

ORIGINAL ARTICLE

Detection of Genetically Modified Protein in Soy-containing Foods

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ABSTRACT The use of genetically modified organisms (GMO) in food is a highly public and controversial issue. We have used an enzyme-linked immunosorbent assay (ELISA) to detect the genetically modified protein 5-enol-pyruvylshikimate-3-phosphate (EPSP) synthase within soy-containing food products from the grocery store. We found that EPSP synthase is detectable in 3 out of 5 food products tested. Of specific interest, we found contamination levels of EPSP synthase (0.36%) in Heinz' Pabulum Soya Cereal, which is currently deemed to be free of genetic modifications by the company as well as by Greenpeace Canada. These results demonstrate that genetically modified organisms are present in foods commonly available for human consumption and that the widespread use of this technology may make it difficult to ensure that any given product is free of all traces of genetically modified protein.

INTRODUCTION

The use of genetically modified organisms (GMOs) in food has been a lightning rod for disagreement across the globe. From violent demonstrations at trade meetings to African nations rejecting GMO food aid during famine, to the embrace of GMO food by American agricultural policy, this is an issue upon which few people are neutral (1, 2). To the opponents of genetically modified food, such modifications are at best untested and at worst dangerous to health and the environment (3). Worse, they are troublesome in spirit: " Frankenfoods " are created by transferring genes between unrelated organisms, making them viscerally unpopular to many. On the other hand, the proponents of GMOs in food argue that such genetic modifications are no worse than the many other additives we already use and that GMO technology has the potential to increase crop yields, decrease pesticide use, increase food's nutritive value, and reclaim land that is currently unusable for agriculture (4, 5). From the global perspective of overpopulation and malnutrition,

proponents of GMO food argue that a new "green revolution" is upon us and that millions of lives are at stake if we ignore such technologies (6). Confounding the question is a lack of trust: governments, corporations, and the public all lack trust in each other to ensure that food production is both efficient and safe. Hence, subsidiary arguments such as those about the mandatory labeling of GMO food have come to prominence in the public arena (7, 8).

However, the biggest reason for the polarization of the GMO food issue is ignorance, which is in part due to the paucity of facts available. Due to the relative youth of the technology, no long-term studies pertaining to the effects of GMO foods on population health or environmental sustainability have been performed. Moreover, since there are no regulations requiring the assessment of short-term risk in large populations, it is difficult to determine if GMO food is correlated to disease or if low incidence effects on health or ecology have occurred. Perhaps as a consequence of this factual void, the public's attitude on GMO food remains quite labile: a recent survey showed that 25% of Americans feel that GMO foods are unsafe, 29% feel that they are safe, and the rest are unsure either way. However, when supplied the statement that GMO foods are already

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prevalent in grocery stores, the number of respondents who believed that GMO foods are safe increased to 48% (9). Furthermore, it seems as though the public's opinion on GMO food is highly dependent upon its depiction in the media (10). Together these studies suggest that a small amount of factual information, responsibly presented, can give a context to this debate that is highly influential.

The most common GMO in food on the market today is genetically modified soy, with 75-80% of American soy crops grown from genetically modified seeds (11); Roundup Ready Soybeans engineered by the Monsanto corporation are the most common variety. These soybeans are genetically modified to withstand the effects of Roundup herbicide; weeds and other undesirable plants are eradicated while the Roundup Ready Soybeans are unaffected. Roundup herbicide contains glyphosate, which inhibits the essential plant enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (EPSP synthase), causing growth suppression and death. Roundup ready soybeans contain the bacterial variant of EPSP synthase, which is not affected by glyphosate, meaning that growth suppression caused by Roundup herbicide is rescued in the recombinant soybeans. In principle this results in greater herbicidal selectivity and increased crop yields. It should be noted that the bacterial variant of EPSP synthase is quite divergent from the plant homologue, with the two enzymes sharing only 26% amino acid homology (12).

