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Phytoalexin-Enriched Functional Foods

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Functional foods have been a developing area of food science research for the past decade. Many foods are derived from plants that naturally contain compounds beneficial to human health and can often prevent certain diseases. Plants containing phytochemicals with potent anticancer and antioxidant activities have spurred development of many new functional foods. This has led to the creation of functional foods to target health problems such as obesity and inflammation. More recent research into the use of plant phytoalexins as nutritional components has opened up a new area of food science. Phytoalexins are produced by plants in response to stress, fungal attack, or elicitor treatment and are often antifungal or antibacterial compounds. Although phytoalexins have been investigated for their possible role in plant defense, until recently they have gone unexplored as nutritional components in human foods. These underutilized plant compounds may possess key beneficial properties including antioxidant activity, anti-inflammation activity, cholesterol-lowering ability, and even anticancer activity. For these reasons, phytoalexin-enriched foods would be classified as functional foods. These phytoalexin-enriched functional foods would benefit the consumer by providing "health-enhanced" food choices and would also benefit many underutilized crops that may produce phytoalexins that may not have been considered to be beneficial health-promoting foods.

KEYWORDS: Functional; soybean; isoflavone; phytoalexin; phytoanticipin; food

INTRODUCTION

There has been recent, increasing interest to identify phytochemicals or plant compounds with health-promoting activities. In vitro screening assays to identify these bioactive compounds cover a broad area of research including antioxidant, anticancer, antiobesity, cholesterol-lowering, and many other activities. Often, successful characterization of a phytochemical can lead to the development of new food products or supplements with health-promoting activities. Over the past decade these foods have been labeled functional foods, which are generally accepted as foods that naturally contain especially healthy qualities. Supplements containing health-promoting activity are referred to as neutraceuticals.

Plants produce a diverse array of over 100,000 low molecular weight natural products known as secondary metabolites (*1*). These secondary metabolites differ from the components of primary metabolism because they are generally considered not essential to basic plant metabolic processes. Most of these compounds are derived from various plant pathways, including the isoprenoid, phenylpropanoid, alkaloid, or fatty acid/polyketide pathways. One group of important secondary metabolites is the flavonoid group. Flavonoids are ubiquitous in many plants and provide utility for the plant as flower pigments to attract pollinating insects, UV protectants, signal molecules to symbionts, and defense against pathogens. Onions, apples, and grapes are examples of foods that naturally contain flavonoids which also contribute to high antioxidant activity. Isoflavones are a subclass of flavonoids and are the primary constitutive secondary metabolites found only in legumes. Important health-promoting activities have been linked to legume consumption, including reduced risk of various cancers (*2*−*7*) and coronary heart disease (*2*−*7*). The only legume to contain nutritionally relevant amounts of isoflavones is soybean. Genistein, daidzein, and glycitein, along with their respective β- and malonyl glycosides, are the predominant isoflavones in soybean. Many soy foods and supplements that are considered to be functional foods have high concentrations of the constitutive isoflavones daidzein and genistein.

Isoflavones belong to another class of compounds now becoming important to nutritionists called phytoalexins. Phytoalexins are low molecular weight antimicrobial compounds

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Table 1. Phytochemicals and Their Food Sources That May Lead to Many Health Enhancing Benefits

