

Engineering Soybeans for Food and Health

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Growth in consumption of soy foods is restricted by negative flavor associations of soybean products and a limitation in the range of possible food and beverage applications resulting from the constraints of soybean storage protein properties. Soybean biotechnology is addressing these issues and creating even healthier soy-based foods.

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Soybean Foods and Biotechnology

There is an increasing consumer interest in achieving better health through diet. The large volume of recent research and clinical trials addressing the role of soy in preventing a wide range of diseases such as coronary heart disease, cancer, and osteoporosis (Messina, Gardner, & Barnes, 2002) has not gone unnoticed by the popular press. This attention is increasing consumer demand for soy-based foods and food ingredients. In response, major food companies are increasingly finding new ways to incorporate soy protein into mainstream products such as energy bars, breakfast cereals, meat alternatives, and beverages. Soy is now commanding an unprecedented position as a preferred food ingredient in the products on the grocery shelves of the United States. A recent joint study by SPINS and Soyatech (<http://www.soyatech.com>) has indicated that US retail sales of soy foods has grown by more than 10% a year for the last seven years to an estimated retail market in 2002 of \$3.65 billion (Soyatech, 2003).

This growth in consumer demand for soy is only limited by the negative flavor connotations of soybeans and soy products. It is not hard to understand why consumers may have difficulty meeting the US Food and Drug Administration's recommended intake of 25g of soy protein per day when the major flavors associated with these products include painty, grassy, beany, metallic, and bitter. Even when consumers have a good understanding of the health benefits, nutritionally educated consumers will not eat something if it does not taste good (Wansink & Chan, 2001). There are, however, a number of ways in which biotechnology could improve soy-based foods and help increase consumption. The obvious example would be to make soy flours, concentrates, and isolates taste better or, at least, remove some of the most objectionable flavors so that flavorists could make the foods taste better. If the compounds causing the volatile and nonvolatile off-flavors could be identi-

fied, then metabolic engineering might be used to prevent or reduce the formation of these compounds. A well-documented example of this is the reduction in beany flavor associated with the removal of lipoxygenase activity from soybeans (Kitamura, 1995).

A less intuitive but equally important approach would be to change the functional properties of soy proteins. These functional properties include solubility, water absorption, viscosity, gelation, emulsification, and flavor binding (Kinsella, 1979). Increasing the solubility of soy proteins at acidic pH ranges, for example, would allow whole, rather than hydrolyzed, proteins to be added to fruit juices with a consequent reduction in the bitter flavors associated with small peptides (Adler-Nissen, 1978). Improving the gelation properties of soy proteins could result in reduced processing and consequently reduce any possible off-flavors developed during the extended processing required for some soy ingredients (Kitamura, 1995).

Finally, because the main driving force of the soy food business is the health properties of soy components, improving or enhancing these health properties may provide an additional incentive to consume soy products. Removing antinutritional compounds such as protease inhibitors (Friedman & Brandon, 2001), indigestible sugars (Hitz, Carlson, Kerr, & Sebastian, 2002), or allergens (Herman, Helm, Jung, & Kinney, 2003) are obvious examples. But one could also envisage increasing the content of pronutritional compounds such as isoflavones (Jung et al., 2000) or augmenting the positive health effects of soy with bioactive soy proteins (Kitts & Weiler, 2003) and bioactive fatty acids (Cahoon et al., 1999; Knapp, Salem, & Cunnane, 2003).

Improving Soy Flavor

Although there may be hundreds of volatile compounds associated with bad flavors in soy preparations (Stephan & Steinhart, 1999), many of these compounds are asso-

ciated with the oxidation of the polyunsaturated fatty linoleic and linolenic acids (Frankel, 1987). These are the predominant fatty acids in soybean oil; their oxidation during bean storage and processing results in the formation of secondary products of lipid oxidation that impart off-flavors to soy protein products. Several years ago, a high-oleic soybean variety was developed in which the polyunsaturated fatty acids were reduced from 70% of the total fatty acids to less than 5% (Kinney, 1996; Kinney & Knowlton, 1998). This was accomplished with a transgenic silencing of a key gene associated with polyunsaturated fatty acid content—the Fatty Acid Desaturase 2 (FAD2) gene.

The initial goal of the FAD2 project was to produce an oxidatively stable oil that did not require hydrogenation and thus to reduce the trans fatty acid content of human diets (Kinney & Knowlton, 1998). In addition to a large increase in the oxidative stability of the oil, a large reduction (5- to 10-fold) in lipid oxidation products, such as 2-pentyl furan and trans 2-hexanal, in flours and protein isolates was also observed. As expected, this resulted in reduced grassy and beany flavors in various protein products made from the high-oleic beans as compared with commodity beans. These volatile compounds are active at very low concentrations, however, and significant grass-like flavors still remained in the high-oleic soy flours and isolates. Thus, current approaches seek to combine lines having reduced-oxidation substrates with other transgenic traits that reduce or remove the enzymes that act on these substrates, such as lipoxygenases (Hildebrand, 1989) and hydroperoxide lyases (Noordermeer, Veldink, & Vliegthart, 2001).

