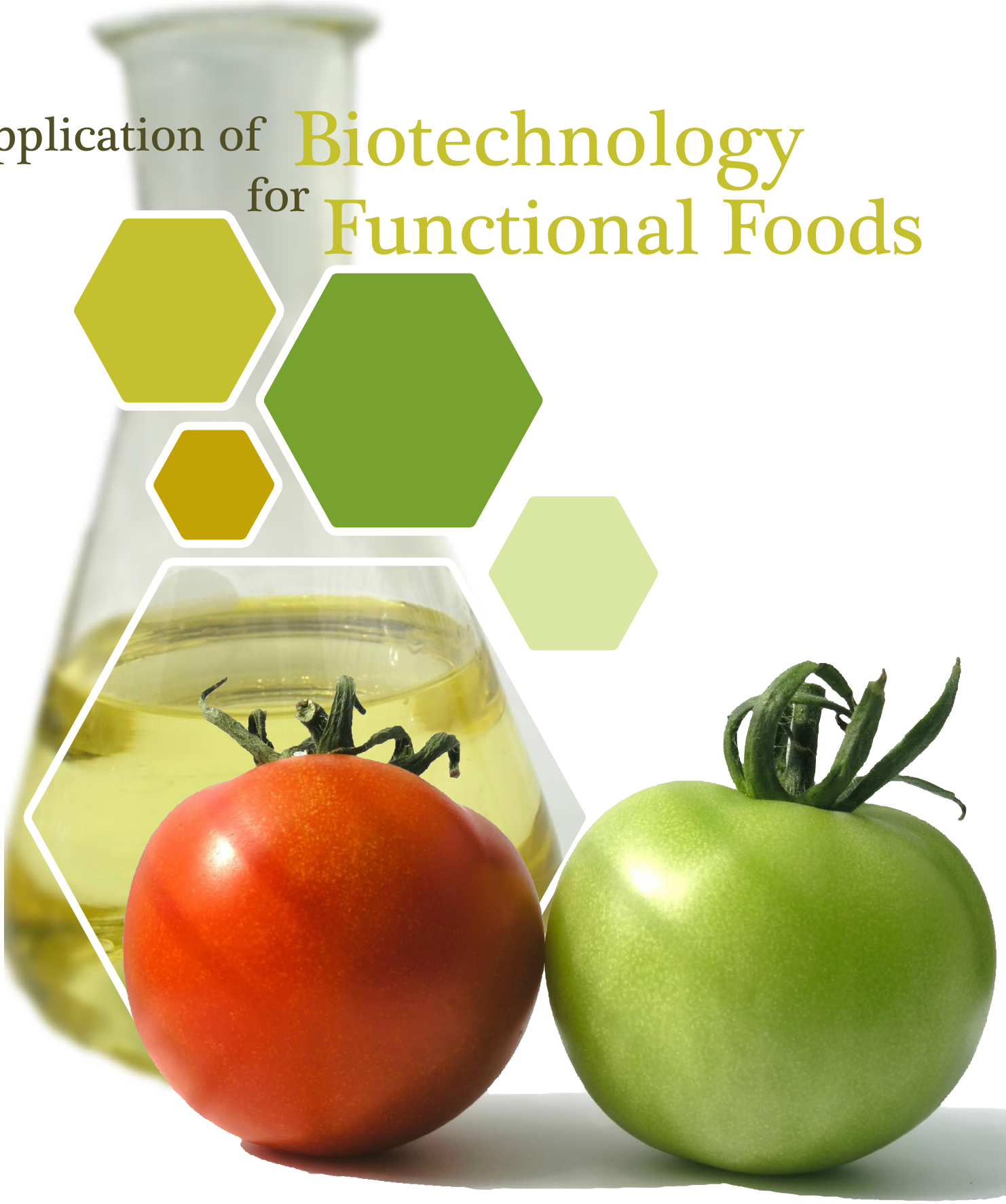
Pew Initiative on Food and Biotechnology

Application of Biotechnology for Functional Foods



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Preface

Since the earliest days of agricultural biotechnology development, scientists have envisioned harnessing the power of genetic engineering to enhance nutritional and other properties of foods for consumer benefit. The first generation of agricultural biotechnology products to be commercialized, however, were more geared towards so-called *input traits*, genetic modifications that make insect, virus and weed control easier or more efficient. These first products have been rapidly adopted by U.S. farmers, and now account for the majority of soybeans, cotton and corn grown in the United States.

Agricultural biotechnology innovations aimed directly towards consumers, sometimes collectively referred to as *output traits*, have been a longer time in development. As the technology advances, and we learn more about the genes and biochemical pathways that control those attributes that could offer more direct consumer benefits, the long-awaited promise of genetically engineered food with more direct consumer benefits moves closer to reality.

One category of potential products aimed at consumers is those products with added health benefits, also known as "functional foods." The term functional food means different things to different people, but generally refers to foods that provide health benefits beyond basic nutrition.

This report looks at the potential to develop functional foods through the application of modern biotechnology. The first section describes some recent scientific advances that could lead to functional foods on grocery store shelves, and the second section analyzes the legal authorities that could govern the use of biotechnology-derived functional foods.

The range of work being done on functional foods described in this report—from oils that product no trans fats or contain heart healthy omega-3 fatty acids, to cassava with increased protein content to help fight malnutrition in developing nations, to foods with enhanced levels of antioxidants—is impressive. This report is not intended to be an exhaustive catalog, however, but is rather a snapshot in time to give readers a sense of the kinds of products that may one day be available.

It should also be noted that much of the work described here is still in preliminary stages, and may never make its way into consumer products for technical, economic or other reasons.

The analysis of relevant statutory authorities suggests that there is ample legal authority to cover the kinds of functional foods currently being explored in laboratories, but that different authorities may come into play for different kinds of foods and that the application of different authorities can have significant consequences for product developers, food manufacturers and consumers. Different authorities impose different safety and labeling standards, have different requirements for regulatory review and clearance or approval, and could result in different levels of transparency to the public. The use of modern biotechnology to produce functional foods will not likely fundamentally challenge existing regulatory structures, but may challenge the boundaries of some regulatory classifications.

The Pew Initiative on Food and Biotechnology's first report, *Harvest on the Horizon* (2001), provided a broad overview of what could be the "next generation" of genetically engineered agricultural products. It is fitting that this, the last of the Initiative's reports, turns again to look at a category of new products on the horizon.

We would like to acknowledge the contributions of Joyce A. Nettleton, who created the scientific review used in the development of this paper; and of Edward L. Korwek, for the review of regulatory authorities that could govern future functional foods.

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Executive Director
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PART 1 Applications of Modern Biotechnology to Functional Food

Applications of Biotechnology for Functional Foods

I. BACKGROUND

A. Functional Foods

A relatively recent concept in the U.S. to describe the broad healthfulness of foods is the term "functional foods." These foods are defined as foods that provide health benefits beyond basic nutrition (International Food Information Council 2004). The Food and Nutrition Board of the National Academy of Sciences described a functional food as, "any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrients it contains" (Food and Nutrition Board 1994). The original concept of functional foods originated in Japan from its development of a special seal to denote Foods for Specified Health Use (FOSHU). More than 270 foods have FOSHU status in Japan. Foods qualify as "functional foods" because they contain non-essential substances with potential health benefits. Examples of the diverse foods and their bioactive substances that are considered "functional foods" are: psyllium seeds (soluble fiber), soy foods (isoflavones), cranberry juice (proanthocyanidins), purple grape juice (resveratrol), tomatoes (lycopene), and green tea (catechins). The broad classification of functional foods carries some irony, as John Milner, Chief of the Nutrition Science Research Group at the National Cancer Institute noted, "It is unlikely that a non-functional food exists."

Bioactive components of functional foods may be increased or added to traditional foods through genetic engineering techniques. An example would be the high lycopene tomato, a genetically modified tomato with delayed ripening characteristics that is high in lycopene, which has potent antioxidant capabilities. This report focuses on biotechnology applications in functional and improved foods, using the National Academy of Sciences definition as a guideline.

B. Applications of Biotechnology in Food Crops

In 1990, the U.S.Food and Drug Administration (FDA) approved the first genetically engineered food ingredient for human consumption, the enzyme chymosin, used in cheese-making. It is estimated that today 70% or more of cheese made in the U.S. uses genetically engineered chymosin. The first genetically engineered food, the FlavrSavr™ tomato, was approved for human consumption in the U.S. in 1994.

C. Transgenic Acreage Expands Steadily

Seven million farmers in 18 countries now grow genetically engineered crops. Leading countries are the U.S., Argentina, Canada, Brazil, China, and South Africa. Cultivation of genetically engineered crops globally has expanded more than 10% per year for the past seven years, according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA, James 2004). Such an expansion rate amounts to a 40-fold increase in the global area of transgenic crops from 1996 to 2003. Thus, in spite of continuing controversy, the technology continues to be adopted by farmers worldwide. ISAAA highlighted its key findings this way:

> In 2003, GM crops were grown in 18 countries with a combined population of 3.4 billion, living on six continents in the North and the South: Asia, Africa and Latin America, and North America, Europe and Oceania…. the absolute growth in GM crop area between 2002 and 2003 was almost the same in developing countries (4.4 million hectares) and industrial countries (4.6 million hectares) … the three most populous countries in Asia—China, India, and Indonesia, the three major economies of Latin America—Argentina, Brazil and Mexico, and the largest economy in Africa, South Africa, are all officially growing genetically engineered crops.

The leading genetically engineered crops globally and in the U.S. are soy, maize (corn), cotton, and canola. In the U.S., transgenic virus-resistant papaya and squash are also cultivated.

D. Agronomic Traits Prevail

Research in plant biotechnology has focused primarily on agronomic traits—characteristics that improve resistance to pests, reduce the need for pesticides, and increase the ability of the plant to survive adverse growing conditions such as drought, soil salinity, and cold. Biotechnology traits developed and commercialized to date have largely focused on pest control (primarily Bt crops) or herbicide resistance. Many plant pests have proven either difficult or uneconomical to control with chemical treatment, traditional breeding, or other agricultural technologies and in these instances in particular, biotechnology has proven to be an effective agronomic tool. Herbicide resistance allows farmers to control weeds with chemicals that would otherwise damage the crop itself.

Varieties combining two different traits, such as herbicide tolerance and insect resistance, have been introduced in cotton and corn. The addition of new traits, such as resistance to rootworm in maize, and the combinations of traits with similar functions, such as two genes for resistance to lepidopteran pests in maize, are expected to increase. In its 2003 report, ISAAA suggested that five new Bt and novel gene products for insect resistance in maize could be introduced.

While the improvement of agronomic characteristics in major crops has been highly successful, few products genetically engineered to meet the specific needs of either food processors or consumers have yet been commercialized. Recently, however, a renewed emphasis on developing agricultural biotechnology applications more relevant to consumers has accompanied continuing efforts to develop crops with improved agronomic traits. Although genetically engineered crops with enhanced health, nutrition, functional, and consumer benefits have lagged behind agronomic applications, research on many such products is in the advanced stages of development. These applications

could improve human and livestock nutrition and health, the nutritional quality of food animals for human consumption, and create ingredients with superior properties for food manufacturing and processing.

II. FOOD APPLICATIONS FOR HUMAN HEALTH

A. Quantity and Quality of Food Oils

Food oils have both nutritional and functional qualities. From a nutritional perspective, fats and oils contribute more energy (calories) than any other nutrient category, about nine calories per gram. This compares with about four calories per gram from carbohydrates and protein. At the same time, specific fatty acids that comprise most of what we call "fat" can affect a person's risk of developing certain chronic diseases such as heart disease. Research over the past several decades has shown that some categories of fatty acids, such as saturated fatty acids, increase the risk of heart disease and other chronic diseases when consumed in excess. Fatty acids also influence how foods behave during manufacturing and processing. For example, saturated fatty acids add stability, texture, and flavor to foods, so they are not simple to replace.

To reduce the saturated fatty acid content of foods, plant breeders and food manufacturers increased their use of vegetable oils rich in polyunsaturated fatty acids and developed food oils low in saturated fatty acids. One example is canola oil with 6% to 7% total saturated

Fats and Fatty Acids – Like Oil for Water

Fats are slippery substances that usually do not dissolve in water. We see them in foods in marbled meat, salad and cooking oils, and spreads such as margarine and butter. Substantial amounts also hide in foods such as cheese, mayonnaise, peanut butter, doughnuts, and chips.

What distinguishes fats from one another is their **fatty acids**. Each fat contains three fatty acids, which may be a combination of three different types. People have been warned for years to limit their intake of saturated fat, the kind rich in saturated fatty acids. These warnings relate to the ability of most saturated fatty acids to raise blood cholesterol levels, thereby increasing the risk of heart disease. Butter, cheese and other dairy foods, and meats are rich in saturated fatty acids.

So-called "good fats" are rich in **unsaturated** fatty acids. These fats or oils are usually liquid at room temperature. Unsaturation refers to the presence of "double bonds" in the fatty acid. The more double bonds there are, the more unsaturated the fatty acid is. Fatty acids with just one double bond are called "monounsaturated" and the amount in a food appears on the nutrition label. Olive oil and high oleic sunflower oil contain mainly monounsaturated fatty acids.

Other vegetable and fish oils are abundant in **polyunsaturated** fatty acids with two to six double bonds. The amount of polyunsaturated fat is also listed on the nutrition label. Heart healthy foods are those having a majority of mono- and polyunsaturated fatty acids.

fatty acids. To improve the stability of vegetable oils rich in polyunsaturated fatty acids, food manufacturers developed partially hydrogenated oils. The process of hydrogenation reduced the polyunsaturated fatty acid content and increased oil stability, but created *trans* fatty acids, which were subsequently associated with adverse health effects. As a result, hydrogenated fats, the main source of dietary *trans* fatty acids, are now being eliminated from foods. Food manufacturers are developing other ways to reduce undesirable saturated fat content while maintaining stability such as using short chain saturated fatty acids and monounsaturated fatty acids.

To date, one functional food oil created with the tools of biotechnology has been commercialized. Calgene's high lauric acid canola, Laurical™, containing 38% lauric acid, is used in confectionary products, chocolate, and non-food items such as shampoo. Conventional canola oil does not contain lauric acid. Laurical™ is a substitute for coconut and palm oils. FDA approved its use in foods in 1995 (FDA 1995). The following section describes research to date focused on developing crop varieties with other unique oil profiles.

B. Strategic Aims of Altered Fatty Acid Profile

Improving the healthfulness and functionality of food oils can be accomplished in several ways. Where traditional plant breeding reaches its limits, biotechnology may be used to:

- Reduce saturated fatty acid content for "heart-healthy" oils
- Increase saturated fatty acids for greater stability in processing and frying
- Increase oleic acid in food oils for food manufacturing
- Reduce alpha-linolenic acid for improved stability in food processing
- Introduce various omega-3 polyunsaturated fatty acids including long-chain forms
- Enhance the availability of novel fatty acids, e.g., gamma-linoleic acid

C. Achievements in Altered Fatty Acid Profile

Reduced saturated fatty acid content: Genetically modified soybeans have been developed that contain about 11% saturates compared with 14% in conventional soybeans (Table 1). In May 2003, scientists reported the development of transgenic mustard greens (*Brassica juncea*) containing 1% to 2% saturated fatty acids, a level significantly less than in the control plants (Yao et al. 2003). The transgenic plants also contained slightly higher amounts of oleic acid, a monounsaturated fatty acid, and higher levels of the polyunsaturates, linoleic and alpha-linolenic acids than the control plants. These results illustrate that alterations in one type of fatty acid may affect the levels of others, suggesting that combined strategies or genetic transformations may be necessary to achieve specific fatty acid profiles.

Palm oil low in saturated fatty acids is currently in development. This tropical oil contains about half saturated fatty acids (49.3%), primarily palmitic acid (16:0, 43.5%). However, with the recent success of biotechnology techniques in palm, transgenic palm oil enriched in oleic and stearic acids is under development (Parveez et al. 2000). Because of the long life cycle of palm and the time required to regenerate the plants in tissue culture, genetically engineered palm is not anticipated for another two decades (Parveez et al. 2000).

Increased saturated fatty acid content: Because saturated fatty acids confer certain functional properties to food fats and oils and are more stable to heat and processing than unsaturated fatty acids, their use in cooking and baking is essential. To avoid the use of animal fats and hydrogenated vegetable oils with trans fatty acids, genetic engineering techniques have been used in canola and soy to develop oils with more short chain saturated fatty acids—12 to 18 carbons long—mainly lauric (12:0), myristic (14:0), palmitic (16:0), and stearic (18:0) acids. For example, Calgene's high lauric acid canola, Laurical™, containing 37% lauric acid, was developed using the enzyme acyl-ACP thioesterase isolated from the California Bay Laurel (*Umbellularia californica*). Conventional canola oil contains no lauric acid, and only about 6% short chain saturated fatty acids. This was the first transgenic oilseed crop produced commercially. High laurate canola is used in confectionary products, chocolate, and non-food items such as shampoo as a substitute for coconut and palm oils. FDA approved its use in 1995.

Enrichment of canola with even shorter chain saturated fatty acids, those with eight and ten carbons, has also been accomplished (Dehesh et al. 1996). Using a palmitoyl-acyl carrier protein thioesterase gene from a Mexican shrub, *Cupea hookeriana*, Dehesh and colleagues developed lines of canola with as much as 75% caprylic (8:0) and capric acids (10:0). These fatty acids are absent in conventional canola oil. When consumed, these water-soluble fatty acids are mainly oxidized for energy.

Soybeans have been genetically modified to produce oil enriched in stearic acid (18:0), a saturated fatty acid that scientists believe does not raise serum cholesterol levels. The stearic acid-rich oil shown in Table 1 had 28% stearic and 20% oleic acids, with lower linoleic acid (18:2) than the conventional oil. Gene transfer technology also boosted the stearic acid content of canola (Hawkins and Kridl 1998). Researchers at Calgene, Inc., Davis, CA, cloned three thioesterase genes from mangosteen, a tropical tree that stores up to 56% of its seed oil as stearate. One of these genes led to the accumulation of up to 22% stearate in transgenic canola seed oil, an increase of more than 1,100% over conventional varieties (Hawkins and Kridl 1998).

Increased oleic acid content: The most recent approach to developing more healthful food oils is increased oleic acid content. High oleic acid oils are lower in saturated and polyunsaturated fatty acids compared with conventional oil. Oleic acid, the predominant monounsaturated fatty acid in seed oils, is abundant in olive (72%), avocado (65%), and canola (56%) oils, but not in others. Like saturated fatty acids, high oleic acid oils are useful in food processing and manufacturing for maintaining functionality and stability during baking and frying. Unlike saturated fatty acids, however, they do not raise blood cholesterol concentrations and are therefore considered more healthful.

Biotechnology offers a means to increase the oleic acid content of vegetable oils, usually at the expense of polyunsaturated fatty acids, and sometimes, saturated fatty acids, depending on the particular transformations used. The concomitant reduction in polyunsaturated fatty acids has the added advantage of increasing the stability of the oil and ultimately the processed food. While traditional plant breeding allowed a modest increase in oleic acid, biotechnology has been necessary to achieve the high levels desired. For example, canola oil moderately high in oleic acid was developed using traditional plant breeding techniques. With the application of biotechnology, oleic acid content increased to 75% (Corbett 2002). Others have developed canola oil with more than 80% oleic acid (Wong et al. 1991, Scarth and McVetty 1999).