phytochemical	food sources	possible health benefit
flavonoids	apples, berries, cherries, citrus fruits, prunes, plums, whole grains, and nuts	may increase HDL cholesterol and help with cancer prevention
stilbenes (resveratrol)	grapes (red and muscadines), red wine, and peanuts	may reduce the risk of heart disease and certain cancers
catechins	green and black tea	may reduce the risk of heart disease, stroke, and certain cancers
allyl sulfides	onions, garlic, chives, and leeks	may lower LDL cholesterol; reduce risk of heart disease and certain cancers
carotenoids (lutein, β-carotene, and lycopene)	deeply colored fruits and vegetables such as tomatoes, sweet potatoes, and spinach	may strengthen the immune system, protect eyes (macular degeneration), and prevent certain cancers (prostate)
indoles	broccoli, cauliflower, cabbage, turnips, and Brussels sprouts	may help reduce the risk of breast and colon cancer
isothiocyanates	broccoli, cauliflower, cabbage, turnips, and Brussels sprouts	may inactivate cancer-causing materials
monoterpenes	citrus fruits and juices (oranges, grapefruits, tangerines, lemon, and lime)	may inactivate cancer-causing materials
isoflavones	soy foods (tofu, soy milk, soy nuts) and legumes (kidney beans, northern beans, and chick peas)	may lower LDL cholesterol and protect against breast, ovarian, colon, and prostate cancer
saponins	potatoes, green vegetables, tomatoes, nuts, soy foods, and legumes	may protect against cancer
β-glucan	oats	may lower total and LDL cholesterol and prevent cardiovascular disease

that are synthesized de novo and accumulate in plants in response to infection or stress due to wounding, freezing, ultraviolet light exposure, and exposure to microorganisms (*8*−*12*). Phytoalexin biosynthesis can be manipulated by application of abiotic (nonliving) or biotic (living) factors that stress the plant into producing or releasing greater phytoalexin concentrations (*8*−*13*). Antifungal, antimicrobial, and antioxidant activities are some of the beneficial activities of phytoalexins that help to enhance the survival of the soybean plant or seed during stress induction (*13*).

Phytoalexins have been well documented in the field of plant defense. Much research has been conducted on the elicitation process, and specific elicitors have been discovered (*8*−*13*). However, only recently are phytoalexins being explored as nutritional components and a source for development of health-promoting food products. These underutilized plant compounds could hold previously unknown potential for antioxidant activity, anti-inflammation activity, cholesterol-lowering ability, and even anticancer activity. For these reasons, an updated system is proposed whereby foods containing enhanced or elicited phytoalexins are considered to be phytoalexin-enriched functional foods. Phytoalexin-enriched foods would benefit the consumer by providing health-enhanced food choices and would also benefit many underutilized crops that may produce phytoalexins that may not have been considered to be a beneficial health-promoting food.

FUNCTIONAL FOODS

Functional Foods Cover a Broad Spectrum. Functional foods are foods that provide health benefits beyond basic nutrition due to certain physiologically active components. Many of these foods may help in disease prevention, reduce the risk of disease, or enhance health. As consumer interest has shifted toward achieving and maintaining good health, interest in functional foods has increased dramatically. Consumers are seeking a greater number of healthy functional foods such as those listed in **Table 1**.

Red Wine and Resveratrol. The “French paradox” showed the incidence of coronary heart disease is relatively low in southern France despite high dietary intake of saturated fats and has been attributed in part to the consumption of red wine. Epidemiological studies found that low incidence of coronary heart disease among wine-drinking populations correlated with resveratrol present in wine (*14*, *15*). Resveratrol is found in grapes, peanuts, and herbal plants (*14*, *15*). Resveratrol is found at high concentrations in the skin of red grapes and is a constituent of red wine. Red wine contains between 0.2 and 5.8 mg/L, depending on the grape variety, whereas white wine has much less. Red wine is fermented with the skins, allowing the wine to absorb the resveratrol, whereas white wine is fermented after the skin has been removed. Resveratrol has antioxidant, anti-inflammation, and anticancer properties (*16*−*19*). Resveratrol has also been shown to extend the life span of several short-living species of animals (*20*) and, more recently, has been shown to improve the health and survival of mice (*21*). These studies on the health benefits of resveratrol spurred consumer interest in red wines, supplements containing resveratrol, and other functional foods containing resveratrol.