The GMO food debate is highly coloured by cultural, social, and economic interests. We have made a scientific enquiry in order to help fill the factual void that exists in current public knowledge. To determine the prevalence of GMO foods in our shared food supply and in order to assess the controversial claims of relative health safety or danger posed by advocates and detractors of GMO foods, we tested common soy-containing grocery store foods for genetically modified soy components.

MATERIALS AND METHODS

Foods were purchased from prominent grocery and health food stores in Montreal, Canada. Foods were tested by ELISA using the GMO4 Soya Test Kit (Strategic Diagnostics Inc.) as per the manufacturer's instructions for toasted meals (13). A nearly identical kit (Strategic Diagnostics, Inc.; not available at the time of our study) has now been validated by the Institute for Health and Consumer Protection at the Joint Research Centre of the European Commission. This kit uses identical reagents except for slightly different protein standards. The quantitative protein standards used in this study were protein isolates containing 0%, 0.3%, 1.25%, or 2.5% EPSP synthase. Foods were selected on

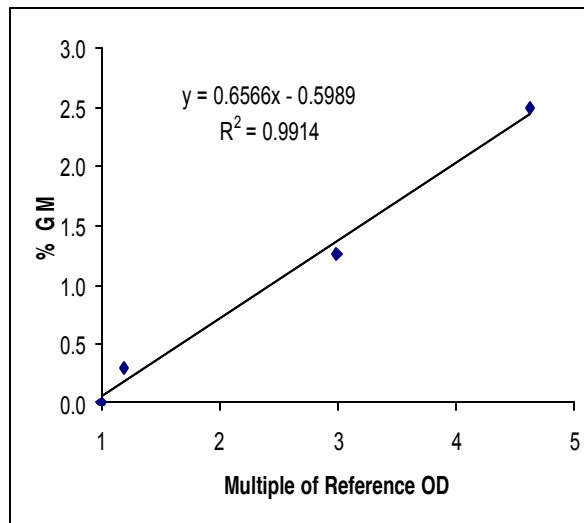


Figure 1. A standard curve of optical density (OD) for bacterial EPSP synthase was created using protein standards containing a known percentage of the protein. See materials and methods for details.

the basis of being sold in a powdered form and having soy as an ingredient on the label, whether as soy or as an oil derived from soy. Each experiment was conducted three times. Since different optical density readers were used for each trial, the absolute optical density had some variation. To weigh all three trials equally, values were normalized by expressing optical density as a proportion of the background reading for that trial. A standard curve was constructed using the normalized optical density readings of known bacterial EPSP synthase protein standards at 0%, 0.3%, 1.25 %, and 2.5%, followed by the use of linear regression (Figure 1). Abundance was quantified by comparing the normalized optical density readings of food samples to the standard curve.

RESULTS

Five foods were tested in total: three foods (Heinz Pablum Soya Cereal for infants, Red Mill whole grain soy flour, and soybean protein powder for bodybuilders) contained whole soybean components, while the other two contained soy lecithin or oils only (President's Choice Organic apple-cinnamon muffin mix and Knorr pasta sauce mix). An ELISA test kit using antibodies against bacterial EPSP synthase was used to detect the prevalence of genetically modified soy protein in these products. Heinz Pablum Soya Cereal was found to contain 0.36% bacterial EPSP synthase, Red Mill soy flour 1.29%, and bodybuilding soy protein 1.07%, while no significant level was detected in the soy oil-based products (Figure 2).