More recently, Buhr and colleagues at the University of Nebraska used genetic engineering to increase oleic acid levels in soybeans by inhibiting the ability of the plant to convert oleic acid to polyunsaturated fatty acid (Buhr et al. 2002). When the conversion enzyme was inhibited, the level of oleic acid increased from 18% in the wild-type seed to 57% in the transgenic seed. When two gene transformations were applied, oleic acid content increased to 85%, with saturated fatty acids reduced to 6%.

Using a different approach, scientists at DuPont used the technique of cosuppression to reduce the production of polyunsaturated fatty acids in soybeans. Cosuppression occurs when the presence of a gene silences or turns off the expression of a related gene. Like Buhr and colleagues, these scientists were able to turn off the production of the enzyme that converts oleic acid to polyunsaturated fatty acids. The result was greatly increased production of oleic acid and reduced production of polyunsaturated fatty acids. Examples of genetically modified high oleic acid oils compared with their conventional counterparts are shown in Table 1.

Gene silencing has also been used to produce high oleic and high stearic acid cottonseed oils (Liu et al. 2002). Cottonseed oil is high in palmitic acid, very high in linoleic acid, and free of alpha-linolenic acid. Conventional cottonseed oil has about 13% oleic acid. When gene silencing was used to transform cotton, the resulting oil had 78% oleic and only 4% linoleic acids, respectively, with palmitic acid reduced from 26% to 15%. Cotton was also genetically modified to produce high stearic oil having 40% stearic and 39% linoleic acids, with 15% palmitic acid. A combination was also developed to have 40% stearic, 37% oleic and only 6% linoleic and 14% palmitic acid. These examples illustrate the power and specificity of this technology to develop tailored seed oils.

Reduced alpha-linolenic acid: Several genetic transformations designed to increase oleic or stearic acid content do so at the expense of the polyunsaturated fatty acids alpha-linolenic and linoleic acids. These fatty acids have desirable nutritional characteristics, but their presence reduces the stability of oils for baking, processing, and frying and increases their susceptibility to oxidation or rancidity. Oils with appreciable amounts of alpha-linolenic acid such as canola and soybean, with about 10% and 8% alpha-linolenic acid, respectively, have been genetically modified to reduce this fatty acid. Such oils would be desirable for the commercial uses mentioned. Pioneer Hi-Bred, a DuPont company, developed low alpha-linolenic acid soybean seeds through conventional breeding techniques with less than 3% alpha-linolenic acid in its oil. Marketed under the brand TREUS™ the company claims that the oil eliminates the need for hydrogenation in food processing. A similar product from Monsanto, Vistive™, offers a similar level of reduction in alpha-linolenic acid.

Omega-3 fatty acids: There is extensive interest in increasing Americans' consumption of omega-3 fatty acids, because they are associated with many health benefits, but are consumed only in small amounts. In 2002, the National Academy of Sciences' Institute of Medicine recognized that omega-3 fatty acids are essential in the diet and established an estimated adequate intake for them (Institute of Medicine 2002). The main food sources of the long-chain omega-3 fatty acids are fish, especially fatty species such as salmon, rainbow trout, mackerel, herring, and sardines. Some plants—mainly canola, soybean, and flax oils—provide the 18-carbon omega-3 fatty acid, alpha-linolenic acid. However, higher plants lack the enzymes to make 20- and 22-carbon polyunsaturated fatty acids needed by mammals. Humans can convert alpha-linolenic acid to the more biologically active long-chain forms, but they do so very inefficiently. Thus, plant foods with alpha-linolenic acid may be insufficient to supply the need for long-chain omega-3 fatty acids, especially during pregnancy and lactation (Pawlosky et al. 2001).

Western diets contain predominately omega-6 polyunsaturated fatty acids found in soybean, corn, sunflower, canola, and cottonseed oils. It is now recognized that diets high in omega-6 fatty acids and low in omega-3 fatty acids may exacerbate several chronic diseases (Simopoulos et al. 2000). Because of the many health benefits associated with the regular consumption of omega-3 fatty acids, several health organizations, including the American Heart Association and the 2005 Dietary Guidelines for Americans, have called for increased consumption of these substances. One limitation to boosting consumption is that they occur naturally mainly in fatty fish and some seeds. Ironically, reducing the level of alpha-linolenic acid in soy and canola oils used in food processing, may actually reduce consumption of this fatty acid, although product developers are working to combine high omega-3 and low alpha-linolenic traits in one product.

Although aquaculture has increased the availability of some fish and shellfish species, increasing worldwide demand has put severe pressure on wild aquatic resources and limited seafood availability. Thus, it would be desirable to increase the availability of these fatty acids or their precursors in a variety of other foods, especially plants. Such foods would also be useful for animal and fish feed.

TABLE 1. Selected fatty acid content of vegetable oils with modified fatty acid profiles compared with the commodity oil.

OIL	Oleic (18:1)	Linoleic (18:2)	Alpha-linolenic (18:3)	Total Saturates
CANOLA				
Conventional	60	20	10	7
High oleic	75	14	3	<7
High oleic	84	5	3	5
Low linolenic	65	22	4	7
Low linolenic P6	78	11–13	2–3	N/A
High myristate/palmitate	34	15	4	43
High laurate (37%)	34	12	7	45
SUNFLOWER				
Conventional	20	65	<1	10
High oleic	82	10	<1	8
Mid oleic	56	33	<1	9
SOYBEAN				
Conventional soy	23	51	7	14
Low linolenic	23	60	2	15
High palmitic (17%)	17	55	8	20
High stearic (28%)	20	35	7	35
High oleic soybean	83	2	3	12
OTHERS				
Conventional safflower	14	75	0	6
High oleic safflower	75	14	0	6
Conventional corn	24	58	<1	13
High oleic corn	70	-	-	-
Olive	75	8	<1	14
Avocado	65	15	1	14

One strategy to increase the availability of long-chain omega-3 fatty acids is to develop oilseed crops such as canola and soybean that contain *stearidonic acid* (18:4n-3). This omega-3 fatty acid occurs naturally in only a few plants such as black currant seed oil and echium. Stearidonic acid is the first product formed when alpha-linolenic acid is converted to eicosapentaenoic acid (EPA), a desirable long-chain omega-3 fatty acid. Usually, this first step limits the amount of EPA produced, but increasing the level of stearidonic acid helps overcome this limitation. Then the body's enzymes convert stearidonic acid to 20-carbon polyunsaturated fatty acids.

Dr. Virginia Ursin and colleagues at Calgene studied the metabolism of stearidonic acid in people (James et al. 2003). Her studies showed that when either stearidonic acid or EPA was consumed the amount of EPA in red blood cells increased significantly. This finding meant that the stearidonic acid was converted to EPA and appeared in red cells just as readily as the preformed EPA. In contrast, when the study volunteers consumed alpha-linolenic acid, there was no change in their red cell EPA content. None of the fatty acids consumed had any effect on cell DHA levels, another long-chain omega-3 fatty acid associated with health benefits. Although the study used supplements, not stearidonic acid from transgenic plants, the findings suggest that plants with stearidonic acid would have potential to provide EPA.

Toward this end, scientists at Calgene, have successfully transformed canola so that it makes stearidonic acid. This genetic engineering feat required two genes from the fungus *Mortierella alpina* and one from canola for the three enzymes needed to produce sufficient stearidonic acid (Ursin 2003). The engineered plants accumulated up to 23% stearidonic acid in the seed oil with a reduction in oleic acid content from about 60% to about 22%. By breeding the transgenic lines with various lines of canola the investigators were able to develop a line of canola containing more than 55% of alpha-linolenic acid and stearidonic acid. Total omega-6 fatty acids remained about 22%, a level similar to conventional canola. Calgene scientists have also developed soybean that contains stearidonic acid (Ursin, personal communication 2004).

The implications of Calgene's work with stearidonic acid are substantial. This is the first demonstration of the incorporation into edible plants of a biologically potent source of long-chain omega-3 fatty acids. This work marks an important advance in the development of plant-based sources of long-chain omega-3 fatty acids that could be consumed directly or incorporated into food products. However, because stearidonic acid contains four double bonds, it is vulnerable to oxidation and would require antioxidant protection. One can imagine that transgenic canola and soybean could be developed using additional traits to boost antioxidant protection, possibly from vitamin E.

In May 2004, a landmark paper announced the production of long-chain polyunsaturated fatty acids—both omega-6 and omega-3 types—in *Arabidopsis thaliana*, a type of cress widely used as a model plant in biotechnology research. Dr. Baoxiu Qi and colleagues at the University of Bristol, United Kingdom, transferred to *Arabidopsis thaliana* three genes encoding for different enzymes in the metabolic pathway from linoleic and alpha-linolenic acids to arachidonic and eicosapentaenoic acids, respectively (Qi et al. 2004). The additional genes were necessary to provide the enzymes to make these long-chain fatty acids. Yields of EPA (13%) and arachidonic acid (29%) in leaves were significantly higher than in conventional cress, which usually does not produce these fatty acids, and accounted for 43% of the total 20-carbon polyunsaturated fatty acids. In addition to the production of EPA and arachidonic acid, the concentration of alpha-linolenic acid was reduced from 48% to 14%. This achievement was also remarkable because it used a pathway seldom found in plants.

This work is important in several regards. One is that it demonstrates the feasibility of developing plants capable of synthesizing long-chain polyunsaturated fatty acids. Another is the relatively high efficiency of conversion of the precursor fatty acids to the long-chain forms. A third advantage is the improved balance of omega-6 and omega-3 fatty acids, with significant reduction in the amounts of the 18-carbon precursors linoleic and alpha-linolenic acid compared with conventional plants. Yet another is the demonstration that plants can be engineered not only with respect to the outcome of final products, but also the pathways for achieving the desired ends. A likely next step will be to apply this technology to seed oil crops such as canola and soybean to see if the long-chain polyunsaturated fatty acids will accumulate in the seed.

Although production of EPA in plants represents an enormous scientific achievement, the question of making Docahexenoic Acid (DHA), a 22-carbon polyunsaturated omega-3 fatty acid important in retina and brain function and other body systems remained unsolved. In mammals, the conversion of EPA to DHA is inefficient and requires several steps. It is possible, in theory, to perform this conversion in a direct manner, but the enzymes to do so are not present in mammals. Several research groups have examined many algae and identified the specific enzymes for this conversion (Sayanova and Napier 2004, Meyer et al. 2004). Once the genes for these enzymes were identified and cloned they could be incorporated into model organisms to see whether DHA would be produced. In late 2004, Amine Abbadi at the University of Hamburg, Germany, working with colleagues in the U.K. and the U.S., reported the successful transformation of yeast that yielded small amounts of DHA (Abbadi et al. 2004). This accomplishment required four gene transformations. The team then went on to develop transgenic flax, a plant with abundant alpha-linolenic acid for conversion to long-chain fatty acids.

Several steps remain before long-chain polyunsaturated fatty acids will be available in commercial crops. However, the demonstration that plants can be modified to make these important nutrients means that many of the scientific hurdles have been conquered. This work gives a large boost to the potential for plants to be an important dietary source of these fatty acids.

Gamma-linolenic acid: This fatty acid is the first step in the conversion of linoleic acid to arachidonic acid in the omega-6 fatty acid pathway. When consumed in evening primrose or borage oils, it is poorly converted to arachidonic acid. For that and other reasons, it may have potential benefit in cardiovascular disease (Fan and Chapkin 1998). Gamma-linolenic acid has been associated with improved skin conditions in human subjects, improved liver function in patients with liver cancer, and with anti-cancer effects in cell culture studies. It was also shown to enhance the effectiveness of tamoxifen, an anti-estrogenic medication used to prevent the recurrence of breast cancer. It is believed to suppress the production of estrogen receptors in cells.

Gamma-linolenic acid is naturally present in appreciable amounts in few plants, notably borage (*Borago officinalis*), evening primrose (*Oenothera biennis*), black currant oil (*Ribes nigrum*) and echium (*Echium plantagineum*). The ability to increase the production of gamma-linolenic acid in tobacco plants by transferring the gene for the delta-6 desaturase enzyme from various sources was first shown in 1996 by Reddy and Thomas at Texas A&M University, and by others in 1997 (Reddy and Thomas 1996, Sayanova et al. 1997). A recent study reported that gamma-linolenic acid content in transgenic canola ranged from 22% to 45% (Wainright et al. 2003). Evening primrose has also been genetically modified for enhanced gamma-linolenic acid content (Wainright et al. 2003). Arcadia Biosciences, Davis, CA, has also reported transgenic safflower plants with 65% gamma-linolenic acid in the oil.

Ursin's study of transgenic canola enriched in stearidonic acid discussed above also reported that a cross between the transgenic line of canola producing stearidonic acid and a canola line high in gamma-linolenic acid yielded a canola containing about 11% gamma-linolenic acid and about 14% stearidonic acid (Ursin 2003). This example illustrates the variety of fatty acids that can be developed in seed oils using a combination of genetic engineering and traditional plant breeding techniques.

III. QUANTITY AND QUALITY OF PLANT PROTEIN

Efforts to improve the protein content and quality of staple foods have been underway for decades. The main focus is crops grown in developing countries, where nutrient shortfalls are widespread and dietary diversity limited. Foods such as potato and cassava, staple foods in several parts of South America and Africa, have less than one percent protein.

Efforts to improve protein quality strive to increase the amount of limiting essential amino acids provided by the protein in the food. The amino acids most often present in inadequate amounts are lysine, tryptophan, and methionine. Improvements in protein quality benefit both human and animal nutrition and increase the feed efficiency of crops fed to food animals. For example, corn is widely fed to cattle but it is limiting in lysine and methionine. Corn with higher levels of these amino acids would significantly improve feed efficiency and lower input costs to farmers. Improved corn varieties consumed by humans would also have nutritional benefits.

There are various ways of improving protein quantity and quality. One is to increase the total amount of protein produced by selecting germplasm with an altered balance of seed proteins. This may be done by traditional cross breeding or genetic engineering. Another approach is to introduce genes from other sources for proteins that have a favorable balance of essential amino acids. An example is the introduction into potato of a gene for seed albumin protein from amaranth. A third approach seeks to increase the production of specific amino acids such as lysine. This approach was used in the development of Quality Protein Maize, discussed below.

William Folk and his team at the University of Missouri, Columbia, MO, pioneered another approach to improve seed protein quality. Their strategy was to substitute more desirable and scarce amino acids for more abundant ones in certain seed proteins (Chen et al. 1998, Wu et al. 2003). They applied this concept to rice by increasing the production of lysine, an essential amino acid, at the expense of the non-essential amino acids, glutamine, asparagine and glutamic acid.

Cassava: A staple food for some 500 million people in tropical and sub-tropical parts of the world, cassava (*Manihot esculenta* Crantz), also known as yucca or manioc, thrives in marginal lands having little rain and nutrient-poor soils. It is widely consumed in Africa, and parts of Asia and South America. Cassava root has less than 1% protein and poor nutritional value. However, the leaves are also consumed and these are a good source of beta-carotene, the precursor of vitamin A.

In 2003, Zhang and colleagues reported using a synthetic gene to increase the protein content in cassava (Zhang et al. 2003). The gene is for a storage protein rich in nutritionally essential amino acids. When the gene was expressed in cassava, transformed plants expressed the gene in roots and leaves, both of which are consumed in human diets. The experiment demonstrated the feasibility of increasing the quantity and quality of protein in cassava.

Cassava also contains cyanogenic glucosides that can produce chronic toxicity if not eliminated or reduced by grating, sun-drying, or fermenting. Efforts to develop cassava varieties low in these toxicants is a high research priority.

Corn: Corn (*Zea mays*) is the predominant staple food in much of Latin America and Africa. Although some varieties may contain appreciable quantities of protein, its quality is poor because of low lysine and tryptophan content. In 1964, it was discovered that corn bearing a gene known as opaque-2 contained increased concentrations of lysine and tryptophan and had significantly improved nutritional quality (Food and Agriculture Organization 1992). However, opaque-2 corn proved to have low yields, increased susceptibility to diseases and pests, and inferior functional characteristics.

At the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, work with the opaque-2 gene continued using both traditional breeding and molecular methods. After at least 12 years' work, CIMMYT researchers succeeded in developing hardy corn varieties that contained twice the lysine and tryptophan content as traditional varieties, but were disease-resistant and high-yielding. Scientists Surinder K. Vasal and Evangelina Villegas of CIMMYT were awarded the World Food Prize in 2000 for their work developing 'Quality Protein Maize'. Quality Protein Maize varieties have been adapted to and released in over 40 countries in Latin America, Africa, and Asia.

Recent researchers at CIMMYT reported the development of transgenic corn with multiple copies of the gene from amaranth (*Amaranthus hypochondriacus*) that encodes for the seed storage protein amarantin (Rascon-Cruz et al. 2004). Total protein in the transgenic corn was increased by 32% and some essential amino acids were elevated 8% to 44%.

In 2004, a team of researchers at the University of California, Riverside, reported that transgenic corn with increased production of the plant regulating hormone, cytokinin, had nearly twice the content of protein and oil as conventional corn (Young et al. 2004). This development resulted from an unusual change in the way the plant developed. Normally, corn ears develop flowers in pairs, one of which usually dies. Under the influence of the additional cytokinin, both flowers developed but yielded only a single kernel. These kernels contained more protein and oil than conventional corn.

Pursuing a different strategy to improve protein quality, researchers at Monsanto, St. Louis, MO, used genetic engineering techniques to reduce the amount of zein storage proteins. These storage proteins constitute over half the protein in corn and are deficient in lysine and tryptophan. Increased production of other proteins in the corn led to higher levels of lysine, tryptophan, and methionine (Huang et al. 2004). The agronomic and nutritional properties of these lines are currently being evaluated.

Researchers at the Max Planck Institute, Germany, have focused on methionine, another limiting amino acid. They elucidated several key steps in methionine metabolism in plants. This work, currently in the preliminary stage, could pave the way for using genetic engineering techniques to improve the methionine content of plants (Hesse and Hoefgen 2003).

Potato: Potato (*Solanum tuberosum*) is a dietary staple throughout parts of Asia, Africa, and South America. Typically, potatoes contain about 2% protein and 0.1% fat. It was reported in 2000 that, as in cassava, transfer of the gene for seed albumin protein from *Amaranthus hypochondriacus* to potato resulted in a "striking" increase in protein content of the transgenic potatoes (Chakraborty et al. 2000). In 2004, researchers at the National

Centre for Plant Genome Research, India, reported the development of a nutritionally improved potato line with 25% higher yields of tubers and 35%–45% greater protein content (ISAAA 2004). Dubbed the "protato," the protein-rich potato had significant increases in lysine and methionine, which enhance the quality of the additional protein (Council for Biotechnology Information 2004). In February 2004, this potato was reported "approaching release" to farming communities.

It should be noted that while potatoes are known for their high starch content, it has been possible to genetically engineer potatoes that contain fat (triglycerides). In July 2004, Klaus and colleagues at the Max Planck Institute of Molecular Plant Physiology demonstrated increased fatty acid synthesis in potatoes (Klaus et al. 2004).

