

ABSTRACT

Title of Document: NUTRACEUTICAL PROPERTIES OF LOW
 α -LINOLENIC SOYBEANS GROWN IN
MARYLAND

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Eight soybean genotypes grown in Maryland were analyzed for total phenolic content (TPC), antioxidant capacity, isoflavone composition, lutein content, tocopherol composition, and fatty acid profile. The soybean samples consisted of seven low α -linolenic (18:3n-3) experimental lines and one non-modified cultivar for comparison. 18:3n-3 levels were negatively correlated with oleic acid (18:1n-9). The isoflavones daidzein and genistein were positively correlated with 18:3n-3 levels. α -Tocopherol, γ -tocopherol, and total tocopherols were negatively correlated with 18:3n-3. Two of the reduced 18:3n-3 lines contained significantly higher lutein levels than the ordinary cultivar. All low α -linolenic lines contained similar antioxidant capacity to the non-modified genotype. The results of this study show that the Maryland-grown low 18:3n-3 soybeans are sources of antioxidants and isoflavones and may be consumed for health benefits. Specific genotypes may be selected for food production to obtain the most desirable combination of nutritional, nutraceutical, and chemical properties.

NUTRACEUTICAL PROPERTIES OF LOW α -LINOLENIC SOYBEANS
GROWN IN MARYLAND

By

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Chapter 1: Literature Review

Introduction

Soybeans and their components comprise a significant portion of animal and human food products in the United States. Soybeans comprise 90% of oilseeds grown in the U.S., and in 2005 were the second-largest crop in area and value (Ash et al., 2006). 98% of soybean meal is used for animal feed, with the remaining meal used in human food products. Soy foods have increased in popularity, most notably after the U.S. Food and Drug Administration allowed a health claim for soy protein in 1999 (Ash et al., 2006).

Soybeans used for human foods have been bred to select for specific traits, especially in the fatty acid profile. Low levels of unstable polyunsaturated fatty acids are desirable to help reduce the need for hydrogenation (Ash et al., 2006). In 2006, the FDA mandated labeling of *trans*-fats in foods, and demand for soybeans with reduced α -linolenic acid (18:3n-3) is increasing (Fehr, 2007).

Selection for one trait may alter other positive aspects of the soybean (Dolde, et al., 1999). However, recent research on modified fatty acid soybeans has indicated that soybeans maintain most of their health benefits when fatty acids are altered (Slavin, et al., 2009). If low α -linolenic soybeans continue to be cultivated for use in foods, it is of interest to determine which genotypes may contain the most health-enhancing properties. The objective of this research is to further examine the variability by genotype of specific nutrient composition and antioxidant level of Maryland-grown low α -linolenic soybeans.

Soybean Composition, Use in Foods, and Nutritional Properties

Soybeans and their components provide significant contributions to the human diet in both natural and processed foods. Traditional soy foods are associated with Asian cuisine and include tofu, tempeh, natto, miso, and soymilk (Goblitz & Jordan, 2006). Soy foods have recently increased in popularity in Western cultures, especially in the production of vegetarian foods and mock meats. Soy components such as oil, protein, lecithin, fiber, and sterols are used in processed foods for both functionality and nutrition (Sugano, 2006).

Soybean Oil

Soybean oil is consumed more than any other type of edible oil in the United States and the rest of the world (Eckel, et al., 2007). It is used to produce cooking oils and margarines, and is used in all types of packaged and processed foods. The ubiquitous use of soybean oil draws concerns over its health effects. Soybean oil consists of a high amount of polyunsaturated fats, which are generally considered more healthful than the saturated fats found in animal products. However, these polyunsaturated fats decrease the oxidative stability of the oil in processed foods. Hydrogenated soybean oil is more resistant to oxidation, so it is included in packaged foods to improve the shelf life (DiRienzo, et al., 2008).

Soy Oil Composition

A triacylglycerol molecule consists of 3 fatty acids attached to a glycerol backbone. Fatty acids contain a carboxylic acid and an aliphatic tail (Nawar, 1996). Short-chain fatty acids have 2-6 carbons, medium-chain fatty acids have 8-12 carbons, and long-chain fatty acids have 14 or more carbons. During exposure to heat or enzymes, the triacylglycerol may be hydrolyzed, and the fatty acids released. The free fatty acids are more susceptible to oxidation than the triacylglycerol (Nawar, 1996).

Soybean contains on average 15 to 20% oil by weight. The major fatty acids consist of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and alpha linolenic (18:3)(Sugano, 2006). The percentage of each fatty acid may vary depending on the genotype of soybean. Some genotypes are bred to contain altered quantities of certain fatty acids. The typical soybean that has not been modified contains 10% palmitic, 4% stearic, 23% oleic, 53% linoleic, and 8% linolenic fatty acids (Sugano, 2006).

Soy Protein Products

Soy protein is available in multiple forms for use in processed foods. Commercial soy protein products are available as soy flour, soy protein concentrate, and soy protein isolate (Paulsen, et al., 2006). Soy flour is higher in protein and lower in carbohydrate than wheat flours. The flour can be produced in full-fat or defatted varieties. Soy flour is added to baked goods to improve moisture content and

texture. Soy protein concentrate and isolate are added to products such as nutritional bars, powdered protein shakes, and meat analogs (Paulsen et al., 2006).

Soy Protein Nutritional Value

Soy protein contains all of the essential amino acids for humans, which makes it unique among plant-based proteins (Paulsen, et al. 2006). Protein makes up 40% dry weight of the soybean. Soy protein prior to processing is not highly bioavailable due to inhibition factors such as trypsin inhibitors, urease, and hemagglutinin. However, soy protein isolate can be processed to have protein digestibility amino acid score comparable to animal protein (Paulsen, et al. 2006). These factors make soy protein a good choice for addition to vegetarian food products and nutritional supplements.

Soy Protein Effect on Cholesterol

Observational studies have shown a reduced incidence of cardiovascular disease correlated with soy protein intake (Vega-Lopez & Lichtenstein, 2005). A 1995 meta-analysis of randomized controlled trials by Anderson et al. showed a significant reduction in total cholesterol, LDL-cholesterol (LDL) and triglycerides from soy protein compared with animal protein (Anderson et al., 1995). The U.S. Food and Drug Administration approved a health claim for soy protein and risk of coronary disease (FDA, 2000). Since the approval of the health claim, a large number of clinical trials have been conducted on dietary intake of soy protein (Balk et al, 2005; Sirtori & Johnson, 2006; Xiao, 2008; Zhan & Ho, 2005). Many studies have

found a larger LDL reduction in hypercholesterolemic subjects as opposed to those with normal cholesterol (Anderson et al., 1995; Zhan & Ho, 2005).

The mechanism of soy protein on LDL reduction has been attributed to reduced absorption of intestinal cholesterol, reduced cholesterol synthesis, or stimulation of LDL receptor transcription (Cho et al., 2007). An in vitro study of soy protein hydrolysate on human liver cell lines showed significant increase of LDL receptor transcription (Cho et al., 2007). This would then increase uptake of LDL-cholesterol in the liver and reduce serum levels. The same study did not find a high bile acid binding capability in soy protein hydrolysate, and found that at high levels the soy protein hydrolysate actually stimulated cholesterol synthesis.

A 2006 analysis by the American Heart Association nutrition committee found that the effect of soy protein on cholesterol levels was not as significant as previously thought (Sacks et al., 2006). The authors found that in well-controlled studies, LDL cholesterol reduced only 2 to 7 percentage points with intake of soy protein compared with animal protein. The announcement of the results cast doubt upon the previous health claim. The AHA nutrition committee concluded that soy remains a heart-healthy food due to its low-saturated fat content. However, the cholesterol-reducing effects may not be sufficient to warrant the previous promotion of soy. Soy protein intake has also not shown clear effect on hypertension, or on the high-density lipoprotein (HDL) (Balk, et al., 2005; Xiao, 2008).

Some evidence from in vitro and animal studies shows that soy protein can reduce adiposity and promote greater percentage of lean body mass (Velasquez & Bhatena, 2007). Mechanisms for the impact of soy protein on obesity include

increased satiety, improved insulin sensitivity, lipid metabolism, and hormonal effects. Studies in humans have not shown a clear reduction in body weight or fat mass with soy protein when compared with animal protein. However, the soy protein diets do improve serum LDL and triglycerides more effectively than animal protein diets (Velasquez & Bhathena, 2007).

Soy Lecithin

Lecithin is a phospholipid found in both plant and animal sources (Figure 1). Phospholipids are acylglycerols with a phosphate group in place of one fatty acid. Due to the polar phosphate group and non-polar carbon tails, the molecule is amphiphilic and has high surface activity (McClement & Dekker, 2008). Soy lecithin is a product of soybean oil degumming, but has multiple uses as a food additive. It serves as an emulsifier, stabilizer, and antioxidant in foods (Li, 2006; Sugano, 2006).

Lecithin contains phosphatidylcholine which is a precursor to the neurotransmitter acetylcholine (Magil et al., 1981). Soy lecithin has also shown anti-cholesterolemic and anti-atherogenic properties (Wilson et al., 1998).

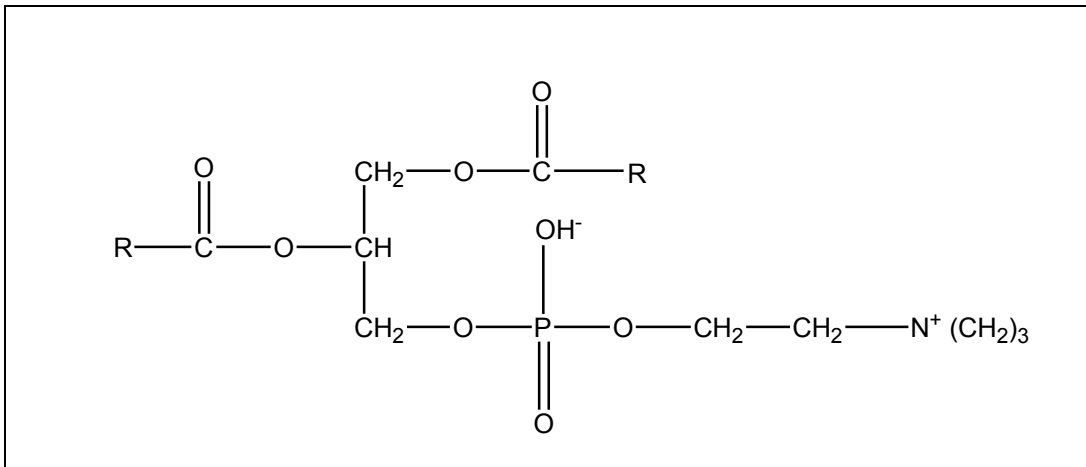


Figure 1. Phosphatidylcholine, a component of soy lecithin and precursor to the neurotransmitter acetylcholine.

Phenolics

Soy has been found to contain several types of phenolic acids (Sakthivelu et al., 2008). Phenolics are compounds consisting of an aromatic ring and hydroxyl group. Phenolic acids are known to possess anti-radical and antioxidant activity, due to donation of the hydrogen from the hydroxyl group (Sroka & Cisowski, 2002). In fact, they are one of the most effective antioxidants from natural sources, more potent in vitro than nutrients such as ascorbic acid, α -tocopherol, and β -carotene (Rice-Evans et al., 1996). The number and position of the hydroxyl groups on the phenolic ring has been correlated with the antioxidant activity of the particular phenolic compound (Sroka & Cisowski, 2002).

Polyphenols in plants are composed of aromatic rings with 2 or more hydroxyl groups, and are associated with plant coloring and defense against environmental stress. Polyphenols in the human diet are linked to health benefits such as prevention of cardiovascular disease (Hooper et al., 2008). The inhibition of LDL oxidation is

an important mechanism by which polyphenols can reduce CVD risk (Rice-Evans et al., 1996). The polyphenols are classified into several subgroups; some of the most well known are flavonoids.

Flavonoids are composed of 3 aromatic rings with other varied structures depending on the group. They are found in various plants and are known for antioxidant and antimicrobial properties. Extensive research has highlighted the beneficial effects of flavonoids on cardiovascular disease, inflammatory diseases, and some cancers (Middleton et al., 2000).

