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Comparative study of transgenic and non-transgenic maize (*Zea mays*) flours commercialized in Brazil, focussing on proteomic analyses

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ABSTRACT

Genetically modified foods are a major concern around the world due to the lack of information concerning their safety and health effects. This work evaluates differences, at the proteomic level, between two types of crop samples: transgenic (MON810 event with the Cry1Ab gene, which confers resistance to insects) and non-transgenic maize flour commercialized in Brazil. The 2-D DIGE technique revealed 99 differentially expressed spots, which were collected in 2-D PAGE gels and identified via mass spectrometry (nESI-QTOF MS/MS). The abundance of protein differences between the transgenic and non-transgenic samples could arise from genetic modification or as a result of an environmental influence pertaining to the commercial sample. The major functional category of proteins identified was related to disease/defense and, although differences were observed between samples, no toxins or allergenic proteins were found.

1. Introduction

Biotech crops, including genetically modified organisms (GMO), have been the most rapidly adopted crop technology in recent history due to their benefits, for example, contributing to the alleviation of poverty and hunger. However, there is little information about transgenic foods available in the literature, e.g., biosafety and food safety, impact assessment and science communication (James, 2012).

In this scenario, Brazil ranks second worldwide, after the USA, in biotech crop cultivation, with 36.6 million hectares, and is emerging as a global leader in this sector. Of the crops grown globally, maize (*Zea mays*) accounts for the second-largest cultivated area, just behind soybean (James, 2012).

In Brazil, many genetically modified (GM) maize events have been authorized since 2007. The MON810 event was the first such maize event that was allowed to be commercialized in the country by MAPA, a Brazilian public agricultural sector institution (BRAZIL, 2012). MON810 is a *Zea mays* line, known as YieldGard, from the Monsanto Company. MON810 is characterized by the inserted gene Cry1Ab, derived from the *Bacillus thuringiensis* bacterium. The product of the expression of the Cry1Ab gene is the Cry1Ab protein, producing the Bt toxin, which is poisonous to insects in the order Lepidoptera, including *Spodoptera frugiperda* and *Helicoverpa zea*, two very common species in tropical areas (Monsanto, 2002). In its free form, the Bt toxin is known as protoxin. It is not active and is therefore harmless. This toxin becomes active only when it enters the stomach and intestinal tract of certain insects. The stomach enzymes of the insects cleave a portion of the Bt toxin, which converts the protoxin into an active toxin. Once activated, the Bt toxin attaches itself to the cell membrane in the stomach and intestinal canal of the insect. There, it disturbs the composition of the cell membrane, and the cells rupture and start leaking. Once the cells rupture, the stomach and intestinal tract can no longer function properly (Schnepf et al., 1998).

GM foods are a focus of concern due to unintended effects that could be dangerous to human health (Frewer et al., 2004). The main strategy proposed to evaluate the safety of GM-derived food is the "substantial equivalence" concept, which is an internationally

Abbreviations: DNA, deoxyribonucleic acid; 2-D DIGE, Two-dimensional difference gel electrophoresis; 2-D PAGE, Two-dimensional polyacrylamide gel electrophoresis; GM, genetically modified; GMO, genetically modified organism; MAPA, Ministério da Agricultura Pecuária e Abastecimento; MW, molecular weight; NCBI, National Center for Biotechnology Information; nESI-QTOF MS/MS, nano-electrospray ionization quadrupole time-of-flight mass spectrometry; NT, non-transgenic; PCR, Polymerase Chain Reaction; pI, isoelectric point; T, transgenic.

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recognized standard that measures whether a biotech food or crop shares similar health and nutritional characteristics with its conventional counterpart. Biotech foods that are substantially equivalent have been determined to be as safe as their conventional counterparts (EFSA, 2006; FAO/WHO, 2000; OECD, 1993). If there is incomplete correspondence between the new GM food and any existing organism, the modified organism is not necessarily considered to be dangerous to human health, but further considerations about safety are required.