Green Tea and Catechins. Tea is the second most consumed beverage in the world, after water. Freshly brewed green, black, oolong, and decaffeinated teas all seem able to promote health. Most of the research focus has been on the polyphenolic constituents of tea, particularly green tea. Green tea extracts have shown anticarcinogenic (*21*) and antimutagenic (*23*) activities. Epidemiological studies linking cancer chemopreventive effects to green tea have produced both positive correlations (*24*) and inconclusive results (*25*). However, the consumption of three or more cups of green tea per day was associated with decreased recurrence of breast cancer in Japanese women (*26*). Also, there is evidence that green tea components have cancer chemopreventive effects (*27*, *28*) and may reduce the risk of cardiovascular disease (*2*, *8*). Much of the beneficial effects of green tea have been attributed to its high antioxidant activity (*29*, *30*) due mostly to the catechins epigallocatechin gallate, epicatechin gallate, epigallocatechin, and epicatechin (*30*−*32*).

Legumes and Isoflavones. The observation that animal diets of clover and soy affected reproduction led to the discovery of phytochemicals with estrogenic activity (*33*, *34*). Since then, more than 300 plants have reportedly caused estrogenic responses in animals, resulting in efforts to identify estrogenic compounds in animal and human food products (*35*−*40*). The estrogenic phytochemicals, which include isoflavones, lignans, phytostilbenes, and enterolactones, appear to primarily function by binding to and activating the estrogen receptor, at 100−1000 greater concentrations than 17β-estradiol (*41*−*43*). Phytoestro-