Rice: Almost half the world's population eats rice (*Oryza sativa* L.), at least once a day (IRRI undated). Rice is the staple food among the world's poor, especially in Asia and parts of Africa and South America. It is the primary source of energy and nutrition for millions. Thus, improving the nutritional quality of rice could potentially improve the nutritional status of nearly half the world's population, particularly its children. Commodity rice contains about 7% protein, but some varieties, notably black rice, contain as much as 8.5% (Food and Agriculture Organization 2004). The most limiting amino acid in rice is lysine. Efforts to increase the nutritional value of rice target protein content and quality along with key nutrients often deficient in rice-eating populations, such as vitamin A and iron. The International Rice Research Institute (IRRI), Philippines, is a primary center for rice research and development of improved varieties.

In 1999, Dr. Momma and colleagues at Kyoto University, Japan, reported a genetically engineered rice having about 20% greater protein content compared with control rice (Momma et al. 1999). Transgenic plants containing a soybean gene for the protein glycinin contained 8.0% protein and an improved essential amino acid profile compared with 6.5% protein in the control rice.

As mentioned briefly above, Dr. William Folk and his team genetically modified rice to increase its content of the amino acid lysine (Wu et al. 2003). They did so by modifying the process of protein synthesis, rather than by gene transfer or the expression of new proteins. They achieved an overall 6% increase in lysine content in the grain (Chen et al. 1998). Although lysine content remained below optimum levels, the scientists suggested that additional transformations and modifications could further boost lysine levels.

Perhaps the most famous genetic transformations in rice are those in "Golden Rice" involving the vitamin A precursor, beta-carotene, and iron. The lead scientist in the golden rice project, Dr. Ingo Potrykus, now retired from the Swiss Federal Institute of Technology, was also involved in applying biotechnology for the improvement of rice protein. Although details are sparse, Potrykus described the work of Dr. Jesse Jaynes, who synthesized a synthetic gene coding for an ideal high-quality storage protein with a balanced mixture of amino acids. The gene, named Asp-1, was transferred to rice with the appropriate genetic instructions for its production in the endosperm or starchy part of the rice grain. The transgenic rice plants accumulated the Asp-1 protein in their endosperm in a range of concentrations and provided essential amino acids but data are not yet available on the concentrations achieved or their nutritional relevance. Precedent for the expression of a synthetic gene in rice grown in cell culture suggests that Jaynes' approach is viable (Huang et al. 2002).

IV. MODIFIED CARBOHYDRATE

A. Starch

Starch from cereals, grains, and tubers contribute a substantial share of dietary calories and in many poor countries, provide the majority of food energy. Starch is also important for feed and industrial purposes. Its use as paste goes back at least 4000 years BCE to the Egyptians who cemented strips of papyrus stems together with starch paste for writing paper.

Besides providing energy, starch confers functional characteristics to foods: texture, viscosity, solubility, gelatinization, gel stability, clarity, etc. These characteristics depend on the proportion of amylose and amylopectin, the main components of starch. Amylose and amylopectin differ from each other in chain length, branching, and degree of polymerization. Amylose is linear and amylopectin is highly branched. How a particular starch will be used in foods, determines what ratio of amylose to amylopectin is most suitable. High amylose starches include high amylose corn (70%), corn (28%), wheat (26%) and sago (26%). In contrast, waxy rice and waxy sorghum contain no amylose. Members of the potato family—potato, sweet potato, cassava—have 17% to 20% amylose.

Many lines of corn have been developed with different characteristics derived from modified starch ratios and increased amylose content. Transgenic high amylose potatoes developed by inhibiting two branching enzymes were reported to yield more tubers and have lower starch content, smaller granules, and increased reducing sugars (Hofvander et al. 2004).

Biotechnology has also been directed to increasing starch content (Geigenberger et al. 2001, Regierer et al. 2002). Potatoes were genetically altered to increase the activity of adenylate kinase, an enzyme involved in the plant's energy metabolism and starch production. The resulting transgenic potatoes had substantially increased adenylates and a 60% increase in starch compared with wild-type plants (Regierer et al. 2002). Unexpectedly, the concentrations of several amino acids were increased 2- to 4-fold, and tuber yield increased.

Considerable publicity was given to potatoes engineered by Monsanto in the early 1990s to have increased starch content. These were touted as more desirable for French fries because they would absorb less fat during frying. They are an example of the type of starch modification that may have secondary health benefits as a consequence of how they are used.

B. Fructan

Fructans are polymers (repeating units) of the sugar fructose. They serve in food products as a low-calorie sweetener, source of dietary fiber, and bulking agent. They may also stimulate the growth of desirable colonic bacteria, such as bifida. Fructans have environmentally friendly non-food applications in the manufacture of biodegradable plastics, cosmetics, and detergents. Fructans are naturally occurring in Jerusalem artichokes (sunchokes) and chicory, but agronomic shortcomings in growing these crops have limited their use.

Inulin, a fructan found in Jerusalem artichokes, was successfully synthesized in potatoes following the transfer of two genes from globe artichokes (*Cynara scolymus*) (Hellwege et al. 2000). The full spectrum of inulin molecules present in artichokes was expressed in the transgenic potatoes. Inulin comprised 5% of the dry weight of the transgenic tubers and did not influence sucrose concentration. However, starch content was reduced.

In a program called the Agriculture and Fisheries Programme, or FAIR, the European Commission funded multidisciplinary research programs in agriculture and fisheries, including a project on fructans for food and non-food uses. Research to date includes the isolation of several genes for fructosyl transferase enzymes involved in the production of fructans. The feasibility of using these enzymes has been demonstrated in model plants and target crops such as sugar beet (Anonymous 2000). In addition, it was reported in 2004 that genes encoding for fructosyl transferases in onion were isolated and transferred to sugar beet, a plant that does not normally synthesize fructans (Weyens et al. 2004). Following the transfer of the genes, onion-type fructans were produced from sucrose without loss in storage carbohydrate.

V. INCREASED VITAMIN CONTENT IN PLANTS

A. Beta-carotene and Other Carotenoids

Beta-carotene belongs to the family of carotenoids and is abundant in plants of orange color. It is the precursor of vitamin A and can be converted to the active vitamin during digestion. Other carotenoids do not have potential vitamin A activity. Humans cannot synthesize carotenoids and therefore depend on foods to supply them. Many staple foods, particularly rice, contain no beta-carotene or its precursor carotenoids. Diets lacking other food sources of vitamin A or beta-carotene are associated with vitamin A deficiency which can result in blindness, severe infections, and sometimes death. According to the World Health Organization, vitamin A deficiency is the leading cause of preventable blindness worldwide. The deficiency affects some 134 million people, particularly children, in 118 countries. Overcoming this nutrient deficiency is an urgent global health challenge.

The development of "Golden Rice," so named because of its yellow color conferred by the presence of beta-carotene, was a landmark achievement in the application of biotechnology to nutrition and public health. Peter Burkhardt, working with Ingo Potrykus and colleagues in Switzerland, was the first to show that transgenic rice, carrying a gene from daffodil, could express phytoene, a key intermediate in the synthesis of beta-carotene (Burkhardt et al. 1997). Subsequently, the Potrykus group reported the application of three transgenes in the development of rice expressing the entire pathway for the production of beta-carotene (Ye et al. 2000, Beyer et al. 2002). Additional work with Golden Rice included the insertion of a gene to increase the iron content (Potrykus 2003). IRRI is currently cross-breeding the nutrient-enhanced transgenic rice with local rice varieties from Asia and Africa, and field-testing the new lines for nutritional value and agronomic performance. Varieties of Golden Rice are not expected to be ready for farmers for several more years.

The development of transgenic plants able to produce a variety of carotenoids is an active area of research. It is clear that production of phytoene, the first product in the pathway for carotenoid synthesis, is the rate-limiting step in generating carotenoids (Cunningham 2002). Using gene transfer technology to increase the expression of phytoene synthase, the enzyme that makes phytoene, increases the synthesis of carotenoids substantially. For example, Shewmaker and colleagues (1999) from Monsanto reported an increase up to 50-fold in carotenoids, mainly alpha and beta-carotene, in canola (*Brassica napa*). However, vitamin E levels decreased significantly, oleic acid content increased, and linoleic and alpha-linolenic acids were reduced compared with non-transgenic seeds. These other changes would have to be modified or evaluated to determine whether they might have meaningful nutrition implications.

In a separate study on canola, the Monsanto group transferred to canola three genes from bacteria that affect the phytoene synthesis pathway. When they included a triple construct—genes for three different enzymes, phytoene synthase, phytoene desaturase and lycopene cyclase—the resulting transgenic canola seeds maintained the same amount of total carotenoids, but increased the ratio of beta to alpha-carotene from 2:1 to 3:1 (Ravanello et al. 2003).

Stalberg and colleagues in Sweden also studied the effects of phytoene synthase on carotenoid synthesis in transgenic *Arabidopsis thaliana*. They examined three keto-carotenoids; transformed seeds had a 4.6-fold increase in total pigment and a 13-fold increase in these three carotenoids (Stalberg et al. 2003). They also reported a 43-fold average increase in beta-carotene (Lindgren et al. 2003). Lutein, another nutritionally important carotenoid, was significantly increased, but zeaxanthin was only increased by a factor of 1.1. They also observed substantial levels of lycopene and alpha-carotene in the seeds, whereas only trace amounts were found in the control plants. However, germination was delayed in proportion to the increased levels of carotenoids.

Others have examined the effect of transgenes affecting phytoene metabolism on carotenoid synthesis. Dr. Peter Bramley's group at the University of London, United Kingdom, transformed tomatoes using a bacterial gene encoding for an enzyme that converts phytoene to lycopene, the precursor of beta-carotene. Tomatoes carrying the bacterial gene had about a 3-fold increase in beta-carotene content, but total carotenoids were not increased (Romer et al. 2000). The altered carotene content did not affect plant growth and development.

Lutein and zeaxanthin are nutritionally important carotenoids for protection of the retina and reduced risk of age-related macular degeneration (Krinsky et al. 2003, Gale et al. 2003). Lutein is found in dark green leafy vegetables such as spinach and collards, and zeaxanthin occurs in yellow foods such as mangoes, corn, and peaches. The latter is not particularly abundant in Western diets. Romer and colleagues at Universitat Konstanz, Germany, were able to use biotechnology to block the conversion of zeaxanthin to another carotenoid and thereby increase its content in potatoes (Romer et al. 2002). With this approach they obtained increased levels of zeaxanthin in potatoes ranging from 4- to 130-fold. Total carotenoids were increased by 5.7-fold, but in some, lutein content was decreased. Alpha-tocopherol (vitamin E) was increased 2- to 3-fold in the transgenic potatoes. Fine-tuning these alterations has the potential to significantly enhance the nutritional value of potatoes.

In another study, Bramley's group transferred the gene that increases carotenoid synthesis from a bacterium to tomatoes and measured total and specific carotenoids in the transgenic fruits (Fraser et al. 2002). Total carotenoids were 2- to 4-fold higher in transgenic fruits than in nontransformed plants, with increases in phytoene, lycopene, beta-carotene, and lutein of ranging from 1.8- to 2.4-fold.

Tomatoes with delayed ripening were produced as a result of inserting a gene encoding for S-adenosylmethionine decarboxylase, an enzyme involved in the ripening process (Mehta et al. 2002). An additional consequence of this transgenic modification was a several-fold increase in lycopene content. Lycopene is normally converted to beta-carotene, but tomatoes with increased lycopene content may have enhanced nutritional value. Lycopene consumption has been linked to reduced risk and spread of prostate cancer, though definitive data are lacking (Etminan 2004, Kristal 2004).

The carotenoid astaxanthin is synthesized by algae and plants and is responsible for the pink color in shrimp and salmon. Humans absorb astaxanthin poorly, but absorption is increased in the presence of fat (Mercke Odeberg et al. 2003). Astaxanthin is of interest because of its strong antioxidant properties *in vitro*. It is less certain whether it is an antioxidant in human health. Astaxanthin is used commercially in feed for cultured salmon and trout.

Production of astaxanthin in flowers and fruits has also been accomplished with the techniques of biotechnology. Using a gene from the alga *Haematococcus pluvialis*, researchers at The Hebrew University of Jerusalem, Israel, transferred the gene into tobacco (*Nicotiana tabacum*). Transgenic tobacco plants produced astaxanthin and changed color (Mann et al. 2000). This ability to manipulate pigmentation in fruits and flowers may have commercial potential and possible implications for increasing the availability of carotenoids for human health.

B. Vitamin E

There is strong interest in vitamin E because of studies linking it to decreased occurrence of several degenerative diseases and cancers, although efficacy remains unproven and data are inconsistent (71, 72). Some recent trials with vitamin E supplementation reported no protection against cardiovascular disease or cancer and some chance of increased risk of heart failure (Eidelman et al. 2004, Lonn et al. 2005, Miller et al. 2005). Also, because vitamin E is an anti-oxidant, it is useful in foods and oils to provide oxidative stability. Vitamin E is found mainly in vegetable oils, wheat germ, and a few other foods not widely consumed. The most potent form of the vitamin is alpha-tocopherol, but the less potent gamma, beta, and delta forms are more widespread in plants. Efforts to increase the content of vitamin E in food plants, particularly cereals and grains, which may have low amounts, have sought to increase the amount of precursor substances by overexpressing the genes for various enzymes involved in the biosynthetic pathway. Vitamin E biosynthesis involves complicated pathways so that multiple genetic manipulations are required.

In September 2003, Dr. Edgar Cahoon of the U.S. Department of Agriculture (USDA) and co-researchers at the Donald Danforth Plant Science Center, St. Louis, MO, announced the development of transgenic corn with increased levels of vitamin E. Insertion of a gene from barley into corn increased the conversion of vitamin E precursors to vitamin E itself (Cahoon et al. 2003). The content of vitamin E and tocotrienol, a closely related substance, was increased up to 6-fold. However, much of the antioxidant produced was tocotrienols rather than vitamin E (Aijawi and Shintani 2004). Tocotrienols, although potent antioxidants *in vitro*, are poorly absorbed in humans; however, they may have cholesterol lowering properties (Theriault et al. 1999). Besides enhancing the potential therapeutic and nutritional value of corn, this alteration may increase its oxidative stability after harvest.

In soybeans, Van Eenennaam and colleagues developed transgenic plants that were able to increase the conversion of the weaker forms of tocopherol typically present in soybeans to the more potent alpha-tocopherol (vitamin E) form. The result was a 5-fold increase in vitamin E activity (Van Eenennaam et al. 2003). This work paves the way for the development of vitamin E-rich oils and plants with potential health benefits.

C. Vitamin C

Vitamin C, or ascorbic acid, is abundant in citrus and other fruits such as strawberry and kiwi, but is very low in cereals and grains. Moreover, it cannot be synthesized by humans nor stored to any appreciable extent. Thus, we depend on regular dietary consumption to meet our vitamin C needs. In areas of the world where foods containing vitamin C are not widely consumed, strategies to increase the vitamin C in cereals and grains hold considerable potential to improve health.

In 2003, Gallie and colleagues at the University of California, Riverside, announced the successful transformation of corn and tobacco that resulted in a 20% increase in vitamin C (Chen et al. 2003). They accomplished this increase by transferring a gene from wheat for an enzyme that recycles vitamin C and prevents its breakdown. Increased expression of this enzyme in transgenic corn and transgenic tobacco resulted in a 2- to 4-fold increase in vitamin C content in the kernel. Application of this approach to other food plants remains to be developed and evaluated.

The ability to increase the vitamin C content of strawberries, a good source of the vitamin, was reported in 2003 by a research team at the University of Málaga, Spain (Agius et al. 2003). Using a gene for the enzyme D-galacturonate reductase transferred to strawberry (*Fragaria spp*) this group showed that vitamin C content in the modified strawberry fruit increased with the expression of the transgene. This study demonstrated the feasibility of using this enzyme to raise the level of vitamin C in plants.

D. Folic Acid

Inadequate intake of the vitamin folic acid, one of a family of folates, is associated with megaloblastic anemia, birth defects, impaired cognitive development, and some chronic diseases. Folates are available in small amounts in a variety of fruits and vegetables, but intakes tend to be low. For this reason, it would be desirable to increase the concentration of folates in dietary staples and foods widely consumed. In the U. S. several foods are fortified with folic acid. In 2004, Hossain and colleagues at Tufts University, Medford, MA, and the Donald Danforth Plant Science Center reported a 2- to 4-fold increase in folates and pterins, the precursors of folates, in transgenic *Arabidopsis thaliana* modified by the incorporation of the transgene for the first step in the synthesis of folic acid (Hossain et al. 2004). Other investigators have developed transgenic tomatoes, also enriched in the same gene, that had twice the amount of folate as control fruit (Diaz de la Garza et al. 2004). This group was able to boost folate content 10-fold by including a second gene transformation to increase the content of another substance, para-aminobenzoate, needed for folate synthesis. These studies provide good evidence of the potential to increase the availability of this vitamin in widely consumed foods.

E. Antioxidants

Vitamins C and E function as antioxidants in the body. However, many other substances widely distributed in foods in small amounts also provide protection against potentially damaging breakdown products arising from oxidation during normal metabolism. Oxidation breakdown products, such as reactive oxygen species, oxidized lipids, and free

radicals have been associated with chronic diseases, so there has been considerable interest in the availability of antioxidants. Caution should be sounded, however, because in small amounts many of these substances are protective; in high doses, they can act as prooxidants and may be harmful. Several examples of the applications of biotechnology for enhanced antioxidant capacity in foods are described below.

Phenolic compounds are the most widespread antioxidants in foods. They include such substances as flavanols, tocopherols, quercetin, resveratrol, and many others. They have become familiar to consumers because they are touted in foods as diverse as berries, wine, tea, olive oil, and many others. Potatoes are a source of antioxidant flavanoids and vitamin C. To enhance the antioxidant content of potatoes, Lukaszewicz and colleagues conducted a series of transformations using one or multiple genes encoding enzymes in the bioflavanoid synthesis pathway (Lukaszewicz et al. 2004). Transgenic plants exhibited significantly increased levels of phenolic acids and anthocyanins, plus improved antioxidant capacity. However, starch and glucose levels were decreased. These findings point to complex relationships between antioxidant content and other compounds, but indicate that antioxidant levels in potatoes can be altered using biotechnology.

Another phenolic antioxidant, chlorogenic acid, accumulates in some crops and is found in apples, green coffee beans, tomatoes, and tea. It is synthesized by the enzyme hydroxycinnamoyl transferase in solanaceous plants (e.g., potato, tomato, eggplant). In 2004, it was reported that transgenic tomatoes carrying the gene for this enzyme accumulated higher levels of chlorogenic acid with no side effects on levels of other phenolics (Niggeweg et al. 2004). The transgenic tomatoes also showed improved antioxidant capacity, suggesting that such enhanced tomatoes might provide additional antioxidants.

Yet another transformation in tomatoes was recently reported to result in the synthesis of resveratrol, an antioxidant not normally found in tomatoes. Resveratrol is usually associated with grapes and wines where it is abundant. In this study, tomatoes incorporating the gene for stilbene synthase, an enzyme in the pathway for resveratrol synthesis, had a resveratrol content of 53 mg/g fresh tomato upon ripening (Giovinazzo et al. 2005). The contents of two other antioxidants, vitamin C and glutathione, were also increased.

VI. TRACE MINERAL CONTENT AND BIOAVAILABILITY

Improving human nutrition by increasing the availability of trace minerals in crops is potentially highly efficient and effective. This strategy may reach more people in developing countries than fortification of foods, because many subsistence farmers grow their own food and are outside the market system. If they have access to and consume improved crop varieties, they will not only improve their nutrient intake, they may improve their crop yields and consequently their economic wellbeing. This is because trace minerals are essential to the plant's ability to resist disease and other environmental stresses (Bouis 2002). Further, plants with improved ability to take up minerals from the soil will not deplete nutrient-poor soils. Such plants are able to unbind minerals in the soil and make them available to the plant, thus making use of an abundant resource in the soil that is otherwise unavailable. Mineral-efficient plants are also more drought resistant and require fewer chemical inputs (Bouis 2002).