In view of these facts, proteomics constitutes an important tool for improving the concept of substantial equivalence. Comparative proteomics has been used as a tool to analyze differences in food (Jin et al., 2014). Comparisons based only on the centesimal composition are not sufficient to answer questions about the differences between food. The proteome is defined as the entire complement of a genome and the result of genetic expression, ribosomal synthesis, and proteolytic degradation (Wilkins, Williams, Appel, & Hochstrasser, 1997). Proteins are of special concern in safety assessments because they could act as toxins (e.g., phytohemagglutinin), antinutrients (e.g., protease inhibitors) or allergens or may be involved in their synthesis. Moreover, in contrast to the genome, which is constant for an organism, the proteome is highly dynamic and depends on the cell cycle, environmental influences, and tissue/cell types (Pastorello et al., 2000). Previous studies (Barbosa, Arruda, Azevedo, & Arruda, 2012; Lehesranta et al., 2005) have found differences between GM and non-GM lines in potato and soybean seeds at the proteomic level, considering only genetic modifications. Wang et al. (2012) reported that the differences resulting from genetic modification in rice can also be influenced by environmental variation, despite being planted at the same location. This occurs because a transgenic plant (more resistant) can experience slightly different growth conditions in an ideal environment compared with its non-transgenic counterpart (more susceptible). The effects of environmental growth conditions on the rice proteome are not significantly different from the effects caused by a single gene insertion (Barros et al., 2010).

In this work, a comparative proteomic analysis between transgenic (T) and non-transgenic (NT) maize flour was performed because these flours are consumed at high rates around the world. Many studies have evaluated the effects of transgenes on the primary food product, but few have evaluated the final product, or the so-called "table product". Food safety must be considered from "farm-to-table". The table product is the product that will be actually consumed by humans and animals, and the importance of its nutritional content is clear. Any GM versus non-GM differences in the final product are important to consumers as, in principle, all of these food should be nutritionally equivalent. Moreover, it is important to evaluate these foods regarding safety, health and nutritional concerns. From a proteomic perspective, the only expected difference between T and NT maize flour should be the presence of the Cry1Ab protein, which is encoded due to a gene insertion. Thus, the aim of this work was to identify any differences between T and NT maize flour through a proteomic approach, due to the commercial importance of these products, considering the genetic modification involved (MON810) and environmental conditions in Brazil.

2. Materials and methods

2.1. Samples

Maize flour samples were obtained from a commercial market in Rio de Janeiro, in the southeastern region of Brazil, by regulatory health authorities as part of a monitoring programme required by legislation specifying the labeling of products containing GMOs. In an appropriate laboratory, free of contamination, the samples were carefully transferred from their original packing to ziplock bags, identified and stored in the refrigerator at an average temperature of 4 °C. One T and one NT sample were selected based on protein mass concentration criteria. In total, fourteen samples were collected, but, in many of these samples, a low protein concentration was found, which may affect gel resolution. For this reason, the sample that showed the highest mass concentration in each category (T or NT) was chosen for running the final gels.

2.2. Detection of the MON810 transgenic event

The amplificability of the extracted DNA was verified, using the primers ZE01/ZE02, specific to intrinsic maize gene zein (Ze 1), to confirm the presence and quality of DNA extracted from maize-containing samples (Matsuoka et al., 2000). The samples showing a positive signal for the zein gene were analyzed to detect the E35S promoter/hsp70 exon–intron cassette of MON 810 maize via nested PCR, using the primer pairs, mg1/mg2 and mg3/mg4 (Zimmermann, Liniger, Luthy, & Pauli, 1998). Visualization of an amplicon of 149 bp in an agarose gel indicated the presence of MON810 maize in the sample.

