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Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans ☆

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ABSTRACT

This article describes the nutrient and elemental composition, including residues of herbicides and pesticides, of 31 soybean batches from Iowa, USA. The soy samples were grouped into three different categories: (i) genetically modified, glyphosate-tolerant soy (GM-soy); (ii) unmodified soy cultivated using a conventional "chemical" cultivation regime; and (iii) unmodified soy cultivated using an organic cultivation regime. Organic soybeans showed the healthiest nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soy. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soy. GM-soy contained high residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively). Conventional and organic soybean batches contained none of these agrochemicals. Using 35 different nutritional and elemental variables to characterise each soy sample, we were able to discriminate GM, conventional and organic soybeans without exception, demonstrating "substantial non-equivalence" in compositional characteristics for 'ready-to-market' soybeans.

1. Introduction

Food and food quality is crucial. Given its significance for human and animal health, we investigate whether plant products from a defined geographical region, produced under different agricultural practices are substantially equivalent or not, in terms of quality indicators like nutritional content, elemental characteristics and herbicide/pesticide residues.

By comparing herbicide tolerant ("Roundup Ready") GM soybeans directly from farmers' fields, with extended references to both conventional, i.e., non-GM soybeans cultivated under a conventional "chemical" cultivation regime (pre-plant herbicides and pesticides used), and organic, i.e., non-GM soybeans cultivated under a "no chemical" cultivation regime (no herbicides or pesticides used), a test of real-life samples 'ready-to-market' can be performed.

Globally, glyphosate-tolerant GM soy is the number one GM crop plant. The herbicide glyphosate is the most widely used herbicide globally, with a production of 620,000 tons in 2008. The world soybean production in 2011 was 251.5 million Metric tons, with the United States (33%), Brazil (29%), Argentina (19%), China (5%) and India (4%) as the main producing countries.

In 2011–2012, soybeans were planted on about 30 million hectares in the USA, with Roundup Ready GM soy contributing 93–94% of the production. Also in the other leading producing countries, this same GM soy dominates the market accounting for 83% and 100% of production, respectively in Brazil and Argentina. Globally, Roundup Ready GM soybeans contributed to 75% of the total soy production in 2011.

The first-generation glyphosate-tolerant GM-soy plant (event 40-3-2), produced and patented by Monsanto Company, has been genetically modified to tolerate exposure to glyphosate-based herbicides during the entire growth season. For herbicide-tolerant GM plants, herbicide co-technology is an integral part of the production system and will always be used by the farmer. However, in early studies of the composition of Roundup-Ready GM soy, the researchers did not spray the tested plants with the recommended herbicide (Millstone, Brunner, & Mayer, 1999). This shortcoming was quickly corrected, and also sprayed GM soybeans were claimed to be substantially equivalent to non-GM soybeans (Harrigan et al., 2007). Still, and surprisingly, even in these studies, the residues of herbicides were not measured.

The concept of 'substantial equivalence' (i.e., close nutritional and elemental similarity between a genetically modified (GM) crop and a non-GM traditional counterpart) has been used to claim that GM crops are substantially equivalent to, and therefore as safe and

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nutritious as, currently consumed plant-derived foods (Aumaitre, 2002). However, we argue that compositional studies that have overlooked (not measured) pesticide residues contain serious shortcomings. Chemical residues, if present, are important because (i) they are clearly a part of a plants composition, and (ii) they may add toxic properties to the final plant product either by itself or by affecting the plant metabolism. This is particularly relevant for herbicide-tolerant varieties.

For the predominantly used GM soy on the market, the 40-3-2 event, herbicide tolerance was achieved by insertion of a transgene construct into the plant genome which constitutively expresses the *Agrobacterium* strain CP4 analogue of the plant enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase). The endogenous plant EPSPS is critically important for the production of certain essential aromatic amino acids. Glyphosate, the active ingredient of Roundup herbicide formulations, is able to bind to all known plant, weed and crop, EPSPS versions. The binding leads to the inactivation of the enzyme and consequently death for the plant. Glyphosate binds the CP4 EPSPS expressed in GM-soy cells in a condensed, non-inhibitory conformation. Hence plants engineered to express the CP4 EPSPS enzyme are tolerant to glyphosate. Accordingly, the farmer may eradicate all kinds of plant weeds by spraying with glyphosate, and not harm the GM crop plants. However, the extensive use of glyphosate over vast land areas may lead to shifts in weed populations and selection of glyphosate-tolerant weeds (Shaner, Lindenmeyer, & Ostlie, 2012). This, in turn, typically triggers the use of higher doses or more applications of glyphosate, which can further accelerate the evolution of glyphosate resistance in weed species (Binimelis, Pengue, & Monterroso, 2009). Such a spiral is clearly not sustainable for farmers, but may also affect the consumer through plant tissue accumulation of glyphosate residues. Evolution of resistance to glyphosate is unfortunately progressing, particularly in the US. System vulnerability to resistance development is enhanced where there is a low diversity in weed management practice coupled with crop and herbicide monoculture.