Isoflavones

Isoflavones are flavonoids found in mainly in legumes, and in highest amounts in soybeans. Soybeans contain from 0.1 to 5 mg total isoflavones per gram of whole bean (Larkin et al., 2008). Many health effects of soybeans are speculated to be related to the isoflavones. Isoflavones are associated with the protein content of the soybean. After protein extraction, the isoflavone level of the protein is similar to that of the whole bean prior to extraction (Larkin et al., 2008).

The isoflavones exist in three isomers: genistein, daidzen, and glycitein (Figure 2). In whole soybeans and many soy foods, isoflavones exist as hydrophilic glucoside conjugates: 6''-O-malonylglucosides, 6''- O-acetylglucosides, and β -glucosides (Larkin et al., 2008). During digestion, these are hydrolyzed to the more bioavailable aglycone form (Cassidy et al., 2006, Koh & Mitchell, 2007). Fermentation and some types of food processing produce the aglycone form prior to food consumption.

The specific isomers of isoflavones may have different bioactive properties. Additionally, different levels of each are noted within soybean seeds. For example, Sathivelu et al. (2008) found that as total isoflavones increased, the percentage of genistein increased compared to daidzein. Reidl et al. (2007) found that the Ohio soybeans had increased daidzein over genistein when total isoflavones increased. This increase in one isomer over the others was proposed to be related to the soybean growing conditions.

Health Benefits of Isoflavones – Cardiovascular

In studies of isoflavones separated from soy protein, the isoflavones do not have significant effect on reducing serum LDL (Sirtori, 2006). However, soy protein without isoflavones retains its hypocholesterolemic properties (Sirtori, 2006). Most evidence shows a synergistic effect of soy isoflavones and soy protein on reduction of LDL cholesterol (Sirtori & Johnson, 2006; Anderson, 1999). A meta-analysis of flavonoids by Hooper et al. (2008) found that soy protein isolate significantly reduced LDL cholesterol and diastolic blood pressure, while other soy products did not.

Soy isoflavones can, however, provide antioxidant activity that may protect against cardiovascular disease. LDL oxidation is known to promote aggregation of fatty streaks in arteries. Inhibition of lipid peroxidation by soy isoflavones may reduce risk of atherosclerosis (Fritz, 2003; Sitori, 2006).

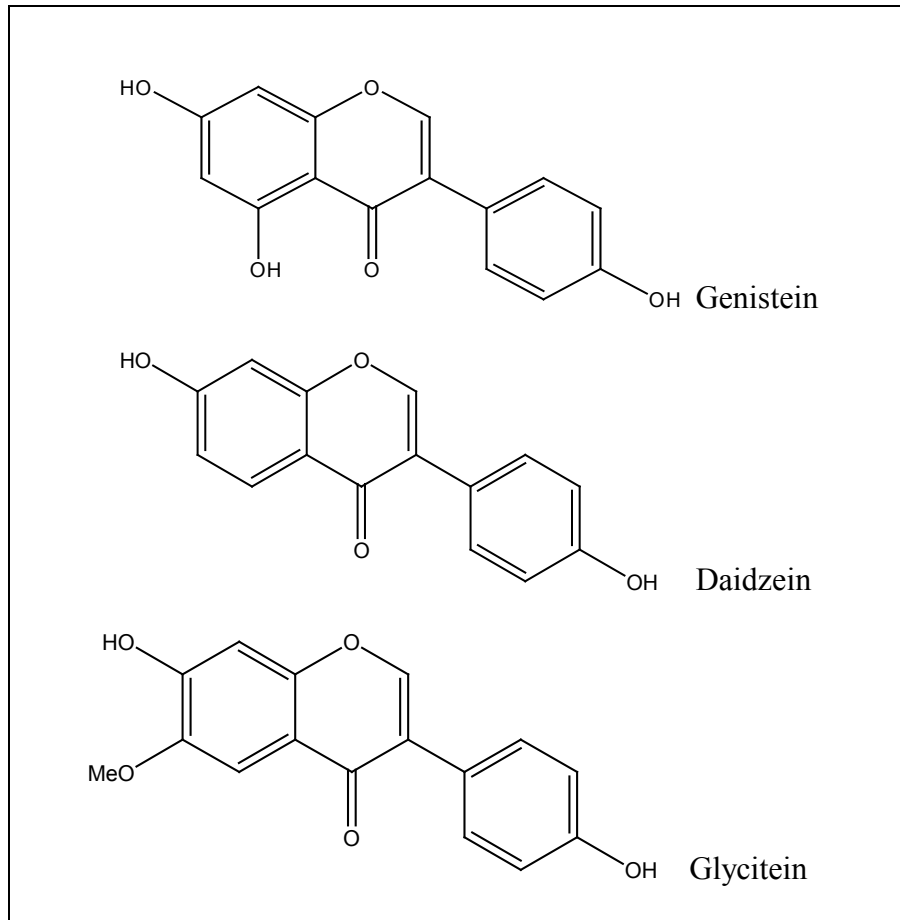


Figure 2. Three isomers of isoflavones in the aglycone form.

Each gram of soy protein contains approximately 1 to 2 mg isoflavones. In a review by Setchell & Cole (2003), 6 to 8g soy protein daily was estimated as the minimum to show cardiovascular benefit in the long-term. This is lower than the 25mg/day recommended by the U.S. FDA to lower reduce risk of cardiovascular disease (Stein, 2000).

Health Benefits of Isoflavones – Cancer prevention

Isoflavones are classified as phytoestrogens that can bind to estrogen receptors in the body. Much of their unique biological activity is related to the ability to act as an estrogen agonist or antagonist (Setchell, 1998). Isoflavones have been shown to lower serum estrogen and lengthen menstrual cycles in premenopausal women (Yamamoto & Tsugane, 2006).

Research has focused on soy in the prevention of some types of cancer, primarily breast, prostate, and colon cancers (Yamamoto & Tsugane, 2006; MacDonald, et al., 2005). The initial association was based on epidemiological studies that showed societies with higher soy intake had lower incidence of certain cancers. In several studies, the isoflavone genistein has shown activity against hormone-related cancers in vitro (Anderson et al., 1999).

Breast cancer risk related to intake of isoflavones focuses on the estrogen-binding activity of these compounds. In vitro studies have shown anti-cancer effects at supraphysiological levels (Yamamoto & Tsugane, 2006), however not at normal physiological levels. Studies of animals have shown dietary isoflavones reduced formation of tumors (Setchell, 1998). Evidence from human studies remains inconclusive (Xiao, 2008; Balk, et al., 2005; Yamamoto & Tsugane, 2006). While many studies have shown an inverse relationship between soy intake and premenopausal breast cancer, the association between soy and postmenopausal breast cancer is unclear (Yamamoto & Tsugane, 2006). Animal studies and one individual-level epidemiological study indicate that intake of soy early in life predicts lower risk of breast cancer as an adult (Yamamoto & Tsugane, 2006; Anderson et al. 1999).

In prostate cancer, isoflavones have shown to delay the growth of tumors in vitro and in animal studies (Anderson, 1999; Messina, 2003). Some epidemiologic studies have shown inverse relationship of soy intake and prostate cancer, however very few populations have a significant intake of soy foods. Most studies of soy intake in Japan are inconclusive, because there is low variation between the highest and lowest levels of soy intake. It is also difficult to conduct case control studies in Western cultures, due to very low overall soy intake (Messina, 2003).

Soy isflavones as a hormone replacement alternative

Isoflavone-rich foods have been studied as an alternative to hormone therapy in menopausal women. However, overall analyses do not show a consistent effect of isoflavone intake on relief of menopausal symptoms (Andersen et al., 1999).

Decreasing estrogen levels at menopause can lead to bone density reduction, and many studies have investigated the effect of phytoestrogens on bone health. There has been epidemiological correlation between soy intake and increased spinal bone density in Asian countries. It is not known if the effects are from soy protein, isoflavones, or both (Messina, 2003). Most intervention studies of soy isoflavones and bone mass have been inconclusive, possibly due to variation in the dose and composition of the isoflavones. Recent research has shown that dietary isoflavones can reduce markers of bone resorption and preserve spinal column bone mass (Ma et al., 2007).

Isoflavones in functional foods

In the United States, foods such as soymilk and soy protein bars are marketed with emphasis on soy isoflavone content. In other nations such as Japan, the amount of available products with isoflavones is more expansive. For example, there are many tea and soft drink beverages containing isoflavones. A soy germ tea product contains 40 mg isoflavones per serving. These beverages are allowed to use a health claim that relates soy to improved bone health (Yamamoto & Tsugane, 2006).

Carotenoids

Carotenoids are pigment compounds in plants, and serve as protective antioxidants to plant tissues (von Elbe & Schwartz, 1996). Certain carotenoids, such as β -carotene, are precursors to vitamin A in animals. Other carotenoids present in mammalian serum include lutein, lycopene, and β -cryptoxanthin. The carotenoid structure consists of an isoprene backbone. A large number of double bonds make the structure susceptible to oxidation. However, this double bond allows scavenging of free radicals through acceptance of a hydrogen molecule (Kiokas & Vassiliki, 2006).

Carotenoids have been researched in prevention of chronic disease with mixed results. Lutein (Figure 3) and zeaxanthin have been associated with prevention of age-related macular degeneration and cataracts (Riyaba-Mercado & Blumberg, 2004). Epidemiological studies show intake of high-carotenoid foods can reduce cardiovascular disease risk, but randomized control trials with dietary supplements have not verified this. In some cases, carotenoid supplementation increased incidence

α -Tocopherol is the primary tocopherol responsible for vitamin E activity in animals (Gregory, 1996) (Figure 4). Tocopherol levels in soybean oil have a maximum concentration at which optimum stability is maintained. Above this level, the tocopherols can form peroxy and oxy radicals, hydroxyl radicals, and singlet oxygen. Thus they become pro-oxidants and the oil can oxidize more rapidly (Kim et al., 2007).

The genes that control tocopherol synthesis have been identified, and now it is possible to modify the tocopherol concentration in oilseeds (Hunter & Cahoon, 2007).

The tocopherol content of soybean oil is also important nutritionally, since Vitamin E is an essential human nutrient. In the United States, the average dietary intake of vitamin E has been shown to be less than the recommended amount (Maras et al., 2004). Some studies have shown vitamin E intake to be preventive against cardiovascular disease and certain cancers. However, other studies have shown that high levels of vitamin E supplementation may have adverse health effects (Dutta & Dutta, 2003).

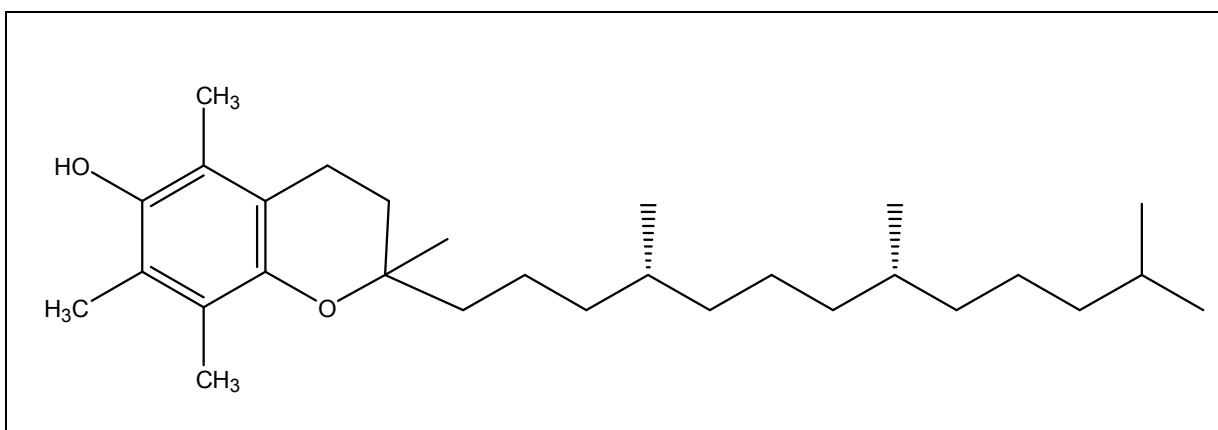


Figure 4. α -Tocopherol, a tocopherol isomer with vitamin E activity.