2.3. Extraction of protein species from maize flour and separation via 2-D PAGE

Protein extraction was carried out, based on the method proposed by Sussulini et al. (2007). Transgenic or NT maize flour (approximately 4 g) was frozen in liquid nitrogen and ground for 30 min. After this step, the sample was mixed with petroleum ether and gently agitated (*ca.* 10 min) to remove oils. Protein extraction was performed, using a buffer containing 50 mM tris(hydroxymethyl)aminomethane (Tris)–HCl, pH 8.8, 1.5 mM KCl, 10 mM dithiothreitol, 1.0 mM phenylmethanesulfonyl fluoride, and 0.1% (w/v) sodium dodecyl sulfate (SDS), in a 10:1 (v/w) ratio. After the mixture had been centrifuged at 5000*g* for 10 min at 4 °C, the supernatant was collected and stored at −20 °C. To precipitate proteins for sample cleaning, a solution of 0.1 M ammonium acetate plus methanol (1:5 v/v) was added to the protein extract and left in contact for 1 h at −20 °C. The proteins were then collected after centrifugation at 4 °C at 5000*g* for 10 min, followed by washing twice with the ammonium acetate–methanol solution, twice with cold 80% (v/v) acetone and, finally, once with cold 70% (v/v) ethanol. The obtained proteins were quantified, using the 2-D Quant Kit (GE Healthcare, Uppsala, Sweden). A total of 800 μg of precipitated protein, which had previously been optimized, was resolubilized in a rehydration buffer solution containing 7 M urea, 2 M thiourea, 2% (m/v) 3-[(3-cholamidopropyl)dimethyl-ammonium]-1-propanesulfonate (CHAPS), 0.002% (m/v) bromophenol blue and 0.5% (v/v) carrier ampholytes and loaded onto immobilized pH gradient strips in the 3–10 pH range. The strips were rehydrated at room temperature for at least 12 h. Protein separation via 2-D PAGE was performed according to the manufacturer's (GE Healthcare, Uppsala, Sweden) recommendations (Berkelman & Stenstedt, 1998). For 2-D SDS–PAGE, the final gel concentration was 13% (m/v) polyacrylamide, and the buffer system consisted of a solution of 25 mM Tris–HCl, pH 8.3, 192 mM glycine and 0.1% (m/v) SDS. The gels were stained with colloidal Coomassie brilliant blue (Candiano et al., 2004), scanned and then analyzed, using ImageMaster 2-D Platinum 6.0 (GeneBio, Geneva, Switzerland) software.

For comparative proteomics analyses, 2D electrophoresis is a platform that allows the analysis of protein maps with a high protein resolution. However, the intrinsic characteristics of this technique (regarding electrophoretic systems, sample preparation strategies, and the possible identification of several proteins from one spot) and natural variations may influence the resultant comparisons (Brandao, Barbosa, & Arruda, 2010). Therefore, to ensure

repeatability and reproducibility (and, thus, the homogeneity of the flour samples), six 2D-PAGE gels were run for each sample before the DIGE procedure. Using an image analysis programme (Master 2D Patinum 6.0), three landmarks were chosen, and a high match percentage was achieved (>70%), indicating that aligned and undistorted gels were obtained.

2.4. 2-D DIGE analysis

For each group of T and NT maize flour protein species, 2-D DIGE gels were prepared in triplicate. Protein samples were labeled according to the manufacturer's protocol for minimal labeling (GE Healthcare). The protein pellet obtained after protein extraction was resolubilized in a lysis buffer (7 M urea, 2 M thiourea, 4% (m/v) CHAPS, and 20 mM Tris, pH 8.8, without any reducing agent) prior to dye labeling. For labeling with CyDye DIGE Fluor minimal dyes, 75 μg (pH range 3–10) amounts of each sample were mixed with 400 pM CyDye DIGE Fluor minimal dye (GE Healthcare) and incubated on ice in the dark for 30 min. For each replicate reaction, one sample was labeled with Cy3, the other sample with Cy5, and the internal standard with Cy2. Two T flour samples and two NT flour samples were labeled with Cy5 and Cy3, respectively. For the third replicate, the CyDyes were swapped to correct for any bias introduced by the different fluorescence characteristics of acrylamide at the different excitation wavelengths of Cy3 and Cy5. The internal standard, labeled with Cy2, consisted of a pooled sample comprised of equal amounts of each T and NT maize flour sample used for each triplicate. The Cy2 dye itself is considered to be a normalizer (Lin et al., 2012), increasing the statistical confidence in quantification among different gels. The reactions were quenched by adding 1 μl of 10 mM lysine, followed by incubation for an additional 10 min. Each labeled protein sample was diluted with rehydration solution. IEF was performed with Immobiline Dry Strips (pH 3–10, 13 cm, GE Healthcare) at 14,600 Vh. The final gels were scanned with an Ettan DIGE Imager Scanner (GE Healthcare) and analyzed for proteome differences. A DeCyder Differential Image Analyzer (GE Healthcare) was used for image analysis, spot detection, matching between the gels, and normalization, based on the pooled internal standard before quantification. A regulation factor of 2.0 (100% variation) was chosen, as determined with the DeCyder 2-D version 7.0 (GE Healthcare) image analysis programme. Differential intensity was considered to be statistically significant, based on Student's *t*-test at $p < 0.05$. After image analysis, 2-D gel electrophoresis was performed as previously described: the gel obtained for a maize flour sample was stained with colloidal Coomassie, and the spots of interest were excised manually and subjected to the identification of protein species via mass spectrometry.

