizes, consistency in analysis methods, and more research are needed to draw further conclusions.

The experimental product was also evaluated for its consumer appeal using consumer acceptance tests. For the acceptability evaluation, two extruded samples of rice (fortified and unfortified extruded rice), and two commercial samples of enriched rice (long-grain white rice) were cooked in a rice cooker and presented to taste panelists. Acceptability of the product was based on a 9-point hedonics test, a just-about-right (JAR) test, and a ranking test. Results suggested greater acceptability by the consumer panel for commercial rice samples. The most sizable difference was seen in the appearance attributes, followed by overall acceptance, and aroma. The addition of vitamins and minerals to extruded rice did not appear to have a large effect on sensory acceptability, with the exception of several appearance attributes. Group differences and group interactions were observed. The South Asian panelist group gave lower scores in several attributes, and the group was generally more critical of (gave lower ratings to) the unfortified extruded rice sample and the parboiled commercial sample than the non-South Asian group. Rice with extruded fortified kernels has potential to be an effective vehicle for fortification in developing countries; however, the challenges in micronutrient retention and acceptability need to be met for optimized utilization.

BIOGRAPHICAL SKETCH

Jessica Hof was born March 15, 1982, in Easton, Pennsylvania to parents, Ellen and Philip Hof. She lived in Easton, Pennsylvania for 18 years. Jessica attended Easton Area High School, where she was a varsity springboard diver, an active member of the marching band, and editor-in-chief of the school newspaper. Her interests were in the sciences, and, as a high school senior, her interest was to pursue a career in veterinary medicine. Jessica was accepted to and matriculated at Cornell University College of Agriculture and Life Science, initially majoring in Animal Science as a pre-veterinary student. It was Jessica's enrollment at Cornell that introduced her to the study of Food Science.

After a few introductory classes, Jessica transferred into the Department of Food Science to pursue her future as a Food Scientist. As a college junior, she worked in the Food Safety Lab, assisting a graduate student in DNA sequence-based subtyping and evolutionary analysis of selected Salmonella enterica serotypes. The following summer, she interned with General Mills, Inc., in Minneapolis, Minnesota. Her duties involved designing and conducting experiments to optimize potato casseroles within Betty Crocker Meals.

In May 2004, Jessica graduated with a B.S. Food Science degree. After a summer internship with Campbell Soup Company doing research and development, she continued her education as a master's student at Cornell University in Fall of 2004. In her graduate program, Jessica became active in the Cornell Food Science Club and the Institute of Food Technologists Student Association (IFTSA). She held the position of president of the Food Science Club for two terms, and she was chairperson for the IFTSA "Fun Run" fundraiser in 2006.

Jessica will pursue her career in Food Science, conducting research and development for Unilever Foods North America.

This work is dedicated to my parents, Ellen and Philip Hof,

who never stop believing in me.

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LIST OF ABBREVIATIONS

A_w – Water Activity

d – day

EDTA - Ethylenediaminetetraacetic Acid

FAO – The Food and Agriculture Organization

Fe – iron

g – gram(s)

HIPEF – High Intensity Pulsed Electric Fields

HIV – Human Immunodeficiency Virus

hrs - hours

HTST – High Temperature Short Time

IRRI – International Rice Research Institute

IU – International Units

JAR – Just About Right

min – minute(s)

mm – millimeter(s)

Na – sodium

psi – pounds per square inch

RAE – Retinol Activity Equivalent

RDA – Recommended Dietary Allowance

UNCTAD – United Nations Conference on Trade and Development

USDA – United States Department of Agriculture

UR – UltraRice

UV – Ultra Violet

vol - volume

Zn – zinc

CHAPTER ONE

INTRODUCTION

I. Micronutrient Deficiencies and Malnutrition

The statistics of malnutrition are alarming. According to the World Health Organization's Fifth Report on the World Nutrition Situation, vitamin A deficiency jeopardizes the health status of 140 million preschoolers and more than 7 million pregnant women every year (United Nations Standing Committee on Nutrition 2004). Iron deficiency anemia among pregnant women is associated with an estimated 111,000 maternal deaths each year, and nearly two billion people (35.2%) worldwide have inadequate iodine nutrition (United Nations Standing Committee on Nutrition 2004). The highest prevalences of anemia and vitamin A deficiency are seen in South Asian countries, such as India (Mason and others 1999). These deficiencies are the result of strict vegetarian diets, lack of diversity in the diet, high rates of infection, and unfavorable socio-economic conditions, especially for women.

The World Summit for Children was held in New York in 1990, and the International Conference on Nutrition was held in 1992 in Rome, where most of the members of the United Nations signed a resolution to eliminate vitamin A deficiency and reduce rates of iron-deficiency anemia by one-third by the year 2000. Yet more than two billion people worldwide still suffer from iron, iodine, and vitamin A malnutrition (Micronutrient Initiative 2005). Zinc deficiency has also been shown to be a significant problem in many developing countries (De Romaña and others 2002).

India continues to face a severe public health crisis today due to these micronutrient deficiencies. Although extensive work has been done to improve India's nutrition status, the statistics show there has not been enough progress. In India, an estimated 75% of children under the age of five suffer from iron deficiency anemia, and an estimated 57% of children under the age of six suffer from sub-clinical vitamin A deficiency (Micronutrient Initiative 2005).

India is not alone in its battle against micronutrient deficiency, also called hidden hunger. Although China is one country that has made vast improvements in its nutrition situation, other areas of Asia have not been as successful. The majority of the world's preschool malnutrition is in Asia, although the locus is shifting to Africa (United Nations Standing Committee on Nutrition 2004). Other micronutrient deficiencies are prevalent as well. Thiamin, riboflavin, calcium, vitamin C, selenium, and magnesium deficiencies also exist in developing countries, but they have been less well-documented. All of these micronutrient deficiencies signify a number of adverse consequences for their victims and the economies of the countries in which they live.

Consequences

Micronutrient malnutrition is one of the leading causes of serious health and economic problems (Mehansho and others 2003). As the largest contributor to disease in the world, malnutrition increases the risk of HIV (human immunodeficiency virus) infection and reduces the survival rate of mothers and children with malaria when compared to conditions where malnutrition does not exist (The World Bank 2006). According to a new World Bank report, malnutrition is costing poor countries up to three percent of their yearly gross domestic product, and malnourished children are at risk of losing up to ten percent of their lifetime earnings potential (The World Bank 2006).

Vitamin A deficiency is a particular problem in children because it can cause partial or total blindness. The greatest burden of deficiency is among children living in South Asia and Sub-Saharan Africa. In fact, one-third of the three million or more children worldwide suffering from vitamin A deficiency live in India. Mild vitamin A deficiency is more common and has been found to reduce resistance to infectious disease and increase morbidity and mortality (Dexter 1998).

Mineral deficiencies can cause a number of adverse effects as well. Severe nutritional anemia from iron deficiency can increase maternal mortality, reduce resistance to infections, alter mental function, and reduce productivity and scholastic performance (Dexter 1998). Adequate iron intakes are required for normal growth, fetal development, and normal physical and mental activities in adults (Dexter 1998). Zinc status can affect height-for-age ratios, resulting in growth stunting when zinc levels are too low (Mehansho and others 2003). Nearly 50% of the global population suffers from poor zinc status because of limited access to animal products and heavy consumption of cereals and legumes high in phytate, a compound known to inhibit zinc and iron absorption (Mehansho and others 2003).

This global prevalence of hidden hunger can be reduced and prevented by improving micronutrient status. In general, three nutritional intervention strategies are currently in use to combat micronutrient malnutrition: (1) increasing the dietary intake of foods rich in micronutrients by dietary diversification, (2) periodic supplementation with target micronutrients, and (3) fortification with one or more micronutrients of commonly consumed dietary items (Lee and others 2000). While dietary modification and supplementation have offered some improvement in developing countries, economic constraints and low rates of compliance are major concerns associated with these strategies. Fortification appears to be the best long-term strategy for controlling most micronutrient deficiencies (De Romaña and others 2002).

History of Fortification

For more than 80 years, food fortification has been successfully implemented throughout the developed world. Consumption of foods fortified with iodine, vitamin D, vitamin B2, and niacin have contributed to the reduction of goiter, rickets, riboflavin deficiency, and pellagra respectively in the United States (Bishai and others 2002). Food fortification has proven to be sustainable in developed countries because of the presence of technical, operational, and financial feasibility, as well as cooperation among governments, the food industry, and consumers. These conditions, however, are not as widespread in the developing world; therefore fortification programs are infrequent and often end after unsuccessful trials (Dary and Mora 2002).

Relative Merits of Fortification

Several fundamental principles underlie successful food fortification schemes and the selection of appropriate foods to fortify with essential micronutrients. As mentioned by Dary and Mora (2002), some of the important criteria for potential food fortification vehicles for public health programs include using a food matrix that (1) is regularly consumed by the target population in predictable amounts, (2) is produced in a centralized fashion, (3) is without sensorial changes compared to the non-fortified equivalent, and (4) is affordable to the target populations. Staple foods such as wheat flour, sugar, oil, and salt have been popular foods used in fortification programs in several developing countries. (Dary and Mora 2002)

In India, rice is an important staple food and has much potential as a food fortification vehicle. However, rice fortification comes with many challenges, and the current fortification programs in India still face much room for improvement. In order to consider rice as a fortification vehicle, it is important to understand rice characteristics and the features of rice fortification.

II. Rice

Rice (genus *Oryza*) is a tall grass crop that is grown more easily in the tropics. It is thought that rice was originally cultivated without water submersion, but mutations have since led to varieties that are semi-aquatic. Although it is tolerant to hot, humid, flooded, dry, and cool conditions and grows in saline, alkaline, and acidic soils, it grows fastest and most vigorously in wet and warm conditions. Water submersion helps control weeds, while also creating an environment for birds and amphibians that help control insect pests.

The rice plant develops a main stem and many tillers, which arch into many clusters of flowers bearing the grains. Of 20 *Oryza* species, *Oryza sativa* is the one most commonly cultivated in the humid tropics of Asia, where it originated. Asian cultivated rice has evolved into three eco-geographic subgroups: *indica* (long-grain), *japonica* (round-grain), and *javanica* (medium-grain). *Indica* varieties account for 80% of cultivated rice and feed about 3 billion people, mainly in developing countries (UNCTAD 2005).

Rice status

Rice is one of the world's most consumed cereals (along with wheat and maize). The world average annual per-capita consumption of milled rice was 57.8 kg per person (1997-1999 average). This average is second to wheat, which averaged 70.8 kg per person from 1997 to 1999 for the world average. Maize averaged 19.0 kg per person as the third most consumed cereal per-capita worldwide from 1997 to 1999. (Childs 2004)

Rice provides at least 50% of the daily calories for more than half of the world population. In Asia alone, more than 2 billion people obtain 60 to 70 percent of their calories from rice and its products (FAO 2004). Sixty-five percent of the total population in India depends on rice as a staple food, and India is the world's second most populous nation: 1 billion and growing at a rate of 1.7% per year. Additionally, agriculture is a large component of India's economy, providing direct employment to about 67% of the working people in the country. Rice also constitutes about 52% of total cereal production in India. Other common food grains include cereals such as wheat, sorghum, pearl millet, and maize, as well as pulses. (Maclean and others 2002)

Culinary traditions and preferences for rice vary from region to region, yet rice is particularly common in areas where population density is high and availability of arable land is very low. For this reason, rice is widespread in Asia and Africa. Low labor costs also contribute to rice's popularity throughout Asia and Africa, since cultivation of rice is labor-intensive. Dietary intake surveys from China and India show an average adult intake equivalent to about 300 grams of raw rice per day (Popkin and others 1993). The rice consumption level in India alone was reported to be 74.2 kg milled rice per person per year in 1999 (Maclean and others 2002).

Rough rice (paddy rice) contains nutritionally-significant amounts of several micronutrients, such as riboflavin, thiamin, niacin, and zinc, as well as lesser amounts of phosphorus, vitamin B6, and copper (Table 1). Small amounts of iron, potassium, and folic acid are found in rice as well. However, many of these nutrients are lost by the time the product reaches the consumer. White rice has limited amounts of the essential micronutrients (previously listed) because of losses during processing. (UNCTAD 2006)

A lack of micronutrients in white rice has become a problem for countries with high consumption rates of white rice. As a result, micronutrient deficiencies are prevalent in many developing countries, and the country's health status and economy may suffer. As mentioned, major concerns are seen in iron, vitamin A, and iodine intake, while low zinc intake is also a problem. One solution to these problems is the implementation of fortification to more foods. Iodized salt has already been seen as an effective strategy to reduce iodine deficiency disorder to a level of 15%, down from 30% in 1990. Because rice is so widely produced and consumed, it has been proposed as an effective vehicle for fortification with a variety of micronutrients.

Table 1 Range of Mean Micronutrient Content (µg/g at 14% moisture) of Rough Rice and Its Milling Fractions[1]

Micronutrient	Rough	Brown	Milled
Riboflavin	0.6 - 1.1	0.4 - 1.4	0.2 - 0.6
Thiamin	2.6 - 3.3	2.9 - 6.1	0.2 - 1.1
Niacin	29 – 56	35 – 53	13 – 24
Zinc	1.7 – 31	6.0 – 28	6.0 – 23
Phosphorus	1,700 - 3,900	1,700 - 4,300	800 - 1,500
Vitamin B6	4.0 - 7.0	5.0 - 9.0	0.4 - 1.2
Copper	2.0 – 11	1.0 - 6.0	2.0 - 3.0
Iron	14 – 60	2.0 – 52	2.0 – 28
Potassium	1,500 - 3,700	600 - 2,800	700 - 1,300
Folic acid	0.2 - 0.4	0.1 - 0.5	0.03 - 0.14

[1] Adapted from Table 3 in Juliano and Bechtel (1985)

Extruded Fortified Rice Kernels

The experimental product used in this study is a low-cost, nutritionally fortified, extruded rice developed using novel technology by the Department of Food Science at Cornell University. It is manufactured using an extrusion process that combines rice flour (which can be ground from broken rice kernels) with a vitamin and mineral premix to deliver intact, nutritionally enhanced rice kernels. This technology may have the potential to address micronutrient malnutrition, while maintaining economic sustainability.

Rice Harvesting and Production

When considering a fortification program, it is important that the food vehicle is widely available and acceptable. In the case of rice, it is possible to obtain from one to four harvests of rice crop per year. In tropical climates rice is generally harvested twice a year, and the factors that must be present for a good harvest include adequate temperatures, ample water, and sufficient labor. (UNCTAD 2006)

Rice Cultivars

Rice is a truly ubiquitous commodity. Although the number of varieties cultivated around the world is debatable, a total of 83,000 varieties are held in the International Rice Research Institute (IRRI) gene bank in the Philippines. Varieties, or cultivars, vary in their morphology (of both grains and plants), grain composition, productivity, and tolerance to biotic factors (weeds, diseases, and insects), as well as abiotic factors (cold, drought, soil acidity, and lack of mineral nutrients in the soil). For example, Jasmine rice from Thailand is long-grain and less sticky than short-grain cultivars, as long-grain rice contains less amylopectin. Chinese *sticky rice* (properly known as *glutinous rice*), which is high in amylopectin, is a short-grain variety. Indian rice cultivars include long-grained and aromatic Basmati (grown in the North), long and medium-grained Patna rice, and short-grained Masoori. Parboiled rice is usually prepared in East India and South India by boiling rough rice in large pans of water immediately after harvesting and before removing the husk. It is then dried, and the husk removed later. Through this process, natural vitamins are driven into the inner layers of the rice kernel helping to retain nutrients during subsequent milling. Additionally, the heat and pressure of parboiling kill fungi and other contaminants, but it may cause an undesirable odor. Parboiled rice is thought to be more digestible and is mostly used by blue collar workers. In South India, it is often used to make *idlis*, dried rice crackers.

Rice Milling

Rice can exist in several forms, depending on the stage of processing. The common forms of rice are referred to as *rough rice, brown rice,* and *white rice.* Rough rice (paddy rice) is composed of 20% hull (the outermost layer), 10% bran and germ, and 70% starchy endosperm (Figure 1). Brown rice (partly milled rice) is paddy from which only the external hull has been removed. The bran and germ layers remain, making it far more nutritious than white rice.

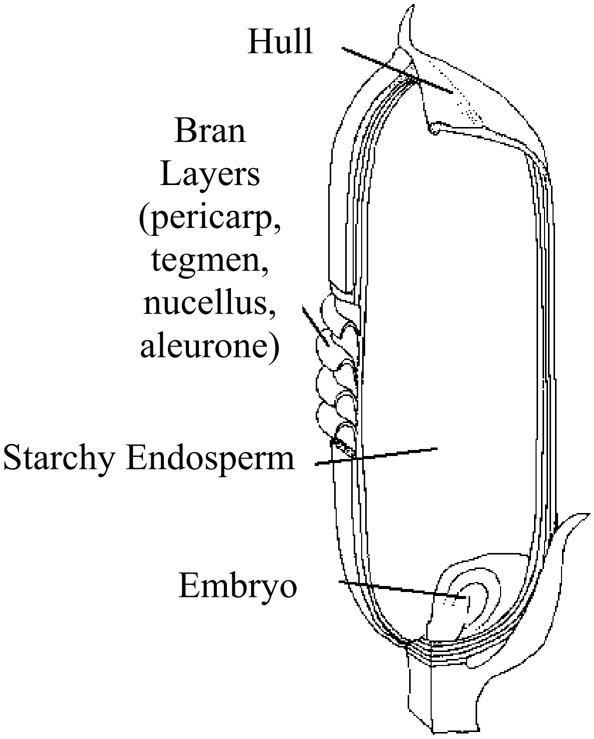

Figure 1 A Paddy Kernel, Showing the Major Parts
(Adapted from Bond 2004)

In much of Asia, however, brown rice is associated with poverty, and in the past was rarely consumed except by the sick and elderly. White rice (milled rice) is produced by removing the outermost hull and the bran layer (pericarp, tegmen, nucellus, and aleurone) along with polish (subaleurone), germ (embryo), and a small part of the endosperm underneath (Champagne and others 2004). White rice can also be buffed with glucose or talc powder, or milled into rice flour.

On average, milling 100 kg of paddy yields about 60 kg of white rice, 10 kg broken grains, 10 kg of bran and germ, and 20 kg of hull (FAO 1994). While the milling process is highly desirable for organoleptic properties, it may cause the loss of many nutrients upon removal of the hull and bran layers. The concentration of vitamins is typically two to ten times higher in brown rice than in milled rice, and the mineral and fiber contents are two to three times higher (Champagne 2004). In some countries, including the United States, white rice is often enriched with iron, niacin, and thiamine so that it can reclaim part of its original nutritive value. In India, however, enriching processes are not as common (UNCTAD 2006).

Determinants of Rice Quality

Cooking, sensory, and processing qualities of rice are highly dependent on the group of people consuming rice and the varieties of rice consumed. In an effort to understand the basis of rice end-use quality, the United States Department of Agriculture (USDA) devised simple tests for rice quality analysis in the 1950’s. Although these quality tests are not necessarily applicable across the globe, they point out some key attributes to consider for rice quality.

Milling quality is an important factor that influences the value of rice. It is derived from the total amount of milled rice kernels and the total amount of whole kernels recovered after milling (Bergman and others 2004). The percentage of broken kernels in milled rice also determines the quality. High-quality rice is considered to be rice with less than 10% broken grains, medium-quality rice is considered to be rice that is 15 to 20% broken, and low-quality rice is considered to be rice that is 25 to 100% broken (UNCTAD 2006). Any irregularity (yellow or chalky kernels and foreign matter) reduces the rice quality grade as well.

The size and shape of kernels is yet another determining factor of rice quality. Long-grain rice kernels (such as Basmati, Jasmine, and Ferrini from Italy) are 7 mm long and 3 times longer than they are wide after milling. When cooked, this grain is light and separates easily, unless it is a stickier variety (as seen in Laos and Thailand). Medium-grain rice kernels are 5 to 6 mm long and 2 to 2.9 times longer than wide, making them shorter and wider than the long grain. Short or round-grain rice kernels are 4 to 5 mm long and 1.9 or less times longer than wide. (Bergman and others 2004)

Hydration during cooking is another important consideration in rice quality. It is the amount of water absorbed by a known quantity of rice when cooked in boiling water for a given time expressed as “water absorption ratio” (grams of water absorbed per gram or 100 grams of rice). People from India prefer firm and fluffy cooked rice (high amylose content) and small, slender grains (high water absorption ratio). (Bergman and others 2004)

Post-Harvest Processing

Another challenge in rice quality preservation is post-harvest processing. In developing countries, post-harvest losses destroy about 15 to 16% of the rice crop. This figure is even greater (as much as 40 to 50%) in countries where there are challenging natural events and climatic conditions, such as regular heavy monsoons. The Food and Agriculture Organization (FAO) advocates a rice post-harvest system concept, an efficient, modern approach that focuses on preventing post-harvest losses and ensuring the quality and safety of the rice crop during its processing and storage.