Sterols

Sterols are lipid compounds that contain three 6-carbon rings, one 5-carbon ring, and an aliphatic tail (McClement & Decker, 2008) (Figure 5). Cholesterol in animals is one of the most well known sterols, and helps to stabilize membranes. However, high serum levels of LDL-cholesterol are undesirable, due to increased cardiovascular disease risk.

Phytosterols maintain membrane fluidity in plants, as cholesterol does in animals (Brufau et al., 2008). Consumption of phytosterols can reduce LDL cholesterol in humans, because they reduce absorption of cholesterol. Soybean oil is a rich source of phytosterols (Brufau, et al., 2008).

In a natural form, plant sterols have low bioavailability, but they may be esterified to improve absorption (Brufau et al., 2008). Plant sterols can be saturated to form stanols, which are also more bioavailable (AbuMweis & Jones, 2008). Recently sterol esters and stanols have been added to food products with the aim to help reduce serum cholesterol. These food products are typically fat-soluble, such as margarine spreads, but also include yogurt, baked goods, juice, and chocolate (AbuMweis & Jones (2008). A relatively low amount of sterol esters, such as 2-3g/day, has shown to be effective in reducing LDL cholesterol (Brufau et al., 2008).

A meta-analysis of randomized control trials by AbuMweis et al. (2008) showed that reduction in LDL cholesterol by dietary sterols/stanols was most significant in subjects with hypercholesterolemia versus those with normal cholesterol levels. The carrier food was important in the bioavailability of the sterols

Soy fiber that is left as a residue from soymilk and tofu production is called okara. Okara contains 20% of the protein and 11% of the oil from the original soybean (Wang & Cavins, 1989). Because okara is a byproduct of food processing, its possible uses have been studied. It has demonstrated water holding capacity and fat holding capacity (Kuan & Liong, 2008). Thus the soy fiber can improve emulsion stability and act as a fat replacement in baked goods. It has been used in a wide variety of reduced-calorie products, including puddings and beverages (Riaz, 2001).

A snack product that contained okara was developed by Katama & Wilson (2008). The product resembled a commercial Japanese snack cracker and contained dried okara powder as a principle ingredient. The okara cracker had increased protein, fiber, calcium, and potassium compared to the original product. The results of sensory tests showed that the okara product was preferred over the control and the subjects did not detect the “beany” odor that is typical of soy. This study demonstrates that the byproduct of soymilk production can be used to enhance the nutritional quality in other foods (Katama & Wilson, 2008).

Okara contains the anti-nutritional factor phytic acid, however fermented okara has shown potential for nutraceutical value (O'Toole, 1999). Fermented okara contains antioxidants such as isoflavones and γ - and δ -tocopherol. In a study by Yokota et al. (1996), okara extract reduced inflammation of gastric ulcer in rats. Jimenez-Escrig et al. (2008) reported the effect of a 10% okara diet on nutritional parameters in rats. Following 4 weeks of the diet, the rats had reduced total cholesterol, reduced weight gain, and higher antioxidant levels in the cecum. These

animal studies indicate that there may be health value when soy fiber is added to foods.

Lipid-modified Soybeans

Oilseeds can be modified through mutagenesis selection programs. Mutants have been developed by genetic engineering to optimize the fatty acid content. Some are bred for low-saturated fat content, some for low α -linolenic content, and others for high-oleic content (Sugano, 2006; Ash et al., 2006). Depending on genotype and growing conditions, large variation can occur in the soybean oil composition (Sugano, 2006).

Low α -Linolenic Soybeans

Lipid Oxidation

Lipid oxidation is a major concern in food production. Lipid oxidation can lead to off-flavors and formation of undesirable compounds such as hydroperoxides, aldehydes, ketones, and dienals (Cherrak, et al. 2003; Wilson, 2004). It also increases the rate of food spoilage. Autoxidation is a self-catalytic reaction with molecular oxygen (Nawar, 1996). This is thought to be the primary reaction in breakdown of lipids. Other mechanisms for oxidation include photochemical reactions, catalysis by metal ions, and enzymatic reactions. The process of lipid autoxidation involves 3 steps: initiation of a free radical, propagation, and termination (Nawar, 1996) (Figure 6).

Antioxidants inhibit the propagation reaction by acting as hydrogen atom donors or acceptors. Phenolic structures are useful antioxidants because they form stable resonance structures and prevent attack of the free radical by oxygen (Nawar, 2004).

Oxidative Stability of Soybean Oil

The high α -linolenic (18:3n-3) content of regular soybean oil makes it susceptible to autoxidation. The 18:3n-3 molecule contains 3 double bonds, and thus it can deteriorate easily during processing and storage. 18:3n-3 oxidizes twice as quickly as linoleic acid (18:2n-6) in stable conditions. At high temperatures during cooking, it can degrade even more rapidly (Wilson, 2004).

The poor stability of natural soybean oil creates a need for hydrogenation in order for the products to remain stable on the shelf or at the high temperatures used in frying (Eckel et al., 2007). The process of hydrogenation changes the double bond structure of the fatty acid, from *cis* to *trans*. The resulting structure is more stable in food products against oxidation and hydrolysis during cooking (Figure 7).

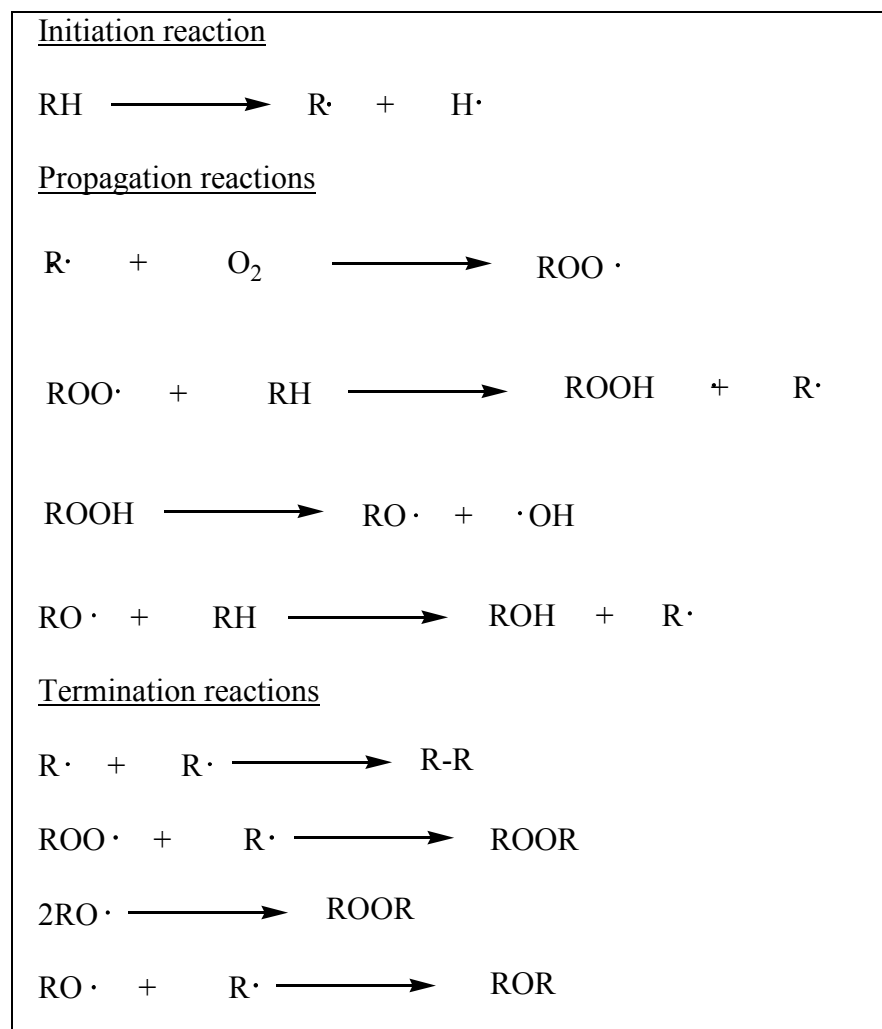


Figure 6. Lipid autoxidation reactions to form hydroperoxides: initiation, propagation, and termination.

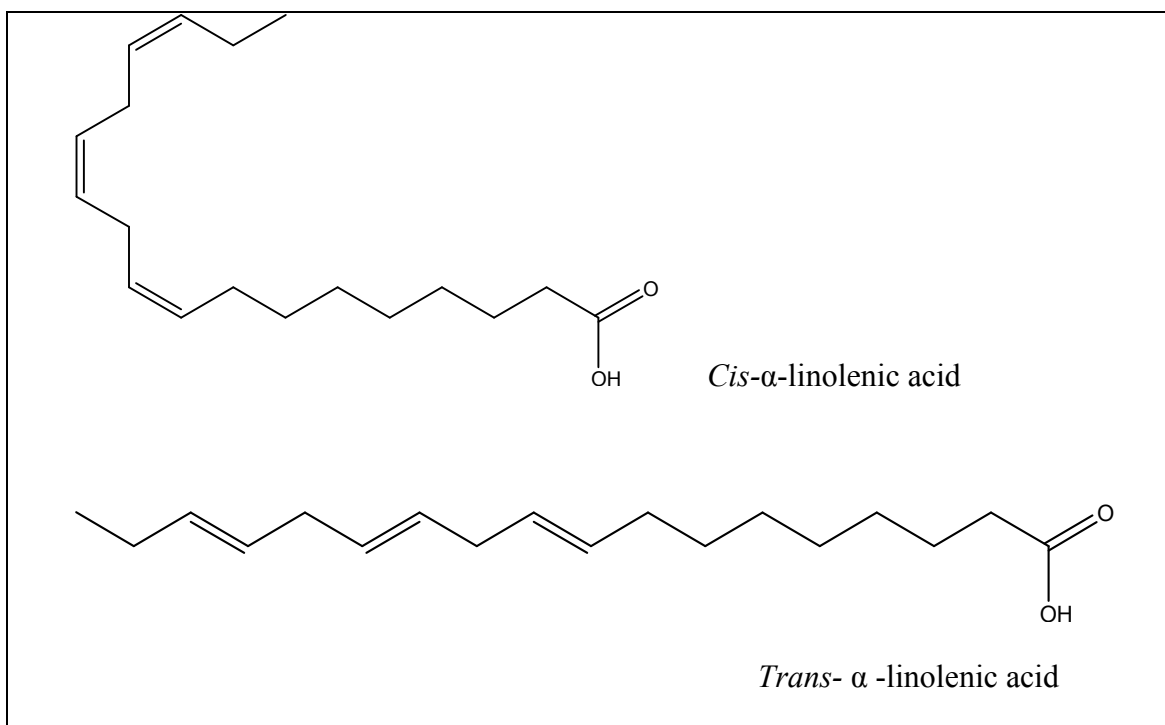


Figure 7. *Cis*- and *trans*- isomers of α -linolenic acid (18:3n-3).

Value of Low α -Linolenic Soybeans

Low α -linolenic soybeans contain less than 3% α -linolenic acid versus the 7% in non-modified soybeans (Sugano, 2006). Some low- α -linolenic cultivars contain less than 1% α -linolenic acid. In commercial baked and fried food products, the desired linolenic acid portion is 2% to maintain stability without hydrogenation (Wilson, 2004) (Table 1). In order to compete with other stable oils, low α -linolenic soybean oil should be used in food production.

Table 1. Desired fatty acid profile for oil used in commercial food processing.

<u>Fatty Acid</u>	<u>Unmodified Soybean Oil</u>	<u>Frying</u>	<u>Baking</u>
Saturated fats (16:0 + 18:0)	15%	7%	42%
Oleic (18:1n-9)	23%	60%	19%
Linoleic (18:2n-6)	53%	31%	37%
Linolenic (18:3n-3)	9%	2%	2%

Comparison of the fatty acid profile of unmodified soybean oil with the desired profile of commercial frying and baking fats. Source: *Soybeans: improvement, production, and uses*, 2004, 621-677.