USDA data document dramatic increases in the use of glyphosate-based herbicides and GM soy is a major driver for this development (Benbrook, 2012). US GM soybeans thus represent a system that is influenced by glyphosate exposure and should be an ideal system in which to test whether crop management practices that include spraying with glyphosate might lead to accumulation of chemical residues, or other compositional differences, in the final soy product. Residue analysis is of particular interest, since there are no programmes in the EU, US or Canada designed to monitor the main herbicides used in transgenic crop production.

In contrast to real-life samples from the market, transgenic crops intended for scientific studies are often produced in well-controlled small experimental plots. In most research studies, application of herbicides has been omitted or has been done at doses lower than those typically used by farmers, giving test materials that are not representative of actual conditions existing in typical agricultural operation, e.g., with regard to glyphosate residues. The knowledge regarding links between glyphosate application rates and soybean nutrient composition is scarce. One study found links between glyphosate application on glyphosate-tolerant soybean and decreased levels of α-linolenic acid (ALA) and iron, and increased levels of oleic acid (Zobiole, Bonini, de Oliveira, Kremer, & Ferrarese, 2010). A 12–14% reduction in phytoestrogen levels in GM soybean strains compared to isogenic conventional strains has been documented (Lappé, Bailey, Childress, & Setchell, 1998). However, Wei et al. showed that GM soybeans may have both a higher and lower content of isoflavones compared to conventional soy (Wei, Jone, & Fang, 2004).

Generally, the suggested key food and feed nutrients found in the OECD consensus documents, are considered in safety evaluations of new varieties of soybeans and risk assessment of GM plants has focused on allergenicity and toxicity resulting from the transgenic product itself, or from the possible unintended effects of the transformation process (Podevin & du Jardin, 2012). However, little attention is given to the residues of herbicides and their metabolites that can potentially accumulate in the final product, and also whether exposure to these herbicides, or other functional alterations related to the genetic modification itself (such as alterations in intermediary metabolism of the GM plant), may affect nutrient and elemental composition.

In the present study, 31 samples of soybeans grown within a defined area within the state of Iowa in the US, were collected. The influence of agricultural practice on (i) residues of glyphosate, AMPA and other pesticide compounds, and (ii) the nutritional and elemental composition of "ready-to-market" soybeans was analysed. We used methods of multivariate analyses, such as cluster and discriminants analyses, and attempted to track differences (if any), both between individual samples and between the three management systems through which they were produced, namely GM, conventional and organic systems.

With H_0 as substantial equivalence between the categories of soy, the following hypotheses were tested:

H_1: The residues of pesticides in soybeans will be influenced by the agricultural practice they have been produced under, specifically:

(a). GM-soybeans contain high residue levels of glyphosate and AMPA due to repeated spraying of the plants with glyphosate-based herbicides throughout the production season. Other pesticides may also be present according to use.
(b). Conventional soybeans contain low residue levels of glyphosate and AMPA due to pre-planting applications. Other pesticides may also be present according to use.
(c). Organic soybeans are expected to represent a control group with zero residues of glyphosate, AMPA and others chemical pesticides. Such pesticides are not allowed in organic farming.

H_2: The detailed nutritional composition and hence, the nutritional quality (i.e., total fat and protein, main sugars, ash, amino acids, fatty acids and micronutrients/basic elements) of soybean samples will be influenced by the agricultural practices under which they have been produced.

2. Materials and methods

2.1. Soy samples and characterisation

Three kg samples of whole soybeans were obtained from $n = 31$ individual fields/sites in Iowa, USA. Seed type (genetic variety), agricultural practice, i.e. whether samples were 'GM' ($n = 10$), 'conventional' ($n = 10$) or 'organic' ($n = 11$), and pesticide use was noted for all samples (Table 1). All individual soybean samples were analysed for their nutritional content, including total protein, total fat, dry matter, starch, ash, minerals, trace elements, vitamin B6, amino acid and fatty acid composition, in addition to the relevant pesticides.

2.2. Proximate composition of the soybeans

Dry matter was analysed by drying at 103 °C for 24 h, ash by weight after burning at 540 °C and lipid after extraction with ethyl-acetate. Nitrogen was measured with a nitrogen determinator (LECO, FP-428, Leco Corporation, St Joseph, MI, USA) according to the Association of Official Agricultural Chemists official methods of analysis and protein calculated as N X 6·25. Glycogen was mea-