The system also includes procedures that add value to both rice and secondary rice products such as rice flour. (FAO 1994)

The rice post-harvest system consists of several steps that lead to the production of polished rice kernels for use as they are or in other dishes. The first post-harvest step is pre-drying rice plants in the field. This is followed by threshing and winnowing to separate the rice kernels from the plants. Subsequent drying and storage stages occur before primary processing (cleaning, hulling, pounding, milling, grinding, sieving, soaking, and/or parboiling) and secondary processing (cooking, baking, frying, blending, and/or fermenting). The last considerations may be packaging, economic and marketing issues, and utilization by the consumer. (FAO 1994)

Some stages in the rice post-harvest system are more critical than others, particularly in tropical and subtropical areas, such as India, where rice is more vulnerable to spoilage and more likely to suffer qualitative and quantitative losses. Among these critical stages, efficient storage is especially important because up to 6% of total rice crops can be lost during storage. Some technological advances have been made in the area of rice storage techniques and equipment, and the FAO is playing an important role by contributing to the transfer of new post-harvest technologies for storage, which include small, metal silos for storing grains at the household level. The use of the small, metal silo is also recommended by the FAO as a feasible and valuable option for reducing small- and medium-scale rice farmers' food losses. (FAO 1994)

Drying is another critical post-harvest operation for rice processing. Drying in the fields is often not enough to prevent spoilage from fungus, and thus commercial drying is necessary. Efforts are being made to improve small rice driers. For example, small portable electric fan driers have been developed by the International

Rice Research Institute (IRRI) and are becoming important to small- and medium-scale rice farmers in terms of increasing their food security and ensuring the safety of their rice crops. Rice farmers are beginning to understand and accept the need to invest in post-harvest technologies because, not only are these technologies affordable, but they also offer the potential to increase profits by adding quality and commercial value to the final products. (FAO 1994)

Rice Markets

Rice is an important commodity, with many advantages beyond the gastronomic benefit. In addition to these advantages, rice is also a symbol of prosperity in many world cultures, and this value of rice helps drive world rice production. In 2003, world production totaled 395 million tons of milled rice. China and India, which account for more than one-third of global population, produce over half of the world's rice.

Rice Enrichment and Fortification

High-fiber cereal grains are inexpensive vehicles for providing basic nutrition to large populations (Hoffpauer 1992). With such high production and consumption rates, particularly for populations living in many developing countries, rice appears to be a reasonable choice for use with fortification. In many developing countries, rice makes up the largest percentage of calories and protein in individuals' diets. Without fortification, however, these individuals cannot receive adequate intakes of many essential micronutrients from rice alone (Brown 2002).

According to Dexter (1998), there are two conventional methods used in rice enrichment and fortification processes currently in commercial use: powder and whole grain enrichment. Powder enrichment involves the use of a pre-blended powder mix such as B vitamins (thiamin, riboflavin, and niacin) and iron (commonly ferric orthophosphate, ferric sulfate, or reduced iron). This vitamin premix can be applied to rice at various points during the milling and packaging process. Powder enrichment is most effective when it is added soon after milling white or parboiled rice because the temperature and moisture at the grain surface are optimal for the powder to adhere to the grain. Brown rice has more oil on the surface, and vitamins and minerals readily adhere to the surface. Powder enrichment is relatively inexpensive compared to other methods of enrichment; however, a significant portion of the nutrients is lost if the rice is rinsed, sifted, or cooked in excess water before consumption. In India, where washing the rice before cooking is common, it is estimated that 20 to 100% of the vitamins are lost depending on the amount of water used in rinsing and the length of cooking time (Hoffpauer 1992).

The second type of commercial enrichment used with rice is known as "grain" type. In this more common method, vitamins and minerals are applied at high concentrations to rice followed by a water insoluble food-grade substance that coats the rice and does not rinse off. These grains are then blended with unenriched grains, at an approximate ratio of 1:200 to attain a desired enrichment level in the final product. Commercial improvements have been made to the "grain" method over the years by companies such as Hoffmann-La Roche and Merck Company. (Dexter 1998)

Another less conventional method of rice fortification is using extrusion technology to produce fortified, extruded rice grains. One such product developed by Lee and others (2000) is called "ULTRA RICE™" (UR). UR is produced by extruding rice flour with all-trans retinyl palmitate (a form of vitamin A) at a fortification level of 2500 IU/g to form UR kernels. One gram of UR is then mixed with 99 g of long grain rice. (Lee and others 2000)

Several studies have been conducted using technology similar to UR. Moretti and others (2005) developed and evaluated iron-fortified extruded rice grains. These rice grains were fortified at a level of 1g Fe/ 100g of rice. The fortified grains were then mixed for the final product at a ratio of 1:100 or 1:200 with natural rice grains. This study revealed several key findings about iron loss and sensorial aspects of extruded fortified rice. Moretti and others (2005) concluded that their extruded, iron-fortified rice closely resembled natural unfortified rice. They also found the final product to be acceptable in sensory tests with very little loss of iron through processing and handling. An evaluation of iron forms pointed to micronized dispersible ferric pyrophosphate as a bioavailable source of iron with minimal sensory effects. (Moretti and others 2005)

Moretti and others (2005) did not consider the cost of iron in their evaluation of iron options, however. They found micronized dispersible ferric pyrophosphate to be a highly bioavailable source with nominal organoleptic changes. Yet micronized iron is not very cost-efficient compared to other iron forms. An estimate by Fortitech Inc. (Schenectady, NY) showed that micronized dispersible ferric pyrophosphate (SunActive® iron) costs approximately $28/lb compared to electrolytic iron and iron sulfate, averaging approximately $8/lb (Fortitech Inc., personal contact, 11/23/05). While extrusion can be done at low cost and high output (Harper and Jansen 1985, as cited by Moretti and others 2005), and the use of rice flour derived from broken rice may further decrease costs, it would not be enough to counteract such a price difference in the iron source. One important criterion of a successful food fortification program is that the product is affordable to the target population. Therefore, using dispersible ferric pyrophosphate is not necessarily the most feasible option for use in developing countries.

Still, this innovative technology has much potential, and it is also an important alternative to genetically-engineered crops. *Golden Rice* is a variety of rice that has been genetically-engineered to produce beta-carotene (pro-vitamin A) in an attempt to combat vitamin A deficiency (Paine and others 2005). The first prototype, Golden

Rice 1, of this genetically-engineered rice was found to achieve a maximum level of 1.6 µg total carotenoids per gram of rice, and Golden Rice 2, the improved prototype, was found to achieve a maximum level of 37 µg total carotenoids per gram of rice. While it has been predicted that approximately 75 grams of dry Golden Rice 2 could satisfy 50% of Recommended Dietary Allowance (RDA) for a young child and substantially reduce vitamin A deficiency, many individuals are wary of the long-term effects of using genetic engineering technology. This, among other reasons, has delayed commercial acceptability and widespread use of biofortification using genetic engineering.

III. Retention of Nutrients in Fortified Products

One major problem when considering fortification programs in developing countries is the stability of micronutrients. Warm, humid climates like those of Sub-Saharan Africa and South Asia (India and Pakistan) are particularly challenging for prevention of heat-labile vitamin degradation. Nutrient stability in fortified products is dependent on many factors including the fortification vehicle and process, the reactive nature of the micronutrients, storage and washing of the product, and cooking conditions (Dexter 1998, Richardson 1993). Therefore, it is virtually impossible to generalize on the exact stability of micronutrients (Richardson 1993). Additionally, food analysis laboratories are prone to variation, due to different methods used (de Jong and others 2000).

Determination of micronutrient loss through processing is important to assure both that the product contains what the label claims and that consumers are receiving the intended amount. Many physical and chemical factors influence the stability of micronutrients in fortified processed foods. For example, vitamin A and vitamin C are both micronutrients sensitive to oxidizing agents, heat, and light. In the case of the experimental product, fortified flour is exposed to temperatures of approximately 90ºC for one to several minutes in the extruder. Following extrusion, the product is left overnight to dry in a cabinet drier where it is exposed to oxygen. Lastly, the experimental product is exposed to ultra-violet (UV) light through normal handling and may even experience harsher conditions in real-life applications.

Vitamin A naturally inherent in foods, as retinyl esters in animal-based foods, is dissolved in a lipid matrix, which offers some protection from oxygen exposure. The presence of antioxidants, such as vitamin E, also helps to protect vitamin A from oxidation (Ball 2006). However, when vitamin A is added to foods as a fortificant, it is often more vulnerable to direct oxidation because it is not protected inside the food matrix. In this situation, retinyl esters may be more exposed to oxygen and free radicals present in the food produced during lipid oxidation (Ball 2006). Factors that accelerate lipid oxidation, such as oxygen, light, and high temperatures, enhance vitamin A degradation as well. Ascorbic acid (vitamin C) is another vitamin that is vulnerable to oxidation. Oxidation of ascorbic acid may be catalyzed by transition metals such as iron and copper or by enzymes (ascorbic acid oxidases) that may be present in the food (Ball 2006).

Extruded fortified rice has an advantage over rice fortified with traditional methods. The vitamins and minerals are pre-blended with rice flour and therefore are incorporated into the food matrix when all ingredients are extruded together. Theoretically, these vitamins and minerals would have reduced exposure to oxidation compared to traditionally fortified rice. However, the extrusion process itself exposes the product to high temperatures and pressures, and the product is more porous than conventional rice, putting vitamins at risk for destruction.

Although only a handful of studies have been published on vitamin loss in extruded fortified products, many fortified foods or foods naturally containing high levels of particular vitamins, have been tested for vitamin retention after a variety of treatments that may cause micronutrient loss. This literature can offer some insight regarding the expected retention of vitamins in extruded fortified rice.

In a study by Dellamonica and others (1978), vitamin A and vitamin C retentions were measured during boiling of a fortified whey-soy drink mix. In the study, the product was boiled for up to 5 minutes and showed approximately 50% loss of the original vitamin content. In another trial, the product was mixed with boiling water and allowed to stand at room temperature. The study demonstrated that vitamin A retention was 92% when first mixed with boiling water, 75% after 1 minute, 51% after the 2- and 4-minute intervals, and 40% after 5 minutes. This trend shows considerable vitamin loss due to heat treatment. Additionally, this study suggests that length of time of heat treatment is a crucial factor in determining vitamin degradation. (Dellamonica and others 1978)

In the study by Whited and others (2002), whole fat, reduced fat (2%), and nonfat milk samples were exposed to fluorescent light at 2000 lux at time intervals of 2 hrs, 4 hrs, 8 hrs, and 16 hrs. The samples were analyzed for vitamin A content to evaluate vitamin loss due to light exposure. Nonfat milk demonstrated observable vitamin loss at 2 hrs, reduced fat milk demonstrated vitamin loss at 4 hrs, and whole fat milk demonstrated vitamin loss at 16 hrs. After 16 hrs, vitamin A content was reduced by 29% in reduced fat milk and by 49% in nonfat milk. This experiment showed significant vitamin A loss due to light exposure. It also demonstrated that vitamin A loss is inversely related to the fat content of the milk (Whited and others 2002). Because rice does not have a high fat content, it may be more difficult to retain vitamin A fortified into the experimental product than other food products with higher fat contents.

Another study investigating micronutrient loss in milk showed that processing milk resulted in 50% vitamin C loss after sterilization (135°C for 5 seconds) (Kon

1972, as cited by Graham 1973). Additionally, authors noted that storage temperature, exposure to light, and oxygen availability appear to be of major importance to vitamin C stability (Graham 1973).

Ascorbic acid stability was assessed by Achinewhu and Hart (1994) in pineapple juice stored at room temperature for two weeks and processed with pasteurization. After the two week storage, ascorbic acid was reduced to between 59 and 65% of the fresh juice (Achinewhu and Hart 1994). Processing the juice by pasteurization reduced the ascorbic acid to between 28 to 46% of the original content (Achinewhu and Hart 1994). A similar study examining heat pasteurization of orange juice exhibited poor vitamin C retention (25 to 43%) in heat-processed juice after 14 days of storage (Elez-Martinez and others 2006). The vitamin C analyzed in these studies was inherent to the juices yet demonstrates significant vitamin C degradation as a factor of processing and storage time.

A study by Yadav and Sehgal (1995) on spinach and amaranth leaves examined the ascorbic acid content before and after several processing operations. The findings demonstrated that ascorbic acid content significantly decreased when these green, leafy vegetables were stored under refrigeration and at 30°C. Higher destruction was observed in leaves stored at 30°C (55% loss after 24 hours) than under refrigeration (an average of 4% loss after 24 hours) due to heat sensitivity. Additionally, ascorbic acid was reduced by 83% in amaranth leaves and 90% in spinach leaves after sun and oven drying, respectively.

The extremely high losses (90%) of ascorbic acid observed in spinach leaves in Yadav and Sehgal's study (1995) can be attributed to the exposure to higher temperatures, as well as increased oxygen exposure. The losses may also be a drying effect. According to Bluestein and Labuza (1988), the relative reaction rate of lipid oxidation gets increasingly higher as the water activity moves from 0.2 to 0. Catalysts for free-radical oxidation are no longer hydrated at this point and their effectiveness may increase (Labuza 1971, as cited by Bluestein and Labuza 1988). However, diffusion of catalysts decreases as moisture is decreased, leading to a decreased rate of losses of nutrients.

Vitamins in the experimental product are exposed to several water activity (A_w) levels during manufacturing. Exposure may cause destruction of vitamins most sensitive at each A_w level. Considering the general picture of reactions (Figure 2), the conditions to be avoided are high temperatures at intermediate- to high-moisture contents, the conditions the experimental product vitamins most likely experience during extrusion processing (Labuza 1972). Additionally, expanded extrudates have high porosity, probably increasing oxidative sensitivity (Cheftel 1986).

Extrusion cooking can be very harsh in that food ingredients can be subjected to a combination of heat sources, including frictional heat, direct steam injection, and heat transfer from steam or water jackets surrounding the extruder barrel. At the end of the extruder, the high-temperature, pressurized, cooked dough is forced through a small opening called a die. Generally, the retention of vitamins in extruded products decreases with several extrusion conditions: increased temperature, increased screw speed, decreased moisture, decreased throughput, decreased die diameter, and increased specific energy input (Killeit 1994). Vitamins A and C are reported as being the least stable vitamins through extrusion processing, compared to other common vitamins used in enrichment and fortification of cereals such as thiamin, riboflavin, niacin, and vitamin B6 (Lorenz and other 1980).

Looking specifically at the stability of vitamin A in extruded rice, a study by Murphy and others (1992) examined vitamin A in ULTRA RICE^{TM} (UR), a synthetic rice fortified with retinyl palmitate at a target level of 2500 IU vitamin A/g rice. In addition to vitamin A equivalent, the UR also contained lipids and antioxidants, such

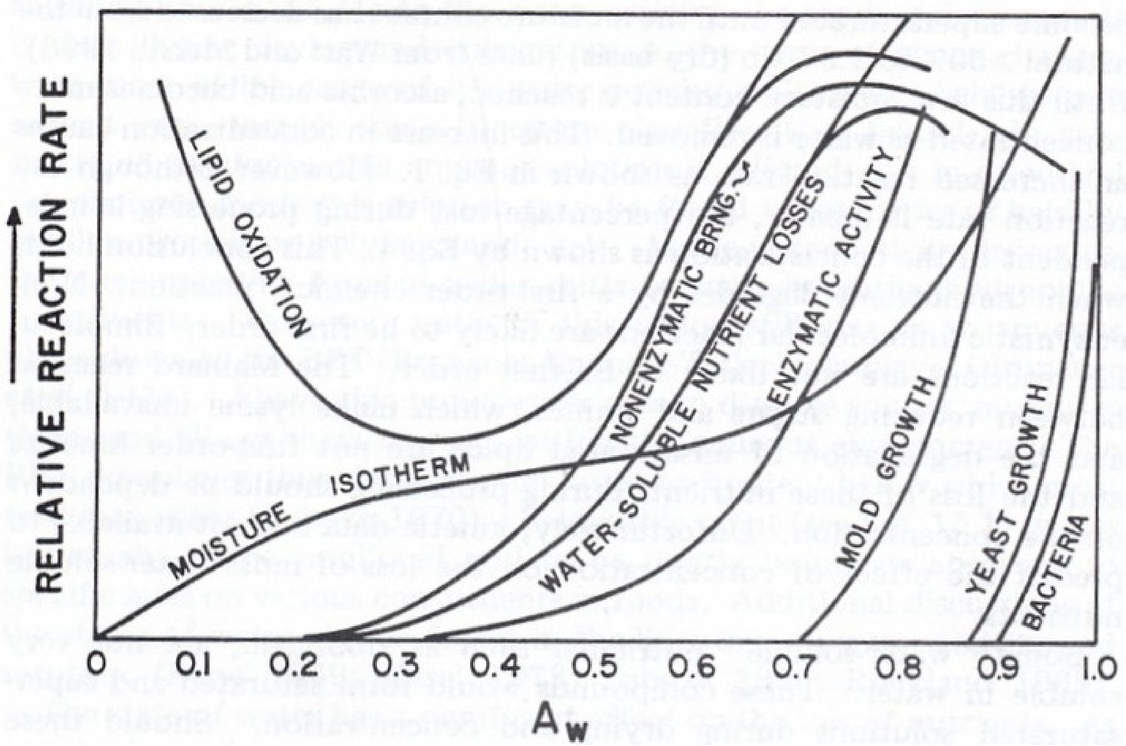

Figure 2 General Scheme of Reaction Rates as a Function of Water Activity (Bluestain and Labuza 1988)

as alpha-tocopherol, BHA, BHT, and ascorbic acid, as minor components. In the study, Murphy and others (1992) performed washing tests and cooking tests to determine vitamin retention in the rice product. The experimenters measured the retention of vitamin A after 5 minutes of stirring rice in cool water and 5 minutes cooking rice in boiling water. Results showed 100% retention of vitamin A in UR during washing, but the cooking trials demonstrated 46 to 94% retention in samples, depending on the oil type used during cooking (corn, olive, coconut, peanut, and soybean) and different antioxidants. UR containing combinations of more saturated oils, tocopherols, and ascorbate were recommended as critical for adequate preservation of vitamin A in the UR premix. Because the extruded fortified rice produced at Cornell University is low in oil, it would be expected to have vitamin A retention in the low range, in this case closer to 46%. The degradation of vitamin A in UR was determined to be a first order process based on the thermodynamics of the degradation. (Murphy 1992)

Flores and others (1994) also examined vitamin A stability in UR using storage and cooking trials. For the storage study, UR premix was stored up to 180 days, in a cool (approximately 26°C), reduced-light environment. An initial loss of about 25% of the vitamin A content was observed, after which the level remained fairly stable. In the cooking trial, the vitamin A loss was measured under normal rice cooking conditions (approximately 5 min boiling followed by 20-25 min under low-heat). The trial revealed a loss of approximately 26% due to cooking. (Flores and others 1994)

Vitamin C also experiences quite a bit of degradation during extrusion. Coelho (1991) reported ascorbic acid as the most sensitive of the vitamins. Twenty to 40 percent losses are consistently observed for ascorbic acid after undergoing extrusion (Cheftel 1986).

From the studies cited, it is evident that a significant loss of both vitamin A and vitamin C occur due to heat processing (such as extrusion), and vitamins are further oxidized during storage, due to increased porosity and light exposure after extrusion (Cheftel 1986). A thorough summary of vitamin stability is found in a paper written by Ritter (1976), manager of analytical services at Hoffmann-La Roche Inc. Recommendations for use of ascorbic acid in fortification of food products include: 1) add the vitamin as late in the process as possible, 2) minimize heating time and temperature, 3) avoid the use of bronze, brass, copper, cold rolled steel, or black iron equipment, 4) minimize head space in containers, and 5) use vacuum deaeration and inert-gas treatment during processing wherever feasible (Ritter 1976).