Safety of Trans Fats

When compared with unsaturated fatty acids, *trans* fats have an undesirable effect on low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol. The ratio of total cholesterol to HDL cholesterol is a marker of cardiovascular disease risk. Multiple studies have shown an unfavorable ratio of cholesterol:HDL when *trans* fats are increased in the diet, even compared to high-saturated fat diets (Eckel et al., 2007). It has been estimated that increasing dietary *trans* fat by 2% may increase coronary disease risk by 23% (Eckel et al., 2007). In recent years, restaurants and food manufacturers have taken measures to reduce *trans* fatty acids in their food products.

The reduction of α -linolenic acid content naturally increases the content of other fatty acids in the soybean. The α -linolenic acid may be replaced with oleic (18:1n-9), linoleic (18:2n-6), stearic (18:0), or palmitic (16:0) fatty acids. For health considerations, an increase in the monounsaturated oleic acid would be desirable. However, when α -linolenic acid is reduced, there is often an increase in saturated fatty acids. This contributes to the stability of the oils, but a higher intake of palmitic

acid may have deleterious health effects (Warensjö et al., 2008). An increase in dietary stearic acid intake has been evaluated as non-significant to cardiovascular risk, even though it is a saturated fatty acid (Baer et al., 2003; Warensjö et al., 2008). Cultivars have been developed with both low palmitic and low-linolenic traits (Cherrak et al., 2003). This would be the ideal combination of traits from the perspective of health value.

Health Effects – Long-Chain Polyunsaturated Fatty Acids (PUFA)

α -Linolenic acid is an omega-3 fatty acid, as the first double bond is at the third carbon from the methyl group on the carbon backbone. Omega-3 fats are the subjects of much research due to their potential for reducing inflammatory diseases in humans (Kris-Etherton et al., 2003). The recommended ratio of omega-6 to omega-3 fats in the human diet is approximately 3 to 1. This ratio in non-modified soybean oil is 7 to 1. When modified to 3% α -linolenic acid, the ratio becomes approximately 18 to 1. From this perspective, reduction of α -linolenic acid will further decrease the intake of beneficial omega-3 fats, particularly in light of the large intake of soybean oil in the American diet. However, the benefit of reducing *trans* fats likely outweighs the harm of reducing α -linolenic acid intake (Lichtenstein et al., 2006).

Health Effects – Tocopherols

Previous research has shown that low α -linolenic soybeans have reduced tocopherol content, and indicated that there is a linear correlation between tocopherol

and linolenic acid (Dolde et al., 1999; McCord et al., 2004; Almonor et al., 1998). McCord et al. (2004) reported that some low-linolenic genotypes did have an equivalent tocopherol level as standard soybeans, and that selection for high levels of tocopherol may be possible. The tocopherol level is important as an antioxidant for oil stability, and for vitamin E activity.

Other Modified Soybeans Through Breeding Efforts

Decreasing saturated fatty acids (18:0 and 16:0) through breeding efforts and genetic modification has been a goal for some soybean producers. Foods must contain 1g or less saturated fat per serving to be labeled as low in saturated fat (Fehr, 2007). Soybean oil should contain less than 7% 16:0 and 18:0 combined to meet this guideline (Fehr, 2007). The first low-saturated soybean oil available commercially was sold in 1997, and was used by the USDA National School Lunch Program. However, canola oil remains lower in total saturated and is less costly to produce. Thus the use of low-saturated soybean oil has not gained commercial popularity (Fehr, 2007).

Increased oleic acid (18:1n-9) is also a goal of soybean breeding programs, due to the stability of this monounsaturated fatty acid (Fehr, 2007). Soybean oil with 50% 18:1n-9 and 1% 18:3n-3 was developed by Iowa State University. This oil demonstrated oxidative stability of 15 hours, while the reduced 18:3n-3 with normal 18:1n-9 content had only 9 hours of oxidative stability (Fehr, 2007). High oleic soybeans have also been produced (80-90% 18:1n-9). This oil has demonstrated good stability, but had low flavor acceptability in sensory tests (Warner & Gupta,

2005). Further research is in progress to develop soybeans with the ideal combination of oleic, saturated, and α -linolenic fatty acids (Fehr, 2007).

Methods For Soybean Research

Gas Chromatography for Fatty Acid Characterization

Gas chromatography (GC) is a commonly used technique for identification of fatty acids in oil. Helium is the carrier gas, and a fused silica capillary column is the stationary phase. A flame ionization detector (FID) fueled by hydrogen gas detects compounds as they are eluted from the column. The FID is best suited to detect carbon-carbon or carbon-hydrogen bonds (Reineccius, 2003). To create a volatile sample, the triacylglycerols are saponified and esterified to form fatty acid methyl esters (FAMES) (Reineccius, 2003).

Compounds analyzed through gas chromatography must be volatilized at high temperatures of 250 degrees C. Lipids are suited to this method after preparation.

An advantage of GC with FID is high sensitivity in analysis. A disadvantage is that time-consuming sample preparation is required, which may alter the sample components (Reineccius, 2003).

High Performance Liquid Chromatography for Isoflavone Analysis

High performance liquid chromatography (HPLC) is a pressurized system that separates compounds based on partition between the stationary phase in the column and liquid solvent(s) (Rounds & Gregory, 2003). In normal phase HPLC, the

stationary phase is polar and the mobile phase is non-polar. Reversed-phase HPLC uses a non-polar stationary phase (such as an octadecyl chain), and a polar mobile phase (Rounds & Gregory, 2003). Water and a polar solvent such as acetonitrile or methanol (Lee et al., 2007; Penalvo et al., 2004) compose the mobile phase. Solutes are retained in the column based on hydrophobicity. Increasing the percentage of organic solvent in the mobile phase will decrease retention time (Rounds & Gregory, 2003). For isoflavone separation, reversed-phase HPLC with ultraviolet-visible (UV-vis) detection is typically used (Wang & Murphy, 1994; Hoeck et al., 2000; Kim & Chung, 2007).

Advantages of HPLC analysis are speed of analysis, and high sensitivity if the appropriate detector is used. A disadvantage is the requirement for volatile solvents in the mobile phase. Another disadvantage is that samples usually require preparation and filtering prior to analysis (Rounds & Gregory, 2003).

Tocopherol and Carotenoid Analysis

Reversed-phase HPLC is also described in the literature for detection of tocopherols and carotenoids. Often samples are saponified to reduce extraneous compounds in the oils prior to analysis. However, the process of saponification exposes the samples to heat and a strong base, which may result in loss of some of the carotenoids. Tocopherols and carotenoids are not soluble in water, so a polar organic solvent is more suitable for the mobile phase (Gimeno et al., 2000).

Antioxidant Analysis

Antioxidant capacity has been defined as the ability of a substance to prevent or delay deterioration by oxygen (Huang et al., 2005). There are multiple types of free radicals and different sources of antioxidants within a biological system. While there are several assays that can determine aspects of antioxidant capacity, but there is not one single assay to determine total antioxidant capability (Prior et al., 2005). There is also lack of standardization in antioxidant assays between laboratories. The results of one assay reported by a laboratory are only true under specific conditions, which may not correlate to a biological system (Huang et al., 2005). Several antioxidant assays are required to determine the scope of antioxidant capacity of a single compound.

Assays for antioxidant capacity typically measure either single electron transfer reactions (SET) or hydrogen atom transfer reactions (HAT) (Figure 8). HAT reactions occur during the chain-breaking reactions during the propagation stage of autoxidation, and thus can inhibit formation of peroxy radical.

Assays involving HAT reactions include oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), Crocin bleaching, and LDL oxidation inhibition. SET reactions include Trolox equivalent antioxidant capacity (TEAC), total phenolic content assay (TPC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Prior et al., 2005; Huang, et al., 2005).

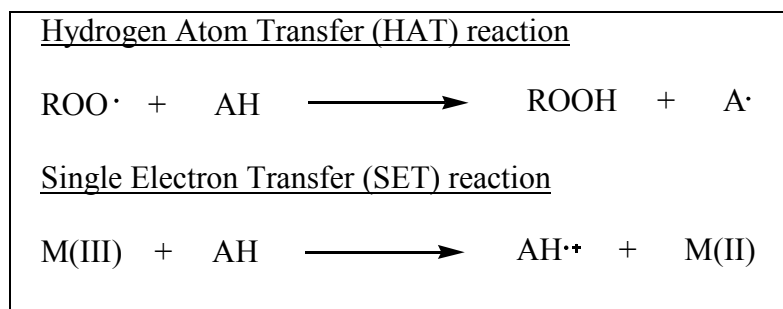


Figure 8. Hydrogen atom transfer and single electron transfer reactions represent mechanisms in antioxidant capacity assays.

Assays have been developed to detect scavenging of ROS or reactive nitrogen species (RNS) using mechanisms other than those previously described. These include hydroxyl radical scavenging capacity (HOSC), singlet oxygen scavenging capacity, and peroxynitrite scavenging capacity.

In measuring antioxidant capacity of food compounds, it is recommended to choose assays that quantify physiological radicals. In this respect, ORAC and HOSC, and LDL oxidation inhibition assays are advantageous. Huang et al. (2005) identified the area under the curve (AUC) quantification method as a recommended measure in antioxidant assays.

The oxygen radical absorbance capacity assay was first developed by Cao and Cutler (1993). The method detects the radical scavenging ability of a chain-breaking antioxidant against peroxy radical. The radical is generated by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (Ou et al., 2001). Cao and Cutler used B-phycoerythrin (B-PE) as a fluorescent probe. Later, Ou et al. developed a method using fluorescein as the fluorescent probe that is accurate when conducted with a 96-well plate reader (Ou et al., 2001). AAPH degrades the fluorescent capacity of

fluorescein over time at 37 degrees C. The antioxidant capacity of a sample is measured by the area under the curve of fluorescein degradation compared with the blank (Ou et al., 2001). Trolox, a vitamin E analog, is used as the standard to compare antioxidant activity of the sample.

The ORAC assay measures scavenging of a physiological radical, which makes it more relevant than some other antioxidant testing methods (Moore & Yu, 2008). It is also conducted at a physiological pH (7.4). The described method measures only hydrophilic antioxidant capacity, whereas another method would be needed to measure hydrophobic antioxidant capacity (Ou et al., 2001).

Total Phenolic Content (TPC)

The total phenolic content is measured in an assay with the Folin-Circlet (FC) reagent (phosphomolybdate and phosphotungstate). The TPC assay is an electron transfer reaction; under basic conditions the phenol becomes a phenolate anion and reduces the FC reagent (Huang et al., 2005). The reagent color turns from yellow to blue upon reduction. After 2 hours, the reaction is measured with a spectrophotometer at 765 nm.

Singleton and Rossi developed a standardized TPC method to produce consistent results (Huang et al., 2005). The method includes reaction time, temperature, and ratio of reagent volumes. The phenolic content of the samples is calculated based on a standard curve using gallic acid.

The TPC assay is widely performed in antioxidant studies and has good correlation with other assays such as Trolox Equivalent Antioxidant Capacity

(TEAC) (Huang et al., 2005). However, many other organic and inorganic compounds can interfere in the reaction (Prior et al., 2005).

Hydroxyl Radical Scavenging Capacity (HOSC)

The hydroxyl radical (OH) is a highly reactive physiological molecule that can damage lipids, proteins, and DNA (Huang et al., 2005). The HOSC assay developed by Moore et al. measures antioxidant capacity against OH radical generated by the Fenton reaction of Fe(II) and H₂O₂ (Moore et al., 2006). Fluorescein is the fluorescent probe, and the reaction can be measured in a 96-well plate reader. The antioxidant prevents degradation of the fluorescein by the OH. The antioxidant activity is calculated by the area under the curve of fluorescein degradation compared with the blank (Moore et al., 2006).