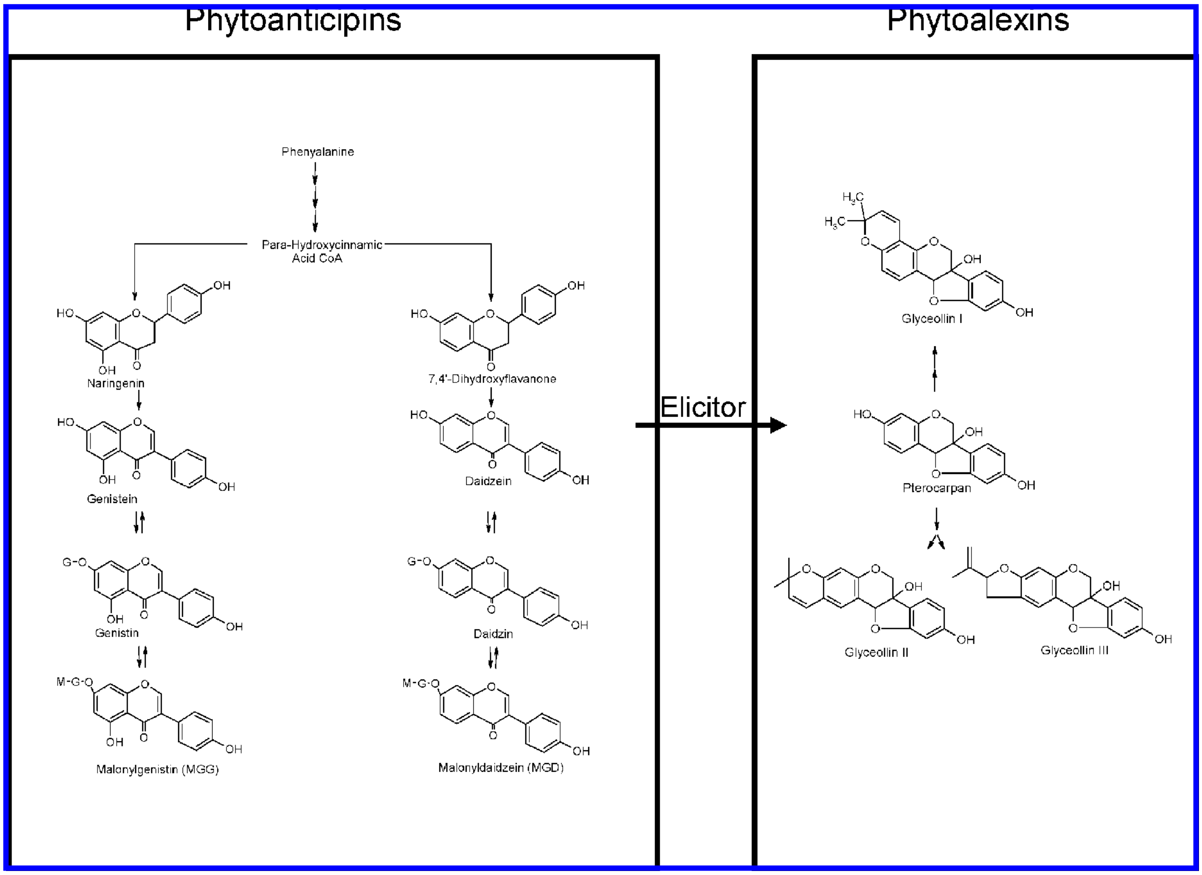

Figure 1. Flavonoid pathway showing the biosynthetic route from phenylalanine to daidzein, genistein, and the conjugated forms daidzin, genistin, MGD, and MGG in soybean. Elicitor activation is required to produce the glyceollins I, II, and III from daidzein.

gens are found in a variety of plants, including fruits and vegetables, but are most abundant in leguminous plants. Legumes are present in most diets throughout the world, and many other parts of the plant are edible in addition to the seeds. The legume that has attracted the most attention is the soybean, which contains high concentrations of the isoflavones daidzein and genistein (**Figure 1**) that are responsible for many of soy's health benefits (*35*−*40*).

The observation that soy isoflavones can function as 17*β*-estradiol agonists is consistent with the observed health benefits of soy foods such as decreased incidence of osteoporosis and cardiovascular disease (*35*−*40*, *44*−*46*). However, the similar decrease in risk of breast cancer would indicate a potential antiestrogenic activity of soy isoflavones (*43*−*46*). Consistent with this information, certain phytochemicals have been reported to exert antiestrogenic effects at higher concentrations (*43*). Studies have shown that phytoestrogens may prevent cancer (*35*−*40*, *44*−*46*), act as antioxidants (*47*−*49*), and lower serum cholesterol (*50*). Isoflavones act as anticancer agents through several mechanisms, one of which may be an ability to function as antioxidants. Isoflavones can inhibit free radical formation (*47*−*49*), reduce lipid oxidation (*51*), and stimulate antioxidant enzymes (*52*). That isoflavones can act as antioxidants is due to an ability to form delocalized unpaired electrons, stabilizing the formed phenoxyl radical after reaction with lipid radicals (*53*).

Fermentation Effects on Soy Isoflavones. Soy isoflavones have been extensively studied because of their potential to promote human health. Growing evidence shows that isoflavones function as antioxidants and free radical scavengers (*47*, *48*, *55*−*60*). Naim et al. (*57*) observed that the number of hydroxyl groups in the isoflavone nucleus positively correlated with antioxidative capacity and that the aglycones had higher antioxidant activities than their glycosides. Other research has demonstrated that the malonyl isoflavones possess strong antioxidant activities, but are very unstable during storage (*61*). Much of this research suggests that techniques to increase the aglycone isoflavones in soy would increase antioxidant activities.

One method to increase the concentration of the aglycone form of isoflavones in soy foods is through the use of fermentation. Many different soy foods are made from fermented soybeans using different strains of bacteria and fungi. Miso, tempe, and soy sauce are all popular foods in Asia and produced from fermented soybeans. The higher levels of isoflavone aglycones that are produced contribute to higher antioxidant activities (*62*−*64*); however, in each study the soybean seeds were initially steamed at high temperatures (typically 120 °C), which prevented further production of phytoalexins during the fermentation process. High-temperature steps applied before fermentation irreversibly denature the majority of enzyme proteins within the seed including those in the isoflavonoid pathway and lyse cells, thus disrupting the biochemical signaling necessary for phytoalexin production. Heating the soy followed by fermentation could affect the levels and health-promoting effects of the isoflavones.