Data examining mineral stability in extruded foods are scarce. Many studies on minerals are partly contradictory, and additional research is needed (Cheftel 1986). It is suggested that, when adding minerals for fortification, the compounds should be highly bioavailable, mixed homogeneously with the other ingredients in the food, and should remain compatible with processing and post-extrusion storage. (Cheftel 1986)

IV. Bioavailability of nutrients in rice

Bioavailability, the amount of a nutrient consumed that is available for absorption and use by the body, is a concern in vitamin and mineral fortification. It is affected by chemical structure, particle size, encapsulation of the nutrient, and the presence of enhancing agents (such as ascorbic acid, an enhancer of iron absorption) or inhibiting agents (such as phytates, an inhibitor of iron absorption) (Monsen 1983). Absorption of iron may be inhibited in rice because of the presence of phytates. In extruded fortified rice samples produced by Cornell University ascorbic acid is added as an iron absorption enhancer to help compensate for the inhibitors in rice.

Different chemical and physical forms of iron have different bioavailabilities. Hoffpauer (1992) recommends the use of ferric orthophosphate as the best iron source for rice because of its relative water insolubility and white color. However, ferric orthophosphate is not very bioavailable (33% to 60% of the bioavailability of ferrous sulfate) (Shah and others 1979). Ferrous sulfate is more bioavailable than ferric orthophosphate, but it can produce undesirable sensory changes in cereals during storage (Peil and others 1981, cited in Dexter 1998). Electrolytic iron is also a commonly used fortificant because of its relatively low cost and minor effect on organoleptic properties. However, according to a study by Swain and others (2003), the average bioavailability of two electrolytic iron powders tested was shown to be 50% of ferrous sulfate. Choosing which type of iron to use in fortification can be challenging because of the variation in the properties of iron options.

V. Sensory Evaluation of Fortified Products

Organoleptic, or sensorial, aspects of a fortified food are important to consider in order for a fortification program to be effective. Testing methods, demographics of consumers, and samples all affect the outcome of sensory evaluation. Del Mundo and Juliano (1981) conducted a consumer preference test to determine major criteria for selecting raw and cooked milled rice. The study was run on consumers 16 years and older in a selected Philippine village. Samples were coded and presented in groups of five in covered wooden trays with dividers. Preference was determined by ranking, and acceptance was specified as yes or no. Results of the study showed that whiteness and hardness were the principal quality criteria for raw rice. For cooked rice, aroma and flavor were found to be most important for the consumer's preference. Amylose content was shown to be the principal determinant of eating quality. (Del Mundo and Juliano 1981)

Suknark and others (1998) examined the use of fish incorporated into extruded snack products and its effect on sensory properties. This study demonstrated that familiarity with a product influences a consumer's perception. Suknark and others (1998) found that aroma, flavor, and overall acceptance of fish snack products were rated significantly higher by consumers from Asia, where fish crackers are popular, than by American consumers. Therefore, it is important to consider the acceptability ratings of consumers with taste preferences from the native area where the product will be marketed compared to other non-native consumers. With the experimental product developed by Cornell University, consumers from South Asia, the target market may have different acceptability ratings and therefore should be included in the panel.

Color, flavor, aroma, and appearance are all components of sensory acceptability of a food product (Lawless and Heymann 1999). In fortified foods, changes in color may occur due to reactivity and concentration of micronutrients used. Iron is a particularly reactive micronutrient. Undesirable color changes are detected in foods fortified with ferrous sulfate, for example, when the product is exposed to conditions of high humidity (Dexter 1998). Moretti and others (2005) found that the fortification of rice with ferrous sulfate resulted in highly discolored (dark brown) rice grains. Rice grains fortified with Na FeEDTA had a brown-reddish color, and fortification with elemental iron resulted in gray rice grains (Moretti and others 2005). Overall, ferric pyrophosphate compounds exhibited the best color match to natural rice in the study (Moretti and others 2005). Color problems can be avoided by changing the fortificant form, by combining it with another source, or by reducing the fortification levels (Dexter 1998).

VI. Objectives

The purpose of this study was to assess the palatability and micronutrient retention of extruded fortified rice. Because the experimental product is a potential solution to hidden hunger and the consequences of micronutrient deficiencies in developing countries, South Asia was considered as the population of interest for this study.

The first objective of this study was to select and adapt analytical methods and conduct analyses for vitamin A and vitamin C in extruded fortified rice samples, as well as use inductively coupled argon plasma emission spectrometry to measure mineral retention. The second objective of this study was to conduct sensory evaluation studies to evaluate acceptability of the experimental product prototypes by consumers and compare acceptability by South Asian (Indian and Pakistani) consumers to that of non-South Asian consumers.

CHAPTER TWO

MATERIALS AND METHODS

Previous attempts to fortify rice by powder enrichment have proven unsuccessful due to loss of nutrients through typical washing and cooking methods employed in most countries (Hoffpauer 1992, as cited in Lee and others 2000). However, recent extrusion technology combining flour made from broken rice kernels with a vitamin and mineral premix to manufacture fortified rice kernels introduces a solution to overcome this hurdle.

I. Sample Treatments

This novel technology was used to develop the experimental product, extruded fortified rice. Eight extruded prototype treatments were developed, and each treatment consisted of a mixture of long-grain rice flour and specific micronutrients (except for the control prototype). The sample names, concentrations of micronutrients, and descriptions of analyses for each prototype are presented in Table 2. Additionally, two commercial, conventional rice samples were used, which are also described in Table 2. Fortification levels for 100 g of experimental product and Recommended Dietary Allowance levels are presented in Table 3.

Sample EF-ZAC/B was manufactured with the addition of 5% defatted rice bran, and EF-ZAC/BRF had 50% of the flour added to the premix as brown rice flour. For all other prototypes, 100% white rice flour was used as the flour base. Micronutrient premixes were obtained from Fortitech (Schenectady, NY). The premixes contained tricalcium phosphate, a filler and a cohesive. For all vitamin and mineral premixes delivered from Fortitech, an overage of 15% for vitamin A, 15% for ascorbic acid, 10% for iron, and 10% for zinc was added to compensate for loss during transportation and handling.

Table 2 Names, Descriptions, and Analyses Performed for Each Sample

Sample Name	**Composition (in 100 g rice)** [1]	**Analyses**
FS-ZAC	18 mg Fe as *ferrous sulfate*, 12 mg Zn as zinc oxide, 70 mg ascorbic acid, and 1 mg retinol activity equivalent (RAE) as retinyl palmitate	Vitamin, Mineral
FS-Z	18 mg Fe as *ferrous sulfate* and 12 mg Zn as zinc oxide	Mineral
EF-Z	18 mg Fe as *electrolytic Fe* and 12 mg Zn as zinc oxide	Mineral
EF-ZAC	18 mg Fe as *electrolytic Fe*, 12 mg Zn as zinc oxide, 70 mg ascorbic acid, and 1 mg RAE as retinyl palmitate	Vitamin, Mineral, Sensory
FEDTA-ZAC	18 mg Fe as *Na FeEDTA*, 12 mg Zn as zinc oxide, 70 mg ascorbic acid, and 1 mg RAE as retinyl palmitate	Vitamin, Mineral
EF-ZAC/B	18 mg Fe as *electrolytic Fe*, 12 mg Zn as zinc oxide, 70 mg ascorbic acid, and 1 mg RAE as retinyl palmitate, 5% bran	Vitamin Stability
EF-ZAC/BRF	18 mg Fe as *electrolytic Fe*, 12 mg Zn as zinc oxide, 70 mg ascorbic acid, and 1 mg RAE as retinyl palmitate, 50/50 white and brown long grain rice flour	Vitamin Stability
CONTROL	Control, no micronutrient premix	Vitamin Stability Sensory
LGPR[2]	Long grain, parboiled Canilla® rice, enriched with iron (ferric orthophosphate), niacin, thiamine (thiamine mononitrate) and folic acid (Goya Foods, Inc., Secaucus, NJ)	Sensory

Table 2 (Continued)

Sample Name	**Composition[1]**	**Analyses**
ELGR[2]	Extra long grain Canilla® rice, enriched with iron (ferric orthophosphate), niacin, thiamine (thiamine mononitrate) and folic acid (Goya Foods, Inc., Secaucus, NJ)	Sensory

[1] Composition per 100 grams rice made from long grain white rice flour unless otherwise specified

[2] Rice samples LGPR and ELGR were purchased from Wegman's Grocery Store in Ithaca, NY, two days prior to the sensory evaluation.

Table 3 Fortification Levels of Sample EF-ZAC in Experimental Product and Recommended Dietary Allowances (RDAs) of Micronutrients

Extruded Fortified Rice				
	Vitamin A[1] (μg/100g)[3]	Vitamin C [2] (mg/100g)	Iron [1] (mg/100g)	Zinc [1] (mg/100g)
EF-ZAC Concentration (per 100g)	1000	60	18	12

Recommended Dietary Allowances				
	Vitamin A (μg/d)[3]	Vitamin C (mg/d)	Iron (mg/d)	Zinc (mg/d)
Children				
1–3 y	300	15	7	3
4–8 y	400	25	10	5
Males				
9–13 y	600	45	8	8
14–18 y	900	75	11	11

Table 3 (Continued)

	Vitamin A (μg/d)[3]	Vitamin C (mg/d)	Iron (mg/d)	Zinc (mg/d)
Males				
31–50 y	900	90	8	11
51–70 y	900	90	8	11
> 70 y	900	90	8	11
Females				
9–13 y	600	45	8	8
14–18 y	700	65	15	9
19–30 y	700	75	18	8
31–50 y	700	75	18	8
51–70 y	700	75	8	8
> 70 y	700	75	8	8
Pregnancy				
≤ 18 y	750	80	27	12
19–30 y	770	85	27	11
31–50 y	770	85	27	11
Lactation				
≤ 18 y	1,200	115	10	13
19–30 y	1,300	120	9	12
31–50 y	1,300	120	9	12

[1] Source: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2000)

[2] Source: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000)

[3] As retinol activity equivalents (RAEs). 1 RAE = 1 µg retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.

For the prototype formulations, each vitamin and mineral premix was dry blended with fine long-grain rice flour (10% moisture content, obtained from Rivland Partnership in Houston, TX) at 0.1 to 0.2% by weight (to achieve desired fortification level). All formulations were blended with white rice flour, except EF-ZAC/BRF, which was a blend of 50% white rice flour and 50% brown rice flour. Distilled monoglycerides were added at a level of 0.5% by weight to all sample treatments as an emulsifier and processing aid. Ingredients were mixed for 20 minutes in a *wheelbarrow style* cement mixer (Kushlan Products, Inc., Goldendale, WA).

Commercially available conventional rice products were purchased from Wegman's Grocery Store in Ithaca, NY, for comparison in the sensory evaluation. *Long grain, parboiled white rice,* enriched with 1.44 mg iron (ferric orthophosphate), 1.6 mg niacin, 225 µg thiamine (thiamine mononitrate), and 60 µg folic acid per serving (47 g), was one commercial variety, sample LGPR. *Extra long grain white rice,* enriched with the same levels of iron (ferric orthophosphate), niacin, thiamine (thiamine mononitrate) and folic acid, was also used, sample ELGR. Both commercial varieties were Canilla brand, distributed by Goya Foods, Inc. (Secaucus, NJ). These samples were not extruded.

Extrusion Conditions

Prototypes of rice with extruded fortified kernels were manufactured on six occasions over a 15-month period, with each extrusion occasion referred to as a *batch.* A pilot-scale, co-rotating twin-screw extruder (Model TX-52, Wenger Manufacturing, Sabetha, KS) with a 28:1 barrel length to diameter ratio was used. The extrusion system was configured to operate at a screw speed of 170 rpm and dry feed rate of 75 kg/h. Pressure at the end of the barrel was maintained at 600 to 800 psi, and product temperature was in the range of 85 to 90°C.

Target feed moisture content was 35%, which was achieved by injection of water and steam directly into the pre-conditioner and the extruder. A die configured with the rice kernel shape resembling long grain rice kernels was set up at the end of the extruder with an appropriate cutting blade system. Extruded rice kernels were collected on stainless steel, mesh trays. Trays were loaded into a cabinet dryer composed of a proofing cabinet (Wear Ever Food Service Equipment, Lincoln Manufacturing Co, Inc.) with a small fan and 120-volt Thermolyne© heating unit circulating air from the bottom of the cabinet to the top. Rice kernels were dried with the heat and fan for one to five hours and left in the cabinet overnight, approximately 12 hours, after which they had a water content of approximately 10g/100g.

To cook samples for vitamin and mineral analyses, a 4:7 water to rice volume/volume ratio was cooked in a Salton® 3-cup rice cooker. Cook time (between 10 and 20 min) was determined automatically by the rice cooker based on a temperature sensor. After cooking, samples were allowed to stand for approximately 5 min before fluffing and serving. Cooked rice kernels were frozen in a -20ºC freezer immediately following cooking. They were then freeze-dried using a Virtis 100 SRC-8 lypholizer (The Virtis Company, Garginer, NY) until kernels reached an approximate water content of 5g/100g.

All ingredients in the experimental products were food-grade, and good manufacturing practices were used during preparation of the products. Wood, glass, and cardboard were prohibited in the processing area (pilot plant) at all times.

Sample Collection and Testing Schedule

The production and collection schedule for the experimental product is presented in Table 4. For each extrusion run, samples were collected at several phases for testing. These phases are also shown in Table 4. For each collection phase, samples were sealed in Ziploc® freezer bags, placed in air-tight plastic containers, and stored in a –20ºC freezer until analyzed. Samples used for vitamin and mineral stability tests were analyzed in triplicate.

Prototype quality and efficiency of collection were considered when determining which samples to analyze for the micronutrient stability and sensory studies. Therefore, during each batch, not all treatments were collected, and samples were not collected at all phases. Instead, treatments of extruded fortified rice varieties were collected from each batch as presented in Table 4.

Dried rice samples were pulverized by gentle grinding with a very small-scale mill (General Electric, Arthur H. Thomas Co., Scientific apparatus). Samples were stored in a –20ºC freezer immediately following grinding and analyzed within 72 hours. All vitamin analyses were performed as soon after the extrusion run as possible. However, because extraction methods were still being developed during the initial runs, analyses of many of these samples were performed within several weeks.

Table 4 Treatment, Batch, and Phase Descriptors for Extruded Samples used in Vitamin C, Vitamin A, Iron, Zinc, and Sensory Analyses

<u>Treatment</u> – Ingredients used in addition to long grain rice flour and 0.5% emulsifier
FS-ZAC – FeSO4, Zn, vitamin A, vitamin C
FS-Z – FeSO4, Zn
EF-Z – electrolytic Fe, Zn
EF-ZAC – electrolytic Fe, Zn, vitamin A, vitamin C
FEDTA-ZAC – Na Fe EDTA, Zn, vitamin A, vitamin C
EF-ZAC/B – electrolytic Fe, Zn, vitamin A, vitamin C, 5% bran flour
EF-ZAC/BRF – electrolytic Fe, Zn, vit A, vit C, 50% flour is brown rice flour
CONTROL – no vitamin and mineral premix used

<u>Batch</u> – Extrusion Manufacturing of Experimental Product

I	Rice Run performed January 6, 2005
II	Rice Run performed June 30, 2005
III	Rice Run performed December 2, 2005
IV	Rice Run performed December 14, 2005
V	Rice Run performed December 16, 2005
VI	Rice Run performed April 7, 2006

<u>Phase</u> – Stage in rice process when sample is collected
Pre-extrusion – flour with premix, before pre-conditioning
Mid-process – product after pre-conditioning
Post-process – product immediately after extrusion
After Drying – Approximately 24 hours after drying cabinet drier
Cooked rice – 1.75 cups rice to 1 cup water, cooked in Salton 3-cup rice cooker

II. Determination of Vitamin Content in Extruded Fortified Rice

Vitamin analyses were performed under reduced-light conditions.

Ascorbic Acid Analysis

Ascorbic acid (vitamin C) levels were determined in triplicate by titration method according to a modified method of the official method 967.21 of the AOAC International (2000). For the extracting solution, metaphosphoric acid-acetic acid was prepared by dissolving, with shaking, 15 g HPO_3 pellets in 40 mL glacial CH_3COOH and diluting to 500 mL with H_2O. 2,6-dichloroindophenol Na salt was added to 50 mL H_2O containing 42 mg $NaHCO_3$ and diluted to 200 mL with H_2O for the titrant. Both the titrant and the extracting solution were filtered, stored in the refrigerator, and used within 7 days of preparation.

For each sample, 1 or 2 g of rice material was weighed into 15 ml plastic, screw cap centrifuge tubes (VWR International). One control sample was spiked with 1 to 2 mg ascorbic acid to determine retention through the extraction process. To each tube, 10 ml of metaphosphoric acid-acetic acid solution was added. Tubes were vortex mixed and allowed to stand 5 min. The mixing step was repeated two more times, with a 5 min standing time between each mixing. Tubes were centrifuged at a relative centrifugal force of 1610 x g with a bench top centrifuge. Four ml supernatant were removed from each tube and mixed with 5 ml metaphosphoric acid-acetic acid in a small (30 ml) beaker.

An ascorbic acid stock solution was prepared by diluting 50 mg L-ascorbic acid (Sigma Chemical Co.) to 50 mL volume with HPO_3-CH_3COOH for immediate use. Standard solutions were prepared by separately delivering 0.5 ml, 1 ml, and 1.5 ml of the stock solution and 5 ml metaphosphoric acid-acetic acid into a small beaker. These samples were used to develop the standard curve.

Each small beaker was titrated rapidly with dye solution until a light but distinct rose pink color persisted for at least 5 seconds. Ascorbic acid concentration was determined based on the ml of dye used to titrate each sample compared to the standard curve.

Vitamin A Analysis

Vitamin A levels based on retinyl palmitate concentrations were determined in triplicate by HPLC according to an adaptation of the *Quantitative Analysis of Vitamin A* methods from the Milk Quality Improvement Program at Cornell University as described in *Fluid Milk Fortification Compliance in New York State* (Murphy and others 2001). Retinyl palmitate (Sigma Chemical Corp., St. Louis, MO) was used as the primary standard.

Stock solution was prepared by weighing approximately 0.05 g of retinyl palmitate into a 250-ml actinic volumetric flask and diluting to volume with HPLC grade hexane. Concentration was determined as IU/ml (a unit commonly used in the chemical industry) based on a 1,600,000 IU Vitamin A/g standard, a conversion provided by Sigma Chemical Corp. Dilutions of 1:5, 1:10, and 1:25 of the standard were prepared by diluting the stock with HPLC grade hexane in actinic volumetric flasks.

For each sample, 0.5 g of rice material was weighed into 15 x 150 mm glass test tubes with Teflon screw caps. To each tube, 5 ml of 190-proof ethanol was added, vortex mixed, and allowed to stand 5 min. To each tube, 5 ml hexane was added. Tubes were vortex mixed and allowed to stand 5 minutes. The vortex step was repeated two more times with a 5-min standing time in between each mixing. Next, 3 ml distilled water was added to each tube and inverted several times. Tubes were centrifuged at 1610 x g with a bench top centrifuge. Two ml of the hexane layer was transferred to a clean 15 x 150 mm test tube. The hexane layer was evaporated to dryness under a stream of N_2 gas. The residue was re-suspended in 200 µl HPLC grade hexane and vortex mixed two times for at least 30 seconds. The residue-hexane solution was transferred to autosampler vials and subjected to HPLC analyses.

HPLC analyses were performed on a Waters 600 Controller with Waters 996 PDA detector and a Waters 717 Plus Autosampler (Waters Associates, Milford, MA). The column was a 4.6 x 250-mm Silica Column (Phenomenex, Inc., Torrance, CA) maintained at 20°C with hexane/chloroform (92:8 vol/vol) as the mobile phase at a flow rate of 1 ml/min. The wavelength of maximum absorbance for vitamin A, 325 nm, was used to quantify the results. Chromatograms of retinyl palmitate extracted from fortified rice flour and extruded fortified rice after drying are presented in Figures A17 and A18 respectively. *Trans*- and *cis*-retinyl palmitate peak areas were integrated by using the Millennium32 Software Package (Waters Associates). Sample vitamin A levels based on *trans*- and *cis*-retinyl palmitate concentrations were calculated as IU per g rice.