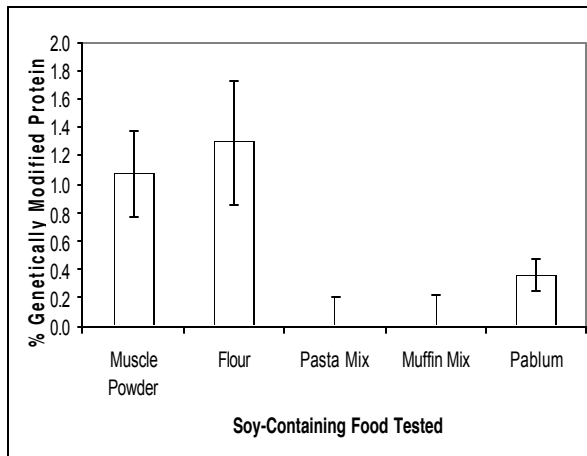


Figure 2. Soy-containing grocery store foods have detectable levels of bacterial EPSP synthase. Quantitative ELISA detected protein levels of bacterial EPSP synthase between 0 and 1.3% in five foods obtained from grocery stores. Error bars represent a 95% confidence interval. Results represent a normalized average of six assays for each food.

DISCUSSION

Using ELISA we have determined that the bacterial protein EPSP synthase is detectable in soy-containing foods consumed by the Canadian population. Three out of five soy-containing products tested contained detectable levels of the protein. Of the two products where genetically modified protein was not detected, soy was only a minor component of the food, being represented in the oil (sauce mix) or lecithin (muffin mix) only. It is possible that these products also contained genetic modifications, but were not detected by the assay due to their low abundance at the protein level. Unexpectedly, Pablum Soya Cereal contained detectable levels of genetically modified protein (0.36%) despite claims by Heinz that their infant foods are GMO free. We attribute this detection to contamination of GMO free soybeans by Roundup Ready soybeans somewhere in the production of the infant cereal. To put the level detected into perspective, the European Parliament recently voted to make the protein threshold of classifying a food as "genetically modified" at 0.5% and above (14). Although foods such as the Pablum Soya Cereal are by this definition GMO free, we have determined that they still contain detectable levels of GMO protein. Unfortunately the classification between "genetically modified" and "contaminated" is a political distinction and not a scientific one, since there is no certainty as to whether there are any health effects of long-term exposure and if these effects depend on the quantity of ingestion. Such a labeling system might be misleading to consumers who truly wish to ingest foods that are free of GMOs, but is preferable for companies that are evidently unable to prevent GMO "contamination."

At the present time there is no evidence to suggest that bacterial EPSP synthase is in any way harmful to health. High dosage acute administration of the bacterial EPSP synthase in mice does not result in noticeable toxicity, and simulated mammalian gastric contents cause rapid degradation of the protein (15, 16). Moreover, the bacterial EPSP synthase does not appear to act as an allergen any differently than the plant version. For instance, examination of IgE-binding proteins in soy does not reveal any difference between the genetically modified and wild type strains (17). It is on the basis of such results that Roundup Ready soybeans have been approved for use in many countries around the world and have found their way into our grocery stores. Furthermore, there has not been a single confirmed clinical report of toxicity or allergenicity to genetically modified soy. However, in the absence of studies monitoring large populations and with nothing known about the long term, many are wary of equating a lack of known harm with general safety. Indeed, several theoretical risks remain (18). Our present results suggest that genetically modified protein is detectable in widely available grocery store foods, but a caveat of our study is our relatively small sample size. An exhaustive study of a much wider range of food products and types of genetically modified proteins would give a better idea as to the prevalence and abundance of genetically modified protein in grocery store foods. Nevertheless, our ability to detect bacterial EPSP synthase in widely available grocery store foods suggests that, at least in the short term, the risk posed to human health is probably low.

It is problematic to generalize to all GMO foods any determinations concerning genetically modified soy. While GMO foods have realized several economic and yield gains, especially in developing countries (19), there is no a priori safety inherent to GMO foods. Each GMO food expresses a different protein, each of which will affect the environment and health in a different way. From a health and scientific point of view, legislators should not expend their efforts on determining thresholds for food labeling or setting up trade barriers to such foods, but rather should be ensuring that these novel foods are as safe as possible. While the uncertainty inherent to science challenges this goal, especially in the long term, other industries such as pharmaceuticals have devised ways to ensure the relative safety of their products. Once safety is ensured, it is then up to society to decide whether such foods are ethical, desirable, or irreconcilable. Given the potential of GMO food technology to increase food yields across the globe, it is important that science work hand-in-hand with society to make sure that the decision is an informed one. Finding GMO foods

surreptitiously spread through the grocery store aisles without societal consultation, as we have found, does not engender the use of reason in the public domain.