ORGANIC FOODS

Organic Foods Contain Altered Levels of Secondary Metabolites. One method that could increase the production of secondary metabolites, particularly flavonoids, in plants is through organic culturing techniques instead of conventional production. Conventional agricultural practices utilize many chemical substances such as fertilizers, herbicides, and insec-

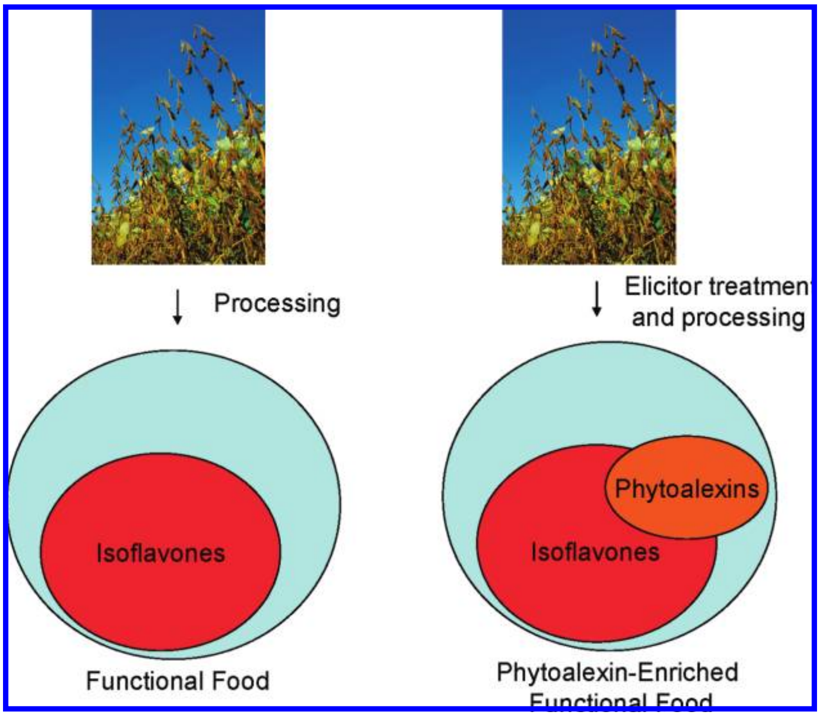

Figure 2. Functional foods provide health benefits from pre-existing phytochemicals or phytoanticipins. The main phytoanticipins in soybean are daidzein and genistein. Phytoalexin-enriched functional foods provide health-enhancing activities from both phytoanticipins and induced phytoalexins (glyceollins) through elicitor treatment.

ticides that both increase and decrease production of the polyphenolic compounds in plants which may be beneficial to health (*65, 66*). Without the use of many protective fungicides and pesticides, plants grown using organic agricultural practices are left vulnerable to more insect and plant pathogen attack, which could alter their concentrations of secondary metabolites. The production of polyphenolics can occur in the edible portion of vegetable and fruit plants, particularly the skin or outer surface, thus contributing to health effects of the food.

Several researchers have demonstrated differences in nutrients and polyphenolics in foods prepared from organic versus conventionally grown crops. Micronutrients in tomatoes are influenced by growing conditions, and several papers have compared their effects on microconstituents. Significantly higher levels of quercetin (30%), kaempferol (17%), and ascorbic acid (26%) were found in organically grown Burbank tomatoes (fresh weight basis), and significantly higher levels of kaempferol (20%) were found in a second tomato variety, Ropreco (*67*). Caris-Veyrat et al. (*68*) found that organically grown tomatoes had higher vitamin C, carotenoid, and polyphenol contents (except for chlorogenic acid) based on fresh matter when compared with conventionally grown tomatoes. However, a study by Rossi et al. (*69*) found organic tomatoes contained more salicylic acid but less vitamin C and lycopene versus conventional tomatoes.