III. Determination of Mineral Content in Extruded Fortified Rice

Iron and Zinc Analyses

To ash each sample, 0.5 g of rice material was weighed into glass culture tubes. One ml of concentrated nitric acid was added to each sample. Tubes were covered with cling film and left at room temperature overnight in a fume hood. Tubes were then transferred to a hot block at 120ºC and heated to dryness. The block temperature was raised to 150ºC, and nitric acid was added 1 ml at a time to the tubes until the heated samples no longer gave off red-brown fumes and the sample was light brown to yellow in color. One ml of $HNO_3/HClO_4$ (50/50) solution was added, and the block temperature was increased to 180ºC. Samples were digested for 1-2 hours. When digests were clear to light yellow in color, the temperature was increased to 240ºC and heated to dryness. The tubes were removed from the block and allowed to cool. The ash was dissolved in 0.25 ml of concentrated HCl. When the ash was completely dissolved, 10 ml of 5% HNO_3 was added and the mixture was allowed to stand for 20 minutes.

Iron and Zinc concentrations were determined in triplicate by inductively coupled argon plasma emission spectrometry (ICAP Model 61E Trace Analyzer, Thermo Jarrell Ash, Franklin, MA). Mineral concentrations were expressed as µg per gram rice.

IV. Sensory Evaluation

The following samples were used for sensory evaluation.

1. *EF-ZAC,* manufactured in batch IV via extrusion in the Cornell Food Science Pilot Plant. Dried samples were stored in refrigerated conditions for one month before sensory study.
2. *CONTROL,* manufactured in batch IV via extrusion in the Cornell Food Science Pilot Plant. Dried samples were stored in refrigerated conditions for one month before sensory study.
3. *LGPR,* commercial long grain parboiled white rice
4. *ELGR,* commercial extra-long grain white rice

Cooking Methods

The commercial rice varieties were cooked using a household rice cooker, using a 1:1 water to rice v/v ratio, as specified by the machine instructions for preparing 2 cups of rice. Extruded fortified rice samples were also cooked in the rice cooker, but a 4:7 water to rice v/v ratio (1 cup of water mixed with 1.75 cups of rice) was used. The ratio of water to rice for the extruded rice was determined in preliminary cooking trials. Extruded rice was cooked to resemble commercial rice and to achieve the least sticky texture. Cook time was automatically set by the rice cooker (approximately 20 minutes).

Consumer Acceptability Test Methods

The sensory evaluation was conducted in the Cornell University Sensory Testing facility in Stocking Hall. Before sensory testing, several preliminary cooking trials were performed for optimization of the water to rice ratio and cooking methods. The methods varied in water to rice ratio, time of cooking, and time before serving.

For the sensory test of acceptability, 102 consumers (ages 18 to 60) were recruited to evaluate acceptance of rice samples. All consumers were pre-screened as regular rice consumers (those who consume rice several times per month). Consumers were South Asian (Indian or Pakistani) (51) and non-South Asian (51) as determined by self-report of ethnicity, and they consisted of staff and students from Cornell University, Ithaca, NY. Recruitment methods included posters circulated around the Cornell University campus and the Ithaca community, emails sent to Indian organizations at Cornell University, and sign-up sheets on Cornell University's campus.

At the time of the test, consumers signed an informed consent form (Figure A19), after reading it and having the opportunity to ask questions, and were offered a copy (Appendix). Each consumer sat at a computer station with Compusense software (Compusense v.5.4, Compusense, Inc. Guelf, Ontario, Canada). They were given no information about the experimental product's formulation or process methods to ensure an unbiased evaluation.

Cooked rice samples were freshly prepared every hour and kept warm (at approximately 50˚C) until ready for serving. A 25 g sample of cooked rice was presented in a cream-colored plastic cup with lid (Polytainers, Inc., Kansas City, MO). Samples were given 3-digit codes and were presented under disguising red lights. Each consumer evaluated all products, presented simultaneously. The order in which the consumers were asked to evaluate the samples was determined using a random block design. Consumers were asked to indicate how much they liked the product presented on a Just-About-Right (JAR) scale (Figure 3). For the JAR test, consumers marked their rating on a 5-point just-about-right scale in which 1 = too soft and too dry for firmness and moistness respectively, 3 = just about right, and 5 = too firm and too moist for firmness and moistness respectively. Consumers also indicated the degree of liking of each sample for several attributes using a 9-point hedonic scale (from 1 = disliked extremely to 9 = liked extremely, with 5 = neither liked nor disliked) (Lawless and Heymann 1999) (Figure 4). The attributes examined in the hedonic scale were overall aroma, firmness, moistness, overall texture, flavor, aftertaste, overall acceptance, color, shape, and overall appearance for cooked samples. Additionally, consumers ranked all four samples in order of overall preference (from 1 = most preferred to 4 = least preferred) (Figure 5).

Please evaluate sample 201 and indicate how much you like the intensity of each attribute.

Firmness

Too soft		Just about right		Too firm
☐	☐	☐	☐	☐
1	2	3	4	5

Figure 3 Just About Right Evaluation Example Question

Please evaluate sample 201 and indicate how much you like each attribute.

Flavor

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

Figure 4 Nine-Point Hedonic Scale of Liking Example Question

Please rank the four samples in order of your overall preference, from *MOST* preferred to *LEAST* preferred.

Rank	**Sample #**
_______	_______
_______	_______
_______	_______
_______	_______

Figure 5 Ranking Test Example Question

In a separate room, consumers evaluated both cooked and raw rice samples for all 4 products by the 9-point hedonic scale under standard (fluorescent) light conditions. The attributes examined in these normal light conditions were color, shape, and overall appearance. All evaluations were carried out in individual booths.

After scoring the acceptability of the rice, consumers were asked to provide demographic information including age, gender, race, and consumption of rice per week. The testing took approximately 15 to 20 minutes. Each consumer was paid $3.00 for his or her assistance.

IV. Data Analysis

Statistical analyses of consumer test data were computed by using the CompuSense software (Compusense v.5.4, Compusense, Inc. Guelf, Ontario, Canada). Analysis of variance (ANOVA) and Tukey HSD tests were performed on the consumer data for multiple comparisons, except the attribute *ranking*. Ranked data were analyzed using the Friedman test and least-squared-difference test (Lawless and Heymann 1999). In addition, data were sorted by ethnic groups, South Asian consumers and non-South Asian consumers, and these data were analyzed for treatment effects. Significance of differences was defined as $p < 0.05$.

One-way ANOVA and Tukey HSD tests were also performed on the micronutrient content data to examine differences between sampling phases and determine micronutrient retention. Significance of differences was defined as $p < 0.05$.

CHAPTER THREE

RESULTS AND DISCUSSION

I. Sensory

9-Point Hedonic Test

The mean ratings, standard errors, and p-values from the two-way ANOVA for multiple comparisons are listed in Table 5. Mean ratings were plotted for all consumer panel data in overall aroma, flavor, aftertaste, firmness, moistness, overall texture, firmness just-about-right (JAR), moistness JAR, overall acceptance, color, shape, overall appearance, and ranking (Figures 6-12). Attributes mentioned throughout the results and discussion refer to cooked rice samples unless specifically distinguished as "of raw kernels."

The largest differences were observed between hedonic ratings of extruded and commercial samples. Both extruded rice samples (EF-ZAC and CONTROL) were rated as lower in liking of aroma, overall acceptance, color of raw kernels, overall appearance of raw kernels, shape of cooked kernels, and overall appearance of cooked kernels. Off-odors and flavors may have been due to some oxidation that occurred during storage, as well as the effect of the vitamin and minerals added to the fortified sample. As expected, the appearance attributes were rated lower in the extruded samples likely due to a darker color and slightly unnatural shape compared to the commercial samples. The darker color may have been due to the greater porosity of the extruded kernels. The vitamins and minerals (particularly iron) in the fortified sample darkened the color as well. Irregular shape is a result of the extruder die, which can be changed to represent a more natural rice shape.

The scales for texture attributes did not discriminate the extruded products from the commercial products as much as the other scales; only the CONTROL sample was rated as lower than a commercial sample for firmness, moistness, and overall texture. The lower liking of the texture attributes in the CONTROL sample may have resulted from increased stickiness due to excessive shearing in the extruder.

When comparing the two extruded samples to each other for effect of fortification on panelists' rating scores, very few attributes discriminated the two samples statistically. The fortified sample (EF-ZAC) scored higher than the CONTROL sample in liking of firmness, moistness, overall texture, and overall acceptance. CONTROL scored higher than EF-ZAC in color of raw kernels, overall appearance of raw kernels, shape of cooked kernels, and overall appearance of cooked kernels. The lower hedonic ratings in appearance for the fortified sample can be attributed to the addition of vitamins and minerals which cause a darker color in the fortified sample, as previously mentioned.

Ranking Test

Panelists ranked each sample from 1-4 (1 being the most preferred overall and 4 being the least preferred overall). Commercial samples scored better (closer to 1) than the extruded samples, with the extruded fortified sample (EF-ZAC) scoring better than the CONTROL sample.

Just-About-Right (JAR) Test

The extruded fortified sample scored closer to "just about right" than the control for firmness and moistness with end anchors of not firm enough and too firm and not moist enough and too moist, respectively.

Table 5 Acceptability Ratings by Consumer Panels: Mean, Standard Error (SE), and P-value (from two-way ANOVA for multiple comparisons, Friedman's test for Ranking). Letters following means indicate results of Tukey HSD tests, least-squared difference test for Ranking. Means with different letters within a row were different.

Attribute Title	p-value	CONTROL Mean	EF-ZAC Mean	ELGR Mean	LGPR Mean
Overall Aroma[1]	0.0001	4.69 b	4.60 b	5.20 a	5.49 a
(SE)		(0.163)	(0.189)	(0.185)	(0.187)
Firmness[1]	0.0000	4.24 c	4.74 bc	5.08 b	5.65 a
(SE)		(.204)	(0.203)	(0.186)	(0.185)
Moistness[1]	0.0000	4.26 c	4.87 b	4.77 bc	5.54 a
(SE)		(0.181)	(0.195)	(0.175)	(0.180)
Overall texture[1]	0.0000	4.40 c	4.46 bc	4.99 b	5.65 a
(SE)		(0.195)	(0.205)	(0.174)	(0.179)
Firmness JAR[2]	0.0000	3.78 a	2.98 b	3.65 a	3.56 a
(SE)		(0.110)	(0.118)	(0.0895)	(0.0837)
Moistness JAR[2]	0.0000	2.14 b	2.72 a	2.20 b	2.49 a
(SE)		(0.089)	(0.103)	(0.0868)	(0.0698)
Flavor[1]	0.0044	4.83 c	4.88 bc	5.50 a	5.39 b
(SE)		(0.165)	(0.190)	(0.169)	(0.189)
After taste[1]	0.0476	4.96 b	5.12 ab	5.50 a	5.11 ab
(SE)		(0.143)	(0.167)	(0.135)	(0.169)
Overall Acceptance[1]	0.0000	4.34 b	4.66 b	5.28 a	5.51 a
(SE)		(0.182)	(0.206)	(0.180)	(0.193)
Color (raw) [1]	0.000	4.168 c	3.416 d	6.960 a	4.861 b
(SE)		(0.157)	(0.152)	(0.121)	(0.193)
Shape (raw) [1]	0.000	5.406 b	4.545 b	6.485 a	6.307 ab
(SE)		(0.175)	(0.197)	(0.155)	(0.143)
Overall Appearance (raw) [1]	0.000	4.842 c	3.495 d	6.772 a	5.436 b
(SE)		(0.167)	(0.166)	(0.131)	(0.175)

Table 5 (Continued)

Attribute Title	p-value	CONTROL Mean	EF-ZAC Mean	ELGR Mean	LGPR Mean
Color (cooked)[1]	0.000	4.990 b	4.802 b	6.792 a	5.208 b
(SE)		(0.161)	(0.168)	(0.128)	(0.185)
Shape (cooked)[1]	0.000	5.564 c	4.743 d	6.663 a	6.168 b
(SE)		(0.159)	(0.188)	(0.133)	(0.192)
Overall Appearance (cooked)[1]	0.000	4.931 c	4.446 d	6.802 a	5.762 b
(SE)		(0.187)	(0.184)	(0.121)	(1.88)
Rank[3]	< 0.05	2.772 a	2.683 a	2.386 ab	2.158 b
(SE)		(0.0953)	(0.119)	(0.107)	(0.113)

[1] Values are scores on a 9-point hedonic scale in which 1 = dislike extremely and 9 = like extremely.

[2] Values are scores on a 5-point just-about-right (JAR) scale in which 1 = too soft and 5 = too firm for firmness, and 1 = too dry and 5 = too moist for moistness. 3 = just about right for both firmness and moistness attributes.

[3] Values are scores on a 4-point ranking scale in which 1 = most preferred and 4 = least preferred.

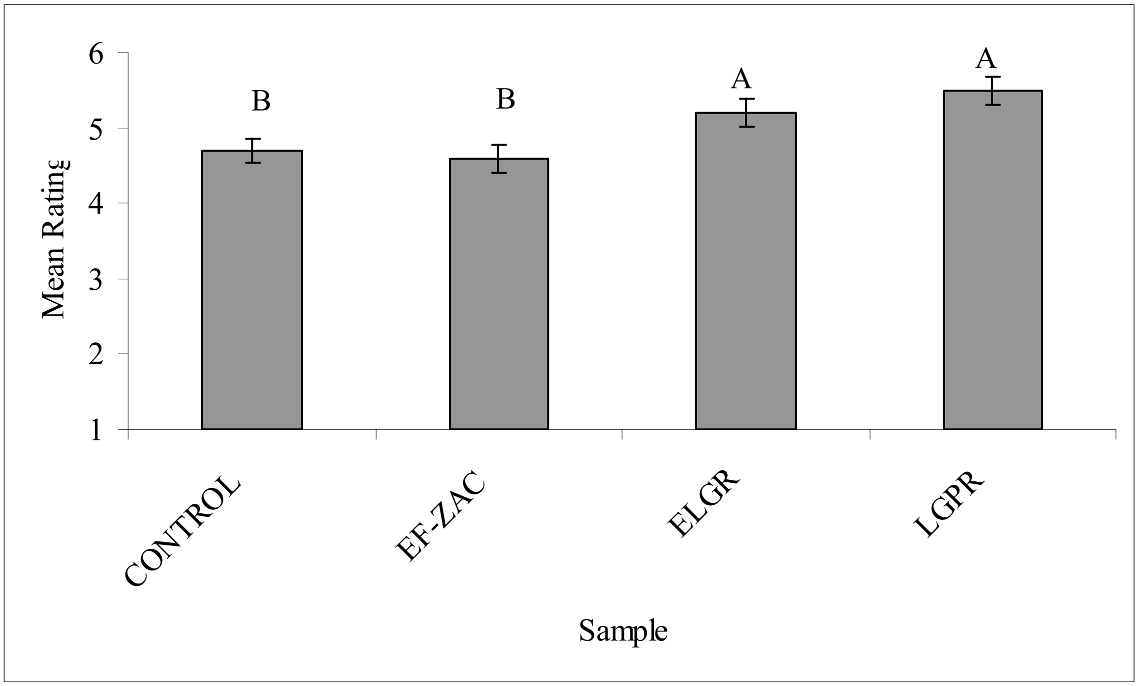

Figure 6 Mean Rating and Standard Error for Aroma by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters were significantly different.

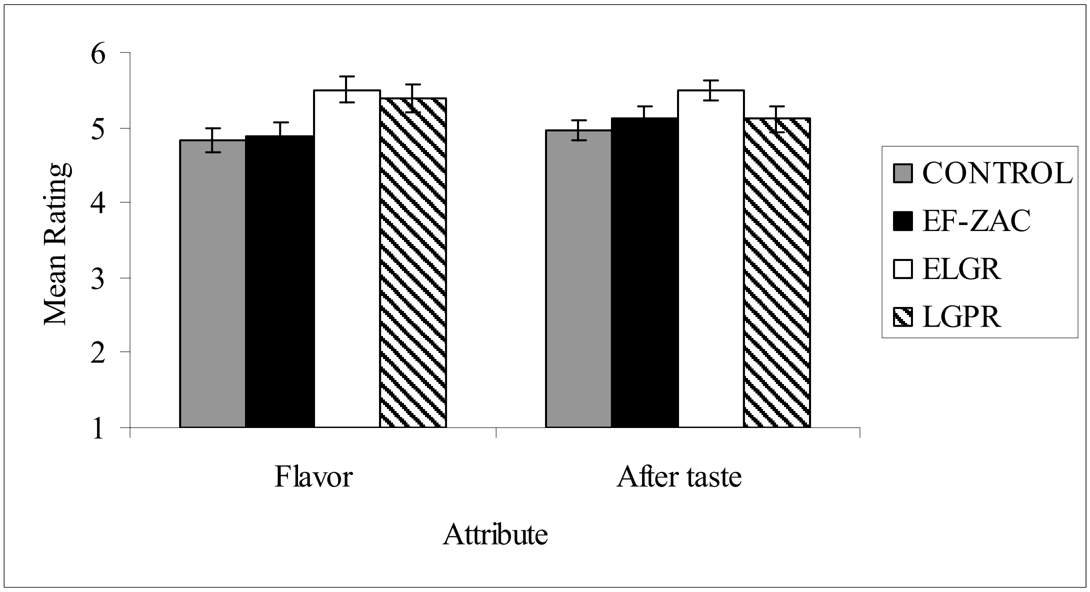

Figure 7 Mean Rating and Standard Error for Flavor and Aftertaste by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters within a group were significantly different.

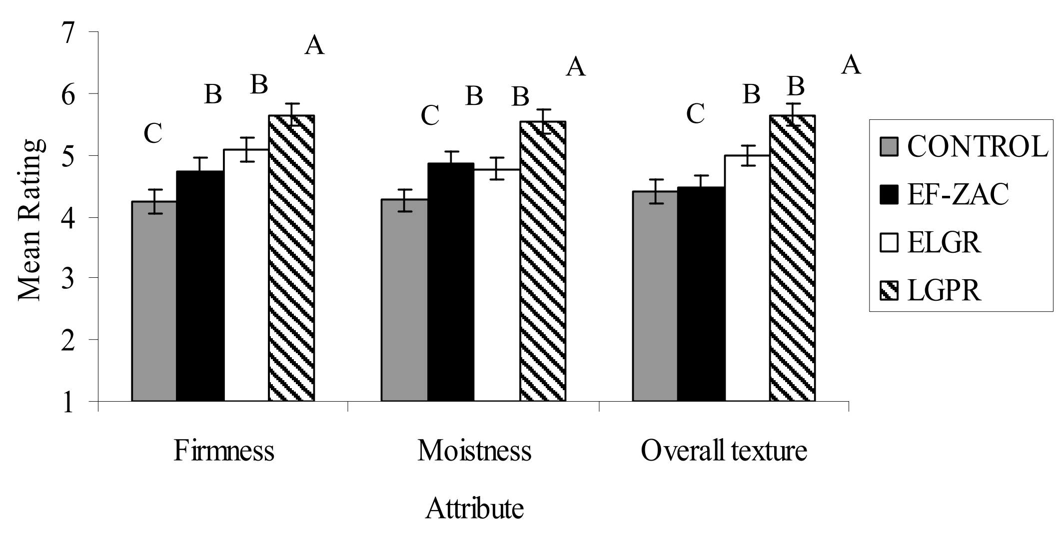

Figure 8 Mean Rating and Standard Error for Firmness, Moistness, and Overall Texture by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters within a group were significantly different.

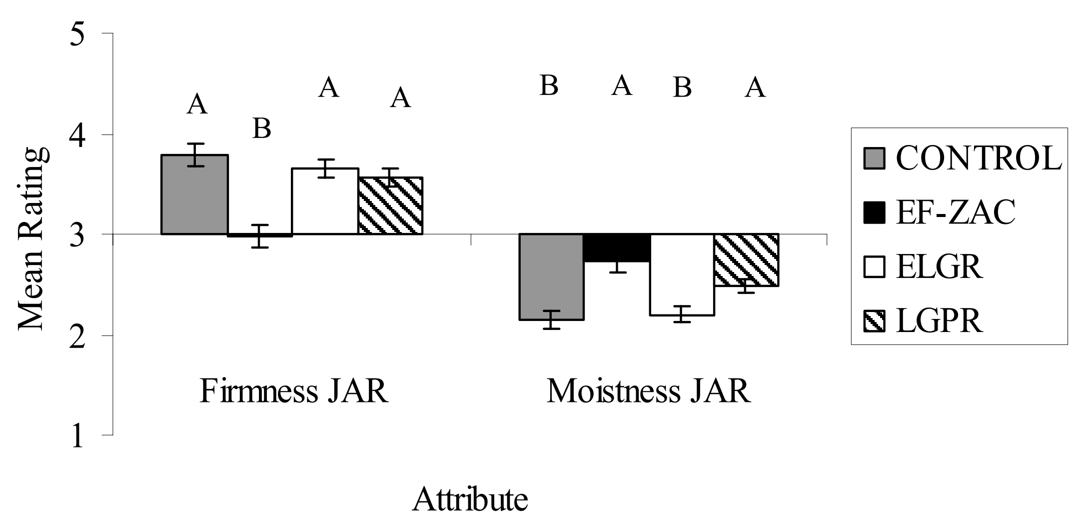

Figure 9 Mean Just-About-Right Rating and Standard Error for Firmness and Moistness by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters within a group were significantly different.