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REFERENCES

1. Adam D. Hostilities resume over future of GM crops. *Nature*. 419:327; 2002.
2. Bohannon J. Zambia rejects GM food on scientist's advice. *Science*. 298(5596):1153-1154; 2002.
3. Flothmann S, van Aken J. Of maize and men. Is the endorsement of GM crops science or politics? *EMBO Reports*. 2(8):644-7; 2001.
4. Huang J, Pray C, Rozelle S. Enhancing the crops to feed the poor. *Nature*. 418(6898):678-84; 2002.
5. Zimmermann MB, Hurrell RF. Improving iron, zinc, and vitamin A nutrition through plant biotechnology. *Current Opinion in Biotechnology*. 13(2):142-145; 2002.
6. Trewavas, A. and Leaver, C. Is opposition to GM crops science or politics? An investigation into the arguments that GM crops pose a particular threat to the environment. *EMBO Reports*. 2, 455-459; 2001.
7. Saegusa A. Japan may require labels on genetic food. *Nature*. 395:628; 1998.
8. Haslberger AG. Monitoring and labeling for genetically modified products. *Science*. 287: 431-432; 2000.
9. Lok C. Americans perplexed by GM food. *Nature*. 410:501; 2001.
10. Frewer LJ, Miles S, Marsh R. The media and genetically modified foods: evidence in support of social amplification of risk. *Risk Analysis*. 22(4): 701-11; 2002.
11. National Agricultural Statistics Service, United States Food and Drug Administration Prospective Plantings Report March 31, 2003 <http://usda.mannlib.cornell.edu/usda/usda.html>
12. Padgett SR, Taylor NB, Nida DL, et al. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *The Journal of Nutrition*. 126(3): 702-16; 1996.
13. Protocol is available at <http://www.sdix.com/PDF/Products/7099999%20v2.5.pdf>
14. Butler D. Europe gets tough on labeling genetically modified foodstuffs. *Nature*. 418:114; 2002.
15. Harrison LA, Bailey MR, Naylor MW et al. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *The Journal of Nutrition*. X126(3):728-40; 1996.
16. Chang HS, Kim NH, Park MJ et al. The 5-enolpyruvylshikimate-3-phosphate synthase of glyphosate-tolerant soybean expressed in *Escherichia coli* shows no severe allergenicity. *Molecules and Cells* 15(1):20-6; 2003.
17. Burks AW, Fuchs RL. Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties. *Journal of Allergy and Clinical Immunology*. 96(6 Pt 1):1008-10; 1995.
18. Alexander TW, Sharma R, Okine EK et al. Impact of feed processing and mixed ruminal culture on the fate of recombinant EPSP synthase and endogenous canola plant DNA. *FEMS Microbiology Letters* 2002 214(2):263-9; 2002.
19. Qaim, M. and Zilberman, D. Yield effects of genetically modified crops in developing countries. *Science*. 299(5608):900-2; 2003.

Tracie Agostino and **Amanda Trnkus** are secondary V high school students at Villa Maria High school in Montreal. This work was presented as a poster at the Villa Maria Science Fair on February 12 and 13, 2003 where the authors received a "Gold" distinction. **Michael D. Jain** completed his B.Sc. in Neuroscience at the University of Alberta. He is currently an MD/PhD student at McGill University. His PhD research is on protein folding in disease models and is supervised by Drs. John Bergeron and David Thomas. He is also vice-president of the Horticultural Club, a Montreal based discussion group pertaining to plants. The collaboration between the authors was organized by Let's Talk Science McGill, a science mentoring program linking McGill graduate students with elementary and high school students around Montreal in the pursuit of science education.