Besides vegetables, many fruits are affected by growing conditions. Carbonaro et al. (*70*) demonstrated a parallel increase in polyphenol content and polyphenol oxidase activity of organic peaches and pears when compared to conventionally grown peaches. Ascorbic acid and citric acids were higher in organic peaches, and α-tocopherol was increased in organic pears (*70*). The authors concluded that organic cultivation practices improved the antioxidant defense system of the plant. A study comparing organic and conventional grapefruit demonstrated that the organic fruit contained higher levels of ascorbic acid and naringin, but lower levels of lycopene (*71*). Also, organic grape juice (white and purple) showed statistically higher values of total polyphenols and resveratrol compared to conventional grape juices from both (*72*).

PHYTOALEXIN-ENRICHED FUNCTIONAL FOODS

Generation of Phytoalexin-Enriched Foods. Phytoalexins were first defined as plant secondary metabolites with antimicrobial activity that were synthesized de novo and functioned as the basis of a disease resistance mechanism (*73*). In 1980, a new working definition assigned phytoalexins as low molecular weight, antimicrobial compounds that are both synthesized and accumulated in plants after exposure to microorganisms (*74*). This new definition excluded antibiotic compounds that are present in plant tissues prior to microbial infection. J. W. Mansfield coined these pre-existing compounds phytoanticipins and defined them as low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are released after infection solely from pre-existing constituents (*75*). This distinction between phytoanticipins and phytoalexins helps to distinguish plant compounds and is based solely on how these compounds are produced. Therefore, resveratrol in grapes and daidzein in soy would be both phytoanticipins (**Figure 2**) and phytoalexins depending on how they were produced. Other compounds such as the glyceollins in soy would be labeled strictly as phytoalexins (*8*−*12*).

Most functional foods would be defined as foods with health-promoting activities based on phytoanticipin content (**Figure 2**). These functional foods are based on pre-existing plant compounds or processing methods that convert these pre-existing compounds to different forms. Phytoalexin-enriched foods would be defined as foods with health-promoting activities based on phytoalexins and would be a subclass of functional foods. Thus, a phytoalexin-enriched food could contain phytoanticipins, but its health-promoting activity would be due in part to its

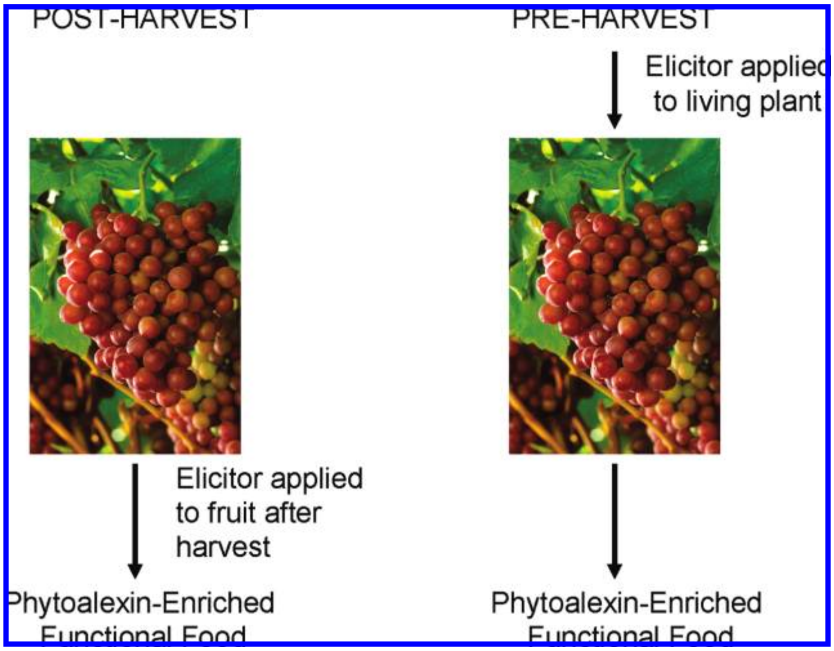

Figure 3. Two techniques for producing phytoalexin-enriched functional foods include both postharvest and preharvest techniques.

phytoalexin content. These phytoalexins could be generated by numerous methods using biotic and abiotic elicitors and other stress-inducing techniques both preharvest and postharvest (**Figure 3**). This change would help clarify future uncertainties in the labeling of foods and supplements containing phytoalexins.