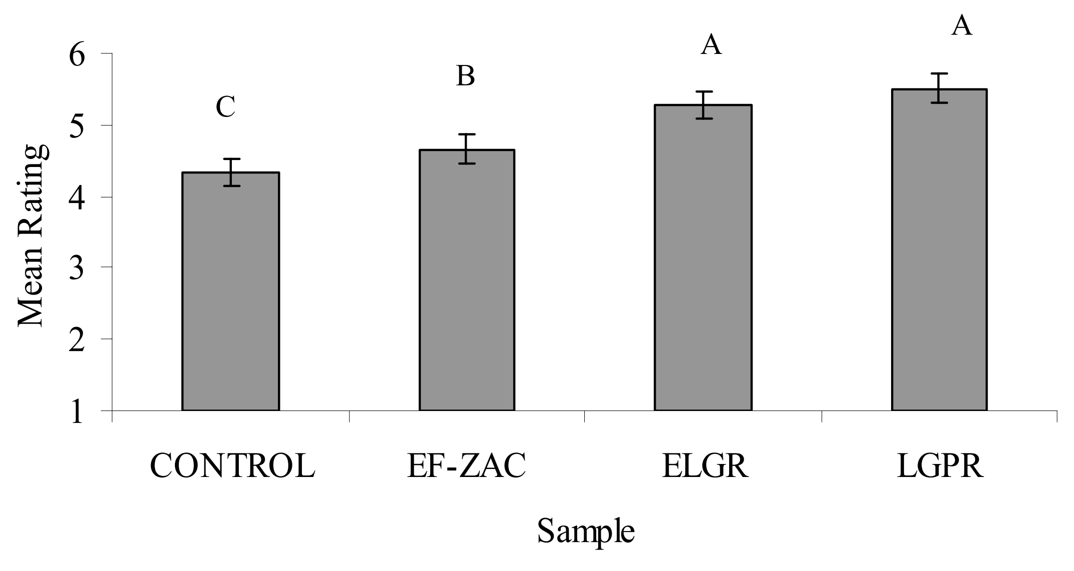

Figure 10 Mean Rating and Standard Error for Overall Acceptance by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters were significantly different.

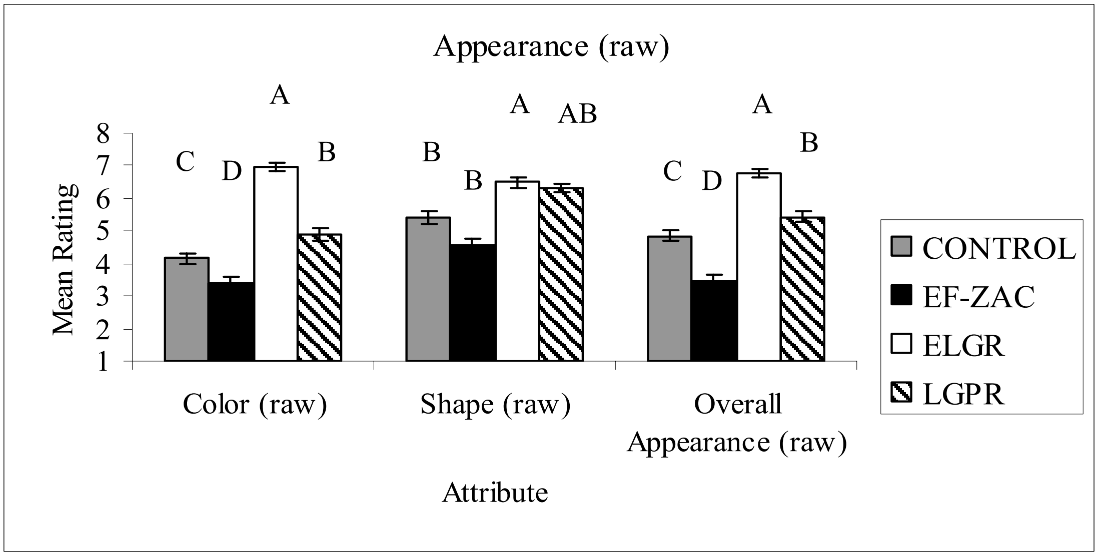

Figure 11 Mean Rating and Standard Error for Appearance Attributes by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters within a group were significantly different.

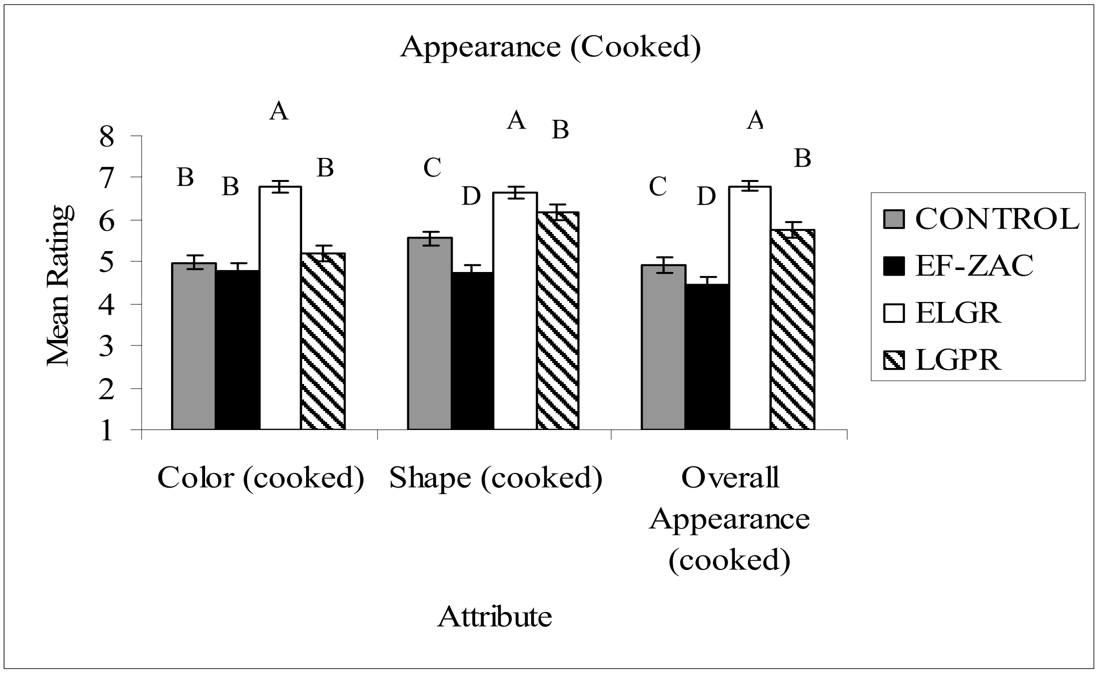

Figure 12 Mean Rating and Standard Error for Appearance Attributes by Consumer Panels. Letters indicate results of Tukey HSD tests. Means with different letters within a group were significantly different.

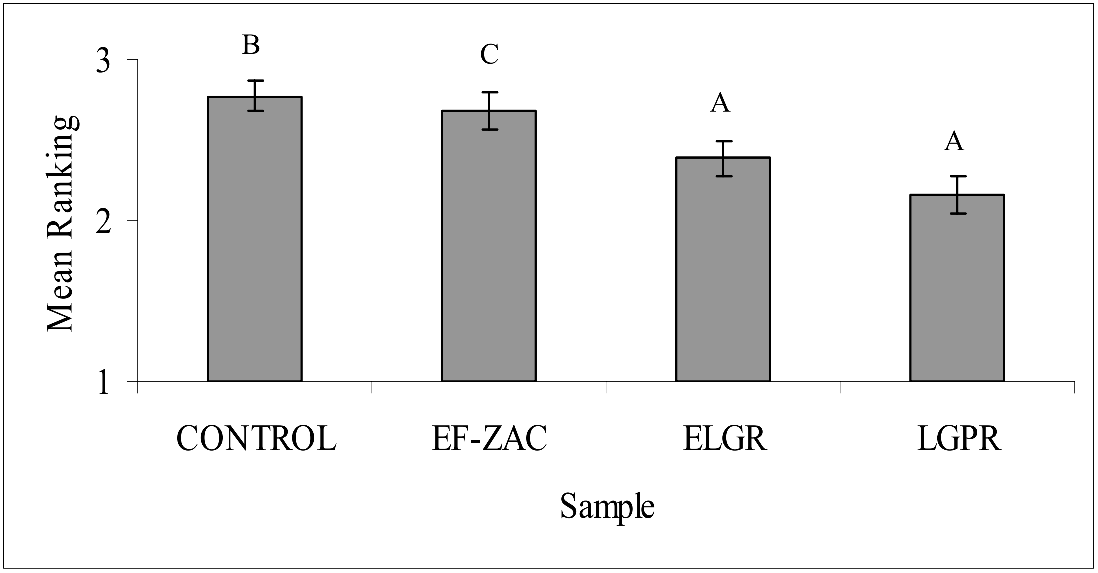

Figure 13 Mean Ranking and Standard Error for Sample Rank by Consumer Panels. Letters indicate results of the Friedman test and least-squared-difference test. Means with different letters were significantly different.

Group Differences and Interactions

Univariate and multivariate repeated measured analysis tested group differences and interactions, comparing the South Asian group to the non-South Asian group. The mean ratings, standard errors, and p-values from the two-way ANOVA for multiple comparisons for South Asian and non-South Asian groups are listed in Table 6 and Table 7 respectively. Mean ratings were plotted for South Asian and non-South Asian consumer panel data in overall aroma, flavor, aftertaste, firmness, moistness, overall texture, firmness JAR, moistness JAR, overall acceptance, color, shape, overall appearance, and ranking (Figures A1-A16).

Table 6 Acceptability Ratings by South Asian Consumer Panels: Mean, SE, and P-value (from two-way ANOVA for multiple comparisons, Friedman's test for Ranking). Letters following means indicate results of Tukey HSD tests, least-squared difference test for Ranking. Means with different letters within a row were different.

Attribute Title	p-value	VitaRice O Mean	VitaRice D Mean	Sample I Mean	Sample H Mean
Overall Aroma[1]	0.108	4.700 a	4.520 a	5.120 a	5.280 a
(SE)		(0.253)	(0.29)	(0.284)	(0.296)
Firmness[1]	0.000	3.980 b	5.280 a	4.900 a	5.260 a
(SE)		(0.32)	(0.29)	(0.271)	(0.285)
Moistness[1]	0.000	3.920 b	5.200 a	4.560 ab	5.160 a
(SE)		(0.32)	(0.273)	(0.251)	(0.270)
Overall texture[1]	0.050	4.520 b	5.000 ab	4.960 ab	5.400 a
(SE)		(0.286)	(0.283)	(0.244)	(0.262)
Firmness JAR[2]	0.000	3.960 a	3.280 b	3.580 ab	3.700 a
(SE)		(0.156)	0.125)	(0.137)	(0.129)
Moistness JAR[2]	0.000	1.940 c	2.620 a	2.160 bc	2.340 ab
(SE)		(0.112)	(0.121)	(0.129)	(0.105)
Flavor[1]	0.100	4.800 a	4.720 a	5.500 a	4.980 a
(SE)		(0.223)	(0.284)	(0.264)	(0.27)
Aftertaste[1]	0.235	4.940 a	5.200 a	5.460 a	5.020 a
(SE)		(0.199)	(0.249)	(0.222)	(0.267)
Overall Acceptance[1]	0.015	4.220 b	4.900 ab	5.200 a	5.180 a
(SE)		(0.251)	(0.309)	(0.283)	(0.29)
Color (raw) [1]	0.000	3.765 c	2.941 d	7.098 a	4.471 b
(SE)		(0.205)	(0.176)	(0.138)	(0.271)
Shape (raw) [1]	0.000	5.020 b	4.157 c	6.373 a	6.059 a
(SE)		(0.257)	(0.289)	(0.213)	(0.219)
Overall Appearance (raw) [1]	0.000	4.627 c	3.157 d	6.824 a	5.118 b

Table 6 (Continued)

Attribute Title	p-value	VitaRice O Mean	VitaRice D Mean	Sample I Mean	Sample H Mean
Color (cooked) [1]	0.000	4.804 b	4.922 b	6.706 a	4.745 b
(SE)		(0.224)	(0.200)	(0.178)	(0.255)
Shape (cooked) [1]	0.000	5.314 bc	4.922 c	6.588 a	5.706 b
(SE)		(0.227)	(0.244)	(0.180)	(0.294)
Overall Appearance (cooked) [1]	0.000	4.686 b	4.588 b	6.725 a	5.333 a
(SE)		(0.284)	(0.244)	(0.156)	(0.259)
Rank[3]	0.000	2.680 a	2.580 a	2.360 a	2.380 a
(SE)		(0.132)	(0.169)	(0.158)	(0.171)

[1] Values are scores on a 9-point hedonic scale in which 1 = dislike extremely and 9 = like extremely.

[2] Values are scores on a 5-point just-about-right (JAR) scale in which 1 = too soft and too dry for firmness and moistness respectively, 3 = just about right, and 5 = too firm and too moist for firmness and moistness respectively.

[3] Values are scores on a 4-point ranking scale in which 1 = most preferred and 4 = least preferred.

Table 7 Acceptability Ratings by Non-South Asian Consumer Panels: Mean, Standard Error (SE), and P-value (from two-way ANOVA for multiple comparisons, Friedman's test for Ranking). Letters following means indicate results of Tukey HSD tests, least-squared difference test for Ranking. Means with different letters within a row were different.

Attribute Title	p-value	VitaRice O Mean	VitaRice D Mean	Sample I Mean	Sample H Mean
Overall Aroma[1]	0.000	4.686 b	4.686 b	5.275 ab	5.686 a
(SE)		(0.209)	(0.247)	(0.241)	(0.230)
Firmness[1]	0.000	4.490 c	4.216 c	5.255 b	6.039 a
(SE)		(0.253)	(0.257)	(0.255)	(0.226)

Table 7 (Continued)

Attribute Title	p-value	VitaRice O Mean	VitaRice D Mean	Sample I Mean	Sample H Mean
Overall texture[1]	0.000	4.275 bc	3.922 c	5.020 b	5.902 a
(SE)		(0.266)	(0.280)	(0.251)	(0.243)
Firmness JAR[2]	0.000	3.608 a	2.686 b	3.725 a	3.431 a
(SE)		(0.154)	(0.191)	(0.116)	(0.106)
Moistness JAR[2]	0.002	2.333 bc	2.824 a	2.2353 c	2.6275 ab
(SE)		(0.133)	(0.165)	(0.117)	(0.088)
Flavor[1]	0.011	4.863 b	5.039 b	5.490 ab	5.784 a
(SE)		(0.244)	(0.254)	(0.214)	(0.254)
Aftertaste[1]	0.201	4.98 a	5.039 a	5.549 a	5.196 a
(SE)		(0.207)	(0.224)	(0.157)	(0.211)
Overall Acceptance[1]	0.000	4.451 b	4.431 b	5.353 a	5.843 a
(SE)		(0.265)	(0.272)	(0.225)	(0.251)
Color (raw) [1]	0.000	4.580 c	3.900 d	6.820 a	5.260 b
(SE)		(0.227)	(0.231)	(0.199)	(0.268)
Shape (raw) [1]	0.000	5.800 b	4.940 c	6.600 a	6.560 a
(SE)		(0.225)	(0.258)	(0.225)	(0.179)
Overall Appearance (raw) [1]	0.000	5.060 b	3.840 c	6.720 a	5.760 a
(SE)		(0.240)	(0.246)	(0.206)	(0.237)
Color (cooked) [1]	0.000	5.180 bc	4.680 c	6.880 a	5.680 b
(SE)		(0.230)	(0.272)	(0.184)	(0.255)
Shape (cooked) [1]	0.000	5.820 b	4.560 c	6.740 a	6.640 a
(SE)		(0.219)	(0.286)	(0.198)	(0.232)
Overall Appearance (cooked) [1]	0.000	5.180 c	4.300 d	6.880 a	6.200 b
(SE)		(0.242)	(0.276)	(0.187)	(0.260)

Table 7 (Continued)

Attribute Title	p-value	VitaRice O Mean	VitaRice D Mean	Sample I Mean	Sample H Mean
Rank[3] (SE)		2.863 a (0.137)	2.784 a (0.169)	2.412 ab (0.146)	1.941 b (0.144)

[1] Values are scores on a 9-point hedonic scale in which 1 = dislike extremely and 9 = like extremely.
[2] Values are scores on a 5-point just-about-right (JAR) scale in which 1 = too soft and too dry for firmness and moistness respectively, 3 = just about right, and 5 = too firm and too moist for firmness and moistness respectively.
[3] Values are scores on a 4-point ranking scale in which 1 = most preferred and 4 = least preferred.

Overall group differences were observed for firmness JAR, moistness JAR, color of raw kernels, and shape of raw kernels. For each attribute with a group difference, the South Asian group was observed to be more critical (rated all samples lower in the 9-point hedonic test and rated all samples farther away from "just about right" in the JAR test). The results for color and shape of raw rice were expected, as Asians consider appearance to have the highest relative importance of sensory characteristics for rice acceptance (Meullenet and others 1998). The South Asian group may have been more sensitive to the JAR scales because they are accustomed to eating different rice varieties than non-South Asians. Therefore, their standard for "just about right" may be higher.

Group interactions were observed in several attributes, demonstrating a difference between the groups' ratings for some but not all samples. The South Asian group rated the CONTROL sample lower for color of raw kernels, moistness, and shape. Additionally, the South Asian group was more critical of (gave a rating farther from "just about right" to) the CONTROL sample for firmness JAR. This group was also more critical of the parboiled commercial sample (LGPR) for appearance, firmness, firmness JAR, moistness, shape, texture, color of cooked kernels, and color of raw kernels. These results are consistent with previous studies with Asian consumers demonstrating a low measured preference for parboiled rice (preference rating between 3 and 3.5 compared to long grain rice at 5.6) (Meullenet and others 1998).

A lesser interaction was observed where the non-South Asian group was more critical than the South Asian group. The non-South Asian group was more critical of (gave lower ratings to) the extruded fortified sample (EF-ZAC) for firmness, moistness, and texture. It is expected that the South Asian group was not as critical of the texture attributes because texture is considered the attribute with the lowest relative importance for rice acceptance by Asian consumers (Meullenet and others 1998).

II. Micronutrient Retention

Vitamin A

Vitamin A concentrations in extruded fortified rice samples are presented in Table 8. All-*trans*-retinyl palmitate was found in the highest amount, and *cis*-retinyl was found in the lowest amount. A significant decrease in vitamin A occurred during extrusion. Of the six batches of experimental product manufactured, batches II - VI were measured for vitamin A retention following extrusion. Due to technical complications in the vitamin A analysis method, the first batch was not analyzed for vitamin A. Batch I samples had been stored several months while the HPLC instrument was repaired, and results would not have been accurate. The average vitamin A retention in extruded rice kernels from batches II – VI after drying was 48% compared to the fortified flour. Only batches IV and VI were available for analysis when the vitamin A methods for cooked rice were secured. The average vitamin A retention in cooked rice kernels (of EF-ZAC samples only) was 37% pre-extrusion samples.