The method described by Moore et al. has been verified by ESR (Moore et al., 2006). The HOSC method is a hydrogen atom transfer assay (HAT), which is similar to the physiological lipid peroxidation reaction. Additionally, the HOSC assay is conducted at a physiologic pH and may be a good reflection of radical scavenging under physiologic conditions (Moore et al., 2006).

DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a commercially available, stable free radical. When reduced by an antioxidant compound, the color of the DPPH[•] solution decreases. The decreased light absorbance is measured by a spectrophotometer over time to quantify the rate of the reaction (Huang et al. 2005). DPPH radical

scavenging value has typically been reported as percent DPPH[·] scavenged over time, however there is little consistency between laboratories due to differences in methods (Cheng et al., 2006). Cheng et al. (2006) reported a high-throughput DPPH[·] scavenging assay measured in Trolox equivalents that can be compared between laboratories. Absorbance of standards and samples is measured at equal time points and the AUC is determined. Trolox equivalent is determined by a standard curve of known Trolox concentration (Cheng et al., 2006).

The DPPH[·] assay can be measured in different solvents, both polar and non-polar (Cheng et al., 2006). It is also a simple and quick assay to perform, if a microplate reader is used. A disadvantage is that it is not a physiological radical, so results do not correlate well with assays such as ORAC or OH radical scavenging. It may also have a reversible reaction with some phenols, such as eugenol, and so the accurate reaction rate may not be determined (Huang et al., 2005).

Objective and Significance

Soybeans are known to possess nutritional, antioxidant, and chemical properties that may enhance human health (Sugano, 2006). Soybeans with reduced α -linolenic acid are in demand for producing food oils with improved stability, and it is of interest to evaluate the impact of oil modification on other soybean components and health properties (Fehr, 2007). This research will examine the effect of lowering α -linolenic acid level through breeding effort on health components and properties in soybeans.

A previous study in our group has evaluated the components (isoflavone, antioxidant, fatty acid, tocopherol, and lutein) of three Maryland-grown, low 18:3n-3 soybeans (Slavin et al., 2009). This research will provide a more in-depth view of several genotypes of reduced 18:3n-3 soybeans. These tests will allow identification of particular soybean lines that may have desirable nutritional, nutraceutical, or chemical properties. Soybean lines with promising characteristics may be selected for future cultivation and analysis.

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Chapter 2: Nutraceutical Properties of Low α -Linolenic Soybeans

Introduction

While traditionally served in some Asian cuisines, soy food products have enjoyed increasing popularity in Western cultures since the 1990's. In 2008, the United Soybean Board reported that 32% of consumers surveyed used soy foods or beverages at least once per month (United Soybean Board, 2008). Soy provides a complete protein for humans with low saturated fat, and therefore is an alternative to many meat products (Anderson et al., 1999). Soy consumption has been associated with health benefits related to its bioactive compounds, including phenolics, isoflavones, and other antioxidant components. (Xiao, 2008; Anderson et al., 1999). According to the United Soybean Board, 35% of soy consumers cited a desire to improve health as a reason for use indicating the importance of health beneficial properties in soybean based food ingredients and ready-to-eat food products (United Soybean Board, 2008).

Soybean oil is recognized as a healthier alternative to animal fats due to its high content of unsaturated fatty acids (Meydani et al., 1991). The significant α -linolenic acid (18:3n-3) content of regular soybean oil makes it a good source of omega-3 fats, which are associated with reduced cardiovascular disease risk (Conner, 2000). However, α -linolenic acid can deteriorate easily during processing and storage, due to its highly unsaturated structure. It oxidizes twice as quickly as linoleic

acid (18:2n-6) under stable conditions (Wilson, 2004). At high temperatures during cooking, it can degrade even more rapidly (Wilson, 2004). The soybean oil is often chemically hydrogenated to improve stability before use in food products.

Unfortunately, partially hydrogenated oils contain *trans* fats and are associated with an increased risk of cardiovascular diseases (Eckel et al., 2006).

Several possible approaches to reduce the level of *trans* fat in food oils have been investigated, including modification of fatty acid composition in edible seed oils through breeding efforts (Tarrago-Trani et al., 2006). Soybean genotypes have been cultivated with reduced levels of α -linolenic acid to improve the stability and avoid the need for hydrogenation (Wilson, 2004). Low α -linolenic soybeans contain less than 3% α -linolenic acid versus the 7% in the conventional soybeans (Slavin et al., 2009; Sugano, 2006). Some low linolenic cultivars contain less than 1% α -linolenic acid. In commercial baked and fried food products, the desired α -linolenic acid portion is 2% for soybean oil to maintain stability without hydrogenation (Wilson, 2004). Due to rising public concerns over *trans* fats, low α -linolenic soybean oil is promising for use in food production.

The reduction of α -linolenic acid content naturally increases the content of other fatty acids in the soybean. The α -linolenic acid may be replaced with oleic (18:1n-9), linoleic (18:2n-6), stearic (18:0), or palmitic (16:0) acids. For health considerations, an increase in the monounsaturated oleic acid would be desirable. However, when α -linolenic acid is reduced, there also may be an increase in saturated fatty acids. This contributes to the stability of the oils, but a higher intake of palmitic acid may have deleterious health effects (Warensjö et al., 2008). An increase in

dietary stearic acid intake has been evaluated as non-significant effect to cardiovascular risk, even though it is a saturated fatty acid (Baer et al., 2003; Warensjö et al., 2008). In addition, one clinical study demonstrated that a diet containing low α -linolenic soy oil improved human lipid profile over a diet containing high amounts of saturated or hydrogenated soy oils (Lichtenstein et al., 2006). Thus the health benefits of reducing the *trans* fats help to justify modification of lipids in soy oil through soy breeding efforts.

Alteration in fatty acid composition may alter other properties of soybeans. For example, previous research indicated that tocopherol levels were decreased in some genotypes of soybeans with modified fatty acid profiles (McCord, Fehr, Welke, & Wang, 2004; Scherder, Fehr, Welke, & Wang, 2006). Our recent study (Slavin et al., 2009) evaluated possible alteration of reducing linolenic concentration on phytochemical profiles and antioxidant properties of soybeans using three Maryland cultivars grown in 2005. The results of this preliminary research indicated the low α -linolenic soybean lines might have antioxidant capacity and nutraceutical compounds similar to the conventional soybean cultivars. The limitation of this research was that only three low α -linolenic acid soy lines from a single location in Maryland were included. To draw a general conclusion, more cultivars from multi-growing conditions are required. As a continuation of our research on low linolenic soybeans, this study was conducted to examine the effects of the breeding effort to reduce α -linolenic acid concentration on desirable health beneficial properties such as isoflavone composition and antioxidant properties. This study focuses on seven Maryland-grown low 18:3n-3 soybean lines and one control soybean variety with

ordinary 18:3n-3 content grown under three different environments representative of Maryland soybean production systems.

Materials and Methods

Materials

Whole soybeans from the 2007 growing season were collected by Dr. William Kenworthy of the Department of Plant Sciences and Landscape Architecture, University of Maryland, College Park. Soybean lines included the Manokin cultivar, Asgrow Vistive varieties (AG2921V and AG3521V), and 5 experimental lines from Maryland. Three soybean samples from each of eight lines or cultivars under each of 3 environments (8 soybean lines \times 3 environments \times triplicate plots under each condition \times duplicate tests = total 72 samples) were selected. Thirty percent ACS-grade hydrogen peroxide was purchased from Fisher Scientific (Fair Lawn, NJ). 2,2'-azobis (2-aminodopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Fluorescein (FL), iron (III) chloride, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Sample Preparations

Oil Extraction

Whole soybeans were ground to particle size 20-mesh using a handheld coffee bean grinder. Five grams of ground soybeans were combined in a tube with 10 mL of petroleum ether. Tubes were vortexed for 15 seconds, and held for 20 hours at ambient temperature in the dark. The supernatant was removed and stored. The extraction was repeated twice (tubes held for 22 hours in the subsequent extractions) and all supernatants were collected and combined. The petroleum ether was evaporated overnight, and the remaining oil was weighed. The oil samples were stored in the dark until further testing.

Antioxidant Extraction

The defatted soy flour was kept in a fume hood overnight at ambient temperature to evaporate all remaining petroleum ether. One g of each soy flour sample was combined in a screw-capped tube with 10 mL of 50% acetone. The tubes were vortexed 3 times for 15 seconds each, and kept in the dark at ambient temperature overnight. The tubes were centrifuged at 1500 rpm for 5 minutes. The supernatant was collected in separate tubes and stored in the dark until further testing.

Fatty Acid Composition

The soybean oil was prepared for gas chromatography (GC) analysis according to a previously described procedure (Yu et al., 2002). The soybean oil was saponified and

methylated to form fatty acid methyl esters (FAME). After cleaning, FAME was quantitatively re-dissolved in isooctane. GC was performed with a Shimadzu GC-2010 with a FID detector. Helium was the carrier gas with a flow rate of 2.2 mL/min. The stationary phase was a fused silica capillary column SPTM-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco Inc. (Bellefonte, PA). Injection volume was 1 μL at a split ratio of 10/1. Oven temperature started at 136 °C, increased by 6 °C/min until 184 °C and held for 3 minutes, then increased by 6 °C/min to a final temperature of 226 °C. Each sample was tested in duplicate. Fatty acids were identified by comparing FAME retention time with that of known standards. The FAMEs were quantified by calculating the area under the curve of each identified peak. Individual FAMEs were reported in g/100g total fatty acids.

Total Phenolic Content (TPC)

The total phenolic content of each soy flour extracts was determined according to a previously described laboratory procedure using gallic acid as a standard (Parry et al., 2005). The final reaction mixture contained 50 μL of soybean extract, 250 μL of Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of ultra-pure water. After 2 hours reaction at ambient temperature, absorbance was read at 765 nm. The reactions were conducted in duplicate and results reported in gallic acid equivalents (GAE) per gram of whole soybean.

Isoflavone Composition

The soy flour extracts in 50% acetone were combined with 0.75 mL 36% hydrochloric acid and heated for 2 hours in a water bath at 55 °C. The acetone was then evaporated under nitrogen. The remaining solution was extracted 3 times with 4 mL ethyl ether/ethyl acetate (1:1, v/v) each time, and the combined ethyl ether/ethyl acetate solution was washed with 3 mL distilled water. The ethyl ether/ethyl acetate was evaporated in a nitrogen evaporator. The remaining soy extract was re-dissolved in 0.5 mL methanol and filtered through a 0.45 µm filter prior to HPLC analysis. HPLC was conducted according to a previously described method (with modifications) (Lee et al., 2007). A Phenomenex C-18 column (150 × 4.6mm, 5 µm) was used. The mobile phase consisted of 99.9% distilled deionized water with 0.1% acetic acid (v/v) (Solvent A) and 99.9% acetonitrile with 0.1% acetic acid (v/v) (Solvent B). The gradient progressed linearly from 25% to 32% solvent B over 20 minutes. The detection wavelength was set at 254 nm. The column was kept at 30 °C. Samples were analyzed in duplicate.

Lutein Content

The soybean oil samples and standards were diluted in methanol/acetonitrile/chloroform (7:7:6, v/v/v) and filtered through a 0.45 µm filter. HPLC was performed according to a previously described method (Su et al., 2002) using a Phenomenex C-18 column (250 × 4.6mm, 5µm). A Shimadzu guard cartridge was attached. The mobile phase was isocratic using methanol/acetonitrile/chloroform

(45:45:10, v/v/v) with 0.05% ammonium acetate (w/v) in the methanol and 0.1% triethylamine (v/v) in the acetonitrile. 50 μ L of each standard and sample was injected and run time was 10 minutes per sample, each conducted in duplicate.

Tocopherol Content

Soybean oil and tocopherol standards were diluted 1:10 in methyl-tert-butyl ether and filtered through a 0.45 μ m filter. Reversed-phase HPLC with UV detection was performed according to a previously described procedure (Darnoko et al., 2000) with modifications. The stationary phase was a C-30 column (250 \times 4.6 mm, 5 μ m).