The 2-D DIGE technique was employed to visualize the protein profiles of the T and NT maize flours, expressed as the result of three biological replicates. Details about this procedure can be found in the literature (Arruda, Barbosa, Azevedo, & Arruda, 2011). This technique allows two samples to be run in the same gel, avoiding the electrophoretic effects that are frequently observed for 2-D PAGE.

2.5. Trypsin digestion of peptides

In-gel digestion of protein spots (from 2-D PAGE), showing changes in intensity, was performed. For this task, the spots (approximately 2 mm) were manually excised from the gel and placed on a micro-SPE plate containing peptide affinity resin, using the Montage® In-Gel digestZP kit (Millipore, Bedford, USA). The digestion and vacuum-elution protocols were performed according to the manufacturer's recommendations. Briefly, a dye removal step, using acetonitrile was first employed, followed by trypsin digestion (using *ca.* 166 ng of enzyme for each spot). Then, a clean-up step was carried out, using 130 μl of 0.2% (v/v) TFA solution. Finally, purified peptides were eluted from the resin using 20 μl of 0.1% (v/v) TFA in 50% (v/v) acetonitrile solution. For vacuum-elution, a Multiscreen® Vacuum Manifold (Millipore) was used (Maciel et al., 2014).

2.6. Identification of protein species

For nESI-QTOF MS/MS analysis, the peptides obtained through enzymatic digestion were dried and resolubilized in deionized water. An aliquot (4.5 μl) of the resulting peptide mixture was separated, using a C18 column (Waters, Manchester, UK, BEH C18, 100 mm × 100 μm), RP-nanoUPLC (nanoAcquity, Waters), coupled to a Synapt HDMS mass spectrometer (Waters) with a nano-electrospray source, at a flow rate of 1.0 μl min^{-1}. The applied gradient was 2–90% acetonitrile in 0.1% (v/v) formic acid over 40 min. The instrument was operated in data-dependent analysis (DDA) mode, in which the equipment acquires one spectrum per second and, when multi-charged species were detected, the three most intense species were fragmented in the collision cell (collision energy set according to precursor *m*/*z* and charge). Spectra were acquired using MassLynx v.4.1 software.

All mass spectra were processed into peak list format, using Mascot Distiller (Matrix Science, London, UK) and subjected to the NCBI database (checked in October 2013). The Mascot Server 2.3 MS/MS search parameters were used for searching for protein identities, which included oxidation of methionine as a variable modification, carbamidomethylation of cysteine as a fixed modification, a ± 0.1-Da peptide and fragment mass tolerance, and a maximum of one missed cleavage. The significance threshold was set at $p < 0.05$, which corresponds to a minimum score of 55.

3. Results and discussion

3.1. Comparative proteomics of transgenic and non-transgenic maize flours

In this study, the 2-D DIGE technique revealed 99 differentially abundant protein spots (47 with higher and 52 with lower abundance) between the T and NT maize flour samples at a 2.0 regulation factor of 100% variation, as shown in Fig. 1. Of these 99 spots, 64 could be observed and collected from the 2-D PAGE preparative gel to identify the proteins through mass spectrometry. This difference could have occurred because 2-D DIGE is a more sensitive technique than is 2-D PAGE, and more spots can be detected/visualized through 2-D DIGE than 2-D PAGE. Statistical information, from *t*-tests and the volume average ratio of the identified proteins spots, is provided in Table 1.