Table 8 Mean, Standard Deviation (SD), and Mean Percent Retention of Vitamin A Concentrations of Fortified Samples (Analyses Performed in Triplicate)

	Sample	Mean (IU/g DWB)	SD (IU/g DWB)	Mean % retention
Batch II	EF-ZAC, pre-extrusion	4.37	0.60	100.0
	EF-ZAC, dried	1.65	0.07	38
Batch III	FEDTA-ZAC, pre-extrusion	11.87	0.69	100
	FEDTA-ZAC, dried	2.00	0.68	17
Batch IV	EF-ZAC, pre-extrusion	13.41	1.60	100
	EF-ZAC, dried	1.60	0.09	12
	EF-ZAC, cooked	4.33	1.08	32
	EF-ZAC/B, pre-extrusion	14.22	2.04	100
	EF-ZAC/B, dried	4.45	0.17	31
Batch V	EF-ZAC, pre-extrusion	6.83	0.54	100
	EF-ZAC, dried	3.20	0.41	47
	EF-ZAC/B, pre-extrusion	9.20	4.13	100
	EF-ZAC/B, dried	4.22	0.13	46

Table 8 (Continued)

	Sample	Mean (IU/g DWB)	SD (IU/g DWB)	Mean % retention
Batch VI	EF-ZAC, pre-extrusion	7.50	0.26	100
	EF-ZAC, dried	2.12	0.01	28
	EF-ZAC, cooked	3.52	0.25	47
	EF-ZAC/BRF, pre-extrusion	6.71	0.44	100
	EF-ZAC/BRF, dried	11.06	0.14	165
	EF-ZAC/BRF, cooked	17.3	0.58	258

Batches IV and VI contained samples of cooked rice kernels for the vitamin A analysis. Results showed a decrease in vitamin A content from fortified flour to extruded fortified kernels but an increase from the extruded kernels to cooked kernels. This observed increase in vitamin A content from the rice kernels to the cooked rice kernels may be due to variability in the extraction method.

EF-ZAC/BRF samples produced in Batch VI showed a dramatic increase in vitamin A levels from rice kernels to the cooked rice kernels. This trend seen in EF-ZAC/BRF may have been caused by the additional brown color of the added brown rice flour or browning that occurred to the flour in the sample. Brown color could cause increased peak area in the HPLC analysis. The brown rice flour may also have had some antioxidants or vitamin preserving agents to reduce the level of loss. Further investigation is required to confirm this observation.

Extrusion processing significantly ($p < 0.05$) reduced the vitamin A content in the samples of extruded fortified rice compared to fortified rice flour (pre-extrusion). Cooking did not appear to have a significant effect on the vitamin A content. HPLC results of all samples showed vitamin A concentrations below theoretical values (values expected based on fortification levels). This is most likely due to an extraction method that does not fully extract all vitamin A from the sample. A more thorough method may need to be used. The loss of vitamin A from pre-extrusion samples to dried rice samples was most-likely due to a heat treatment effect, although light and lipid oxidation also have a large effect. As seen in Figure 2, the relative reaction rate of lipid oxidation is very high at high water activity levels, such as those of rice flour in an extruder. The overall average of 48% retention is comparable to studies of vitamin stability and heat treatment observed in literature (previously mentioned).

In all batches, vitamin A levels in CONTROL samples for flour, rice kernels, and cooked rice kernels were observed to be virtually zero (Table 6).

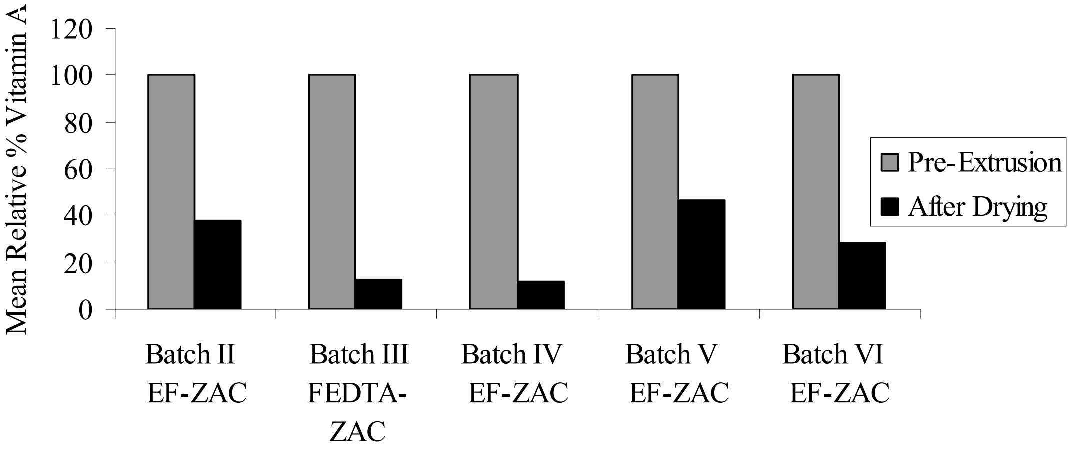

Figure 14 Vitamin A Mean Relative % Retention Pre-Extrusion and after Drying for Extruded Fortified Rice Samples

Vitamin C

Ascorbic acid concentrations were analyzed in all batches of the experimental product manufactured. Results of vitamin C assays are presented in Table 7. As similarly seen from the vitamin A assays, the stability of vitamin C appeared to be more affected by the extrusion process than cooking. The average vitamin C retention in extruded fortified rice after drying was 53% compared to fortified flour (pre-extrusion). Only batches IV and VI were available for analysis when the vitamin C methods for cooked rice were secured. The average vitamin C retention in cooked rice kernels was 48%, which was not significantly different from extruded kernels after drying ($p < 0.05$).

In batch I, the vitamin C levels were analyzed pre-extrusion, mid-extrusion, and post-extrusion in all samples manufactured. Comparisons of the relative percent retention of vitamin C in samples taken from Batch I are presented in Figure 15. Vitamin C assays demonstrated loss with each sampling phase. A great deal of the ascorbic acid was lost in the pre-conditioning stage of the extrusion process (between pre-extrusion and mid-extrusion) and further lost through the extruder barrel.

Levels of vitamin C were shown to be higher for FS-ZAC samples than EF-ZAC samples. This is most likely due to the interaction of $FeSO_4$ with the titration dye, causing a falsely high reading in the ascorbic acid analysis method. Vitamin C levels were virtually zero for EF-Z samples, which were not fortified with vitamin C, however FS-Z (which was also not fortified with vitamin C) was observed to have trace amounts due to the falsely high reading from $FeSO_4$. FS-Z was used as a basis to normalize the levels of vitamin C found in FS-ZAC to account for any interaction effects of $FeSO_4$. When FS-ZAC samples were normalized, the vitamin C levels pre-extrusion were not significantly different from the theoretical value (the value based on the fortification level of vitamin C).

Subsequent batches of extruded fortified rice samples showed similar trends in vitamin C loss to that of Batch I. Comparisons of the relative percent retention of vitamin C in samples from Batches II, III, IV, V, and V are presented in Figure 16. The trends show that very little vitamin C was lost through the cooking process, but most vitamin C was destroyed during extrusion, drying, and storage before analysis.

The measured vitamin C levels for pre-extrusion were not statistically different from the expected concentration of 77.78 mg vitamin C/kg DWB. In all batches, vitamin C levels in CONTROL samples for pre-extrusion, after drying, and cooked rice kernels were observed to be virtually zero, except in FS-Z samples, which showed small amounts of vitamin C, a falsely high reading due to interaction from iron sulfate.

Table 9 Mean, Standard Deviation (SD), and Mean Percent Retention of Ascorbic Acid in Extruded Fortified Rice Samples

	Sample	Mean (µg/g DWB)	SD (µg/g DWB)	Mean % retention
Batch I	FS-ZAC pre-extrusion	1.03	0.09	100
	FS-ZAC mid-extrusion	0.90	0.10	87
	FS-ZAC post-extrusion	0.30	0.04	29
	FS-Z pre-extrusion	0.07	0.03	100
	FS-Z mid-extrusion	0.08	0.05	118
	FS-Z post-extrusion	0.01	0.01	16
	EF-Z pre-extrusion	0.01	0.01	100

Table 9 (Continued)

	Sample	Mean (µg/g DWB)	SD (µg/g DWB)	Mean % retention
	EF-Z mid-extrusion	0.00	0.00	13
	EF-Z post-extrusion	0.01	0.01	45
	EF-ZAC pre-extrusion	0.99	0.09	100
	EF-ZAC mid-extrusion	0.69	0.03	69
	EF-ZAC post-extrusion	0.44	0.02	44
Batch II	EF-ZAC pre-extrusion	926.60	161.20	100
	EF-ZAC after drying	276.15	52.40	30
	EF-ZAC/B pre-extrusion	785.21	53.10	100
	EF-ZAC/B after drying	457.00	223.00	65
Batch III	FEDTA-ZAC pre-extrusion	805.40	29.57	100
	FEDTA-ZAC after drying	318.10	0.83	39
Batch IV	EF-ZAC pre-extrusion	820.43	57.44	100
	EF-ZAC after drying	325.76	48.42	40
	EF-ZAC after cooking	252.59	20.03	28 [1]
	EF-ZAC/B pre-extrusion	821.79	39.07	100
	EF-ZAC/B after drying	645.96	21.62	22

Table 9 (Continued)

	Sample	Mean (μg/g DWB)	SD (μg/g DWB)	Mean % retention
Batch V	EF-ZAC pre-extrusion	821.18	213.10	100
	EF-ZAC after drying	581.96	14.80	71
	EF-ZAC/B pre-extrusion	858.21	64.40	100
	EF-ZAC/B after drying	614.71	69.10	72
Batch VI	EF-ZAC pre-extrusion	821.43	161.30	100
	EF-ZAC after drying	469.86	48.34	57
	EF-ZAC after cooking	464.89	38.34	57
	EF-ZAC/BRF pre-extrusion	751.91	56.33	100
	EF-ZAC/BRF after drying	577.53	31.14	77
	EF-ZAC/BRF after cooking	448.25	7.21	60

[1] Percentage compared to 896.0 μg/g

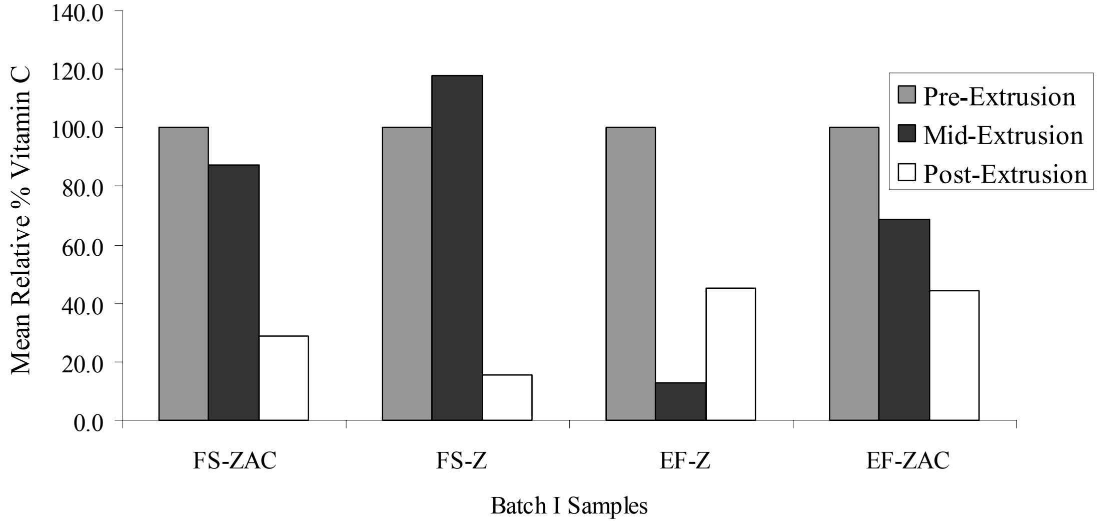

Figure 15 Vitamin C Mean Relative Percent Retention Pre-, Mid-, and Post-Extrusion

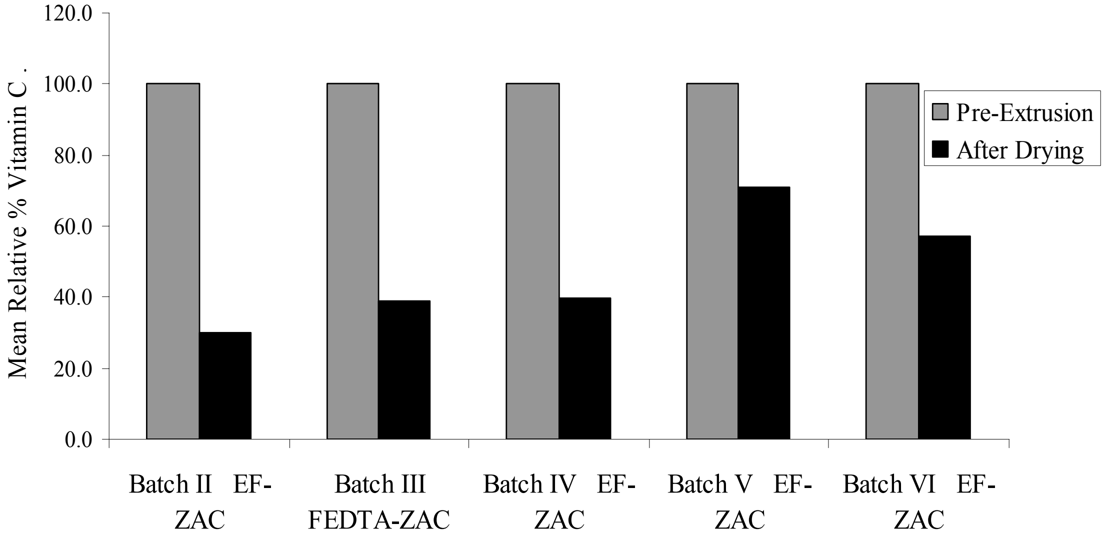

Figure 16 Vitamin C Mean Relative Percent Retention Pre-Extrusion and After Drying for Extruded Fortified Rice Samples

Iron and Zinc

In Batch I, iron and zinc contents of fortified flour (pre-extrusion) and extruded fortified rice (rice after drying) were relatively similar, as expected. However, some loss was observed in successive batches. Iron and zinc retentions both averaged 84% for rice kernels after extrusion (cooked and non-cooked data combined) compared to fortified flour.

Mean iron and zinc concentrations are presented in Tables 10 and 11 respectively. In batch I, retention of both iron and zinc was very high. These results were expected because iron and zinc are not as sensitive to heat, light, and oxygen as the vitamins in the experimental product.

Table 10 Mean, Standard Deviation (SD), and Mean Percent Retention of Iron

	Sample	Mean iron (µg/g DWB)	SD (µg/g DWB)	Mean % retention
Batch I	FS-ZAC, Pre-Extrusion	230.80	3.75	100
	FS-ZAC, After Drying	232.60	1.20	101
	FS-Z, Pre-Extrusion	241.90	2.97	100
	FS-Z, After Drying	240.40	4.83	100
	EF-Z, Pre-Extrusion	258.20	5.19	100
	EF-Z, After Drying	247.90	3.67	96
	EF-ZAC, Pre-Extrusion	252.40	5.19	100
	EF-ZAC, Pre-Extrusion	253.80	7.48	101

Table 10 (Continued)

	Sample	Mean iron (µg/g DWB)	SD (µg/g DWB)	Mean % retention
Batch II	EF-ZAC, Pre-Extrusion	216.80	2.64	100
	EF-ZAC, After Drying	165.20	3.40	76
Batch IV	EF-ZAC, Pre-Extrusion	205.08	2.53	100
	EF-ZAC, After Drying	124.53	4.79	61
	EF-ZAC, Cooked	121.99	11.60	60
Batch V	EF-ZAC, Pre-Extrusion	210.90	8.91	100
	EF-ZAC, After Drying	165.60	5.41	79

Table 11 Mean, Standard Deviation (SD), and Mean Percent Retention of Zinc

	Sample	Mean (µg/g DWB)	SD (µg/g DWB)	Mean % retention
Batch I	FS-ZAC, Pre-Extrusion	167.60	6.79	100
	FS-ZAC, After Drying	167.60	0.64	100
	FS-Z, Pre-Extrusion	169.30	2.13	100
	FS-Z, After Drying	168.80	2.48	100
	EF-Z, Pre-Extrusion	186.00	1.49	100.0
	EF-Z, After Drying	183.70	2.42	99

Table 11 (Continued)

	Sample	Mean (µg/g DWB)	SD (µg/g DWB)	Mean % retention
Batch I	EF-ZAC, Pre-Extrusion	181.60	2.15	100
	EF-ZAC, After Drying	177.80	0.70	98
Batch II	EF-ZAC, Pre-Extrusion	153.30	1.03	100
	EF-ZAC, After Drying	100.94	0.37	66
Batch IV	EF-ZAC, Pre-Extrusion	154.40	0.50	100
	EF-ZAC, After Drying	104.00	0.56	67
	EF-ZAC, Cooked	95.06	6.38	62
Batch V	EF-ZAC, Pre-Extrusion	164.65	4.66	100
	EF-ZAC, After Drying	129.66	2.07	79

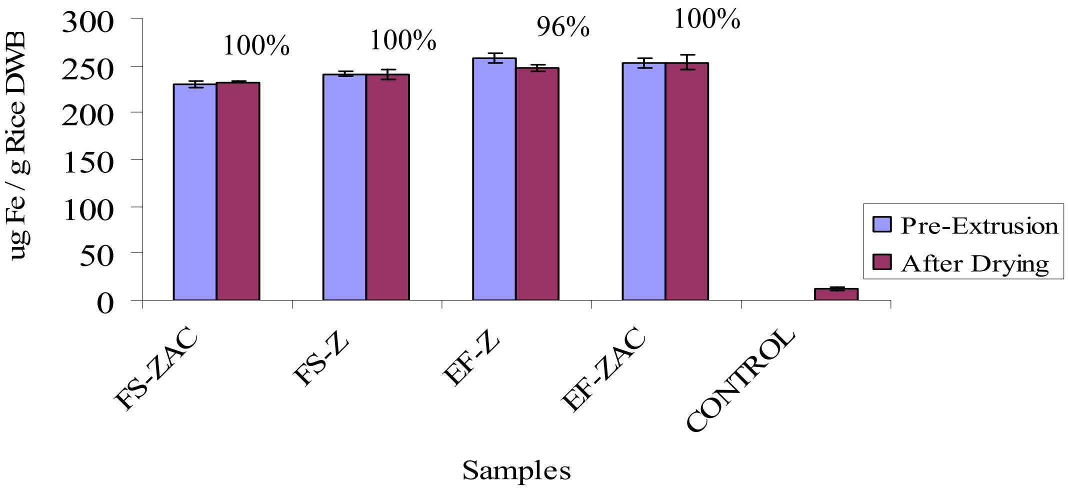

Figure 17 Relative Percent Iron Retention Compared to Pre-Extrusion and Iron Content in Extruded Fortified Rice (Pre-Extrusion and After Drying)

In batches II - VI, a significant decrease in minerals was observed between pre-extrusion and after drying samples. This observed loss of iron and zinc could be attributed to a settling of minerals in the extruder or pre-conditioner, causing the extruded samples to contain a lower concentration of minerals. Another speculation is that the loss is due to sampling extruded rice kernels that were not a representative sample. In other words, the flour sample was a homogenous mixture of rice flour, vitamins, and minerals, while the rice kernel samples contained a greater portion of rice flour. For future studies, a thorough mixing process must be implemented prior to extrusion to ensure representative samples.

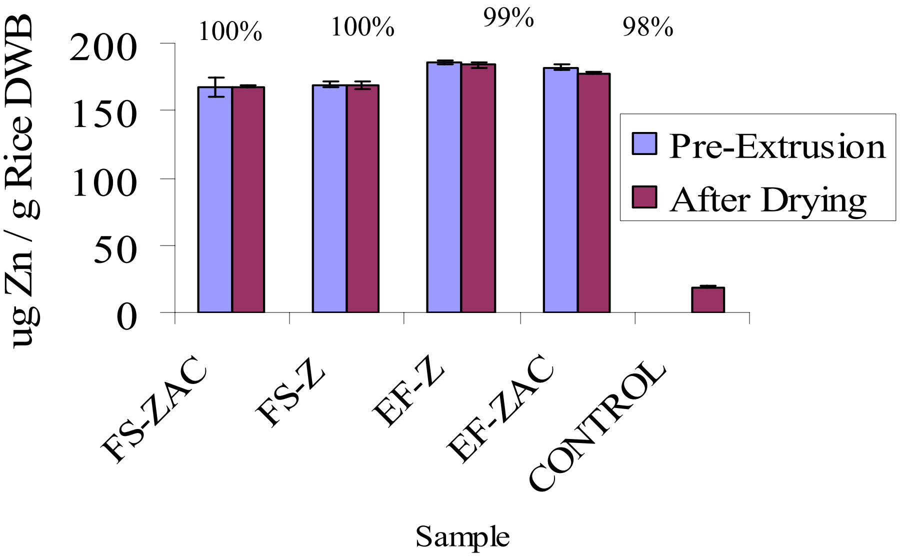

Figure 18 Relative Percent Zinc Retention Compared to Pre-Extrusion and Zinc Content in Extruded Fortified Rice (Pre- Extrusion and After Drying)

CHAPTER FOUR

FUTURE RESEARCH

While extrusion technology offers an innovative alternative to traditional methods of fortification of rice, it is evident that many hurdles must be overcome before the technology can be applied and implemented in real-life situations. Some suggestions for the continuation of research include optimizing the experimental product to produce a higher acceptability score, as well as examining shelf-life and degradation of rice during storage (oxidation, spoilage microorganisms) organoleptically and nutritionally. The shelf-life studies could also be conducted using extreme storage conditions (40°C, 80% humidity) similar to those existing in India.