Solvent A of the mobile phase consisted of methanol/MTBE/water, (81:15:4, v/v/v), and Solvent B was MTBE/methanol (91:9, v/v). The mobile phase was run from 0 to 16% solvent B in 13 minutes, 100% Solvent B from 13 to 23 minutes, and re-equilibrated with 100% Solvent A from 23 to 32 minutes. Flow rate was 1.0 mL/min., and injection volume was 30 μ L. The UV detector wavelength was set at 295 nm. Each standard and sample was run in duplicate.

Relative DPPH[•] Scavenging Capacity (RDSC)

DPPH[•] scavenging capacity was determined according to a previously described laboratory procedure (Cheng et al., 2006), using a Victor³ multilabel plate reader (PerkinElmer, Turku, Finland). DPPH[•] solution was prepared in 50% acetone and filtered through a medium porosity P5 paper filter (Fisher Scientific, Waltham, MA). Trolox was used as the standard. The final reaction mixture contained 100 μ L

soybean extract or Trolox standard or 50% acetone (the control), and 100 μ L 0.2 mM DPPH solution. The absorbance was read at 515 nm every minute for 40 min. Each sample was tested in duplicate. The radical scavenging capacity (RDSC) was calculated from the area under the curve and reported in Trolox equivalents (TE) per g whole soybean.

Hydroxyl Radical Scavenging Capacity (HOSC)

The HOSC was examined using a previously reported laboratory procedure (Moore et al., 2006). Fluorescein (FL) was used as a fluorescent probe and the assay was performed using a Victor³ multilabel plate reader (PerkinElmer, Turku, Finland). Iron (III) chloride and hydrogen peroxide were prepared in ultra-pure water. FL was prepared in 75mM sodium phosphate buffer (pH 7.4). The reaction mixture consisted of 170 μ L of 9.28×10^{-8} M FL, 30 μ L of sample, standard, or blank, 40 μ L of 0.20 M hydrogen peroxide, and 60 μ L of 3.43 M iron (III) chloride. The fluorescence was recorded every 4 minutes for 4 hours. Trolox prepared in 50% acetone was used as the standard. The assay was conducted in duplicate for each sample. Antioxidant capacity was calculated by area under the curve (AUC) described by Moore et al. (2006). Results were reported as μ mol TE/g of whole soybean.

Oxygen Radical Absorbance Capacity (ORAC)

The ORAC values were determined following a previously reported laboratory procedure (Ou et al., 2001), with fluorescein (FL) as a fluorescent probe. Trolox

standards were prepared in 50% acetone and other reagents were prepared in 75 mM pH 7.4 phosphate buffer. Samples were analyzed in duplicate. In the initial reaction, FL was combined with 30 μ L of sample, standard, or solvent in a 96-well plate. The plate was heated at 37 °C for 20 minutes in a Victor³ multilabel plate reader (PerkinElmer, Turku, Finland). Twenty-five μ L of 0.36 M AAPH was added to each well and the fluorescence of the mixture was recorded every 2 minutes over a 40-minute period at 37 °C. Excitation and emission wavelengths were 485 and 535 nm respectively. The results were reported as μ mol TE/g whole soybean, based on AUC calculations.

Statistical Analysis

Data were analyzed using SPSS (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL). Data were reported as mean \pm standard deviation (n = 18). Differences between means were determined by analysis of variance (ANOVA) with Tukey's HSD post hoc test. A two-tailed Pearson Correlation Coefficient test was used to determine correlations among means. Significance was declared at $P < 0.05$.

Results and Discussion

Oil Content and Fatty Acid Composition

The tested soybean lines and cultivars significantly differed in their oil content (Table 2). Oil content of the soybeans with lower 18:3n-3 ranged from 14.7 to 17.3

g/100 g under the experimental conditions, while the Manokin soybean, the control soybean cultivar with ordinary 18:3n-3 level, showed oil content of 15.7 g/100 g. Soybean cultivars on average contain 19 to 20% oil by weight (Sugano, 2006). The extraction procedure used in this research may not have yielded all oil present in the soybeans. Oil content can also vary due to environmental differences during soybean growth (Sugano, 2006; Bennett et al., 2004). A negative correlation between oil content and 18:3n-3 was detected ($r = - 0.19$, $P = 0.024$). Therefore, reduction of 18:3n-3 content might increase the total oil concentration of the soybeans.

Fatty acid compositions of these soybeans were also examined. The oil from Manokin soybeans had an average of 7.5% 18:3n-3, which is similar to many U.S cultivars. The other cultivars and experimental lines had lower 18:3n-3 content as expected. MD 04-6006, MD-05-5656 and MD 04-5217 had about 3% α -linolenic acid, while MD 05-6377 had 1% α -linolenic acid (Table 2). In addition, AG2921V and AG3521V had 2.3 and 2.6% α -linolenic acid, respectively. MD 05-6377 contained a relatively high level of oleic acid (18:1n-9) (33%) compared to standard soybeans and very low α -linolenic acid (1%). MD 05-6377 also contained the lowest palmitic acid (4.4%) of these tested lines. High oleic and low palmitic acids are a desirable trait for cardiovascular health (Warensjo et al., 2008). Taken together, these data indicated that MD 05-6377 has desirable fatty acid composition for human health and oil oxidative stability, indicating excellent potential value of this line in food oil production. Interestingly, two-tailed Pearson correlation test showed that 18:1n-9 content was negatively correlated with 18:3n-3 level ($r = - 0.388$, $P < 0.001$),

suggesting that reduction of 18:3n-3 might increase 18:1n-9 content. In addition, content of total saturated fat was correlated with 18:3n-3 level, indicating the possibility to reduce total saturation of soybean oil while reducing 18:3n-3 content. This is likely the result of breeding efforts to reduce total saturated fats in these soybean lines. These data suggested that reduction of α -linolenic acid and saturated fats through breeding effort may result in soybean lines rich in oleic acid. The resulting soybean oils may have improved oxidative stability and benefit to human health.

Table 2. Oil content and fatty acid composition of soybeans (g/100 g oil)

	AG2921V	AG3521	MD 04-6006	MD 05-5656	MD 05-6377 (1% 18:3n-3)	MD 05-6381 (1% 18:3n-3)	MD 04-5217	Manokin (non-modified)
Oil	17.3d ± 1.1	17.0cd ± 1.2	15.1ab ± 2.3	14.7a ± 1.6	16.0b-d ± 0.8	16.3b-d ± 0.9	16.8cd ± 0.7	15.7a-c ± 0.8
16:0	10.9f ± 0.3	10.5e ± 0.1	7.1c ± 0.4	5.9b ± 0.2	4.4a ± 0.3	5.7b ± 0.3	9.5d ± 0.3	10.9f ± 0.3
18:0	5.0a-c ± 0.9	4.5a ± 0.6	5.5bc ± 0.7	5.7c ± 0.9	5.1a-c ± 0.5	4.9ab ± 0.6	5.5bc ± 1.0	4.9ab ± 0.5
18:1	30.1bc ± 2.5	24.8a ± 2.2	35.3de ± 5.8	38.9e ± 1.5	33.0cd ± 4.6	28.6b ± 1.7	33.8d ± 5.5	22.7a ± 1.3
18:2	52.5b ± 2.4	57.6d ± 2.0	48.7a ± 4.9	46.1a ± 1.8	56.4cd ± 4.4	58.6d ± 1.8	48.1a ± 4.6	54.0bc ± 1.1
18:3	2.4b ± 0.2	2.6c ± 0.2	3.5e ± 0.3	3.4e ± 0.2	1.1a ± 0.1	2.2b ± 0.2	3.1d ± 0.3	7.5f ± 0.5
SFA	15.9	15.1	12.5	11.6	9.5	10.6	15.0	15.9
MUFA	30.1	24.8	35.3	38.9	33.0	28.6	33.8	23.7
PUFA	54.8	60.3	52.2	49.5	57.5	60.8	51.2	61.5

*Data are expressed as means ± standard deviation (n = 18). Values in the same row with different letters are significantly different ($P < 0.05$).

Total oil is expressed as g/100 g soybean. Fatty acids are expressed as g/ 100g oil. All genotypes are low α -linolenic except Manokin, which has standard soybean fatty acid composition. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 3. Isoflavone, lutein, and tocopherols content of soybeans.

	AG2091V	AG3521V	MD 04-6006	MD 05-5656	MD 05-6377 (1% 18:3n-3)	MD 05-6381 (1% 18:3n-3)	MD 04-5217	Manokin (non-modified)
Daidzein	35.3ab ± 7.7	32.9a ± 4.7	52.9b ± 11.8	73.5c ± 20.1	33.5ab ± 6.7	39.1ab ± 6.7	41.4ab ± 10.6	82.6c ± 46.5
Genistein	31.9a ± 10.0	33.0a ± 5.7	36.7ab ± 11.9	52.6bc ± 15.7	24.5a ± 9.6	25.2a ± 5.6	34.8a ± 8.9	64.4c ± 36.4
Glycitein	74.9c ± 21.7	63.4bc ± 17.7	61.1bc ± 27.4	56.6a-c ± 17.6	47.6ab ± 18.4	38.2a ± 14.5	64.7bc ± 21.4	38.3a ± 22.6
Total ISF	0.52a-c ± 0.10	0.47ab ± 0.08	0.56b-d ± 0.12	0.68cd ± 0.18	0.39ab ± 0.08	0.38a ± 0.06	0.52a-c ± 0.10	0.70c ± 0.39
Lutein	16.8bc ± 1.5	15.8b ± 2.0	17.2bc ± 2.1	11.2a ± 1.9	16.3bc ± 1.7	24.1d ± 4.6	23.6d ± 3.1	19.2c ± 4.1
α-Tocopherol	282.5ab ± 20.3	288.7b ± 24.9	284.4b ± 13.1	273.6ab ± 11.2	280.0ab ± 10.7	279.0ab ± 14.4	306.9c ± 16.3	266.8a ± 12.2
γ-Tocopherol	356.5a ± 49.2	400.3ab ± 57.4	469.9cd ± 57.7	378.0ab ± 57.3	492.6d ± 39.6	419.1bc ± 51.8	410.3ab ± 79.5	364.0ab ± 43.0
δ-Tocopherol	379.1b-d ± 46.8	334.6ab ± 45.2	406.9cd ± 60.5	425.6d ± 48.9	367.1a-c ± 47.8	321.7a ± 37.4	372.1bc ± 52.1	371.0bc ± 44.5
Total Toco.	2.5ab ± 0.2	2.5ab ± 0.2	2.9d ± 0.2	2.7cd ± 0.2	2.8d ± 0.2	2.5ab ± 0.2	2.7cd ± 0.2	2.4a ± 0.2

* Data represent the average by genotype ± SD (n = 18). Values in the same row with different letters are significantly different ($P < 0.05$). All samples are low-linolenic genotypes except Manokin, which has standard fatty acid composition. Daidzein, glycitein, and genistein are expressed as μg/g soybean. Lutein, α-, δ-, and γ- tocopherols are expressed as μg/g oil. Total isoflavones (ISF) are expressed as μmol/g whole soybean, and total tocopherols (Toco.) are expressed as μmol/g oil. Genotypes labeled as low α-linolenic contain 3% or less α-linolenic acid.

Total Phenolic Content (TPC)

The soybean cultivars and lines analyzed in this study contained TPC levels between 1.32 and 1.66 mg gallic acid equivalent (GAE)/g soybean (Figure 9a). These levels are comparable to that of 1.51 to 4.36 mg GAE/g soybean reported in previous studies, however fall at the lower end of the overall reported range (Sakthivelu et al., 2008; Xu & Chang, 2008; Slavin et al., 2009). The line AG3521V had the lowest TPC, and Manokin contained the highest level on average. No correlation between 18:3n-3 concentration and TPC value was observed, suggesting that reduction of 18:3n-3 level might not significantly alter total phenolic contents in the soybeans.

Soybeans are known to contain multiple phenolic compounds, including isoflavones (Xu & Chang, 2008). Phenolics contribute to total antioxidant capacity, and sub-classes of phenolics such as soy flavonoids are associated with reduced risk of several aging-related chronic human diseases (Sroka & Cisowski, 2003; Arts & Hollman, 2005). Our previous research on modified-lipid soybeans found that there was not a difference in the TPC content of reduced 18:3n-3 soybeans and the non-modified soybeans (Slavin et al., 2009). This data agrees with those previous findings.

