

Biotechnology and the soybean^{1,2}

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ABSTRACT Glyphosate-tolerant soybeans (GTSs), the first biotechnologically improved soybeans to be marketed, became available commercially in 1996. The safety of GTSs was assessed in 2 ways: study of the introduced protein and of the soybean seed and selected processing fractions. Because soybeans are a major source of protein in most farm animals' diets, animal feeding studies in wholesomeness were done to complement the analyses. Analysis of the expressed protein in GTSs [3-phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19)] showed it to be readily digestible and to possess no allergenic concerns. In addition, comparison of the composition of seeds and selected processing fractions from 2 GTS lines with the parental line showed that the lines are equivalent. Feeding studies in various animal species confirmed that the feeding value of GTS lines is comparable with that of the parental line. These studies support the conclusion that GTSs are as safe and nutritious as traditional soybeans marketed currently and can be incorporated safely into feed and food products in the near future. *Am J Clin Nutr* 1998;68(suppl):1330S–2S.

KEY WORDS Soybeans, glyphosate, 3-phosphoshikimate 1-carboxyvinyltransferase, 5-*enol*pyruvylshikimate-3-phosphate synthase, biotechnology, wholesomeness, feeding value, farm animals

INTRODUCTION

The demands on our global agricultural resources never have been greater. Demographers expect the world's population to double to >10 billion people in our children's lifetime (1). The growing need for food and fiber to feed and clothe this many people will challenge today's farming methods and natural resources.

For centuries, humans have identified new ways to grow more high-quality food and fiber. Agricultural advances such as irrigation, plant breeding, and pesticides have contributed to the current stable, diverse, and abundant food supply. The next step in agriculture is to produce more food and protect our shrinking agricultural land base; this leads to the inevitable conclusion that we must develop and promote more sustainable agricultural practices.

Plant biotechnology, an extension of traditional plant breeding, offers one way to boost food production and preserve the environment for future generations. Like plant breeding, biotechnology introduces new traits with specific benefits into plants; unlike plant breeding, it introduces these traits in a selective, precise, and controlled manner.

Biotechnology will ultimately lead to desirable improvements in the oil and protein contents of the soybean. Today, in the United States alone, there is a substantial market for soy ingredients and food products. Moreover, several health and consumer trends suggest that the soy market will continue to grow significantly if the taste and shelf life problems of soy products are solved. Current biotechnology research in soybeans indicates that it will be possible to develop soybeans with an increased nutrient density (eg, amino acids and energy) and improved oil content by increasing their oxidative stability and modifying their saturated fat content.

Since the early 1980s, several companies have researched the possibilities of plant biotechnology for improving the world's food supply. The mid-1990s marked the beginning of the realization of this vision. After extensive periods of discovery, research, testing, and worldwide regulatory review, crop seeds with built-in herbicide tolerance and insect protection became available to growers in 1996. It was then estimated that >4 million acres of soybeans, corn, cotton, canola, and potatoes enhanced through biotechnology were grown in North America (Monsanto, St Louis and Biotechnology Industry Organization, Washington, DC, unpublished observations, 1996). These crops were harvested and the resulting products have already made their way to processors worldwide.

The companies introducing plant biotechnology products have worked for years with US and international regulatory agencies to show that crops such as herbicide-tolerant soybeans and insect-protected corn produced through genetic modification are equivalent to crops currently on the market. Determining safety is a routine part of the US regulatory process for plant biotechnology. This regulatory process, which applies to the development of foods both through traditional methods and biotechnology, ensures that products developed through biotechnology are both environmentally sound and safe to use and consume without placing unnecessary regulations on the biotechnology industry.

This article addresses food safety aspects associated with plant biotechnology. Glyphosate-tolerant soybeans (GTSs; Roundup Ready soybeans, Monsanto), the first biotechnologically improved soybeans to be marketed, became available commercially in 1996. GTSs were given food-safety approval by reg-

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ulatory agencies around the world and will be used as the example here.

BACKGROUND

In 1996, GTSs were harvested commercially and sent to processors worldwide for incorporation into food and feed products. GTSs are genetically modified to tolerate commercial applications of the nonselective, broad-spectrum herbicide glyphosate [the active ingredient in Roundup herbicide (Monsanto)]. These soybeans promise to offer growers a new, environmentally sound method of weed control in soybeans, which is critical to maintaining harvests of high yield and quality. Trials in the Midwest showed an increased yield of $\approx 5\%$ (2 bushels/acre, or 155 kg/ha) when GTS soybeans were treated with glyphosate instead of conventional herbicides (Monsanto, unpublished observations, 1997).

Monsanto has been involved in the development of glyphosate-tolerant crops since the early 1980s (2). Researchers developed a method of tolerance known as the target-site approach in which an herbicide-insensitive target protein is identified and introduced into soybeans through genetic engineering. Glyphosate specifically binds to and blocks the activity of its enzyme target (3-phosphoshikimate 1-carboxyvinyltransferase or EPSPS; EC 2.5.1.19), an enzyme of the aromatic-amino acid biosynthetic pathway. Glyphosate inhibition of EPSPS prevents the plant from synthesizing the aromatic amino acids essential for protein synthesis.

EPSPS is the only physiologic target of glyphosate in plants. It is present in all plants, bacteria, and fungi, but not in animals. Animals do not make their own aromatic amino acids but obtain them from plant-, microbe-, or animal-derived foods.

The introduction of EPSPS through genetic modification confers the glyphosate tolerance. EPSPS is derived from *Agrobacterium* sp. strain CP4 (CP4 EPSPS), a common soil microorganism. Glyphosate treatment does not affect GTS plants because the continued action of the introduced glyphosate-tolerant CP4 EPSPS enzyme meets the plants' needs for aromatic amino acids derived from this pathway.