Areas for improvement include changes to the processing method, analysis methods, and sensory methods. First, the formulation and processing method should be optimized to achieve more desirable sensory attributes. Formulation improvement would require the development of many different prototypes and a series of sensory tests on these samples. The formulas could include ingredients to improve texture and appearance attributes. Several changes could be made to improve processing. Particularly, changes could be made to the extrusion die such as coating it with Teflon or changing the shape of holes in the die. Teflon may reduce shear on the rice, leading to a less sticky product, and improved hole shapes may create a more realistic appearance for the rice kernels.

Suggestions were mentioned throughout regarding improvements in the vitamin analysis methods. Namely, a more efficient extraction method should be used for vitamin A extraction. In the vitamin A analysis, saponification of rice grains may have given a more effective extraction. Saponification, because of its severity, is considered to be an adequate extraction to break the rice kernel matrix (Lee and others

2000). Repeatability and reproducibility of the vitamin and mineral assays should also have been assessed for the fortified rice kernels, as was performed by Lee and others (2000). Beta-hydroxyanisole (BHA) and beta-hydroxytoluene (BHT) are potential additives that could stabilize retinyl palmitate, as was done by Favaro and others (1991). All trans-retinyl palmitate stabilized with BHA and BHT is a recommended form of vitamin A for the fortification or enrichment of foods (Favaro and others 1991).

In sensory evaluation of the product, the use of a food action rating scale may offer more insight as to whether consumers would accept the product (Lawless, personal contact, 6/9/05). A food action rating scale is a behaviorally-oriented method of measuring food acceptability (Lawless and Heymann 1999). Because the sensory testing facility is not the same as the environment in which a consumer will be using the food, a food action rating scale can be used to demonstrate a consumer's actual behavior in different contexts. Additionally, presenting the nutrition labels or concept information to consumers in a market research study may offer additional information and direction for the product.

Information regarding influences on the acceptability of rice is offered by Del Mundo and Juliano (1981). For example, amylose content and cooking style may affect consumer preference (Del Mundo and Juliano 1981). Amylose can be controlled in the choice of rice flour. A variety of cooking methods can be tested and optimized for delivering high rice quality. Additionally, the palatability of rice is affected by cooking time, amount of water used in cooking, amount of starch in the rice, and the consistency of the rice after it is cooked (Schutz and Damrell 1974).

CHAPTER FIVE

CONCLUSION

In summary, vitamin and mineral analyses demonstrated 48% retention in vitamin A for extruded fortified rice kernels after extrusion, 52% vitamin C retention in extruded fortified rice kernels after extrusion, and 84% iron and zinc retentions in extruded fortified rice kernels after extrusion (both cooked and non-cooked data combined). The vitamin retentions are consistent with studies on vitamin retention in extruded products. However, mineral retention is lower than expected. Larger sample sizes, consistency in analysis methods, and more research are needed to draw further conclusions.

The results of the sensory evaluation suggested a greater acceptability by consumer panelists for commercial rice samples. The most sizable difference was seen in the appearance attributes, followed by overall acceptance and aroma. The addition of vitamins and minerals to extruded rice did not appear to have a large effect on sensory acceptability, with the exception of several appearance attributes. Group differences and group interactions were observed. The South Asian panelist group gave lower scores in several attributes, and the group was generally more critical of (gave lower ratings to) the CONTROL extruded sample and the parboiled commercial sample (LGPR) than the non-South Asian group.

The scope of knowledge surrounding fortification is ever-growing, and further studies are necessary to enhance and expand micronutrient relief in India and developing countries with outstanding public health crises. Research is needed to address fortification and analysis methods that are both effective and efficient. Fortification and extrusion, as well as other innovative methods of rice fortification, could address many of the health concerns in developing countries. However, as seen, the challenges in micronutrient retention, acceptability, and bioavailability of micronutrients used for fortification need to be overcome before commercialized utilization can take place.

APPENDIX

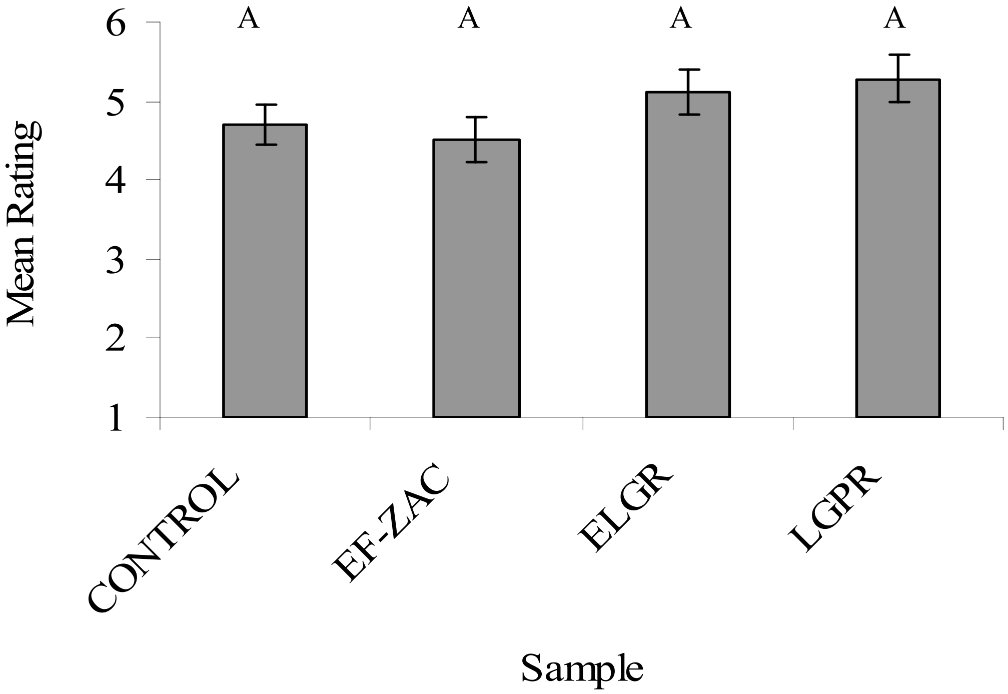

Figure A1 Mean Ratings for Aroma by South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, $p \leq 0.05$)

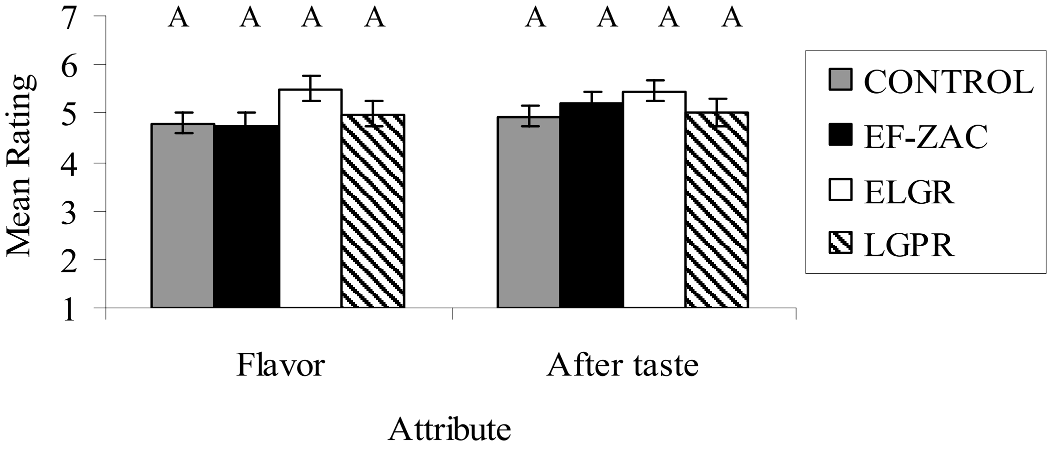

Figure A2 Mean Ratings for Flavor and Aftertaste by South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

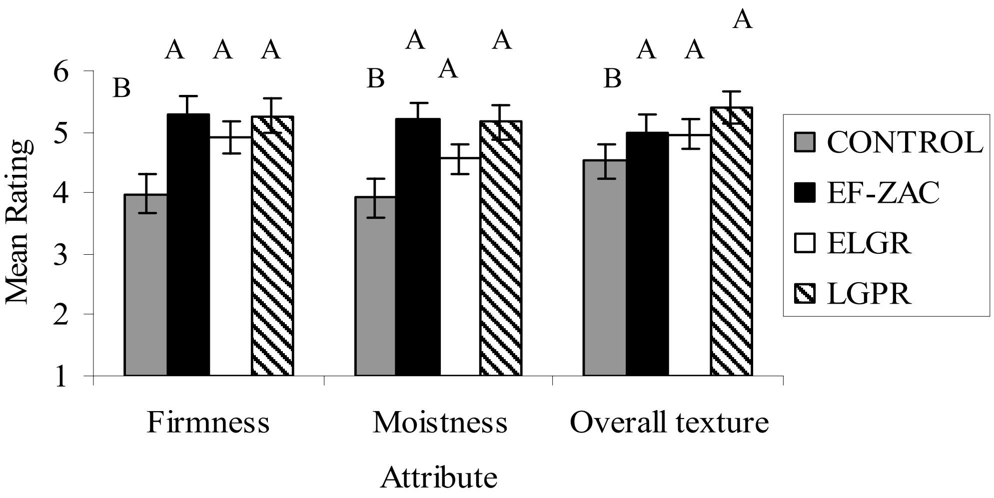

Figure A3 Mean Ratings for Firmness, Moistness, and Overall Texture by South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

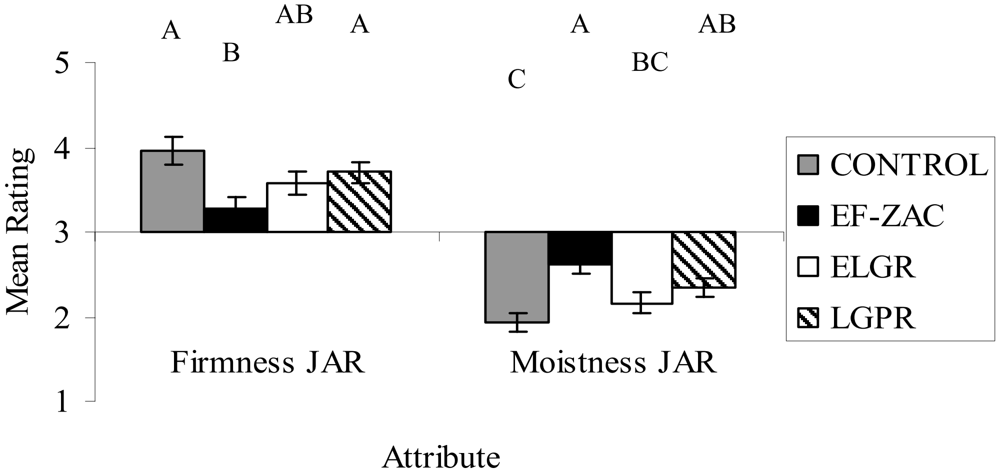

Figure A4 Mean Just-About-Right Ratings for Firmness and Moistness by South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

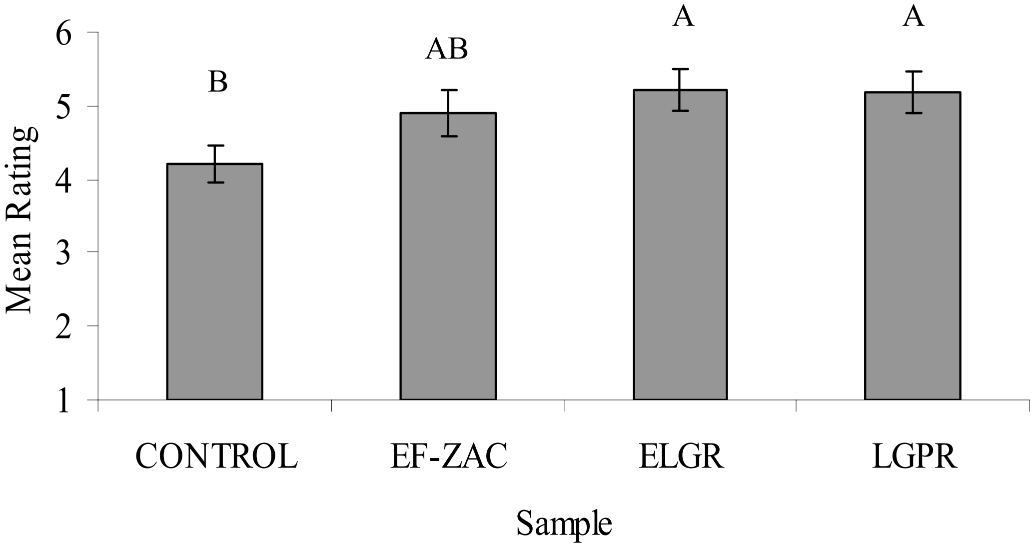

Figure A5 Mean Ratings for Overall Acceptance by South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, p ≤ 0.05)

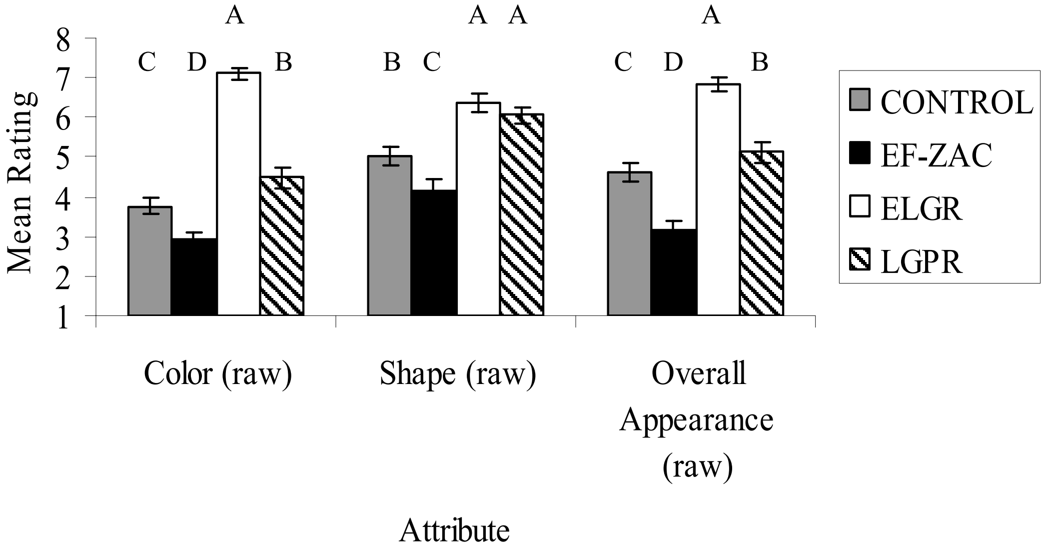

Figure A6 Mean Ratings for Appearance Attributes by South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

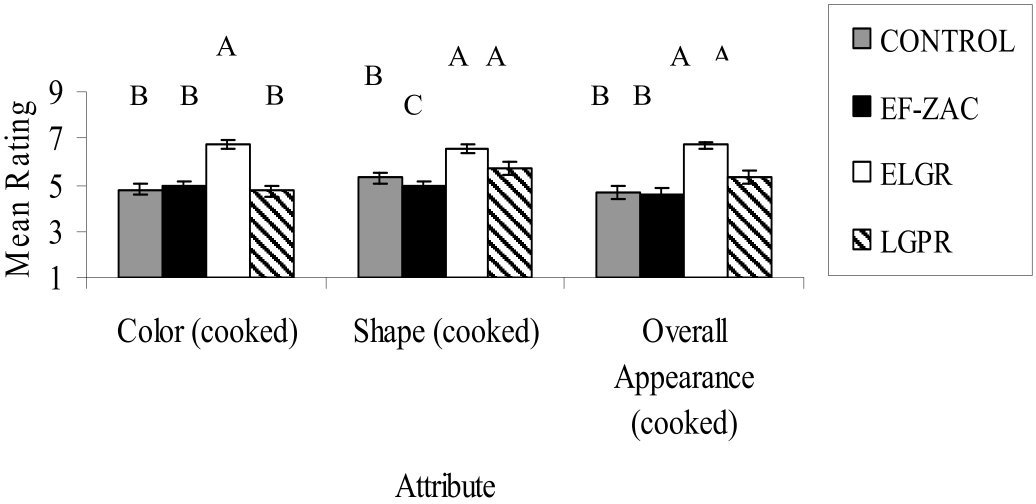

Figure A7 Mean Ratings for Appearance Attributes by South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

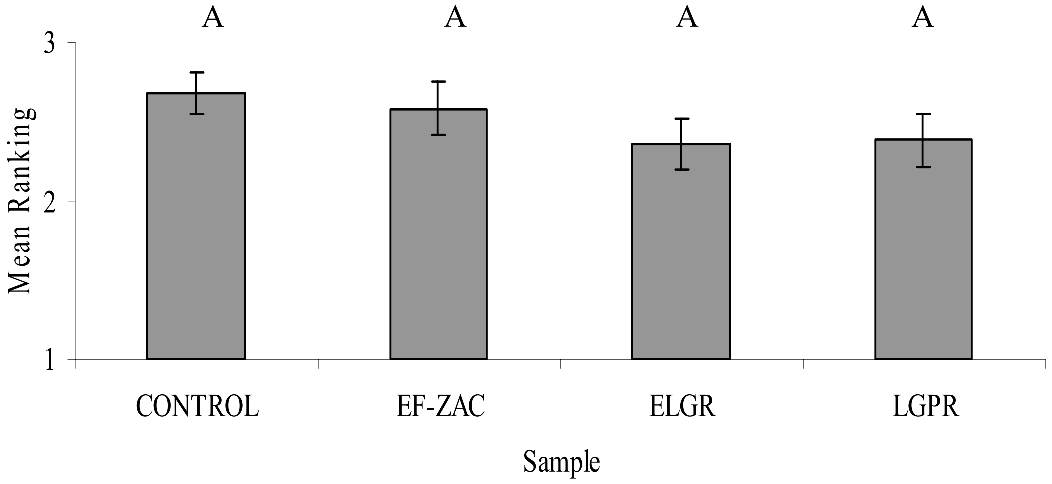

Figure A8 Mean Rankings for Samples by South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, p ≤ 0.05)

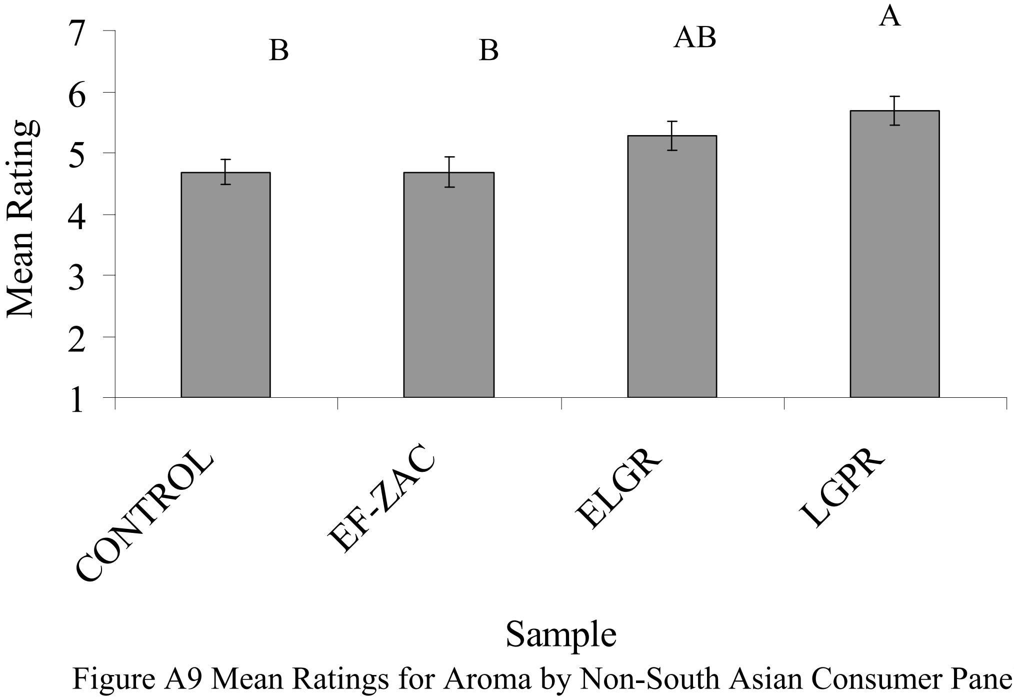

Figure A9 Mean Ratings for Aroma by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, p ≤ 0.05)