A. Iron

Iron deficiency anemia is one of the most widespread nutritional deficiencies in the world. The United Nations estimates that over three billion people in developing countries are iron-deficient (Administrative Committee 2000). The problem for women and children is more severe because of their greater need for iron. For this reason, the enrichment of staple foods, especially those consumed in poor countries, is one of the top priorities in international agricultural and nutrition research. In rice-eating populations, iron deficiency anemia is caused by insufficient dietary iron, absorption inhibitors such as phytate, and lack of enhancing factors for iron absorption such as ascorbic acid.

Although much is known about the uptake of iron and zinc in roots and transport of minerals to and from vegetative parts of the plant, some plants accumulate very little trace minerals in the grain (Holm et al. 2002). For example, in wheat only 20%, and in rice just 5% of the iron in leaves is transported to the grain. In cereals, much is stored in the husk and subsequently lost during milling and polishing. Thus, strategies to increase the iron content of cereals and grains face the challenge of targeting iron storage in a form and location in the plant where it will be bioavailable when consumed.

A significant breakthrough in improving the iron content of cereals was achieved by Ingo Potrykus and colleagues. One of the genes transferred to Golden Rice came from the common bean, *Phaseolus vulgaris*. This gene encoded for the iron storage protein, ferritin, and when expressed in the transgenic rice increased the iron content twofold (46). The bioavailability of iron in transgenic rice varieties containing ferritin was shown to be as good as ferrous sulphate, commonly used in iron supplements, as reflected in biochemical indices of iron status in iron-deficient laboratory rats (Murray-Kolb et al. 2002).

A different source of ferritin genes, soybean, was used in the transformation of rice to increase iron content (Goto et al. 1999). Researchers at the Central Research Institute Electric Power Industry, Japan, transferred the gene for ferritin from soybean into rice and confirmed the stable incorporation of the ferritin subunit in the rice seed. Iron content in the transgenic rice seeds was up to threefold greater than in non-transgenic control plants. Others have used recombinant soybean ferritin under a different promotor to increase the iron content in wheat and rice. In this case, iron was significantly increased in vegetative tissues but not in seeds (Drakakaki et al. 2000). Thus, the experimental conditions, type of promotor used, mineral transport and storage in the plant, and other conditions have substantial effects on the outcome of genetic engineering experiments to increase mineral content.

Iron transport and uptake in plants is carefully regulated. This is because iron has low solubility and is toxic in excess. Recent studies have examined the function of iron transporter proteins in transgenic plants. These proteins have been shown to increase iron uptake into roots when iron is deficient (Eide et al. 1996). The iron transporter protein, IRT1, first isolated from *Arabidopsis thaliana*, also transports other metals such as zinc, manganese, lead, and cadmium; the latter two can be toxic. Researchers in the laboratory of Dr. Mary Lou Guerinot at Dartmouth College, Hanover, NH, have shown that slight changes in the amino acid composition of the transporter protein affects the selectivity of metals transported into the plant (Rogers et al. 2000). This finding introduces the possibility of engineering plants with the ability to take up desirable minerals while excluding toxic and undesirable ones.

A second iron transporter protein, IRT2, has also been identified in the roots of *Arabidopsis*. When the gene for IRT2 was incorporated into iron- and zinc-deficient plants, iron uptake was increased (Vert et al. 2001). Unlike the IRT1 transporter, however, IRT2 did not transport manganese and cadmium when it was expressed in yeast. This observation suggests ways in which selective genetic transformations might be used to enhance the uptake of some minerals while excluding others.

It should be noted that iron and zinc levels tend to be present together in many plants, although the average content of each differs. For example, in screening over 1,000 varieties of common beans, a nutritional staple in many countries, scientists at the International Institute of Tropical Agriculture, Nigeria, found that iron content averaged about 55 mg/g iron, but some varieties from Peru averaged more than 100 µg/g iron (Gregorio 2002). Zinc content, averaged 35 µg/g. When varieties were selected for their iron content, higher zinc levels were obtained as well. These observations suggest that genetic modification of selected varieties to further increase iron content might boost zinc levels too.

As in beans, iron and zinc concentrations differ across varieties of rice. Aromatic rice tends to have the highest iron levels and several varieties have been successfully crossed with elite rice lines having excellent agronomic characteristics and grain qualities. These micronutrient traits were shown to be stable across different growing environments and could be crossed with high yielding varieties to improve the nutrient density. High iron rice developed from traditional breeding is currently being tested for iron bioavailability and effects on iron nutrition status in young women in the Philippines (World Bank 2000). Results are not yet available.

Another approach to improving the availability of iron for infants was reported by Suzuki and colleagues (2003) at the University of California, Davis. These investigators developed transgenic rice in cell culture that expressed the gene for human lactoferrin, a milk protein that binds iron. When they compared the recombinant lactoferrin with native human lactoferrin both proteins retained functional activity after mild heat treatment, high acidity, and *in vitro* digestion. Their findings suggest that recombinant lactoferrin grown in plant culture may be a functional alternative to human lactoferrin in infant formula and provide another way to improve iron availability during infancy.

B. Zinc

Zinc is one of several trace minerals that can be deficient in human diets, especially where meat is not consumed. Zinc deficiency is associated with impaired growth and reproduction, anorexia, immune disorders, and a variety of other symptoms. Zinc is also an important constituent of more than 100 enzymes. Absorption of zinc from cereals and grains can be impaired or blocked by the presence of some substances such as phytate.

Increasing the zinc content of cereals and grains, especially where soils are low in zinc, may be an effective way to improve human nutrition and at the same time increase plant yields. Ramesh and colleagues (2004) in Australia, studied the effect on seed zinc content in barley (*Hordeum vulgare* cv Golden Promise) in plants transformed to increase the expression of zinc transporter enzymes. Multiple transgenic lines exhibited higher zinc and iron contents in their seeds compared with control plants (Ramesh et al. 2004). When grown under zinc deficient conditions, zinc uptake in the transgenic lines was higher in the short term compared with control plants. When zinc content was restored, uptake of zinc decreased

in both transgenic and control plants, suggesting that the transporter proteins may be degraded when zinc is adequate. This study suggests that increasing the production zinc transporter proteins may be one approach to increasing the zinc content of cereals.

C. Selenium

Selenium is an essential trace mineral incorporated into plants from soil. Consumption of selenium has been linked to reduced risk of all cancers, but particularly those of the lung, colo-rectum and prostate (El-Bayoumy and Sinha, 2004, Combs 2004). Selenium is also important for specific enzymes and proteins in the brain and is necessary for proper immune function. However, selenium is toxic at levels only a little greater than those required in a healthy diet, so caution is warranted with supplementation and increased intakes. Areas where soils are deficient in selenium are well known and low to deficient intakes have been observed among human and animal populations in these regions.

Genetic engineering technology offers considerable potential for increasing the uptake of selenium from soils and incorporating the mineral into non-toxic compounds in the edible parts of plants. Plants genetically modified to absorb above average quantities of minerals could be used to improve human or animal nutrition. For example, a study from the University of California, Berkeley reported that genetically engineered *Arabidopsis thaliana* and Indian mustard (*Brassica juncea*) were able to incorporate more selenium from soil and convert it to non-toxic methylselenocysteine than wild type plants (LeDuc et al. 2004). Researchers at Purdue University, West Lafayette, IN, also showed that plants not normally accumulating selenium, such as *Arabidopsis*, can be transformed to do so (Ellis et al. 2004). These studies demonstrate the feasibility of developing crop plants with improved ability to take up selenium from the soil and store it in a non-toxic form. Thus, selenium at appropriate concentrations would be safe for the plant and for human consumption.

VII. PHYTONUTRIENTS AND NOVEL SUBSTANCES

Intense research activity is being devoted to the identification and study of phytonutrients in plants with an eye to their ability to protect health, improve immune function, and reduce the risk of chronic diseases ranging from heart disease and cancer to age-related macular degeneration. Examples of phytonutrients include: phytoestrogens, polyphenols, and isothyocyanates. In spite of their cumbersome technical names, these various categories of substances appear to hold significant potential health benefits when consumed in modest amounts. Because they are widely distributed in fruits and vegetables, diets rich in these foods are likely to furnish generous amounts of many of these phytonutrients.

Unfortunately, there is insufficient scientific data from carefully controlled studies that adequately demonstrate safety and efficacy of substances with potential promise. For many substances—lycopene, isoflavones, resveratrol—to name a few, data appear promising, but are not consistent or conclusive. Extensive media and manufacturer publicity about many of these compounds generated expectations exceeding scientific justification. For these reasons, the enhancement of foods with particular phytonutrients usually lacks sufficient scientific grounding to justify the development of foods with enhanced levels. However, a few examples can be cited.

Isoflavones: Isoflavones are a type of phytoestrogen, so named because they bind to estrogen receptors and mimic some of the effects of the hormone estrogen. However, the biological effects of isoflavones differ markedly from estrogen and many are non-hormonal.

Soybeans are the richest food source of isoflavones, but isoflavones occur in other legumes such as broadbeans, and in many vegetarian ("meatless") foods made from soy products (USDA 2002). They have been linked with easing menopausal symptoms, improving bone health, reducing cardiovascular risk, and possibly reducing the risk of prostate cancer. The main soy isoflavones are genistein, diadzein, and glycitein. Their concentration in legumes is greatly affected by growing conditions and climate, and these variables could potentially override genetic modifications.

Currently, isoflavones are abundant and readily available, so genetic modifications to increase isoflavone content might not be expected to be a high priority. However, Yu and colleagues (2003) at the DuPont Company, Wilmington, DE, reported the application of genetic engineering techniques to increase the isoflavone content of soybeans. By activating genes in the phenylpropanoid pathway, diadzein levels increased and genistein levels fell. By blocking the anthocyanins branch of this synthetic pathway, the investigators obtained higher concentrations of isoflavones. Thus, it is possible to increase the level of isoflavones in soybeans.

Phytosterols and Phytostanols: Phytosterols and their saturated derivatives, phytostanols, are plant sterols found in small quantities in vegetable oils. When consumed in sufficient amount, they are effective in reducing blood cholesterol levels. Sufficient data of their efficacy and safety exist that the FDA has permitting food manufacturers to claim a role for plant sterols or stanols or their esters in reducing the risk of coronary heart disease (see section on health claims below for a further description).

In 2003, researchers at Unilever Research, Netherlands, reported the generation of transgenic tobacco seeds with enhanced total seed sterol level (Harker et al. 2003). Following the insertion of the gene for a key enzyme in sterol biosynthesis, total seed sterol levels increased by 2.4-fold. The additional sterol was present as fatty acid esters and several intermediate sterols accumulated. This group also developed transgenic tobacco carrying two genes involved in regulating carbon flux through the sterol biosynthesis pathway (Holmberg et al. 2003). The two transgenes increased total seed sterol content more than with either gene expressed singly (2.5-fold vs. 1.6-fold).

Researchers at Monsanto reported the application of genetic engineering to modify the ratio of phytosterols to phytostanols in rapeseed (*Brassic napus*) and soybean (*Glycine max*). Plants were transformed with a gene from yeast for the enzyme 3-hydroxysteroid oxidase (Venkatramesh et al. 2003). Seeds from both types of plants exhibited conversion of the major phytosterols to phytostanols and no other functionalities were affected. Several novel phytostanols were obtained as well. Because these substances are hydrogenated they would be expected to be more stable during food processing, yet still confer cholesterol-lowering benefits.

Most recently, Enfissi et al. (2005) reported the development of transgenic tomatoes with a 2.4-fold increase in phytosterols. Increases were greatest for phytoene and beta-carotene. Such an alteration in a widely consumed food would potentially increase the consumption of these substances in a large share of the population.

Probiotics: The term "probiotics" refers to live microorganisms that have a health benefit when consumed in adequate amounts. They are usually bacteria selected from species found in the intestinal tract. Probiotic microorganisms may be concentrated and added directly to a food or added to a milk product in small amounts and allowed to grow. The most common foods having probiotic organisms are fermented dairy products such as yogurt containing *Lactobacillus acidophilus*.

Many health benefits have been attributed to probiotics, including resistance to infectious diseases, prevention of vaginitis, production of antimicrobial metabolites and nutraceuticals, immunomodulation, and others (Ahmed 2003). Foods containing probiotic bacteria are abundant in Japan and parts of Europe, but are less developed in the U.S. Probiotics have been used as dietary supplements and oral agents for intestinal disorders. A genetically engineered strain of *L. lactis* subsp. *diacetyllactis* is used as a buttermilk starter culture in the U.S. (Renault 2002).

Probiotics have been used to treat inflammatory bowel disease by creating more host-friendly gut flora. Selective use of probiotic bacteria can create an environment where stimulation of the immune system is restrained and intestinal inflammation reduced (Guarner et al. 2002). Some strains of *Lactobacilli* have prevented the development of colitis in genetically susceptible mice. Other genetically engineered bacteria have been used to secrete the anti-inflammatory cytokine IL-10 (Guarner et al. 2002). Considerable research is being devoted to the identification and effects of probiotic bacteria and their use as therapeutic agents, but few products have reached application in the U.S.

Genetically engineered probiotic bacteria have been used to overcome problems associated with more traditional technologies for developing such bacteria. In foods, genetically engineered bacteria have been used to improve the flavor and stability, or to block the formation of unwanted flavors. Genetic engineering should make it possible to strengthen the effects of existing bacterial strains and create new ones (Steidler 2003).

VIII. ANTI-NUTRITIONAL SUBSTANCES

Many plants contain substances that inhibit nutrient uptake in various ways or are toxic themselves. They may interfere with intestinal cell function, reduce the ability to break down complex molecules such as proteins and starch, or may be toxic if consumed in sufficient quantity. Some of these substances are rendered harmless with cooking or processing, but others are resistant to digestion, heat treatment, or other forms of processing. Examples of anti-nutritional substances are shown in Table 2.

Although anti-nutrient substances reduce nutrient availability, many benefit human health and plants. For example, dietary fiber improves colon function and reduces plasma cholesterol levels; polyphenols and other substances have anti-carcinogenic properties. Many of these substances are important in plant metabolism and provide resistance to environmental stress and pests. Further, some of the adverse effects on nutrient availability may diminish over time or with different levels of consumption, and this suggests that humans may be better able to adapt to these substances than was once thought (Welch 2002). The question of improving micronutrient availability by reducing anti-nutritional substances through plant breeding, biotechnology, and other means requires careful consideration of the complexities.

TABLE 2. Anti-nutrients in plant foods that reduce nutrient bioavailability, or impair health

ANTI-NUTRIENT	EFFECT	DIETARY SOURCE
Phytic acid (phytate)	Binds minerals, K, Mg, Ca, Fe, Zn	Whole legume seeds, cereal grains
Fiber, e.g. cellulose, hemicellulose, lignin	Decreases fat and protein digestibility; may decrease vitamin & mineral absorption	Whole cereal grains, e.g., wheat, corn, rice
Trypsin inhibitor	Reduces the activity of the enzyme trypsin and other closely related enzymes that help digest protein	Legumes, e.g., soy; cereals, potatoes
Polyphenolics, tannins,	Form complexes with iron, zinc, copper that reduces mineral absorption	Tea, coffee, beans, sorghum;
Hemaglutinins, e.g. lectins	Interfere with cells lining the gastrointestinal tract causing acute symptoms; can bind metals and some vitamins; can be toxic	Legumes
Cyanogens or glycoalkaloids	Inhibit acetylcholinesterase activity which impairs nerve transmission; can damage cell membranes	Cassava, linseed, peas, beans
Glucosinolates	May adversely affect thyroid activity	Cabbage, broccoli
Saponins	May irritate the gastrointestinal tract and interfere with nutrient absorption	Soybeans, peanuts, sugar beets
Goitrogens	Suppress thyroid function	Brassica and Allium foods, e.g., broccoli, garlic
Heavy metals, e.g., cadmium, mercury, lead	May have toxic effects, e.g., high levels of Hg impair fetal brain developmnet	Contaminated leafy vegetables
Gossypol	May harm kidney function and reduce sperm counts; can be toxic	Cottonseed
Oxalic acid	Binds calcium to prevent its absorption	Spinach leaves, rhubarb
Phytotoxins, e.g. solanine	Can be toxic; affects gastrointestinal and nervous systems	Green parts of potato
Mycotoxins, e.g., aflatoxin, fumonisin	Toxins produced by certain molds; toxic to humans and animals; can be carcionogenic	Grain, peanuts, other crops

There are several ways of reducing anti-nutritional substances, including plant breeding, heat, processing, fermentation and drying, and genetic engineering. Examples of the various anti-nutritional substances that have been reduced by the application of biotechnology in different plants are described below.

Phytate: Phytic acid is a phosphorus storage compound found in the seeds of many edible crops, e.g., wheat, corn, barley, rice. Phytic acid forms salts (phytates) of potassium, magnesium, calcium, iron, zinc, and other minerals that cannot be absorbed. Phytic acid-containing foods bind minerals in the intestinal tract rendering them unavailable. When

these minerals are limiting in the diet, the presence of phytic acid can contribute to mineral deficiencies, particularly in the case of iron and zinc. This is a particularly important consideration in the diets of women and children where legumes and cereals are staple foods. In animal nutrition, especially for poultry and swine, phosphorus may have to be supplied in a more available form to overcome the loss due to binding with phytic acid. An additional consequence is the production of high phosphorus animal waste with adverse environmental effects.

Lines of corn, barley, rice, and soybean with slightly different phytic acid characteristics have been used to develop varieties with reduced seed phytic acid (Raboy 2002). Reduction in phytate in the range of 50% to 66% has been achieved with these mutant lines. In soybeans and corn, 80% reduction has been achieved. However, several hybrids developed with the mutant strains exhibited lower yields. It has now been shown that low phytate mutant corn is linked to the reduced expression of the enzyme myoinositol phosphate synthase, the first enzyme in the synthesis pathway for phytic acid (Shukla et al. 2004). This finding was confirmed recently by Italian researchers who developed a mutant corn with 90% less phytic acid and a 10-fold increase in seed-free phosphate (Pilu et al. 2003). Proof that zinc absorption from low phytate corn compared with wild-type varieties was significantly greater was reported by Hambridge et al. (2004) who fed corn tortillas to six healthy adults. Zinc absorption from the 80% phytate-reduced corn was three times greater than from the wild-type corn, 4.9 mg/day compared with 1.5 mg/day.

Although genetically engineered low phytate crops have not been commercialized, biotechnology has been used to express the enzyme phytase in plants (Chier et al. 2004). This enzyme allows animals to metabolize phytic acid and eliminates the need to supplement feed with phosphorous. Consequently, using animal feed engineered to produce phytase addresses many of the problems associated with high phytate levels in animal feed. Phytase has been successfully incorporated into soybean and wheat and is biologically active when the plants are used as animal feed (Brinch-Pedersen et al. 2000). In a study of broiler chickens, consumption of transgenic soybeans containing phytase led to a 50% reduction in phosphorus excretion compared with a diet supplemented with an intermediate level of nonphytate phosphorus (Denbow et al. 1998). Feeding the transgenic soybeans resulted in an 11% greater reduction in phosphorus excretion than feeding with conventional soybeans to which the enzyme is added. Similarly, low-phytate corn and barley fed to broiler chicks resulted in 33% lower phosphorus excretion compared with wild-type grain diets and reduced the need for supplemental phosphorus (Jang et al. 2003).