Elicitor Treatments Enhance Resveratrol Content of Grapes. Several studies have demonstrated that elicitor treatment can increase resveratrol concentrations of postharvest grapes (*76*−*80*). The ability of UV irradiation to elicit the phytoalexin resveratrol was first demonstrated using leaf disks and immature berries (*76*). Further experiments using UV irradiation showed that mature grape berries produced higher concentrations of resveratrol 48 h after UV light exposure (*77*). The potential of UV irradiation to create a resveratrol-enriched table grape was also demonstrated by Cantos et al. (*79, 80*). A serving of irradiated grapes (unpeeled) could supply the resveratrol content equivalent to three glasses of red wine (≈1 mg of resveratrol per glass). In both the title and conclusion of the publication, the authors ask if this is a new "functional" fruit (*79*). By the definitions created here we believe that this resveratrol-enriched fruit points to a new subclass of health-promoting functional foods that can be engineered by inducing higher concentrations of targeted phytoalexins.

Elicitor Treatments Enrich Antioxidant and Antiestrogenic Activities of Soy Extracts. Research in the area of plant defense over the past several decades has fostered identification of many phytoalexins throughout a vast number of plant species (*8*−*13*). Of particular interest to many groups were the isoflavone phytoalexins produced by soybean and other legumes (*8*−*13, 81*−*86*). The glyceollins (I, II, and III) are the predominant soybean phytoalexins with antimicrobial activity against numerous soybean pathogens (*8*−*13*). Recent research from our laboratory has shown that the glyceollins have antiestrogenic and anticancer activities (*82*−*84*). Further work has shown that extracts from elicitor-treated soybeans have higher antioxidant activities when compared to untreated controls (*85*). Soy isoflavone phytoalexins, long known only as plant defensive antimicrobials, are now being viewed as beneficial plant compounds that can be considered alongside other soy isoflavones when anticancer activity and other health-promoting properties are evaluated (**Figure 4**). Although the soy glyceollins are currently not present in any commercial soy food products, the potential exists for a new subclass within functional foods that could be termed phytoalexin-enriched foods. Phytoalexin-enriched foods are defined as any food prepared from plant material that contains higher concentrations or de novo levels of phytoalexins resulting from elicitor treatment. Elicitor treatments range from biotic elicitors such as microorganisms (*Aspergillus sojae*, *Aspergillus oryzae*, and *Rhizopus oligosporus*), microorganism cell wall extracts, and carbohydrates to abiotic elicitors including UV induction and wounding (cutting). Recently, Feng et al. (*87*) showed that black soybeans germinated under fungal stress with food grade *R. oligosporus* could be utilized for the production of soy milk and soy yogurt containing glyceollins and oxylipins. Also, germination of black soybean with *R. oligosporus* for 3 days was sufficient to reduce stachyose and raffinose (which cause flatulence) by 92 and 80%, respectively. This research serves as proof of principle that phytoalexin-enriched foods or foods containing phytoalexins can support a niche in food research.

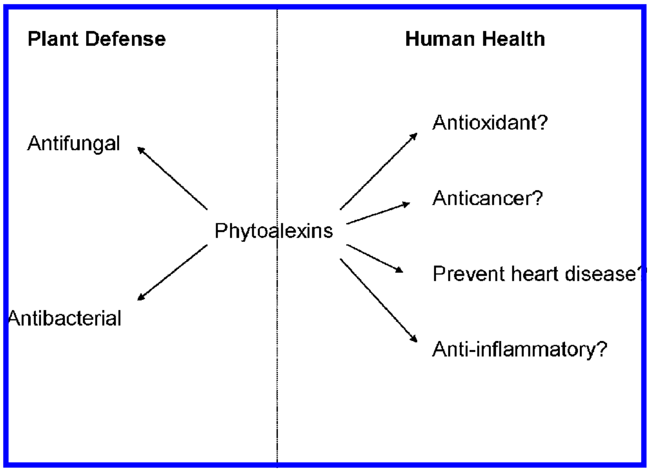

Figure 4. Diversity of the benefits of plant phytoalexins.