STUDIES IN GTSS

The safety of GTS was assessed in 2 ways: a study of the introduced protein and a study of the soybean seed and selected processing fractions. Because soybeans are a major source of protein in most farm animals' diets, animal feeding studies in wholesomeness were done to complement the analyses.

The first study was of the safety of CP4 EPSPS (3). Another purpose of this study was to determine whether the allergenicity potential of GTSs was different from that of traditional soybeans. In the second study, researchers compared the composition of seeds and selected processing fractions from 2 GTS lines with those of the parental line (4). To ensure that the wholesomeness, or feeding value, of GTSs was equivalent to that of traditional soybeans, the feeding value of 2 GTS lines was compared with that of their parental line (5).

Protein study

The amount of CP4 EPSPS needed for the safety assessment experiments was more than could be readily isolated from GTS seeds, so the CP4 EPSPS protein was produced in an *Escherichia*

coli expression system. The *E. coli*-expressed CP4 EPSPS test material was compared with plant-expressed CP4 EPSPS and characterized with respect to molecular weight, immunoreactivity, and absence of glycosylation and enzymatic activity to establish equivalence (3).

An acute oral toxicity study in mice was done with the *E. coli*-expressed CP4 EPSPS test material to establish its safety. Digestive fate studies were done in simulated mammalian gastric and intestinal fluids. Such *in vitro* studies are used widely to investigate the digestibility of plant and animal proteins for the assessment of protein quality and a variety of other animal digestion and pharmaceutical applications.

To assess the allergenicity potential of GTSs, researchers first compared the amount and kind of endogenous soybean protein allergens present in commercially available soybeans with those present in GTSs and found no difference. Next, they compared the biochemical properties of known allergenic proteins with those of the CP4 EPSPS protein. They determined CP4 EPSPS poses no concerns with respect to allergens.

Supplementary information about allergenic potential came from comparisons of the amino acid sequence of CP4 EPSPS with sequences in a database of known protein allergens. An important issue for humans, allergenicity is also a concern for young calves consuming milk containing soy isolates (in milk replacers). Usually, allergenicity is not an issue for older animals.

The study showed that CP4 EPSPS degrades readily in simulated gastric and intestinal fluids, indicating that the mammalian digestive tract will degrade this protein on ingestion as a component of food or feed. There were no detrimental effects from the acute administration of CP4 EPSPS to mice by gavage at a high dose of 572 mg/kg body wt. This dose exceeds by 1000-fold the anticipated human exposure to CP4 EPSPS from food product consumption.

Composition study

Two GTS lines were compared with their parental line (4). Nutrients measured in the soybean seeds included macronutrients by proximate analyses (protein, fat, fiber, ash, and carbohydrates), amino acids, and fatty acids. Antinutrients measured in either the seed or toasted meal were trypsin inhibitor, lectins, isoflavones, stachyose, raffinose, and phytate. Proximate analyses were also performed on batches of defatted toasted meal, defatted nontasted meal, protein isolate, and protein concentrate prepared from both GTSs and control soybean seeds. In addition, the team produced refined, bleached, and deodorized oil along with crude soybean lecithin from GTSs and control soybeans.

The GTS lines were substantially equivalent to the parental conventional soybean line. No significant differences were found in the proximate analyses or in the concentrations of any of the 18 amino acids measured, including aromatic amino acids. Also comparable were the fatty acid compositions, trypsin inhibitor content, urease activity, and lectin activity. No significant differences were detected in any of the isoflavones measured (bound, free, and total). The phytate concentrations in the samples of GTS toasted meal were similar to those in the control samples, as were the stachyose and raffinose concentrations. Protein, ash, fat, fiber, and carbohydrate in defatted nontasted meal; protein isolate; and protein concentrate made from GTSs also were comparable with amounts in fractions from the parental soybean control plant.

Wholesomeness study

Animal feeding studies are not usually used to evaluate the quality of new varieties of soybeans generated by conventional plant breeding. However, because of the large percentage of soybeans in animal feed, these studies were included in the GTS safety assessment (5). GTS meal was incorporated into the diets of rats, catfish, and broiler chickens, and unprocessed whole GTS seeds were included in the diets of lactating dairy cows at concentrations used in commercial practice. Because many dairy cattle are fed raw, unprocessed soybeans, 10 g cracked soybeans per 100 g feed was used, which is on the high end of what is normally fed commercially.

In a separate study, laboratory rats consumed 5 and 10 g of unprocessed soybean meal in a 100-g diet. Processed and ground soybeans were fed separately in 2 trials, although ground soybeans normally are not fed to nonruminants because of the presence of antinutritional factors that are inactivated by processing. The processed soybean meal fed to catfish and broiler chickens is a normal component of their commercial diets.

The studies lasted 4 wk for rats and dairy cows, 6 wk for broiler chickens, and 10 wk for catfish. Growth (in all animals), feed conversion (in rats, catfish, and broiler chickens), fillet composition (in catfish), breast muscle and fat pad weights (of broiler chickens), and milk production, milk composition, rumen fermentation, and nitrogen digestibility (in dairy cows) were compared. In all studies, measured variables were similar in animals fed the GTS lines and animals fed the parental line, indicating that the feeding values of the 2 GTS lines were comparable with that of the parental line. The animal feeding studies confirmed that no major changes occurred with GTS.

FUTURE RESEARCH

Our future research in soybeans will focus on the alteration of soybean components that are linked to health benefits. This could result in an altered fatty acid profile to produce heart-healthy margarines, low-energy oils, oils with more desirable properties (eg, better flavor and longer shelf life), an improved amino acid balance for soy-protein products (eg, flours, concentrates, and isolates), and greater amounts of potentially beneficial isoflavones. 

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