Transgenic phytase-containing wheat was developed by Holm and colleagues at the Danish Institute of Agricultural Sciences, Denmark (Brinch-Pedersen 2000, Holm et al. 2002). Transgenic plants exhibited up to 4-fold higher phytase activity compared with wild-type seeds. However, a drawback of such a transformation for human consumption is that the enzyme is inactivated above 60°C and would be destroyed by cooking (Holm et al. 2002). It is possible that more heat-stable forms of the enzyme could be used, but when more heat-stable enzymes were incorporated into rice, which was then cooked, only 8% of the activity remained (Holm et al. 2002).

Transgenic rice expressing phytase derived from modified yeast genes has also been reported (Hamada et al. 2005). By selectively modifying the genes, the investigators were able to increase the enzyme activity above that of the original yeast gene.

Reducing the phytate content of plants, particularly soybean, has direct implications for human nutrition. For example, soy protein used in infant nutrition may limit mineral absorption because of its phytate content. To investigate this question, Davidsson and colleagues at the Swiss Federal Institute of Technology compared regular and dephytinized soy formula in nine infants 69 to 191 days old. Regular and dephytinized formula contained 300 and 6 mg phytic acid/kg liquid, respectively. The investigators reported that zinc absorption was significantly greater from dephytinized formula compared with regular formula, 22.6% compared with 16.7% absorption (Davidsson et al. 2004). Absorption of iron, manganese, copper and calcium did not differ between the two formulas. These findings suggest that use of dephytinized soy protein improves zinc absorption and can be recommended for infant foods.

Another approach to solving the phosphorous uptake problem in animal production was undertaken by researchers at the University of Guelph, Canada. In this case, swine were engineered to produce the enzyme phytase (Golovan et al. 2001). These pigs expressed the enzyme phytase in their saliva and exhibited complete phytate digestion, required no dietary inorganic phosphorus and excreted 75% less phosphorus.

Gossypol: Cottonseed contains the polyphenolic compound gossypol, long known to be toxic to humans and animals. Interestingly, gossypol also appears to have anti-cancer properties toward several human prostate and breast cancer cell lines (Liu et al. 2002, Jiang et al. 2004). Martin and colleagues at Texas A&M University, College Station, TX, created a transgenic cotton (*Gossypium vitifolium*) using the antisense gene technology for a key enzyme in the synthesis of gossypol. Transformed cotton plants had up to 70% less gossypol in their seeds compared with non-transformed plants (Martin et al. 2003). These findings suggest that biotechnology can be used to reduce the gossypol levels to render the seed oil more suitable for feed and food.

Cyanogens: Cassava (*Manihot esculenta* Crantz) produces various *cyanogenic glycosides* such as, linamarin, lotaustralin, and acetone cyanohydrin in its roots and leaves. These potentially toxic substances are only present in small amounts in "sweet" varieties of cassava, but are sufficiently abundant in "bitter" varieties to require removal (Padmaja 1996). Boiling and drying reduce these substances to safe levels in low cyanogen varieties, but those with higher levels require, soaking, grating or maceration, fermenting, and sun-drying to reduce cyanogens adequately. The toxicity of these cyanogens is exacerbated by low protein intakes, a characteristic of countries where cassava is a staple.

In 2003, Drs. Siritunga and Sayre at the Ohio State University, Columbus, OH, reported the development of transgenic cassava with a 99% reduction in linamarin in the roots and between 60% and 94% reduction in leaves (Siritunga and Sayre 2003). These plants were transformed by inhibiting the expression of two genes that catalyze the first step in the synthesis of linamarin. The following year, this group reported that transgenic cassava roots expressing a different gene contained significantly less acetone cyanohydrin levels compared with wild-type plants (Siritunga et al. 2004). These accomplishments open the door to the development of cassava truly safe for human and animal consumption.

Steroidal glycoalkaloids: Potatoes (*Solanum tuberosum* spp) contain potentially toxic steroidal glycoalkaloids, the best known of which is solanine. This substance is found in the green tissue of potato just under the skin. While these compounds protect the plant from pests, they reduce food quality and safety. Glycoalkaloids are synthesized from cholesterol.

Recently, Arnqvist et. al. (2003) showed that the inhibition of plant sterol synthesis in transgenic potatoes reduced the synthesis of glycoalkaloids by 41% in leaves and 63% in tubers. Other investigators used antisense technology to create transgenic potatoes with up to 40% less steroidal glycoalkaloids in the tubers (McCue et al. 2003). These studies indicate that substantial improvements in the reduction of steroidal glycoalkaloids in potatoes may be close at hand. It remains to be seen whether pest resistance in these transgenic potatoes is affected by the reduction in glycoalkaloids.

Mycotoxins in corn: An important risk to the safety of grains (corn, wheat, barley), groundnuts (peanuts), tree nuts (almonds, walnuts, pistachios) and cottonseed is the production of toxins by fungi. Certain types of fungi—*Aspergillus flavus* and *Fusarium*—are notorious for their deadly products. The substances produced by these organisms cause disease in plants and potentially serious illness in people and animals consuming the infected crops. Aflatoxin, a particularly dangerous cancer-causing mycotoxin produced by the fungus *Aspergillus flavus*, is sometimes found in peanuts and corn. Fumonosins produced by Fusarium fungi are thought to be carcinogenic and harmful to the immune system. Agricultural practices and grain storage conditions help to minimize growth of these fungi, but weather also contributes to their development. Breeding crop varieties with increased resistance to fungi may reduce the production fungal toxins. Researchers at the U.S. Department of Agriculture developed transgenic walnut trees that displayed increased resistance to aflatoxin synthesis (USDA 2004). Partial resistance to *Fusarium* disease was reported in transgenic wheat (Okubara et al. 2002) and in transgenic bacillus thuringiensis (Bt) corn (Bakan et al. 2002). The potential for genetic engineering strategies to reduce the production of fungal toxins in food crops has been discussed by Duvic (2001) and Munkvold (2003).

Oxalates: Oxalic acid is present in spinach, tomato, groundnut, soybean, and chick pea. It binds several minerals including calcium and prevents their absorption. Scientists at the National Centre for Plant Genome Research (NCPGR), India, seek to reduce the oxalic acid content in these foods through the transfer of the gene for oxalate decarboxylase (OXDC), the enzyme that degrades oxalic acid. Genetically engineered tomatoes bearing the OXDC gene have been successfully grown and are currently undergoing field and biosafety tests (ISAAA, 2004).

IX. ALLERGENS

Food allergy, although relatively rare, can provoke severe, sometimes fatal, responses in susceptible people. Specific food proteins can trigger the immune system and provoke an allergic response. Risk of such reaction is greatest in the first two years of life, and by age five about 80% of allergic infants will lose their food allergies.

Eight types of foods account for nearly 90% of all food allergies. These are: milk, eggs, fish, crustacea, wheat, peanuts, tree nuts, and soy. People allergic to one type of food are frequently, but not always, allergic to others. Many proteins associated with food allergies in these foods have been identified, but many remain unknown.

Soybean: In people with soy allergy, as many as 28 proteins may bind with IgE, a type of antibody involved in allergic responses, suggesting that many soy proteins have allergenic potential. More than half of soybean allergic reactions are attributable to a single protein, known as P34 (Cordle 2004, Wilson et al. 2005).

Scientists at USDA and the Donald Danforth Plant Science Center have succeeded in silencing the gene for P34 and created soybeans without this protein (Herman et al. 2003). However, two other proteins that trigger allergic reactions may have to be removed before soybeans could be sold as hypoallergenic. Dr. Anthony Kinney, a researcher involved in the project, commented that removing the other proteins should not be difficult because wild species lacking the genes for the other proteins are already known. Careful plant breeding with the genetically modified soybeans may be able to produce the hypoallergenic soybeans. Other genetic engineering strategies that alter the composition of the allergenic proteins may render the proteins harmless to sensitive people. The ability to eliminate the most hazardous allergens in soy would have substantial benefit for infant formula feeding and for those who are allergic to soy.

Peanut: Allergy to peanut proteins can be fatal. The major allergens in peanut have been identified as Ar h1, Ar h2, and Ar h3, and their genes have been isolated. The protein Ar h2, a Kunitz trypsin inhibitor, is believed to be the most potent of these three (Koppelman et al. 2004). Recombinant versions of these allergens produced in bacteria were heat-killed and used in a vaccine (Li et al. 2003). The vaccine was given in three different doses to allergen-susceptible mice. Animals were challenged after 2, 6, and 10 weeks. Treated mice produced no anaphylactic or histamine response when challenged. Animals given the medium and high doses remained protected for 10 weeks. This particularly encouraging research suggests that protection against peanut allergy may be in the foreseeable future.

Rice: Rice is generally consider a hypoallergenic food, and for that reason is one of the solid foods first introduced to infants. Nonetheless, some people, particularly in Japan, are allergic to rice proteins. The major allergen(s) in rice have been identified and hypoallergenic varieties of rice developed using antisense genetic engineering techniques to suppress the synthesis of the predominant allergen (Nakamuro and Matsuda 1996). Allergen content in the transgenic seeds was reduced from about 300 micrograms/seed to about 60 to 70 micrograms/seed. However, when the hypoallergenic rice was tested in sensitive patients, not all allergenic potential had been eliminated. Extremely sensitive patients were also sensitive to other proteins, so that a single genetic transformation was insufficient to overcome their allergic responses. EuropaBio (2002) reported that several laboratories in Japan are working to develop hypoallergenic rice, but efforts have provided only partial success, as it is not possible to eliminate all allergens.

Shrimp: Allergic reactions to shrimp and other crustacea (lobster, crayfish) are among the most common food allergies. People with hypersensitivity responses to crustacea may also be allergic to mollusks, and some arthropods such as house dust mites and cockroaches. To date there have been no ways to overcome these allergies. The major allergen in shrimp is the muscle protein tropomyosin, known as Pen a 1. Recent analysis of this allergen revealed five IgE-binding regions or epitopes (Ayuso et.al. 2002). These regions contained 15 to 38 amino acids from whose sequence the corresponding gene sequence can be determined. Dr. Samuel Lehrer of Tulane University, New Orleans, LA, has located the gene sequence that encodes these epitopes and has suggested that by altering these epitopes by a single amino acid, IgE binding could be halted. This work suggests that safe recombinant allergens could be synthesized for immunotherapy of those who are allergic to shrimp and related substances.

X. MISCELLANY

A. Beer and Wine

Applications of biotechnology are finding their way into brewing and wine-making. One application in grapevines (*Vitis vinifera* L.) has been increased content of the antioxidant resveratrol. This substance is of special interest in human health for the reduction of oxidized lipids. It is also thought to improve plant resistance to fungal disease. Gonzalez-Candelas et al. (2000) also reported increased resveratrol in wine through the use of transgenic yeast. In a different approach, Giorcelli et al. (2005) developed transgenic grapevines using the gene for stilbene synthase, an enzyme that leads to the production of resveratrol. The plants increased their production of resveratrol, but showed no improved resistance to a leading fungal pathogen.

Transgenic yeast also has potential for modifying the flavor of beer (Vanderhaegen et al. 2003) and sake (Aritomi et al. 2004). Genetic engineering also holds considerable potential for the production and regulation of diverse flavors and aromatic substances, as recently described (Dudareva and Negre 2005).

B. Decaffeinated Coffee

Japanese scientists have succeeded in silencing the gene responsible for caffeine production in coffee plants (Jameel 2003, Ogita et al. 2003). Caffeine content in the transgenic plants was reduced by 70%. The researchers aim to modify Arabica coffee plants, the most popular coffee grown. Toward this end, Ogita et al. (2004) reported additional progress on modifying the pathways in Arabica and canephora coffee varieties which resulted in caffeine reductions ranging from 30% to 50%. This genetic modification would eliminate the need for decaffeination processing.

PART 2 Applications of Modern Biotechnology to Functional Food

Legal and Regulatory Considerations Under Federal Law

I. INTRODUCTION

"Functional food" is touted as a convenient means for consumers to promote optimal health, including the prevention of disease. The focus of much attention in recent years, functional food has been the subject of numerous articles, reports, and consumer education materials, such as a "Functional Food Guide Pyramid"—a modified version of the USDA Food Guide Pyramid that identifies functional food from each major food group. A considerable array of food has been described as "functional" in one or more respects, including calcium-fortified orange juice, whole grains, fruits and vegetables (and components thereof, including lycopene, polyphenols, indoles, and other phytochemicals), soybeans, omega-3 fatty acids, phytosterols, and cocoa.

Despite the widespread attention, the "functional food" concept eludes precise definition. As previously noted, the Food and Nutrition Board of the National Academy of Sciences has suggested that a "functional food" is "any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Food and Nutrition Board 1994). Others argue that a functional food is any food promoted or consumed for a specific health effect, regardless of whether the food has been modified in some fashion. A frequent criticism is that all food is, in some sense, functional.

From a legal perspective, there is no separate regulatory category for functional food in the United States. Food that is deemed functional, therefore, is subject to the same regulatory requirements as any other food. This means that a functional food may be regulated as "conventional food," a "dietary supplement," a food for "special dietary use" (including infant formula) a "medical food," or a "drug," depending upon its positioning in the marketplace, including claims made for it. Significantly, federal requirements for "functional food" apply regardless of how the food is produced, such as through mechanical or genetic methods (e.g., product formulation, modern biotechnology techniques, or other means). Thus, rice that has been genetically enhanced to provide beta carotene is subject to basically the same statutory and regulatory framework as rice to which beta carotene is added through product formulation.[1] The difficulties associated with FDA regulation of functional food historically have been related to whether such food is subject to the more onerous drug provisions of the law.

1 The use of modern biotechnology is subject to regulation by several federal agencies, including the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). This report focuses on safety and labeling requirements enforced by FDA and USDA's Food Safety and Inspection Service (FSIS) pursuant to certain food laws administered by those agencies. Food produced through the use of modern biotechnology is handled somewhat differently by FDA in that it is subject to a voluntary consultation process. *See* 57 Fed. Reg. 22984 (1992).

This section of the report describes how functional food is regulated under certain federal laws governing food in general.[2] To place the regulation of functional food in an appropriate context, this report begins with an overview of the basic statutory framework pertinent to food. It then examines the application of this basic framework to functional food, first in the context of food generally, and then in the context of meat and poultry products, eggs and egg products, and animal feed, including pet food. As appropriate, the discussion also identifies select areas of criticism and issues concerning functional food regulation in the United States.

II. THE FFDCA FRAMEWORK—OVERVIEW AND BRIEF HISTORY

In the United States, food products other than meat and poultry and alcoholic beverages are regulated primarily by the Food and Drug Administration's (FDA) Center for Food Safety and Applied Nutrition (CFSAN) pursuant to the Federal Food, Drug, and Cosmetic Act (FFDCA).[3] In addition to providing for the regulation of "food," the FFDCA establishes comprehensive requirements for the marketing of drugs, medical devices, and cosmetics. Food and other articles regulated by FDA under the FFDCA cannot be adulterated or misbranded. Adulteration refers generally to aspects of a product that typically relate to quality or safety and misbranding refers generally to false or misleading labeling.

Although the concept of "functional food" was not contemplated at the time Congress enacted the FFDCA, the law has evolved—through flexible agency interpretations as well as statutory amendments—to accommodate new products and advances in science. The emergence of functional food as a unique and important category for marketing purposes has led some to question whether the current legal and regulatory framework is adequate.

A. The Statutory Foundation: "Food," "Drug," and Food for "Special Dietary Use"

At the time of its enactment in 1938, the FFDCA established requirements for only three product categories of relevance to functional food regulation. In addition to covering "food" and "drugs," the 1938 Act introduced the concept of food for "special dietary uses." Although this law contained no definition of "special dietary uses," the legislative history reveals that such uses were considered, at that time, to include "infant foods, invalid foods, slenderizing foods, and other dietary [products] intended for special nutritional requirements" (S. Rep. No. 493, 73d Cong., 2d Sess. 12 (1934)). FDA later defined "special dietary uses" by regulation to mean "particular (as distinguished from general) uses of food," including uses that may arise from disease or certain health-related conditions, age-related nutritional needs (e.g., infancy), or a desire to supplement or fortify the diet with any vitamin, mineral, or other dietary property. A food for special dietary use is deemed mislabeled or misbranded under the law unless its label bears information adequate to inform purchasers of its vitamin, mineral, or other dietary content.[4]

2 Under applicable law, food refers to use for human beings or other animals and includes (non-human) animal feed. "Functional food" is primarily intended for human use, as other animal feed is subject to typically different requirements.

3 As discussed more fully below, products with meaningful amounts of meat or poultry (generally 2–3%) are regulated by the U.S. Department of Agriculture (USDA) pursuant to the Federal Meat Inspection Act (FMIA) or the Poultry Products Inspection Act (PPIA). Alcoholic beverages are regulated primarily by the Bureau of Alcohol, Tobacco and Firearms of the Department of Treasury.

4 This report does not address infant formula, a "special dietary use" food for which Congress and FDA have established unique and prescriptive requirements. "Infant formula" is defined to mean "a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk." FFDCA § 201(z), 21 U.S.C. § 321(z).

B. Accommodations of Advancing Science

For nearly thirty years after enactment of the FFDCA, the basic categories of "food," "drug," and food for "special dietary use" remained largely unchanged. During this time period, FDA interpreted almost any use of food for a targeted nutritional purpose to be a "special dietary use." Indeed, as recently as 1971, FDA's framework for the regulation of food for special dietary uses (commonly referred to as "special dietary food" at that time) included requirements addressing all uses of fortification (i.e., the addition of nutrients to food or ingredients thereof), vitamin and mineral supplements, and food purported for use in sodium-restricted diets, among other food or uses deemed to be "special." Underlying this framework was an assumption that food for targeted nutritional purposes (i.e., "special" uses) was of little or no relevance to the general population. Paradoxically, certain products that seemed to more naturally fit the special dietary use category, such as specialty infant formulas, were strictly regulated as drugs.

With advances in nutrition and related sciences, the special dietary use concept was gradually reformed to accommodate evolving views concerning the relationship between diet and health. These reforms ultimately led to new and distinct categories of "food" and new types of claims. Three regulatory developments are of particular relevance to the regulation of functional food, and demonstrate the progressive blurring of the legal distinction between "food" and a "drug" since 1938: (1) the advent of "medical food" in 1972, (2) the authorization of certain nutrition and health-related claims for food in the Nutrition Labeling and Education Act (NLEA) of 1990, and (3) authorization of a separate regulatory scheme for dietary supplements in the Dietary Supplement Health and Education Act (DSHEA) of 1994.

In 1972, FDA created the "medical food" category by deciding on its own initiative to reclassify the specialty infant formula Lofenalac®, which had been regulated as a drug, as a distinct type of food for special dietary use. Intended for the dietary management of phenylketonuria (PKU), an inborn error of metabolism, Lofenalac® was recognized by FDA to meet distinctive nutritional requirements—namely, an impaired ability to metabolize the essential amino acid phenylalanine—and therefore more appropriately regulated as a food. In a regulation issued shortly thereafter, which exempted medical food from nutrition labeling requirements, FDA described the new category as "foods represented for use solely under medical supervision in the dietary management of specific diseases and disorders" (38 Fed. Reg. 2124, 2126 (1973)). As discussed more fully in Part III, FDA today treats "medical food" as a wholly unique category from food for special dietary use, and has developed very specific criteria for the marketing of such products.