3.2. Protein identification and functional evaluation

Sixty-four protein spots excised from the 2-D PAGE gels were subjected to mass spectrometry to identify the proteins that they contained. The use of the Mascot programme to search protein identification databases generated multiple possible IDs for some of the spots. The criterion employed to select the most likely related protein was based on higher scores and coverage. From the 64 differentially abundant spots identified, 34 exhibited both the highest scores and coverage, but only 30 could be matched with the theoretical and experimental pI/MW (Table 1). Thus, 53% of the spots present in the gels were identified in the database, and 46% were successfully identified as maize (*Zea mays*) proteins or as homologues of proteins from another cereal, such as sorghum (*Sorghum bicolor*), and could be considered in the analysis, indicating that

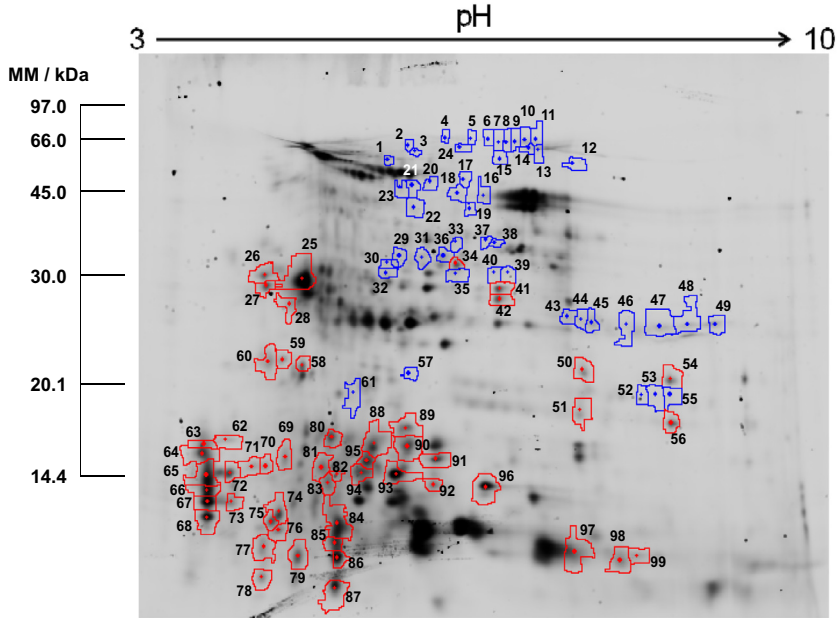

Fig. 1. 2-D DIGE gels of protein species from T maize flour, in blue, and NT maize flour, in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

good efficiency was achieved in this identification according to previous studies (Xu, Garrett, Sullivan, Caperna, & Natarajan, 2006).

The observed MW of the low molecular weight heat shock protein precursor (spot 9) was almost three times higher than its theoretical MW, which may be explained by the formation of trimers consisting of three identical subunits (Hauser & Paulsson, 1994). Eukaryotic translation initiation factor 5A (spot 63) exhibited the same theoretical and observed MW, but a different pI, thus indicating a possible isoform of this protein. Heat shock protein 70 (spot 80) showed an observed MW that was almost four times lower than its theoretical MW and a good score (113) and coverage, likely indicating that a fragment of this protein was identified.

The identified proteins were classified into categories according to their biological activities (Bevan et al., 1998; UniProt, 2013), as seen in Table 1 and Fig. 2. In this study, 40% (12 spots) of the identified proteins were related to disease/defense functions, 7% (two spots) to energy, 3% (one spot) to intracellular traffic, 3% (one spot) to signal transduction, 10% (three spots) to protein synthesis, 7% (two spots) to metabolism, 7% (two spots) to cell structure, 3% (one spot) to cell growth/division and 3% (two spots) to protein destination and storage; 13% (four spots) presented an unclear classification.