Induction of Phytoalexins Preharvest. Most work inducing phytoalexins in food products is done postharvest (**Figure 3**). The phytoalexin resveratrol is induced to higher concentrations in grapes postharvest using several elicitors (*76*−*80*). Most research inducing glyceollins and other soy phytoalexins is done postharvest using seeds, pods, cotyledons, or hypocotyls. However, preharvest techniques are being developed to induce phytoalexins in plants.

Resveratrol concentrations can be increased preharvest using several different techniques (*88*, *89*). Jeandet et al. observed 3−5-fold increased concentrations of resveratrol in grapes harvested under conditions that encouraged the development of gray mold caused by *Botrytis cinerea* (*89*). Iriti et al. (*88*) observed increased polyphenolic and resveratrol contents in preharvest grapes using the plant activator benzothiazole (BTH, 0.3 mM). Field treatments with BTH induced resistance against gray mold caused by *B. cinerea* and caused a 110% increase in the phytoalexin resveratrol. These experiments demonstrate the potential for preharvest techniques to produce a resveratrol-enriched functional food.

Besides inducing resveratrol in grapes, other experiments have demonstrated the potential for the preharvest induction of phytoalexins. Greenhouse experiments demonstrated that seed and foliar applications of biotic elicitors increased total isoflavone concentrations in soybean (*90*). Total isoflavone (genistein, daidzein, and glycitein) increases ranged from 16 to 93% in the mature seed using a foliar application of chitosan; however, the phytoalexin glyceollin was not detected. Glyceollins can be induced in soybean seedling tissues using UV light in the living plant (*91*, *92*). The preharvest application of biotic elicitors to the soybean plant induces the phytoalexins genistein and daidzein within the seed (systemic), but is not expected to induce glyceollin in the mature seed that is protected by the soybean pod. Other preharvest techniques need to be developed to induce glyceollin in soybean, including the production of a glyceollin-enriched seed.

Cell Culture and Hydroponics Produce Phytoalexins. Plant cell cultures have long shown that phytoalexins can be produced using elicitors (*93*−*96*). Cell cultures and hydroponics using either elicitors or electrical current could also be utilized to enhance phytoalexins in different plants. Recently, VanEtten et al. showed that pisatin could be produced at higher concentrations using both elicitors and electrical stimulation applied to the developing root systems of hydroponically grown pea seedlings (*97*). They found that exposing pea plants to certain sublethal doses of electric current produced 13 times higher amounts of pisatin than plants that were not exposed to electricity. The researchers observed similar increases in plant chemicals produced by a variety of other plants when exposed to electricity. There were no adverse effects on the plants. Likewise, similar treatments could be applied to root systems of soybean and other legume seedlings during hydroponic growth to elicit production of phytoalexins with possible health benefits.

CONCLUSION

Most functional foods rely on constitutive plant compounds or phytoanticipins to provide health-promoting benefits. Recent trends have shown increased interest by consumers in many health-promoting foods and supplements. Organically grown foods have been reported to contain higher levels of health-promoting compounds due to exposure to “naturally” occurring challenges from plant pests that induce defensive compounds (phytoalexins) that may have additional health benefits. It is tempting to speculate that in modern agriculture we are limiting at least to some extent the production of health-promoting compounds in our diets that may be present at higher levels in organically grown foods or have been at higher levels in foods grown before the advent of modern agricultural pest control. We propose a new area within functional food research called phytoalexin-enriched foods that utilize induced plant compounds or phytoalexins created either pre- or postharvest that have been traditionally viewed only as plant defensive compounds, but have beneficial health effects. Research from our laboratory and others has shown that many plants can produce higher levels of beneficial compounds under conditions of stress or elicitor treatment. By employing the plant’s own enzyme factory, many of these compounds can be produced at increased levels and readily incorporated into food products.

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