Second, in 1990, Congress passed the NLEA, which establishes a framework for the regulation of "health claims" and "nutrient content claims," among other requirements. "Health claims" are statements that characterize the relationship between a food (or a substance in the food) and a disease or health-related condition (21 C.F.R. § 101.14(a)(1)). "Health claims" are permitted only if approved or otherwise authorized by FDA, the claim addresses the ability of the food, as part of an appropriate diet, to reduce the risk of disease or a health-related condition (as opposed to disease treatment, mitigation, or similar concepts), and the food meets certain qualifying criteria established by FDA. NLEA also authorized "nutrient content claims," which are statements characterizing the level of a nutrient in food, such as "sugar free" and "low sodium." Following NLEA, FDA determined that many nutrition-related claims were of general use to the public, and thus were no longer indicative of "special dietary uses."

Third, in 1994, Congress enacted DSHEA, which establishes comprehensive requirements for dietary supplements, including safety and labeling requirements. Although DSHEA provides that dietary supplements are still "food" for many purposes, the new law creates a unique framework for supplement regulation.

These important developments—DSHEA, NLEA, and FDA's "medical food" policy—helped to shape FDA's regulation of "food" and nutritional claims, and therefore functional food. Particularly, they created new regulatory categories and permitted many products to bear claims that would ordinarily have triggered "drug" regulation. These new categories and claims are described more in Section III and relate to dietary supplements, medical food, health claims, and nutrient content claims.

C. Requests for Reform

The emergence of functional food as a unique and commercially important marketing category has led some to question whether the current regulatory framework is adequate. Some groups, including the Government Accountability Office (GAO) and the Center for Science in the Public Interest (CSPI), have criticized FDA's oversight of ingredient safety and labeling. GAO and CSPI have suggested that companies should be required to notify FDA before using certain "functional" or "novel" ingredients and before making certain claims about the effect of such ingredients on the structure or function of the body (GAO, 2000; CSPI 2002). Other groups, such as the Institute for Food Technologists (IFT) have recommended increased flexibility and policy changes to better allow for claims based on scientific advances (IFT 2005). For example, IFT has proposed that FDA adopt a notification procedure for health claims. As proposed by IFT, companies wishing to use a health claim could convene an expert panel to evaluate the science supporting the claim; if the panel found the claim to be "generally recognized as efficacious," the claim could be notified to FDA and used within 90 days if the agency did not object.

These and other calls for reform led FDA, in October 2006, to announce that the agency would hold a hearing to allow consumers, industry, and other interested parties to provide comments on approaches to the regulation of functional food. Affirming its flexible approach to the statute, FDA stated that "[a]lthough we are confident that the existing provisions of the act are adequate to ensure that conventional foods being marketed as 'functional foods' are safe and lawful, we believe that it would be in the best interest of public health to begin a dialog with industry, consumers, and other stakeholders regarding the regulation of these products" (71 Fed. Reg. 62400, 62401 (Oct. 25, 2006))[5] FDA's views of the current framework for regulating functional food are described in detail next.

III. CLASSIFICATION OF FUNCTIONAL FOOD UNDER THE FEDERAL FOOD, DRUG, AND COSMETIC ACT

The regulatory classification of a product has substantial implications for its marketing and use, as it influences the legal requirements pertaining to safety, labeling, and whether

5 FDA did not specifically seek comment regarding the regulation of dietary supplements. Although there are important legal distinctions between conventional food and dietary supplements, the functional food concept is often considered to include both categories; accordingly, both are addressed in this report.

the product is subject to regulatory pre-market clearances or approval. Accordingly, to determine how a specific "functional food" is regulated under the FFDCA, careful consideration of the pertinent regulatory categories is required. Following a brief review of the pivotal definitions of "food" and "drug" (Section A), which form the basis for much of the regulatory landscape for functional foods, a review of each regulatory category into which a functional food may fall is provided. These regulatory categories are drug (Section B), conventional food (Section C), dietary supplement (Section D), food for special dietary uses (Section E), and medical food (Section F). An overview of the basic categories and claims available for each is presented in Table III-1.

TABLE III-1 Regulatory Categories and General Summary

Category	Scope	Safety Standard	Claims
Drug	Product intended to diagnose, prevent, treat, or mitigate disease	New drugs must be demonstrated to be safe and effective	Must be approved by FDA
Conventional Food	Product used primarily for taste, aroma, or nutritive value, and in a conventional food form	Components must present "reasonable certainty of no harm"; must be FDA-cleared unless determined to be generally recognized as safe (GRAS)	Health and nutrient content claims must be FDA-authorized; other claims must not be false or misleading in any particular
Dietary Supplement	Product intended to supplement the diet; must contain one or more "dietary ingredients" (e.g., vitamins, herbs, substances found in the diet), be intended for ingestion, labeled as a dietary supplement, not represented for use as a conventional food, and not approved as a drug, among other criteria	Must not present a "significant or unreasonable risk of illness or injury"; new ingredients must be notified to FDA	Health and nutrient content claims must be FDA-authorized; structure/function claims must be notified to FDA; other claims must not be false or misleading in any particular
Food for Special Dietary Use	Food intended for supplying particular dietary needs (e.g., those relating to disease, pregnancy, lactation, underweight, or overweight)	See conventional food standard above	Claims of special dietary usefulness (e.g., usefulness in reducing body weight)
Medical Food	Formulated food intended for use under the supervision of a physician for the dietary management of disease with distinct nutritional requirements	See conventional food standard above	Claims for the dietary management of disease

A. Key Definitions: "Food" and "Drug"

Two key definitions form the basis for much functional food regulation: "food" and "drug." An understanding of these overarching regulatory categories is particularly important to appreciate the scope of permissible claims.

"Food" is defined to mean—

(1) articles used for food or drink for man or other animals,

(2) chewing gum, and

(3) articles used for components of any such article (FFDCA § 201(f), 21 U.S.C. § 321(f)).

The food definition is based on a product's actual (as opposed to intended) use. It has been interpreted to mean that articles used primarily, but not exclusively, for taste, aroma, or nutritive value are "food":

> When the statute defines 'food' as 'articles used for food,' it means that the statutory definition of 'food' includes articles used by people in the ordinary way most people use food—*primarily* for taste, aroma, or nutritive value. To hold . . . that articles used as food are articles used *solely* for taste, aroma, or nutritive value is unduly restrictive since some products such as coffee or prune juice are undoubtedly food but may be consumed on occasion for reasons other than taste, aroma, or nutritive value.[6]

FDA actions against weight loss products marketed as starch blockers illustrate the scope of the "food" definition. In one such case, *Nutrilab, Inc. v. Schweiker*, FDA asserted a starch blocking product to be a "drug" within the meaning of section 201(g)(1)(C). The court agreed that the product, which contained substances that were claimed to inhibit enzymes necessary for starch digestion, was a drug. The court reasoned that the product was intended to affect digestion, a bodily function, and was not "food" because it was not consumed primarily for taste, aroma, or nutritive value. In other words, the product affected a bodily structure or function, not by way of its consumption as food, but through a pharmaceutical mechanism of action.[7]

Unlike food, the drug category is based on intended use. A product is deemed to be a drug, in most relevant part, on the basis of its intended use to diagnose, cure, mitigate, treat, or prevent disease, or intended use to affect a bodily structure or function. Specifically, the term "drug" is defined in section 201(g)(1) of the FFDCA to include—

> (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and
>
> (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and
>
> (D) articles intended for use as a component of any article specified in clause (A), (B), or (C).

6 *Nutrilab, Inc. v. Schweiker*, 713 F.2d 335, 338 (7th Cir. 1983) (emphasis added).

7 Today, starch blocker products are positioned as dietary supplements, which are authorized to bear structure/function claims without regard to their nutritive content or positioning as "food." *See infra* section III.C.

Intended use is determined objectively, based on labeling claims made for a product, instructions for use, advertising, and other materials as appropriate. Thus, by controlling claims made for a product, a manufacturer may exercise substantial control over a product's classification. The "food" and "drug" categories are not mutually exclusive, meaning that a product that appears to be a "food" but that is promoted for therapeutic purposes falls within the drug definition and can be regulated as such. Examples include phytosterol-enhanced rapeseed oil (i.e., canola oil) promoted for cholesterol lowering properties and yogurt promoted for probiotic properties that are suggested to prevent or treat infectious disease.

Significantly, the structure/function prong of the drug definition provides a broad exception for food.[8] In other words, because food obviously affects bodily structures and functions through nutrition, a food product is not subject to regulation as a "drug" merely because it bears a structure/function claim (e.g., "Calcium builds strong bones"). Rules for structure/function claims for conventional food and dietary supplements are explained more fully in Sections III.C.2 and III.D.2.

B. "Drug"

1. Scope

Based on the statutory definitions described above, including the exemption for structure/function claims that appear on food, the "disease" prong of the "drug" definition is the primary factor determining whether a functional food will be regulated as a "drug." The line between "disease claims" that render a food a "drug" and structure/function claims can be difficult to discern.

To help identify impermissible disease claims that cannot be used on food, FDA has defined "disease" to mean "damage to an organ, part, structure or system of the body such that it does not function properly (e.g., cardiovascular disease), or state of health leading to such dysfunctioning (e.g., hypertension)," excluding diseases resulting from nutrient deficiencies. (21 C.F.R. § 101.93(g))[9] FDA has further identified by regulation eight types of statements that would cause a product—whether positioned in the marketplace as a conventional food or as a supplement—to be regulated as a drug. These so-called "disease claim" categories are featured in Table III-2.

8 The definition specifically provides that the term "drug" includes "articles *(other than food)* intended to affect the structure or any function of the body of man or other animals." FFDCA § 201(g)(1)(C) (emphasis added). Thus, for example, a food could bear a structure/function claim without FDA approval, but a cosmetic bearing a structure/function claim would be regulated as a drug because there is no "cosmetic" exemption.

9 Although this definition was adopted to aid FDA in the regulation of dietary supplement claims, it is commonly used in evaluating claims for conventional food as well.

TABLE III-2 Disease Claims that May Trigger "Drug" Status

Type of Claim	Example of Impermissible Claim
Effect on a specific disease or class of diseases	"Reduces the pain and stiffness of arthritis"
Effect on an abnormal condition associated with a natural state or process, if the abnormal condition is uncommon or can cause significant or permanent harm	"Support for Cystic Acne"
Effect on the characteristic signs or symptoms of a specific disease or class of diseases, using scientific or lay terminology	"Reduces joint pain"
Effect on disease through statements regarding product name, formulation, supportive scientific references, symbols, or use of the term disease	Name: "Hepatacure" Formulation: Contains digoxin Scientific references: Serial Coronary Angiographic Evidence That Antioxidant Vitamin Intake Reduces Progression of Coronary Artery Atherosclerosis (if placement in labeling suggests disease use) Symbols: EKG Disease: "Prevent the onset of disease"
Product belongs to a class of products that is intended to diagnose, mitigate, treat, cure, or prevent a disease	"Antidepressant"
Product substitutes for a product that is a therapy for a disease	"Herbal Prozac"
Product augments a particular therapy or drug action that is intended to diagnose, mitigate, treat, cure, or prevent a disease or class of diseases	"Use as part of your diet when taking insulin to help maintain a healthy blood sugar level"
Has a role in the body's response to a disease or a vector of disease	"Supports the body's ability to resist infection"
Product treats, prevents, or mitigates adverse events associated with a therapy for a disease and manifested by a characteristic set of signs or symptoms	"Help maintain intestinal flora for individuals on antibiotics"

If a functional food is promoted using statements that FDA considers to be disease claims, it is subject to regulation as a drug. In contrast, drug regulation can be avoided through the use of general health and nutrition-related claims that do not implicate disease, or through claims specifically authorized as outside of the drug category (e.g., FDA-approved health claims).

2. Implications of Classification

In general, products regulated as "drugs" are subject to greater regulatory scrutiny than food. New drugs must undergo a lengthy approval process before marketing, must be demonstrated to be safe and effective for their intended use (generally through one or more well-controlled clinical trials), and may bear only FDA-approved labeling. All drugs must be produced in accordance with prescriptive good manufacturing practice requirements

for pharmaceuticals. In contrast, food, including food ingredients, is subject to pre-market review and clearances in more limited circumstances, and generally enjoys greater flexibility in labeling and production.

C. Conventional Food

1. Scope

In the absence of an expressed or implied intent to diagnose, cure, mitigate, treat, or prevent disease, a product is likely to be regarded as a conventional food if the product is in the form of an "ordinary" food or beverage, and is consumed primarily for taste, aroma, or nutritive value. Although not a legally defined term, "conventional food" as used here denotes products in a traditional food form that are intended for consumption by the general population, including products produced through modern biotechnology. Food in traditional form includes fats and oils used in food preparation, vegetables, grains, beverages, dairy products, nuts, legumes, meat, poultry, or any combination of the above. Such products are subject to all the requirements applicable to food products generally, including safety and labeling requirements enforced by FDA.

2. Implications of Classification

Safety. Of the food safety standards set out in the FFDCA, two are of central importance. First, all food, regardless of the regulatory category into which it may fall, must not bear or contain any added poisonous or deleterious substances that may render the food injurious to health. (FFDCA § 402(a)(1), 21 U.S.C. § 342(a)(1))[10] Historically, the statutory prohibition against added poisonous or deleterious substances has served as the primary tool FDA has used to ensure the safety of all foods, including plant-based products of modern biotechnology.

Second, food ingredients must, as a general rule, be regulated "food additives" or be generally recognized as safe (GRAS) for their intended use or otherwise they and food products containing them are adulterated under the law.[11] If a substance is a "food additive," it requires pre-market clearances from FDA on the basis of safety data and other information submitted in a food additive petition. A legal term of art, "food additive" is defined in pertinent part to mean—

> any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component of or otherwise affecting the characteristic of any food (FFDCA § 201(s), 21 U.S.C. § 321(s))

The term does not include color additives and certain other substances such as pesticides. Food additives include preservatives such as BHA and BHT, nutrients such as folic acid, sweeteners such as sucralose, and the kanamycin marker gene product, the only recombinant DNA product to be regulated as such (See 21 C.F.R. § 173.170).

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10 If a poisonous or deleterious substance occurs naturally in a product, and is not increased to abnormal levels due to mishandling or other intervening acts, the substance must not be "ordinarily injurious" to health, a less stringent standard. *Id.*

11 For a discussion of FDA's authority to require pre-market clearances for food components, including the important distinction between food components and whole foods, see Edward L. Korwek, *FDA Regulation of Biotechnology as a New Method of Manufacture*, 37 Food Drug Cosm. L.J. 289 (1982).

Food substances that are GRAS are exempt from the definition of "food additive" and do not require pre-market clearances from FDA. In order to achieve GRAS status, however, there must be evidence supporting the safe use of the ingredient that is generally available (i.e., published) and accepted by experts qualified by scientific training and experience to evaluate food safety. Under the GRAS standard, a determination of safety may be based on scientific procedures or, for substances used prior to January 1, 1958, through experience based on common use in food. GRAS determinations can be made independently, without FDA input.[12] For purposes of both GRAS determinations and food additive clearances, "safety" means that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 C.F.R. § 170.3(i)).

Functional food derived from modern biotechnology is subject to the same FDA safety framework as other food, meaning that such products similarly must contain only components that are cleared food additives or GRAS. In guidance, FDA has advised that it is the transferred genetic material and the intended expression product that are subject to the food additive and GRAS standards (57 Fed. Reg. 22984, 22990 (May 29, 1992)). Thus, if a plant were modified to provide a particular nutrient or phytochemical, the nutrient or phytochemical would need to be either an FDA-cleared food additive or GRAS for its intended use.

FDA has cleared relatively few "functional" food ingredients as "food additives," so many—if not most—of these ingredients are currently used in food on the basis of self-determined GRAS positions. Those that are regulated food additives include Vitamin D, folic acid, fish protein isolate, and amino acids. In a letter to industry, FDA broadly questioned the legal basis for using "botanicals and other novel ingredients" in conventional food, asserting that many of "these added ingredients are not being used in accordance with an applicable food additive regulation and may not be GRAS for their intended use" (CSFAN 2001). The need for FDA clearance as a food additive or for the establishment of GRAS status therefore represents an area of caution for many functional food ingredients, including those produced through methods of modern biotechnology. Indeed, the safety of functional food ingredients has been a target of critics of the current regulatory framework. GAO recommended that Congress amend the FFDCA to require companies marketing functional food to submit advance notification to FDA of ingredients determined to be safe (GAO 2000). CSPI has urged FDA to implement GAO's proposal based on existing statutory authority (CSPI 2002).

Fortification. Conventional food is subject to FDA's fortification policy, a set of principles intended to serve as "a model for the rational addition of nutrients" to food (21 C.F.R. Part 104). The policy, which is nonbinding in most circumstances, recommends a relatively narrow set of conditions under which FDA believes it is appropriate to add nutrients to

12 FDA has established a voluntary procedure for notifying the agency of independent GRAS determinations, affording the agency an opportunity to object to, or "question" the status of a substance claimed to be GRAS. *See* 62 Fed. Reg. 18937 (1997). FDA has accepted GRAS notifications for many functional ingredients, including lutein, fish oil, phytosterols, and inulin. GRAS notifications are not yet accepted for animal feed ingredients, although FDA's Center for Veterinary Medicine (CVM) has traditionally addressed the safety of feed ingredients through the issuance of so-called "no objection" letters. *See infra* section IV.C.

food. For example, the policy suggests that vitamins and minerals may be added to food to correct a recognized dietary deficiency or to restore nutrients lost in processing. The policy does not address nontraditional substances that are not vitamins or minerals. Application of the policy to functional food is uncertain, especially with respect to non-traditional nutrients.

Labeling Generally. Nutrition and health-related claims for conventional food are subject to prescriptive requirements under the FFDCA, as amended by the NLEA. These requirements, which address "health claims," "structure/function claims" and "nutrient content claims," apply to product labels and to "labeling." The reach of these requirements is broad, as "labeling" is generously defined to include "all labels and all other written, printed, or graphic matter (1) upon any article or any of its containers or wrappers, or (2) accompanying such article" (FFDCA § 201(m), 21 U.S.C. § 321(m))[13] FDA considers a product to be "accompanied" by written, printed, or graphic material when the material supplements the product label or otherwise explains the product.[14] Thus, the agency has characterized brochures, pamphlets, point-of-purchase signage, internet pages, press releases, and in certain circumstances, even books and journal articles, as labeling.

Health Claims. Conventional food is permitted to bear statements regarding the relationship between a food and disease only if such statements are an FDA-authorized "health claim." A "health claim" is defined as—

> any claim that expressly or by implication characterizes the relationship between a food or a substance therein and a disease or health-related condition (FFDCA § 403(r), 21 U.S.C. § 343(r); 21 C.F.R. § 101.14).

FDA may authorize health claims by issuing a health claim regulation, or by accepting a health claim "notification" based upon an authoritative statement of a scientific body with responsibility "for public health protection or research directly relating to human nutrition." To date, FDA has approved 12 health claims by regulation, and has authorized additional health claims through the statutory notification process.[15]

13 The same definition of "labeling" applies for all categories of FDA-regulated products.

14 *See, e.g., Kordel v. United States*, 335 U.S. 345 (1948) (holding that the phrase "accompanying such article" is not restricted to materials that are physically on or in an article or package, and reasoning that it is the textual relationship between a material and a product, not physical attachment, that is dispositive to a finding that the material is "labeling").