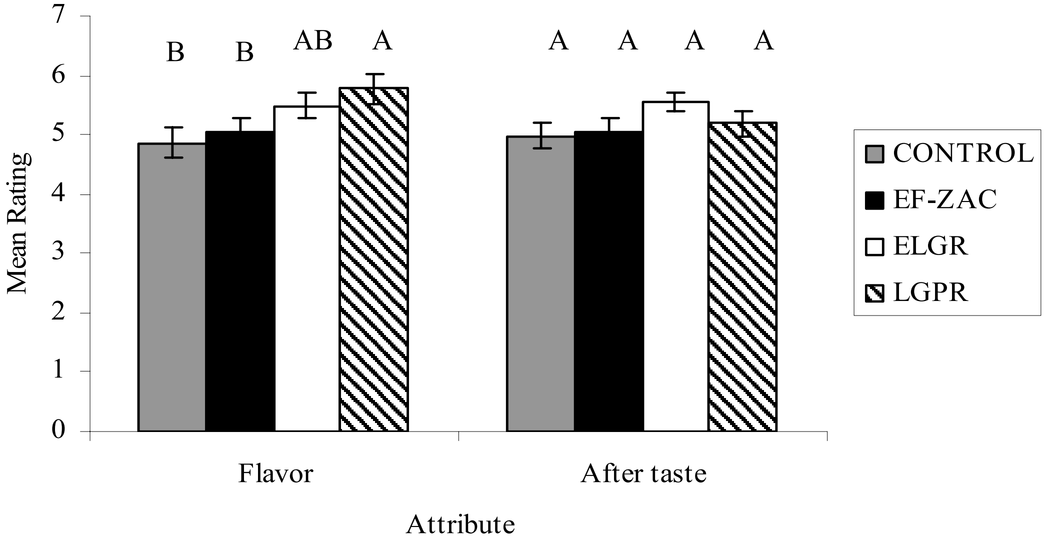

Figure A10 Mean Ratings for Flavor and Aftertaste by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

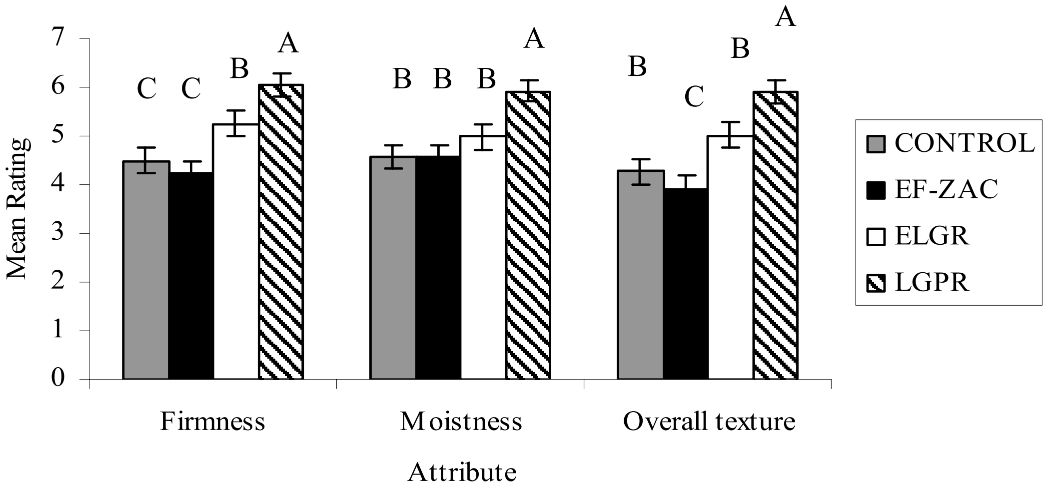

Figure A11 Mean Ratings for Firmness, Moistness, and Overall Texture by Non- South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

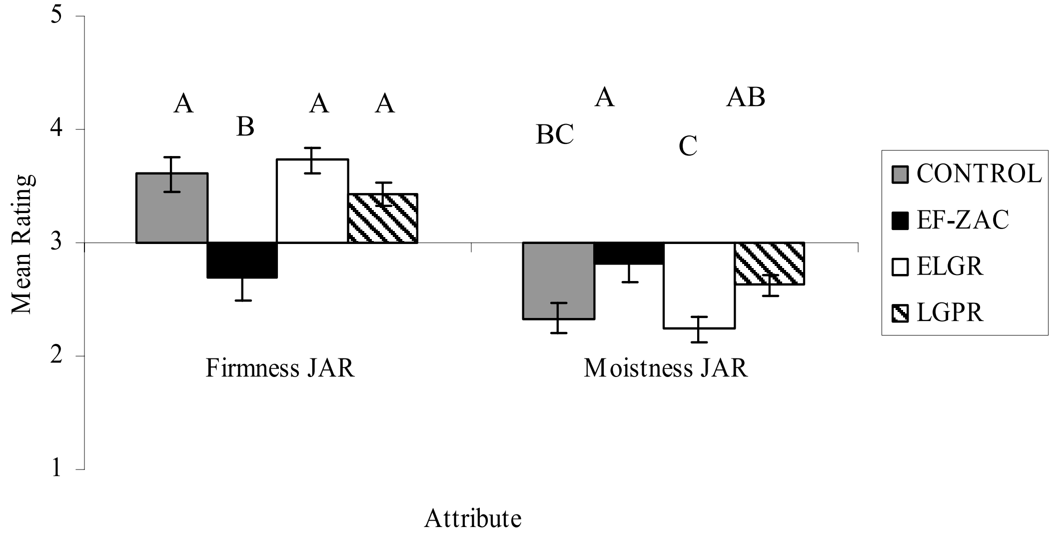

Figure A12 Mean Just-About-Right Ratings for Firmness and Moistness by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, p ≤ 0.05)

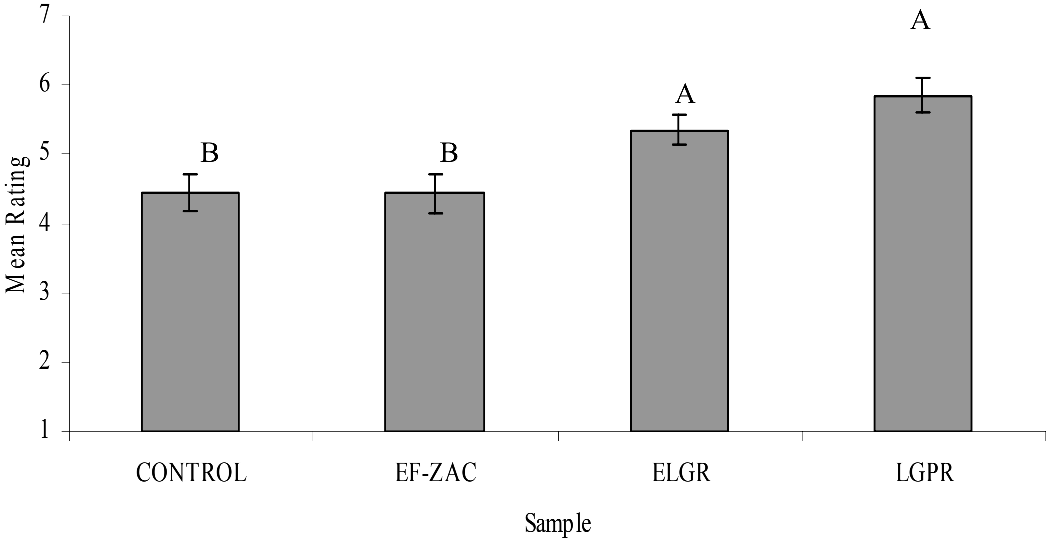

Figure A13 Mean Ratings for Overall Acceptance by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, p ≤ 0.05)

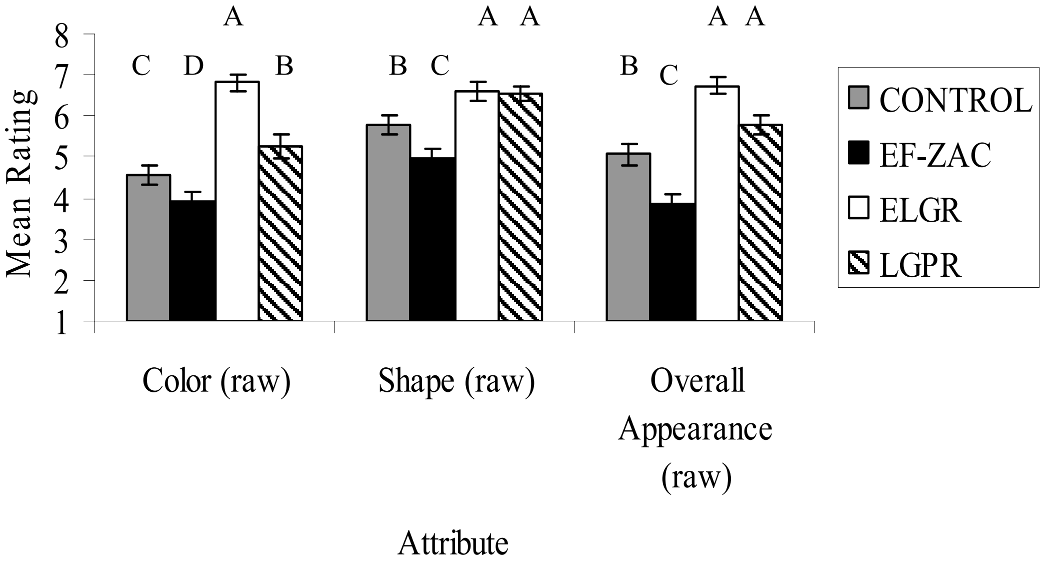

Figure A14 Mean Ratings for Appearance Attributes of Raw Samples by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, $p \leq 0.05$)

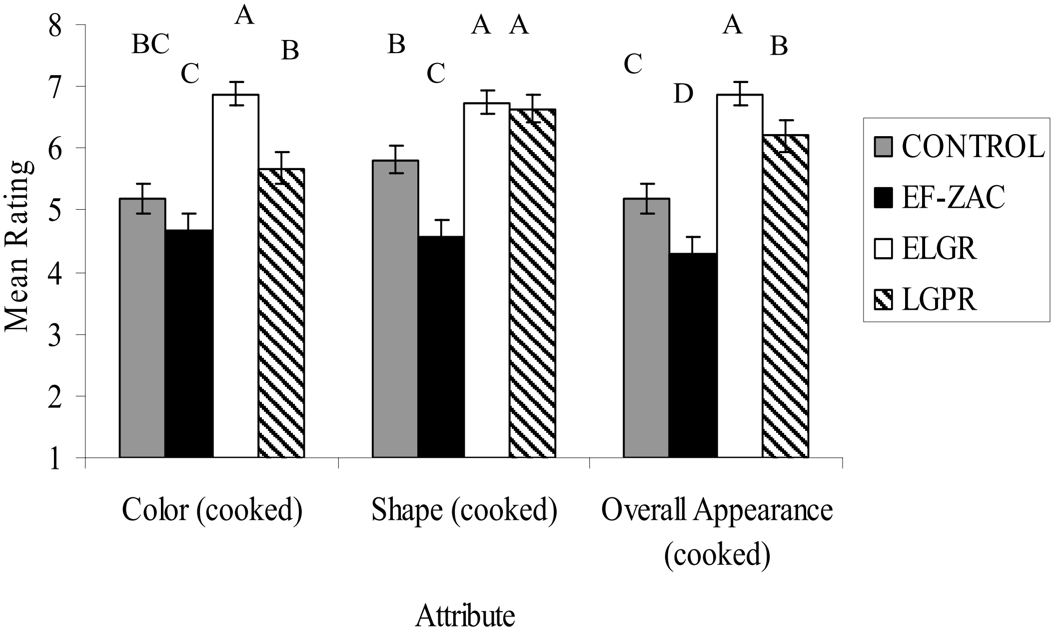

Figure A15 Mean Ratings for Appearance Attributes of Cooked Samples by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters within a group were significantly different. (Tukey HSD, $p \leq 0.05$)

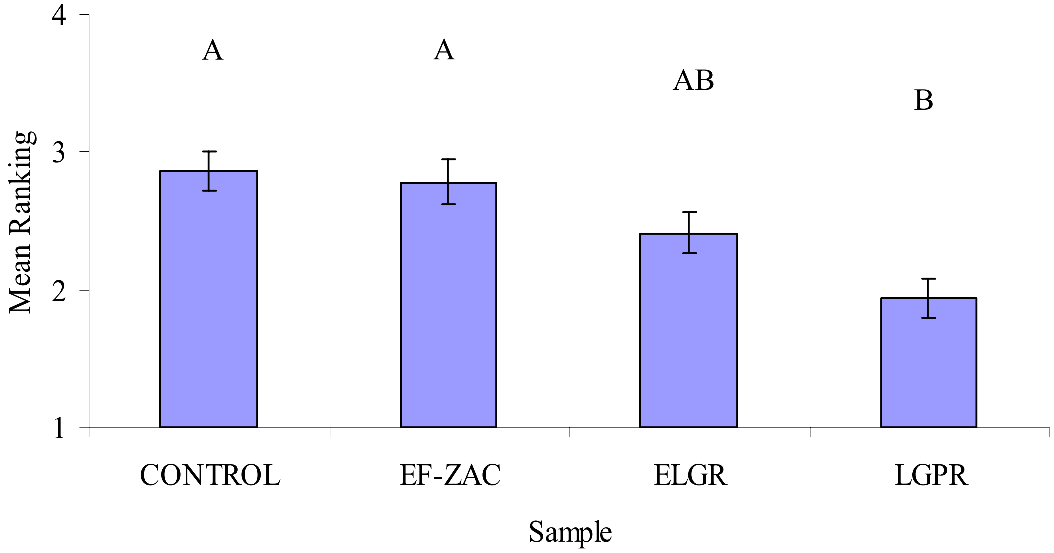

Figure A16 Mean Rankings for Samples by Non-South Asian Consumer Panels. Error bars represent standard error. Means with different letters were significantly different. (Tukey HSD, $p \leq 0.05$)

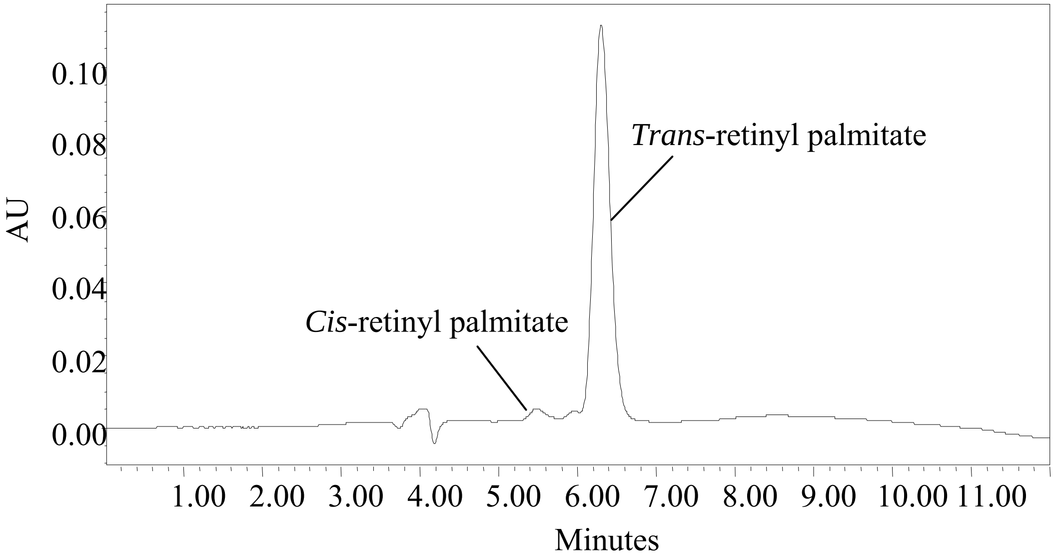

Figure A17 Chromatogram of Retinyl Palmitate Extracted from Sample EF-ZAC, Batch V, pre-extrusion

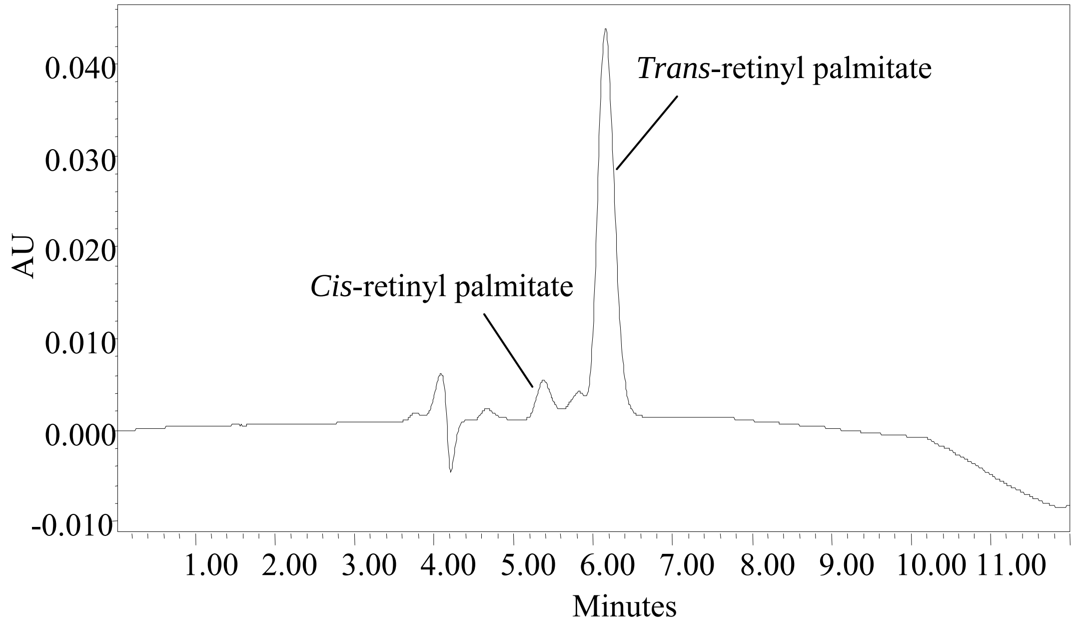

Figure A18 Chromatogram of Retinyl Palmitate Extracted from EF-ZAC, Batch V, Dried

Panelist number_____

INFORMED CONSENT

The purpose of this study is to investigate the sensory properties of different rice varieties. If you agree to be in this study, there will be a series of acceptability tests (you will rate characteristics of rice products on a 9-point scale of liking).

There should be no ill effects from the samples. Water will be provided to rinse your mouth. We do not anticipate any risks for you participating in this study, other than those encountered in day-to-day life. There are no direct benefits to participating. Indirect benefits to participation include contribution to knowledge in the field of Sensory Evaluation. You will be paid $3 for participating in this one-day study.

Your participation is strictly voluntary. You have the right to leave the study at any time you wish, without any penalty or hard feelings. You may skip any questions you don't feel comfortable answering. Such a decision will not influence any other relationship that you may have to the sensory staff in any way.

There are no right or wrong answers in these tests. It is your perceptions about the flavor and texture of rice that we are interested in. Your personal data will never be displayed in any presentation or publication with your identity revealed by name or initials.

The researcher conducting this study is Jessica Hof. Please ask any questions you have about the study at this time. If you have questions later, you may contact her at 607-255-7901, 216 Stocking Hall, jrh36@cornell.edu. If you have any further questions or concerns about your rights as a subject of the study, please contact the University Committee on Human Subjects (UCHS) at 607-255-5138, or access their website at http://www.osp.cornell.edu/Compliance/UCHS/homepageUCHS.htm.

Statement of Consent: I have read the above information, and have received answers to any questions I asked. I consent to participate in the study.

Name (Print)____ Email_____________-
____

Signature____
Date______________________________________

The consent form will be kept by the researcher for at least three years beyond the end of the study and was approved by the UCHS on [date].

Figure A19 Informed Consent Form Sample

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