Isoflavones

The 50% acetone extracts were hydrolyzed with concentrated HCl to cleave glycoside bonds and allow measurement of isoflavone aglycones. Each individual isoflavone, including daidzein, genistein, and glycitein, was measured in μg and total isoflavones were reported in μmole per gram soybean since the different isomers vary in molecular weight. This study used 50% acetone as the extraction solvent since it has been previously used for total antioxidant extraction from soybeans and soybean antioxidants are the focus of this study, (Slavin et al., 2009), although acetonitrile with 0.1N HCl has been used as a solvent for isoflavone extraction in several previous studies (Wang & Murphy, 1994; Reidl et al., 2007; Sakthivelu, et al., 2008; Lee et al., 2003). Daidzein, genistein, and glycitein were detected in all the tested soybean cultivar and lines regardless of 18:3n-3 content. The soybean lines and cultivar differed in their daidzein, genistein, and glycitein compositions (Table 3). Glycitein was the primary isoflavone compound in six of the seven tested low 18:3n-3 soybean lines, whereas daidzein was the major isoflavone in MD 05-5656 and Manokin soybeans. 18:3n-3 content was correlated to daidzein ($r = 0.571$, $P < 0.001$) and genistein ($r = 0.577$, $P < 0.001$) levels, and negatively correlated with glycitein content ($r = - 0.210$, $P < 0.001$). Taken together, these data suggested that breeding efforts to reduce α -linolenic acid may significantly alter the isoflavone compositions and content in soybeans. It is widely accepted that changes in the composition of soybeans may occur over different growing seasons (Hoeck et al., 2000; Lee et al., 2003). Additional research is required to investigate the effects of selected growing conditions such as solar radiation and

irrigation, genotype, and the interaction between genotype and individual growing conditions on soybean isoflavones.

The tested soybean lines and cultivar differed in their total isoflavone contents (Table 3). The Manokin genotype (non-modified fatty acid composition) and MD 05-5656 (18:3n-3) had the highest total isoflavone content of 0.70 $\mu\text{mol/g}$ compared with the other low 18:3n-3 lines. The lowest total isoflavone level of about 0.37 $\mu\text{mol/g}$ was detected in MD 05-6377 and MD 05-5656, each of which contained about 1% 18:3n-3. Reidl and others (2007) reported 1.5 to 7.1 $\mu\text{mol/g}$ total isoflavones in Ohio-grown soybeans with acidic acetonitrile as the extraction solvent at ambient temperature. The lower total isoflavone levels in the current study may be partially related to the extraction solvent used (50% acetone), which could not extract insoluble bound isoflavones from the soybean matrix (Slavin et al., 2009). Total isoflavone content was correlated with the level of 18:3n-3 ($r = 0.421$, $P < 0.001$), suggesting the possibility of decrease in total isoflavones due to the reduction of 18:3n-3 level through breeding effort.

Lutein

Lutein has been previously identified as the major carotenoid in mature yellow soybeans (Monma, Terao, Ito et al., 1994; Kanamaru, Wang, Abe, et al., 2006; Slavin, et al., 2009). Lutein has shown beneficial health effects for humans including prevention of age-related macular degeneration and protection of skin from ultra-violet damage (Heinrich et al., 2003; Mares-Perlman et al., 2002). All soybean samples tested in this study contained lutein ranging from 11.1 to 24.1 $\mu\text{g}/100\text{ g oil}$ (Table 3). Two of the reduced 18:3n-3

lines had a significantly higher lutein level than the non-modified line, whereas two low 18:3n-3 lines had significantly lower lutein concentration than the non-modified soybean, and the remaining three low 18:3n-3 lines contained the same lutein level as the non-modified soybean. This observation was in agreement with the findings from an earlier work from our group (Slavin et al., 2009) that found variation in the level of lutein among both modified and non-modified oil soybeans. In addition, research by Wang et al. (2007) demonstrated that lutein levels are heritable in soybeans. The reduced 18:3n-3 lines identified with high lutein levels may be further cultivated to maximize health benefits in soy oil. Lutein level was positively correlated with total oil content ($r = 0.475$, $P < 0.01$) and the level of linoleic acid ($r = 0.272$, $P < 0.01$), but not correlated with 18:3n-3 level in the soybeans. Lutein content was negatively correlated with total isoflavones ($r = -0.233$, $P < 0.01$).

Tocopherols

Tocopherols provide antioxidant activity for soybean oil to prevent lipid oxidation, and α -tocopherol provides dietary vitamin E (Wilson, 2004). This study quantified α -, γ -, and δ - tocopherol isomers in the soybean oils. The soybean oils differed in their tocopherol compositions, with δ - tocopherol as the primary isomer in two of the low α -linolenic acid and ordinary soybean oils and γ -tocopherol as the major isomer in five low linolenic acid lines under the experimental conditions (Table 3). Total tocopherols ranged from 2.5 to 2.9 $\mu\text{mol/g}$ oil (Table 1). The levels of α -, γ , and total tocopherols were all negatively correlated with 18:3n-3 ($P < 0.01$). The oil from the Manokin

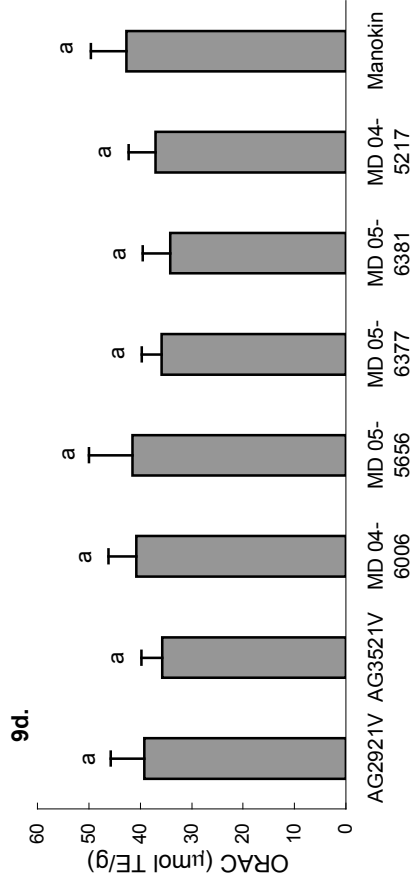
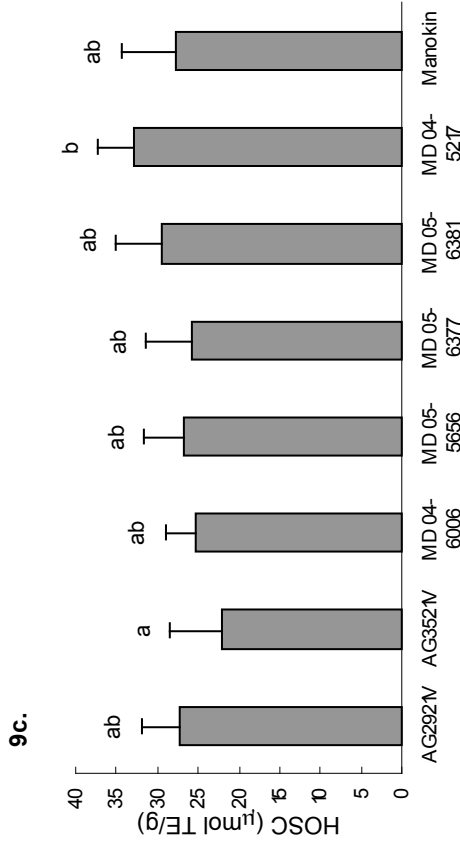
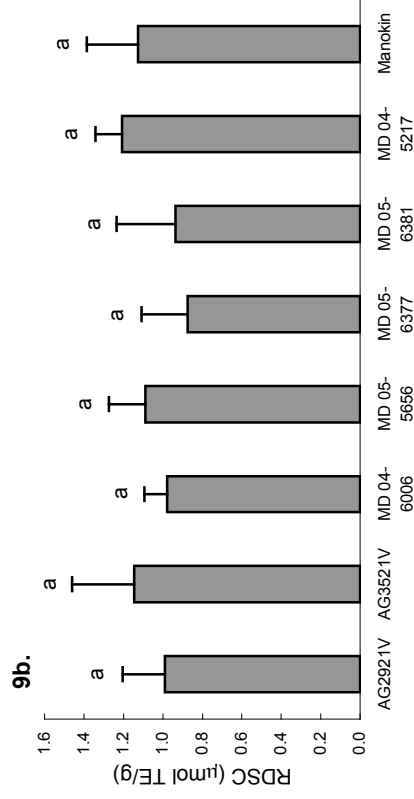
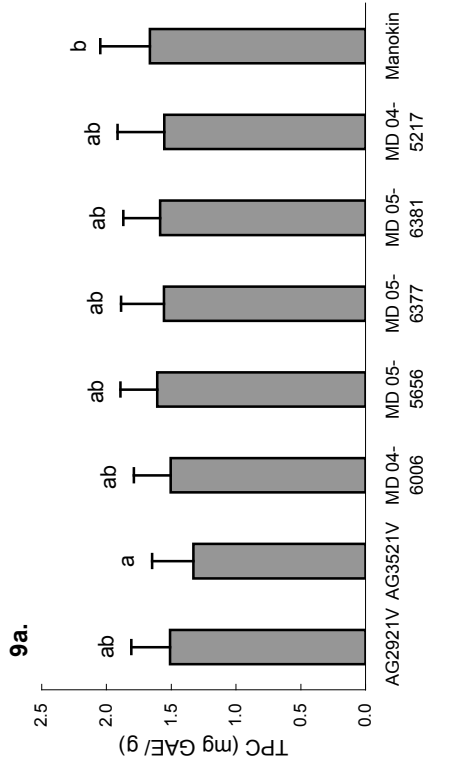
soybeans with the non-modified fatty acid profile contained the lowest total tocopherols compared to the other genotypes. These results suggest that lowering 18:3n-3 levels may result in similar or higher tocopherol levels in these particular genotypes. This conclusion was supported by our previous findings that some reduced 18:3n-3 genotypes had tocopherol levels comparable to the non-modified genotypes (Slavin et al., 2009). This conclusion is in contrast to the observation in another study by Dolde et al.(1999). Dolde et al. reported that reduced 18:3n-3 soybean genotypes contain lower tocopherols than non-modified soybeans under the same growing conditions (Dolde et al., 1999). In addition, there was no correlation between δ -tocopherol content and 18:3n-3 level, but δ -tocopherol content had positive correlation with oleic acid (18:1n-9) ($r = .536, P < 0.001$).

Relative DPPH• Scavenging Capacity (RDSC)

There are multiple types of free radicals and different sources of antioxidants within a biological system. While there are several assays that can determine aspects of antioxidant capacity, there is not one single assay to determine total antioxidant capability (Prior, Wu, & Schaich, 2005). Two or more antioxidant assays are required to determine the scope of antioxidant capacity of a single compound or antioxidant preparation. Assays for antioxidant capacity typically measure either single electron transfer reactions (SET) or hydrogen atom transfer reactions (HAT). Oxygen radical absorbing capacity (ORAC) and hydroxyl radical scavenging capacity (HOSC) are examples of HAT reaction assays, while the DPPH• assay is considered a SET reaction

assay (Prior et al., 2005; Huang, Ou, & Prior, 2005). Three assays have been selected in this study to show the range of antioxidant capacity.