15 The health claim notification process was created in 1997 to streamline the authorization of claims that are based upon findings (specifically, "authoritative statements") of certain scientific bodies, such as the National Academy of Sciences (NAS), with responsibility "for public health protection or research directly relating to human nutrition." A party that wishes to base a claim on an "authoritative statement" must notify FDA of the claim at least 120 days before food bearing the claim is first introduced into interstate commerce. The claim will be permitted so long as the agency does not object to its use, which it may do by issuing a regulation prohibiting or modifying the claim, or by finding that the applicable statutory conditions have not been satisfied.

TABLE III-3 Select Relationships that May Be the Subject of FDA-Approved Health Claims

Calcium and osteoporosis
Dietary lipids and cancer
Sodium and hypertension
Dietary saturated fat and cholesterol and coronary heart disease
Fiber-containing grain products, fruits, and vegetables and cancer
Folate and neural tube defects
Soy protein and coronary heart disease
Plant sterol/stanol esters and coronary heart disease
Whole grains and coronary heart disease and cancer

FDA has advised that two elements must be present before a statement will be classified as a "health claim" (58 Fed. Reg. 2478, 2479-80, 1993). First, the statement must be reasonably understood to refer to a food (e.g., specific fruits and vegetables, such as apples) or a specific substance in a food (e.g., fiber). Second, the statement must refer to a "disease or health-related condition." FDA has defined "disease or health-related condition" to mean "damage to an organ, part, structure, or system of the body such that it does not function properly (e.g., cardiovascular disease), or a state of health leading to such dysfunctioning (e.g., hypertension) (21 C.F.R. § 101.14(a)(5))."

If a statement does not identify both a specific food or substance and a disease or health-related condition, it is not a health claim. For example, the statement "5-a-Day for Better Health" as applied to fruits and vegetables is not a health claim because it does not reference a specific disease or health-related condition; the statement "diets rich in fruits and vegetables may reduce the risk of cancer" is not a health claim because it does not reference a specific food. FDA considers such claims to be in the nature of general dietary guidance, not health claims. General dietary guidance claims are permitted so long as they are not false or misleading in any particular.

Several important limitations restrict the circumstances under which health claims may be used. First, FDA traditionally has required that health claims be based on "significant scientific agreement." The "significant scientific agreement" standard requires a finding that "based on the totality of publicly available scientific evidence … there is significant scientific agreement, among experts qualified by scientific training and experience to evaluate such claims, that the claim is supported by such evidence" (FFDCA § 403(r)(3)(B), 21 U.S.C. § 343(r)(3)(B)). Although "significant scientific agreement" does not require a consensus or incontrovertible evidence, on a continuum between emerging science and consensus, it lies closer to consensus, according to FDA.

Second, for each health claim that is currently permitted, detailed criteria concerning the conditions for using the claim are spelled out in the relevant authorizing regulation or notification. These criteria address the elements that must be present in each claim and

the nature of the food that may bear the claim. For example, the authorized health claim concerning saturated fat, cholesterol, and heart disease must state that heart disease risk depends upon many factors, and may appear only on food products that meet FDA's definitions for "low fat," "low saturated fat," and "low cholesterol" (21 C.F.R. § 101.75). In all cases, the claimed health benefit must be described as a potential reduction in disease risk that may occur when the relevant food or substance is consumed as part of an appropriate diet. FDA has interpreted the health claim provisions not to permit claims that a food or substance may treat, cure, or mitigate disease.

Third, in addition to the claim-specific criteria, all health claims are subject to certain general principles set forth in FDA's regulations, in 21 C.F.R. § 101.14. Perhaps the most significant of these is the "disqualifying" nutrient concept. Specifically, food products that contain fat, saturated fat, cholesterol, or sodium at levels that FDA has deemed to be of nutritional concern are generally "disqualified" from bearing a health claim. The disqualifying levels for fat, saturated fat, cholesterol, and sodium are 13 grams, 4 grams, 60 milligrams, and 480 milligrams, respectively.

In its call for reform of FDA's regulation of functional food, IFT recommended that FDA streamline the agency's review of health claims by adopting a notification procedure for claims that are "generally recognized as efficacious" (GRAE) (IFT 2005). As proposed by IFT, companies wishing to use a health claim could convene an expert panel, described by IFT as a GRAE panel, to evaluate the science supporting the claim. If the panel found the claim to be GRAE, the claim could be notified to FDA and used within 90 days if the agency did not object. FDA, however, does not believe it has the legal authority to adopt such a system (71 Fed. Reg. 62400, 62404, Oct. 25, 2006).

Qualified Health Claims. In a 2003 policy change, FDA adopted an interim process that cleared the way for use of certain "qualified" health claims that are not supported by significant scientific agreement. The new policy was prompted by a series of court decisions that held FDA's implementation of the "significant scientific agreement" standard for health claims to violate First Amendment principles.[16] As a result, FDA is not permitted to automatically reject health claims simply because the claims are not supported by significant scientific agreement; rather, the agency must first consider whether a disclaimer could be used to convey the relative strength of the science and eliminate potential deception.

Under its interim policy, FDA will entertain requests for the use of claims not supported by significant scientific agreement if the following conditions are met:

- The claim is the subject of a health claim petition that is filed for comprehensive review with FDA;
- Credible scientific evidence supports the claim;
- The claim is appropriately qualified to reflect the extent of the scientific evidence; and
- The claim otherwise meets the general regulatory requirements for health claims.

For claims that meet the criteria in the new health claim policy, FDA will allow the claim through the exercise of "enforcement discretion" (i.e., an agreement to not take action against the claim). An example of a qualified health claim that FDA has allowed for conventional food and dietary supplements containing certain omega-3 fatty acids is

16 *See, e.g., Pearson v. Shalala*, 164 F.3d 650 (D.C. Cir. 1999).

"Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease. One serving of [name of food] provides __ gram of EPA and DHA omega-3 fatty acids. [See nutrition information for total fat, saturated fat, and cholesterol content.]"

At the time of its adoption, the qualified health claims process was both praised and criticized for increasing the flexibility of health claim regulation and making possible new claims based on emerging evidence. FDA's implementation of the process, however, has raised questions about its utility. For instance, several "approved" claims (i.e., claims for which FDA agreed to exercise enforcement discretion) characterize the subject diet-disease relationship in a negative manner:

> "Three studies, including a large clinical trial, do not show that calcium supplements reduce the risk of preeclampsia during pregnancy. However, two other studies suggest that calcium supplements may reduce the risk. Based on these studies, FDA concludes that it is highly unlikely that calcium supplements reduce the risk of preeclampsia" (FDA 2005a).

> "One weak and limited study does not show that drinking green tea reduces the risk of prostate cancer, but another weak and limited study suggests that drinking green tea may reduce this risk. Based on these studies, FDA concludes that it is highly unlikely that green tea reduces the risk of prostate cancer" (FDA 2005b).

In addition, consumer perception studies have called into question whether consumers are able to understand disclaimers intended to convey the strength of scientific evidence supporting a claim.[17]

Structure/function claims. A food may bear, without FDA pre-market review or approval, substantiated claims identifying the effect of the food on a bodily structure or function. As explained previously, the legal basis for structure/function claims is the "drug" definition in section 201(g)(1)(C) of the FFDCA, which defines "drug" in pertinent part to mean "articles (other than food) intended to affect the structure or any function of the body of man or other animals." By exempting food promoted for structure/function effects from the definition of "drug," Congress in effect exempted food bearing structure/function claims from the FFDCA's stringent requirements applicable to new drugs.

To avoid "drug" status based on disease claims, structure/function claims must be written in a manner that presumes the intended audience is healthy and seeks solely to maintain, promote, or otherwise support that state of good health. An example of a "structure/function" claim is "conjugated linoleic acid supports a healthy immune system." Structure/function claims for conventional food do not require FDA approval or review, nor has the agency identified specific criteria (e.g., disqualifying criteria) for their use. FDA has issued, however, a draft guidance to address the scientific data and information that industry should have to substantiate structure/function claims as not false or misleading in any particular (FDA 2004).[18]

17 *See* Brenda M. Derby and Alan S. Levy, Working Paper: Effects of Strength of Science Disclaimers on Communication Impacts of Health Claims (Sept. 2005); International Food Information Counsel (IFIC), Qualified Health Claims Consumer Research Project Executive Summary (Mar. 2005) (available at http://www.ific.org) (accessed Oct. 2005).

18 Although the draft guidance applies on its face only to dietary supplements, the principles set out in the guidance apply equally to conventional foods.

For conventional food, such as cranberries or cranberry juice suggested to promote urinary tract health, FDA has taken the position that structure/function claims must be based upon nutritive value. FDA's reasoning for this position is based on the statutory exemption from "drug" regulation for food (i.e., the "other than food" language quoted above): because the statute exempts food intended to affect bodily structures/functions from regulation as a drug, and a food can affect a bodily structure or function only through nutritive value, a structure/function claim should be tied to such nutritive value, and not a pharmacological effect. FDA, however, has not clearly explained the meaning of nutritive value in this context, and appears to have moved away from a strict application of this standard in recent years. In the agency's view, FDA has "provided significant flexibility in determining whether a substance possesses nutritive value" (71 Fed. Reg. 62400, 62405 (Oct. 25, 2006)).[19]

For dietary supplements, DSHEA specifically authorizes the use of structure/function claims (FFDCA § 403(r)(6), 21 U.S.C. § 343(r)(6)). Accordingly, dietary supplements may bear structure/function claims without regard to nutritive value. As a result of DSHEA and FDA's policy on structure/function claims for conventional food, the "disease" provisions of the drug definition are of greater practical importance in assessing potential drug status.

Because structure/function claims can be made with relative ease (assuming adequate substantiation exists), they are commonly used on functional food products, and have been the target of critics of functional food regulation, who suggest that more stringent controls are needed. The GAO has recommended that the FFDCA be amended to require marketers of functional food in conventional food form to follow the same procedures for structure/function claims as dietary supplements (GAO 2000). In other words, GAO suggests that conventional food marketers should be required to notify FDA regarding the use of structure/function claims, and to provide a disclaimer that claims made in labeling have not been reviewed by FDA. The GAO has also recommended that FDA develop regulations or other guidance concerning the scientific evidence needed to support structure/function claims. These recommendations have been supported by groups such as CSPI (CSPI 2002).[20]

Nutrient content claims. A claim that characterizes the level of a nutrient in a food, such as "low fat" or "high in calcium," is a "nutrient content claim." A statement is deemed to "characterize" the level of a nutrient in food if it states or implies that the level has some nutritional significance (i.e., suggests that there is "a lot or a little" of the nutrient relative to a dietary recommendation). Like health claims, nutrient content claims may be expressed or implied, and cannot be used unless authorized by FDA in a regulation or a nutrient content claim notification (FFDCA § 403(r), 21 U.S.C. § 343(r); 21 C.F.R. § 101.13). Express nutrient content claims may be phrased in absolute or relative terms (i.e., by comparing the nutrient value of one food to another).

Examples of FDA-approved nutrient content claims are provided in Table III-4. As a general rule, a product may use these terms in food labeling only if the product meets the FDA definition and criteria for the term.

19 The IFT has asked FDA to replace the "nutritive value" concept with a more inclusive definition, allowing structure/function claims based on either nutritive value or any "physical or physiological effect that has been scientifically documented or for which a substantial body of evidence exists for a plausible mechanism." IFT Report, at 51.

20 *See also* CSPI Reports, *Functional Foods: Public Health Boon or 21st Century Quackery?* (March, 1999).

TABLE III-4 Select FDA-Approved Nutrient Content Claims

Good source, Contains, Provides
High in, Excellent Source of
More, Fortified, Enriched, Added, Extra, Plus
Antioxidant
Light or lite
Calorie free, low calorie, reduced calorie
Sodium free, very low sodium, low sodium, reduced sodium
Sugar Free, No Added Sugar
Lean

Of particular importance to many functional food products, a nutrient generally may be the subject of a nutrient content claim only if the nutrient has an established reference intake, either in the form of a daily value that may be used in nutrition labeling, or a Dietary Reference Intake adopted by the Institute of Medicine or another authoritative body. Thus, because FDA has established a daily value for vitamin E, a peanut that is enhanced to provide "more" vitamin E than conventional peanuts may be promoted on this basis if the increased vitamin E satisfies FDA's regulation for the use of "more" claims. In contrast, spinach that is enhanced to provide increased lutein, for which there is no reference value, cannot be claimed to be a "good source" of lutein, or to contain "more lutein than conventional spinach," no matter how much lutein the finished food contains. The only nutrient content claim currently available to such a food would be a factual statement concerning the amount of lutein in the product (e.g., 2 mg of lutein per serving).

Labeling—Other Considerations. In addition to the requirements for nutrition and health-related claims detailed above, two additional labeling considerations may have relevance to functional foods prepared using modern biotechnology methods: the requirement for food products to bear appropriate statements of identity; and the mandate for labels to reveal "material" facts about a food.

Labels of conventional food products must bear appropriate statements of product identity. By regulation, these statements should reflect (1) the name required by a federal regulation, if any, (2) the common or usual name of the food, or if none, (3) an appropriately descriptive term, in that order of preference (21 C.F.R. § 101.3). If a bioengineered food is significantly different from its conventional counterpart such that the traditional name no longer adequately describes the new food, then a new product identity must be developed. Accordingly, depending upon the nature of the modification, functional and other food produced through modern biotechnology may require unique product identity statements. An example is oil that is altered to contain significantly higher levels of unsaturated fats, in which case the product identity would highlight the predominant fats that are present, such as "high oleic acid soybean oil." Where terms such as "high" are necessary to describe the basic properties of a food, they are not subject to regulation as nutrient content claims.

In addition, under 201(n) of the FFDCA, a food label must reveal all facts that may be material in light of representations made in labeling or in light of consequences that may result from its suggested or customary conditions of use. Consequences that are deemed material typically relate to substantial issues of safety or usage to which consumers should be alerted. For example, if a modified fruit includes an allergen at a level that could trigger an adverse reaction, and consumers would not expect the allergen to be present based on the name of the food, the presence of that allergen must be disclosed (57 Fed. Reg. at 22991).

D. Dietary Supplements

1. Scope

In 1994, Congress amended the FFDCA to create a new regulatory category for "dietary supplements." A dietary supplement is defined as—

> [A] product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients:
>
> (A) a vitamin;
> (B) a mineral;
> (C) an herb or other botanical;
> (D) an amino acid;
> (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or
> (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E) … (FFDCA § 201(ff), 21 U.S.C. § 321(ff))

In addition to containing one or more dietary ingredients, a dietary supplement must be intended for ingestion (as opposed to topical application, inhalation, or other routes of application that do not involve the gastrointestinal tract), must not have been first approved as a "new drug" or as an "investigational new drug" for which substantial public investigations were conducted, must be expressly identified as a "dietary supplement," and must not be represented for use as a conventional food or meal replacement.[21]

The restriction on representations for conventional food uses means that a dietary supplement cannot be promoted as a product to be consumed primarily for taste or aroma (e.g., "Delicious and refreshing beverage") or for conventional food uses (e.g., as a salad dressing). FDA has challenged several "functional food" products that attempted to combine a dietary supplement identity with attributes closely aligned with conventional food. For example, FDA rejected arguments that a spread fortified with phytosterols was a dietary supplement, where promotional materials for the product emphasized taste and depicted the product being used with toast and other conventional food. At the same time, many dietary supplements have been marketed in the form of a bar or liquid beverage-type product without challenge, demonstrating possible overlap between the dietary supplement and conventional food categories.

21 A dietary supplement also must not have been first certified as an antibiotic or licensed as a biologic, or authorized for investigation as a new antibiotic or biologic for which substantial public clinical investigations have been conducted.

Functional food products and dietary supplements are often treated as distinct categories. As suggested above, there is no bright line test for distinguishing functional food from dietary supplements, nor is there an absolute prohibition against marketing what might be a functional food as a dietary supplement. Accordingly, there may be circumstances under which a "functional food" might be marketed as either a conventional food or a dietary supplement. For certain products, such as products that can only be consumed with conventional food (e.g., spreads), a dietary supplement position may not be feasible; moreover, it may not be desirable to refrain from marketing a food on the basis of its taste or flavor. For other products, such as those in bar and certain liquid forms, manufacturers have arguable discretion to position the products as either conventional food or dietary supplements.

2. Implications of Classification

Safety. Safety requirements for dietary supplements differ substantially from those for conventional food. In contrast to conventional food ingredients, which must be determined to present a "reasonable certainty of no harm," the safety standard for dietary supplements provides that dietary supplements must not present "a significant or unreasonable risk of illness or injury" under the conditions of use (FFDCA § 402(f)(1), 21 U.S.C. § 342(f)(1)). The safety standards for dietary supplements are widely considered to be less stringent than conventional food standards.

Application of the "significant or unreasonable risk of illness or injury" standard requires a case-by-case assessment. In its 2004 rulemaking to ban ephedrine alkaloid supplements, FDA determined a supplement to present an "unreasonable" risk if the risks posed by the product outweigh the substantiated benefits. Although FDA's interpretation was initially rejected in a legal challenge, the agency's approach was ultimately upheld on appeal.[22] In upholding FDA's interpretation, the appellate court found the statute to impose two distinct safety standards that may cause a supplement to be adulterated: a "significant risk" of illness or injury, which the court found to imply a "great danger; and an "unreasonable" risk, which the court found to require an accounting of whether the claimed benefits justify the risk.[23]

As for pre-market review, dietary ingredients are exempt from the definition of "food additive," so pre-market clearance is not required, nor are such ingredients subject to the GRAS standard. If a dietary ingredient is "new," however, FDA must be notified of the basis for its safe use before it may be marketed (FFDCA § 413, 21 U.S.C. § 350b). A new dietary ingredient notification is required for any dietary ingredient that was not marketed in the United States before October 15, 1994, unless the new dietary ingredient has been present in the food supply as an article used for food in a chemically unaltered form.

Labeling. Labeling requirements for dietary supplements are similar to requirements for conventional food in many respects. Like conventional food, dietary supplements may bear nutrient content claims and health claims only if such claims are expressly authorized by FDA. Dietary supplements also may bear structure/function claims, although FDA must be notified of the use of the claims, and the claims must be accompanied by a disclaimer stating that—

22 *Nutraceutical Corp. v. Von Eschenbach*, 459 F.3d. 1033, 1038 (10th Cir. 2006)(holding that Congress "unambiguously" required FDA to conduct a risk-benefit analysis when assessing whether a supplement presents an unreasonable risk).

23 *Id*. at 1040.

> This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease (*Id.* § 403(r)(6)(C), 21 U.S.C. § 343(r)(6)(C)).

Dietary supplement manufacturers are required to notify FDA of their intended use of structure/function claims. If FDA objects to the notified claims, it responds to the manufacturer in a so-called "courtesy letter" that explains the basis for the agency's objection. Statements that have been the subject of courtesy letters include "helps maintain healthy cardiac risk ratios," "cholesterol protection formula," "support normal body function during the cold season," "treatment for anxiety, anorexia, and depression," and "helps maintain healthy blood sugar levels," among numerous other claims deemed objectionable by FDA.