The nonvolatile compounds that contribute to the bitter and astringent flavors of soy proteins are less well understood. Bitterness may be imparted by small peptides, as well as by several classes of secondary plant compounds including flavonoids, phenols, and saponins (Drewnowski, 2001). Soy and soy products are rich in many of these secondary compounds (Dalluge, Eliason, & Frazer, 2003); preventing their synthesis in the seed by transgenic methods may help reduce the bitter flavor of soy products. A certain degree of bitterness, however, may be the price we have to pay for health—many of these secondary compounds have also been associated with positive health effects in soy (Messina et al., 2002).

Soy Protein Functionality

Another approach to improving soy food has focused on the functional properties of soy proteins. These proper-

ties determine the effective end use of soy protein in products ranging from beverages to meat substitutes. The bulk of soy protein (more than 80%) is contributed by two major classes of storage protein, conglycinin (11S globulins) and beta-conglycinin (7S globulins). It follows then that the properties of soy protein concentrates and isolates in various applications are mostly related to the properties of these particular storage protein classes. Because 7S and 11S proteins have differing physical properties (Maruyama et al., 2002; Mohamed Salleh et al., 2002), one would expect that the properties of soy protein isolates with different ratios of these protein classes would also have differing functional properties. That is generally the case (Nagano, Fukuda, & Akasaka, 1996; Yagasaki, Takagi, Sakai, & Kitamura, 1997), although interactions with other soy components, such as minerals and free amino acids, do affect the gelling, foaming, and emulsification properties of both 7S and 11S proteins (Garcia, Torre, Marina, & Laborda, 1997; Riblett, Herald, Schmidt, & Tilley, 2001).

Although 7S and 11S proteins are encoded by large gene families (Harada, Barker, & Goldberg, 1989; Beilinson et al., 2002), it has been possible to produce transgenic lines with either no 7S or no 11S protein using gene-silencing techniques (Kinney & Fader, 2002). In these lines, the reduction in one protein class is compensated by an increase in the other, with the total protein content remaining constant. Thus, 11S null lines can be thought of as high-7S lines and vice versa. These lines provide ideal starting material to investigate the effects of a particular storage protein class on the properties of soy protein products and may provide the basis for a whole new class of soy protein products with unique functional properties.

Although these high-7S and high-11S lines represent a major advance in the genetic manipulation of soy protein functionality, the potential applications of products derived from these lines are still limited by the intrinsic functional properties of 7S and 11S proteins. Protein engineering techniques have become increasingly sophisticated, and the molecular structures for these storage proteins are now known (Maruyama et al., 2001; Adachi et al., 2003). It is now possible to redesign storage proteins with specific functional properties in mind (Utsumi, Katsube, Ishige, & Takaiwa, 1997); it is likely that the next generation of genetically enhanced soy proteins will have unique functional properties that further extend the range of applications for soy protein in food.

Augmenting Health Properties: The Future of Soy Food

Soy foods are one of the most potent dietary tools known for reducing blood low-density lipoprotein (LDL) cholesterol concentrations in humans (Sirtori & Lovati, 2001). This positive health effect appears to be directly related to the soy storage proteins rather than other components (Weggemans & Trautwein, 2003). Although there have been some reports that the 7S proteins and their breakdown products are the bioactive proteins involved (Lovati et al., 1996), it is not known for certain which particular protein class, if any, mediates the cholesterol-lowering response. There are also other positive health effects of soy (such as the reduction of bone loss in postmenopausal women) that have been correlated with the interaction of soy proteins and soy isoflavones (Potter et al., 1998).

The availability of new soybean lines containing only 7S or 11S proteins should provide the material for clinical studies to confirm the role of a particular soy protein class in lowering LDL cholesterol in humans. Similarly, the availability of engineered soy lines that contain either no isoflavones or a greatly increased isoflavone content (Jung et al., 2000) will provide material to test the effect of these phytoestrogens in a soy protein background in which the isoflavone content is the only variable that has been changed.

In addition to protein, oil is the other component of soybeans that is used in many food applications (Kinney, 1996). Engineered soybean lines that are rich in oleic acid (producing a stable oil that does not need to be partially hydrogenated and is thus free of trans fatty acids) and lines lower in saturated fatty acids have been produced (Kinney, 1996; Kinney & Knowlton, 1998; Buhr et al., 2002). Although these high-oleic and low-saturate soy oils may indicate the potential for the positive impact of biotechnology on human health, they are only the beginning. It is now known that there are many bioactive fatty acids, not normally found in soy oil, that have dramatically positive effects on human health. These polyunsaturated fatty acids of various forms are known to mediate their heart-healthy effects by mechanisms independent of those of soy protein (Martin & Valeille, 2003; Kelley & Erikson, 2003; Knapp et al., 2003). Bioactive polyunsaturated fatty acids are also known to have many other positive health effects in humans (Knapp et al., 2003). Thus, it may be possible to combine the existing health benefits of soy with the complementary benefits of bioactive lipids and other compounds.

Genetic engineering allows the possibility of introducing completely new fatty acid biosynthetic pathways into soybeans from exotic plants and various microorganisms (Cahoon et al., 1999; Wallis, Watts, & Browse, 2002). The possibility that biotechnology will allow us to produce a wide variety of great tasting soy-based foods and drinks that also confer multiple health benefits on the human population is very exciting. This is surely the future of soy foods.

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