The DPPH• scavenging capacity ranged from 0.9 to 1.2 $\mu\text{mol TE/g}$ soybean and the tested soybean samples had no significant difference in their DPPH radical scavenging capacities (Figure 9b). Because of the varying laboratory methods for measuring and reporting DPPH• scavenging activity (Cheng et al., 2006), it is difficult to compare the current results with many other analyses of soybeans. There was high variation in DPPH• scavenging capacity between different samples within the same soybean lines and cultivar. The data represents nine samples from each line taken from different plots. It was reported that growing conditions such as solar irradiation and temperature during selected growing period altered antioxidant properties in wheat grain (Moore et al., 2006). The high variation of the DPPH• scavenging capacity data may have occurred from unknown differences in soil or other growing factors. No correlation between 18:3n-3 content and DPPH• scavenging capacity was detected under the experimental conditions, suggesting less possibility of altering DPPH• scavenging capacity in soybeans because of breeding effort to reduce α -linolenic acid level. No correlation between linolenic content and DPPH• scavenging capacity was detected under the experimental conditions, suggesting less possibility of altering DPPH• scavenging capacity in soybeans because of breeding effort to reduce 18:3n-3 level.



Figures 9a. Relative DPPH radical scavenging capacity (RDSC), 9b. Oxygen radical absorbing capacity (ORAC), 9c. Hydroxyl radical scavenging capacity (HOSC), and 9d. Total phenolic content (TPC) of soybeans. RDSC, HOSC, and ORAC are expressed as μmol TE/g soybean. TPC is expressed as mg GAE/g soybean. Vertical bars represent SD (n = 18). Different letters represent significant difference within assay ($P < 0.05$).

Hydroxyl Radical Scavenging Capacity (HOSC)

The hydroxyl radical ($\cdot\text{OH}$) is a highly reactive molecule that may be generated under physiological conditions and can damage lipids, proteins, and DNA (Huang et al., 2005). The HOSC assay developed by Moore et al. (2006) measures antioxidant capacity against OH radical generated by the Fenton reaction of Fe(II) and H_2O_2 (Moore et al., 2006). All tested soybean extracts demonstrated hydroxyl radical scavenging capacity (HOSC) (Figure 9c). The greatest HOSC value was observed in the MD 04-5217 soybeans which had about 3.1% 18:3n-3 in the total fatty acids, whereas the lowest hydroxyl radical scavenging capacity was detected in the AG3521V soybeans containing 2.6% 18:3n-3. The hydroxyl radical scavenging capacity of soybeans was not correlated with their 18:3n-3 content, suggesting that reduction of 18:3n-3 level may not alter HOSC in soybeans.

The HOSC values ranged from 22.04 to 32.78 $\mu\text{mol TE/g}$ soybean (26.4 to 39.3 TE/g soy flour) under the present experimental conditions. This HOSC value range is lower than that of 68 to 104 $\mu\text{mol TE/g}$ soy flour previously reported in Maryland soybeans by our group (Slavin et al., 2009). It needs to be pointed out that the focus of this study was to evaluate the effects of reducing 18:3n-3 content through breeding effort on health beneficial properties in Maryland-grown soybeans, and many experimental conditions were selected to handle large numbers of samples but not to determine the maximum value of each property. Changes in extraction conditions might attribute to the lower $\text{HO}\cdot$ scavenging capacity range observed in this study. It was also possible that variation in growing season and environments might affect the antioxidant capacity between crop years. Growing season has shown significant impact on composition of soybeans in previous research (Hoeck et al.,

2000; Lee et al., 2003). Changes in the amount of phenolics, for example, would have an effect on the total antioxidant capacity of the soybeans.

Oxygen Radical Absorbing Capacity (ORAC)

The ORAC assay measures scavenging activity against peroxy radical, which may be formed under normal physiological conditions and involved in many harmful reactions in biological systems such as lipid peroxidation (Ou et al., 2001). ORAC in the evaluated soybean samples was between 34.2 and 42.7 $\mu\text{mol TE/g}$ using 50% acetone as the extraction solvent (Figure 9d). The ORAC levels are within the range reported by Xu and Chang (2008) in 50% acetone extract of yellow soybeans from North Dakota (22.1-91.2 $\mu\text{mol TE/g}$). A previous study by Xu and Chang (2007) found that extraction with 70% ethanol resulted in significantly higher ORAC values for yellow soybeans compared with 50% acetone extracts. This was contradicted by the results of Slavin and others (2009) in which 50% acetone was preferred over 70% ethanol as an extraction solvent for soybean ORAC determination. Furthermore, there was not significant difference among ORAC values of the soybean lines and cultivar at $P < 0.05$ in the present study. This might be due to the high standard deviation among soybean lines and cultivar, suggesting the possible effects of unknown factors related to growing conditions. In addition, no correlation was observed between ORAC value and 18:3n-3 content under the experimental conditions, indicating less concerns of decreasing ORAC in soybeans through breeding effort for reducing 18:3n-3 content.

Conclusion

This study indicates that breeding effort to reduce α -linolenic acid content in soybeans may result in preferred soybean lines and cultivars with preferred fatty acid profile such as lower in palmitic and total saturated fatty acid contents and rich in antioxidants and glycitein, although they may have lower level of total isoflavone, daidzein, and genistein. Additional research is needed to evaluate how genotype, individual growing conditions, and the interaction between the genotype and growing conditions on soybean oil content, fatty acid profile, antioxidant properties, and health beneficial phytochemicals.

Appendix

Table 1. Correlation between 18:3n-3 and other soybean components.

	18:3n-3
16:0	.519**
18:0	<i>NS</i>
18:1n-9	-.388**
18:2n-6	<i>NS</i>
Daidzein	.571**
Genistein	.577**
Glycitein	-.210*
Total Isoflavones	.421**
Lutein	<i>NS</i>
α-Tocopherol	-.245**
γ-Tocopherol	-.318**
δ-Tocopherol	<i>NS</i>
Total Tocopherol	-.240**

Correlations expressed as Pearson correlation coefficient (r-value).

** ($P < 0.01$), * ($P < 0.05$), *NS* = not significant at $P < 0.05$

Table 2. Correlation between antioxidant assays and isoflavones.

	TPC	ORAC	HOSC	RDSC
Daidzein	.282**	.239**	<i>NS</i>	<i>NS</i>
Genistein	.284**	.208*	<i>NS</i>	<i>NS</i>
Glycitein	<i>NS</i>	<i>NS</i>	<i>NS</i>	.189*
Total ISF	.263**	.185*	<i>NS</i>	<i>NS</i>

Correlations expressed as Pearson correlation coefficient (r-value). ** ($P < 0.01$),

* ($P < 0.05$), *NS* = not significant at $P < 0.05$. ISF = Isoflavone.

Table 3. Wye Research and Education Center soybean information.*

WYE RESEARCH & EDUCATION CENTER	
Queen Annes County - Queenstown, MD	
Tests:	Full Season Standard Varieties Maturity Groups III, IV, IV-S, and V
Planting Date:	June 11
Row Spacing:	24 inches
Soil Type:	Matapeake silt loam
Soil Test:	pH 6.1, P Index- 57, K Index- 75
Previous Crop:	Corn
Fertilizer:	10 Gal/A 23-18-0 starter
Lime:	None
Herbicide:	Preemergence: 1.5 Pt/A Dual Magnum, 0.8 Lb/A Lorox DF Post: 1.5 Pt/A Basagran, 1.5 Pt/A Blazer, surfactant
Plots:	4 rows, 20 feet long
Seeding Rate:	6.5 seeds/foot except Maturity Group V entries= 6 seeds/foot
Tillage:	Conventional

Table 4. Poplar Hill full seed soybean information.*

LOWER EASTERN SHORE RESEARCH & EDUCATION CENTER-POPLAR HILL FACILITY	
Wicomico County - Quantico, MD	
Tests:	Full Season Standard Varieties Maturity Groups III, IV, IV-S, and V
Planting Date:	June 7
Row Spacing:	24 inches
Soil Type:	Mattapex silt loam
Soil Test:	pH 6.3, P Index- Very High, K Index- High
Previous Crop:	Corn
Fertilizer:	600 Lbs/A of 2-4-12 liquid fertilizer
Lime:	1 Ton/A
Herbicide:	Preemergence: 1.5Pt/A Dual 8E, 12 Oz/A Lorox DF, 3 Oz/A Canopy XL Post emergence: 1.5 Pt/A Storm, 1 Oz/A 2,4-DB, 3 Oz/A Blazer + Surfactant
Plots:	4 rows, 20 feet long
Seeding Rate:	6.5 seeds/foot except Maturity Group V entries= 6 seeds/foot
Tillage:	Conventional

Table 5. Poplar Hill double crop 2007 soybean information.*

Tests:	Double Crop Standard Varieties Maturity Groups III, IV, IV-S, and V
Planting Date:	June 22
Row Spacing:	15 inches
Soil Type:	Mattapex silt loam
Soil Test:	pH 6.4, P Index- Very High, K Index- High
Previous Crop:	Winter barley
Fertilizer:	None on soybeans
Lime:	None on soybeans
Herbicide:	Preemergence: 1.5 Pt/A Roundup Ultra Max, 1.6 Pt/A Dual, 5 Oz/A Canopy, 8 Oz/A 2,4-DB Post emergence: 1.5 Pt/A Storm, 1 Oz/A 2,4-DB, 3 Oz/A Blazer + Surfactant
Plots:	5 rows, 20 feet long
Seeding Rate:	6 seeds/foot
Tillage:	None

Table 6. Wye Research and Education Center 2007 soybean variety yield and maturity date*

Performance of standard soybean varieties planted at Queenstown.						
BRAND - ENTRY	Seed Yield, Bu/A			2007		
	2007	2006	2-Year	Maturity Date	Height, Inches	Lodging Score*
EXPERIMENTAL - MD 05-6377	17.6	-	-	9-25	16	1.2
*ASGROW - AG 2921V (GP II)	29.1	-	-	9-24	18	1.0
*ASGROW - AG 3521V	35.3	-	-	9-26	19	1.0
EXPERIMENTAL - MD 04-5217	39.3	-	-	9-26	23	1.5
EXPERIMENTAL - MD 04-6006	27.5	37.0	32.3	9-27	17	1.2
EXPERIMENTAL - MD 05-6381	26.7	-	-	9-27	21	1.0
EXPERIMENTAL - MD 05-5656	21.4	-	-	10-08	28	2.8

Table 7. Poplar Hill full seed 2007 soybean variety yield and maturity date.*

Performance of standard soybean varieties planted full season at Quantico.						
BRAND - ENTRY	Seed Yield, Bu/A			2007		
	2007	2006	2-Year	Maturity Date	Height, Inches	Lodging Score*
EXPERIMENTAL - MD 05-6377	30.6	-	-	9-22	27	1.0
*ASGROW - AG 2921V (GP II)	41.5	-	-	9-17	23	1.0
*ASGROW - AG 3521V	46.9	-	-	9-24	28	1.3
EXPERIMENTAL - MD 04-5217	42.3	-	-	9-23	27	1.0
EXPERIMENTAL - MD 04-6006	39.9	55.8	47.9	9-23	25	1.2
EXPERIMENTAL - MD 05-6381	34.7	-	-	9-25	28	1.2
EXPERIMENTAL - MD 05-5656	37.6	-	-	10-07	39	3.7
PUBLIC - MANOKIN	33.2	55.4	44.3	10-05	32	1.8
EXPERIMENTAL - MD 04-6006	37.2	49.5	43.4	10-05	29	1.5

Table 8. Poplar Hill double crop 2007 soybean yield and maturity date.*

Performance of standard soybean varieties double cropped at Quantico.						
BRAND - ENTRY	Seed Yield, Bu/A			2007		
	2007	2006	2-Year	Maturity Date	Height, Inches	Lodging Score*
EXPERIMENTAL - MD 05-6377	31.4	-	-	10-04	27	1.3
*ASGROW - AG 2921V (GP II)	30.3	-	-	10-01	18	1.0
*ASGROW - AG 3521V	37.0	-	-	10-02	23	1.0
EXPERIMENTAL - MD 04-5217	35.6	-	-	10-05	31	2.2
EXPERIMENTAL - MD 05-6381	33.3	-	-	10-05	26	1.0
PUBLIC - MANOKIN	32.8	56.6	44.7	10-15	35	2.8
EXPERIMENTAL - MD 05-5656	31.5	-	-	10-18	37	2.3
EXPERIMENTAL - MD 04-6006	37.2	49.5	43.4	10-05	29	1.5

*Data obtained from Maryland Cooperative Extension Agronomy Facts No. 32, January 2008.

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