E. Food for Special Dietary Uses

1. Scope

Perhaps the most ambiguous category of all, "special dietary uses" is defined to mean—

> [P]articular (as distinguished from general) uses of food, as follows:
>
> (i) Uses for supplying particular dietary needs which exist by reason of a physical, physiological, pathological or other condition, including but not limited to the conditions of diseases, convalescence, pregnancy, lactation, allergic hypersensitivity to food, underweight, and overweight;
>
> (ii) Uses for supplying particular dietary needs which exist by reason of age, including but not limited to the ages of infancy and childhood;
>
> (iii) Uses for supplementing or fortifying the ordinary or usual diet with any vitamin, mineral, or other dietary property. Any such particular use of a food is a special dietary use, regardless of whether such food also purports to be or is represented for general use (21 C.F.R. § 105.3(a)(1)).

Although this language could, on its face, be broadly interpreted to cover numerous food products—including, perhaps, all functional food—its reach has been gradually restricted over the years. Two FDA interpretations have played a particularly important role in shaping the special dietary use category.

First, FDA has taken the position that needs relating to general dietary guidelines are not "special dietary uses." Thus, FDA has determined that most statements concerning nutrients such as sodium and sugars are most appropriately regulated under the nutrient content claim provisions of the FFDCA, since advice concerning restrictions of these nutrients are applicable to the general public. This determination demonstrates a unique aspect of special dietary use regulation—a food can lose its special dietary usefulness once a need related to a particular condition has broad applicability to the public at large. Needs relating to the prevention of diet-related chronic diseases of broad concern seem most apt to fall into this category, and thus to be considered of general usefulness. Among the food products retaining "special" usefulness are products relating to food allergy and sensitivities (e.g., gluten intolerance, peanut allergy), products relating to intake problems common to several diseases or conditions (e.g., dysphagia, or difficulty swallowing), and products intended for weight loss or weight management programs.

Second, FDA has provided by regulation that fortified food is deemed to be for a special dietary use if vitamins or minerals added to the food provide 50% or more of the applicable daily values in a single serving (*Id.* § 101.9(a)(4)). / This determination appears to eliminate many fortified products from the special dietary food category. Because the interpretation applies only to nutrients with a reference value, however, it does not address many functional food products, which may contain phytochemicals and other substances for which a reference intake has not been established.

2. Implications of Classification

Safety. Like ingredients of conventional food, ingredients used in food for special dietary use must generally be regulated food additives or GRAS for their intended use.

Labeling Generally. Food for special dietary use is subject to several unique labeling requirements. The central requirement provides that a food for special dietary use is deemed misbranded unless its label bears information adequate to inform purchasers of its value for—

> such information concerning its vitamin, mineral, or other dietary properties as the Secretary determines to be, and by regulations prescribes as, necessary in order fully to inform purchasers as to its value for such uses (FFDCA § 403(j), 21 U.S.C. § 343(j)).

In a few instances, FDA has issued regulations addressing the labeling information that must be provided for food for special dietary use. For example, food produced through modern biotechnology and intended for use as a hypoallergenic food, an infant food, or a weight loss product would be subject to specific labeling requirements as provided for in FDA's special dietary use regulations. In all other cases, the informational labeling requirement for special dietary uses is subject to interpretation on a case-by-case basis.

In addition, FDA has advised that statements that appear in labeling pursuant to a special dietary use regulation will not be subject to the agency's requirements for health claims and nutrient content claims. The agency has suggested that the exemptions apply only if the claim is made in compliance with a "specific provision" of the special dietary use regulations, and is made solely to note the special dietary usefulness of the product. The reach and practical application of these exemptions, however, is unclear. Following NLEA, FDA suggested that it would initiate rulemaking to clarify the relationship between special dietary use claims and health and nutrient content claims, but the agency has not done so to date.

Labeling—Hypoallergenic Food. There is some promise that modern biotechnology may be used to produce food, such as peanuts, without allergenic proteins. If promoted as such, these products would be subject to FDA's regulations for food represented to be for a special dietary use by reason of "the decrease or absence of any allergenic property" (21 C.F.R. § 105.62). The following labeling information technically would be required under FDA's regulation for hypoallergenic claims:

- The common or usual name and quantity or proportion of each ingredient;
- A qualification of the name of the food, or the name of each ingredient thereof, to reveal the specific plant or animal that is the source of each ingredient; and
- An "informative statement of the nature and effect of any treatment or processing of the food or any ingredient thereof," if the changed allergenic property results from processing.

Adopted many years ago, these requirements, particularly the first two, seem more naturally suited to multiple-ingredient, processed food than single-ingredient commodities produced through modern biotechnology. Their application to food produced using modern biotechnology would need to be assessed on a case-by-case basis.

F. Medical Food

1.Scope

The term "medical food" has been defined to mean—

> [F]ood which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation (Orphan Drug Amendments of 1988, Pub. L. No. 100-290 (amending 21 U.S.C. 360ee); 21 C.F.R. § 101.9(j)(8)). /

As noted previously, the first product recognized as a "medical food" by FDA was the infant formula Lofenalac®, which is intended for the dietary management of PKU. Initially regulated as a drug, Lofenalac® was recognized by FDA to meet distinctive nutritional requirements—namely, an impaired ability to metabolize the essential amino acid phenylalanine—and thus was reclassified as a distinct type of food for special dietary use. The impetus for this reclassification was a practical one: FDA recognized the value that specialty formulas and other highly specialized products bring to a small but vulnerable population, and did not wish to discourage the development or marketing of such products by subjecting them to stringent requirements for drug products.

Now a distinct category from food for special dietary use, medical food is defined in an extremely narrow manner. Specifically, FDA has provided by regulation that a product can be classified as a medical food only if the product is—

- A specially formulated and processed product, as opposed to a naturally occurring food in its natural state;
- A product intended for the partial or exclusive feeding of a patient by means of oral intake or enteral feeding by tube;
- Intended for the dietary management of a patient who, because of therapeutic or chronic medical needs, has limited or impaired capacity to ingest, digest, absorb, or metabolize ordinary food or certain nutrients, or who has special medically determined nutrient requirements that cannot be achieved via modification of the diet alone;
- Intended to provide nutritional support for the management of unique nutrient needs that result from a specific disease or condition, as determined by medical evaluation;
- Intended for use under medical supervision; and
- Intended only for patients receiving active and ongoing medical supervision.

An example of a product positioned as a medical food is a moderate protein, low electrolyte, low fluid, high calorie formula intended to provide balanced nutrition for dialyzed patients with chronic or acute renal failure.

1. Implications of Classification

Safety. Like ingredients of conventional food, ingredients of medical food must generally be regulated food additives or GRAS for their intended use. For example, in providing for the use of the food additive folic acid in food, FDA specifically authorized its use in medical food products.

Labeling. In recognition of their unique status, medical food products are expressly exempt from FDA requirements for nutrition labeling, nutrient content claims, and health claims. Although these exemptions make a medical food positioning extremely attractive, the criteria detailed above reveal that FDA has interpreted the category in a very narrow manner. As a practical matter, few functional food products will be in a position to be marketed as a medical food in substantial compliance with the FFDCA.

IV. FOOD SUBJECT TO ADDITIONAL OR UNIQUE REQUIREMENTS: MEAT AND POULTRY, SHELL EGGS AND EGG PRODUCTS, AND ANIMAL FEED, INCLUDING PET FOOD

Although the FFDCA provides a broad framework that is of central importance to most functional food products, certain products are subject to additional or unique requirements under the FFDCA and other laws. A comprehensive discussion of such additional or unique requirements is beyond the scope of this report; a brief overview of three regulatory schemes of particular interest to functional food products is provided below: meat and poultry products, shell eggs and egg products, and non-human animal food, including pet food.

A. "Meat Food Products" or "Poultry Products"

Products that are "meat food products" or "poultry products" are regulated by the Food Safety and Inspection Service (FSIS) of USDA pursuant to the Federal Meat Inspection Act (FMIA) and Poultry Products Inspection Act (PPIA), respectively. A "meat food product" includes any product capable for use as human food made from the carcass of cattle, sheep, swine, or goats. "Poultry products" include human food products made from the carcass of any domesticated bird. A functional food would be subject to regulation by FSIS if it is meat or poultry, or it is a product that contains a meat or poultry ingredient, at more than a de minimis level (generally, greater than 2 to 3%), and in a form of the type generally considered to be a product of the meat or poultry industries. As is the case with other food under the FFDCA, meat and poultry products cannot be adulterated or misbranded under the FMIA and PPIA.

Although FDA and FSIS requirements are similar in many respects, FSIS generally takes the position that it must specifically approve the use of all meat and poultry product ingredients and labels. Thus, FSIS requires that all ingredients used in meat and poultry products be specifically approved as safe and suitable, and that most labels (and claims) be reviewed and approved before use. Complicating the situation, FSIS has approved the use of many nutrient content claims by regulation (e.g., "low fat," "lean"), but has issued no regulations or formal policies concerning health claims or structure/function claims. As a general rule, FSIS has evaluated labels containing proposed health claims and structure/function claims on a case-by-case basis, and has permitted such claims under limited circumstances. For example, FSIS has allowed the labels of appropriate products to bear the American Heart Association Heart Check program logo and the FDA-approved health claim concerning dietary saturated fat and cholesterol and risk of coronary heart disease.

Of particular relevance to functional food, FSIS has historically taken the position that meat or poultry, per se, is not suitable for fortification. It has permitted certain meat and poultry products to contain FDA-regulated ingredients that are fortified in accordance with FDA requirements (e.g., enriched pasta), if the actual fortification occurs outside of a FSIS-inspected meat or poultry plant. FSIS' limits on fortification would generally be expected to limit the marketing of "functional" meat or poultry products, including products to which functional ingredients are added through genetic means or product formulation.

FSIS has not yet taken a public position concerning the status of transgenic animals that are intended to produce functional food in the form of meat or poultry for human consumption. If presented with a transgenic functional food, such as a ground beef product promoted as containing a favorable fatty acid profile due to genetic enhancement, FSIS would be expected to follow its usual pre-market approval approach. Thus, the agency would likely engage in a review of the suitability of such enhancements (e.g., the effect on the characteristics and cooking properties of the resulting meat, as well as a review of whether the enhancements constitute impermissible "fortification"), the product's safety (regarding which USDA would defer to FDA, but would likely require a specific statement from the agency), and the product's labeling, including substantiation for any claims made.

B. Shell Eggs and Egg Products

Jurisdiction over shell eggs and egg products is shared by FDA and USDA. FDA bears primary responsibility for the safety and labeling of shell eggs for consumer use under the FFDCA and the Public Health Service Act. Processed egg products, such as liquid eggs, are similarly subject to regulation by FDA; the production and labeling of such products, however, is also subject to USDA oversight pursuant to the Egg Products Inspection Act (EPIA).

Shell eggs are subject to the FFDCA provisions concerning safety and labeling, and thus must meet the same basic requirements as all other FDA-regulated food. For example, a shell egg that contains a functional component, such as the omega-3 fatty acid, docosahexaenoic acid (DHA), at elevated levels, must be safe under the FFDCA. FDA would likely take the position that DHA, the targeted and desirable component that differentiates such products from other eggs, must be either GRAS or specifically cleared by FDA as a food additive for its intended use.[24] Additionally, the labeling of such a product is subject to FDA's rules for health claims, nutrient content claims, and other labeling statements. Because eggs are most reasonably classified as conventional food, shell eggs enhanced in a particular way would be regulated as either conventional food or food for special dietary use, depending on the types of alterations and claims that are made.

An "egg product" is defined to mean "any dried, frozen, or liquid eggs, without or without added ingredients, excepting products which contain eggs only in a relatively small proportion or historically have not been … considered by consumers as products of the egg food industry" (9 C.F.R. § 590.5)[25] Like shell eggs, egg products must comply with the safety and labeling requirements of the FFDCA. At the same time, pursuant to the EPIA, egg products must be produced under continuous inspection by FSIS, and must use labels and formulations that are reviewed and approved by that agency, with possible consultation with FDA. The EPIA scheme for egg products results in greater oversight of this industry segment.

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24 *See, e.g.,* FDA, Letter Regarding Eggs with Enhanced Omega-3 Fatty Acid Content and a Balanced 1:1 Ratio of Omega-3/Omega 6 Fatty Acids and Reduced Risk of Heart Disease and Sudden Fatal Heart Attack (Docket No. 2004Q-0072, Apr. 5, 2005) (assessing the petitioner's assertion that eggs enriched with omega-3 and omega-6 fatty acids were GRAS, though not reaching a conclusion on the issue, due to denial of the petition on other grounds). In a recent letter announcing the agency's intent to exercise enforcement discretion and permit a health claim concerning the omega-3 fatty acids DHA and EPA, FDA concluded that certain uses of DHA and EPA are safe and lawful, provided that daily intakes of DHA and EPA from conventional food and dietary supplements do not exceed 3.0 g per person per day. As discussed below, if DHA content is enhanced through the addition of DHA ingredients to animal feed, FDA's Center for Veterinary Medicine (CVM) would likely assert that a separate basis for use of DHA-enriched feed would need to be established and provided for the review of that Center.

25 By regulation, FSIS has exempted several foods from regulation as "egg products," including egg substitutes and "dietary foods." The scope of the FSIS exemption for "dietary foods" is unclear.

C. Animal Feed, Including Pet Food

All food, including pet food, falls within the definition of "food" under the FFDCA, although in common usage often non-human animal food is distinguished as "animal feed." Animal feed, however, is subject to quite different regulatory requirements. Some of the differences are statutory: for example, the NLEA scheme for health and nutrient content claims does not apply to animal feed, nor does the DSHEA framework for dietary supplements.[26] Other differences can be explained in large part by the different Centers within FDA responsible for the regulation of human and other animal food products and their interpretation of the law: human food is regulated by FDA's Center for Food Safety and Applied Nutrition (CFSAN), while other animal food is regulated by FDA's Center for Veterinary Medicine (CVM). Oversight of feed by state feed control officials provides yet another explanation for the disparate regulation. Most, if not all, states require such food, including pet food, to be manufactured or distributed within their borders pursuant to a registration or license, and subject such feed to oversight under state laws similar to the FFDCA but that require registration or licensing of feed.

Regarding safety, the FFDCA and state feed laws require that substances added to food either be, as a general rule, regulated food additives or GRAS for their intended use. In the human food industry, self-determinations of GRAS status are common, and are the basis upon which many food ingredients are used. In contrast, CVM, as well as state feed control officials, routinely take the position that a substance cannot be used in feed unless it has been formally or informally evaluated by FDA. Since very few ingredients are cleared as feed additives and since CVM does not have a GRAS notification approach,[27] feed ingredients that have been evaluated by CVM are typically the subject of so-called "no-objection" letters. Once an ingredient is favorably reviewed by CVM, it is generally allowed for use in feed, including pet food, under state law. As a result of CVM and state oversight, the regulatory requirements for using functional ingredients is typically greater for feed products than for human food products because new feed ingredients must be proven to be safe and to have utility to FDA's satisfaction before a "no objection" letter can be obtained. Examples of a functional feed are those formulations that contain phytase. CVM has allowed limited claims for such feed after having issued no objection letters for a few phytases that are produced through the use of recombinant DNA methods.

Another important difference between CFSAN and CVM regulation concerns the application of the "drug" definition, especially the "structure/function" exception for food. CVM treats most structure/function claims as drug claims; thus, "production" claims such as increased milk production, increased leanness, improved growth, and efficiency of weight gain are viewed as drug claims and feed ingredients with such claims are subject to regulation as unapproved new animal drugs (CVM 1998). Accordingly, feed containing such unapproved new drug ingredients cannot be legally marketed. CVM has permitted structure/function feed claims in limited instances, but only after review and approval. Examples include "urinary tract health" and "dental health" claims on cat food products (CVM 2004).

26 *See* 61 Fed. Reg. 17706 (1996). CVM has indicated that it would not object to the marketing of nutritional supplements for companion animals in limited circumstances. FDA Compliance Policy Guides, CPG 7126.04 (sec. 690.100)(Rev. 03/1995). CVM has issued no regulations to govern special dietary uses for feed products, and there is no formal "medical food" category for feed products.

27 *See supra* note 21.

In summary, CFSAN and CVM approach functional food, including functional ingredients, in a different manner, resulting in distinct safety and labeling requirements for feed products. CVM's prescriptive approach to functional feed, coupled with the lack of a feed-specific framework for nutrient content claims, health claims, and dietary supplements, limits the circumstances under which functional feed ingredients may be marketed without their being treated as new veterinary drugs.

Summary

The application of biotechnology to foods expressly to improve nutritional and health characteristics holds great potential, as demonstrated by many research accomplishments. Food enhancements cover a wide range, including improved fatty acid profiles for more heart healthy food oils, improved protein content and quality for better human and animal nutrition, increased vitamin and mineral levels to overcome widespread nutrient deficiencies throughout the world, and reduction in anti-nutritional substances that diminish food quality and can be toxic. On the horizon are efforts to increase the concentration of various antioxidants and functional substances such as phytosterols and probiotic bacteria. Improvements in the digestibility of animal feed through the reduction of phytic acid, gossypol and glycoalkaloids also have potential to enhance the safety of human foods. Although biotechnology has made some advances in reducing allergenic proteins in some foods, the complexity of allergenic responses, differences in sensitivity among people, and the presence of multiple allergens in a single food make this challenge particularly daunting. It appears that food oils with improved fatty acid profiles are the closest to reaching commercialization, once regulatory clearance has been obtained.

Just what form those regulatory clearances may take is not entirely certain. There is no regulatory scheme for functional food, *per se*, but functional food products are clearly subject to federal regulation. If a functional food product is marketed for a therapeutic purpose (e.g., to treat a disease), it will be subject to regulation as a "drug." If a product is subject to regulation as a "food," it may be further classified as a conventional food, dietary supplement, food for special dietary use (including infant formula), or medical food, again depending upon its intended use and other factors.

How a product is categorized has substantial implications for how it is marketed, the safety and labeling standards it must meet, and what requirements it must address for regulatory review and clearance or approval. A conventional food may be freely marketed on the basis of taste, and enjoys some flexibility regarding certain types of claims, such as structure/function claims, which need not be presubmitted to FDA nor accompanied by a disclaimer. Conventional food, including food ingredients, however, must meet safety standards requiring a

"reasonable certainty of no harm." The safety standard for dietary supplements is less stringent, but supplements are restricted to marketing in certain forms (e.g., as a tablet or powder, and in some circumstances, as a bar or liquid), and can make structure/function claims only if such claims are submitted to FDA and accompanied by the DSHEA disclaimer.

Food for special dietary use enjoys some flexibility as to nutrient content and health claim requirements, but the extent of this flexibility, and of the special dietary use category itself, is unclear and probably very fact-specific. Medical food is entitled to the most flexibility of all, but is permitted in extremely narrow and carefully defined circumstances. Certain food products, including meat and poultry, egg products, and animal feed, including pet food, are theoretically eligible for marketing in a functional food form. Such products are, however, subject to distinct regulatory requirements and oversight that may limit functional food opportunities as a practical matter. Animal feed, in particular, has been regulated by CVM in a manner that restricts the types of ingredients and promotional claims that may be used, despite the statutory classification of animal feed as "food." The use of modern biotechnology to enhance human and other animal food will likely not change these regulatory paradigms, but may challenge the boundaries of some of the regulatory classifications.

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