Single gene insertions may affect a limited number of proteins (e.g., 47 spots in a previous study; Arruda, Barbosa, Azevedo, & Arruda, 2013). In contrast, a large number of potential protein changes may be expected as a result of significant environmental changes. In this work, a substantial number of spots (99) showed differential abundance (2.0 regulation factor) between the T and NT maize flour, which is two times greater than when considering only genetic modifications. Our findings are in agreement with a study using rice seeds (Wang et al., 2012), in which a significant number of proteins was shown to be differentially regulated by both environmental and genetic modification. The levels of approximately 21 proteins were observed to be differentially modulated as a consequence of environmental influence, whereas 22 differentially expressed proteins were found in T seeds in comparison with the corresponding NT lines. Thus, half of the identified differentially regulated proteins are due to genetic modifications and half to environmental conditions. This finding shows that the final products consumed are not nutritionally equivalent, as believed by consumers. This difference is clear and can be attributed to genetic modification and/or environmental influence, which is also evident when taking into account changes at the proteomic level.

The major functional category of proteins identified (40%) was related to disease/defense, as observed in a previous study conducted to identify proteins regulated by ABA (abscisic acid) in response to combined drought and heat stress in maize roots; the authors characterized 26.3% of the proteins as fitting into this category, which were highly abundant (Liu et al., 2013). The identified proteins could not be linked to genetic modification because seven spots were from transgenic maize flour and five from its counterpart. Some of these proteins could result from the mechanism of defense induced by the mechanical stress experienced during the collection (Ben Thabet et al., 2010) and processing procedures applied to obtain the final product (flour).

Some proteins (13%) could not be classified, due to a lack of information. In the UniProt Knowledgebase (UniProt, 2013), there are five types of evidence for the existence of a protein: evidence at the protein level, evidence at the transcript level, inferred from homology, predicted and uncertain. These proteins with unclear classifications are of the predicted and uncertain types, which means that they lack evidence at the protein, transcript, or homology levels, or that the existence of the protein is unsure, respectively.

This study showed that there are differences between T and NT maize flours commercialized in Brazil at the proteomic level. These differences can be attributed to genetic modification and/or the environmental influences. Previous observations reported in the literature indicate that unintended effects of transgenes have very little impact, particularly when compared with the large differences observed between lines produced through conventional breeding approaches (Baudo et al., 2006; Catchpole et al., 2005; Lehesranta et al., 2005) or in response to varying environmental factors (Barros et al., 2010; Coll et al., 2010; Zolla, Rinalducci, Antonioli, & Righetti, 2008). These findings also support the idea that the differences between two conventionally bred varieties are larger than those between a GM variety and its non-GM counterpart.

In this work, the main differences observed were in the proteins classified into the disease/defense functional category, followed by

Table 1
Characterization of the identified protein species, showing changes in abundance, using a regulation factor of 2.0 (100% variation).

Spot	Protein name	Protein accession number	Theoretical pI/MW (Da)	Observed pI/MW (Da)	Mascot score[a]	Coverage (%)	Matched peptides[b]	*t*-test	Volume average ratio[c]
Functional category: disease/defense									
1	Hypothetical protein SORBIDRAFT_04g033510 [*Sorghum bicolor*]	gi|242066550	6.07/65,470	5.62/53,683	63	5	3	0.0007	2.63
6	LOC100285569 [*Zea mays*] - "Heat shock protein STI"	gi|226531388	6.26/65,791	6.52/63,220	315.9	22	11	1.3×10^{-3}	2.97
7	Hypothetical protein SORBIDRAFT_04g033510 [*Sorghum bicolor*]	gi|242066550	6.07/65,470	6.59/63,629	55	5	3	8.6×10^{-4}	2,93
9	Low molecular weight heat shock protein precursor [*Zea mays*]	gi|162458147	6.47/23801	6.90/63,629 trimer	130	23	6	0.0007	2.70
29	Hypothetical protein SORBIDRAFT_04g027330 [*Sorghum bicolor*]	gi|242065900	6.79/24,208	5.79/33,217	82	6	1	8.9×10^{-4}	2.23
35	Hypothetical protein SORBIDRAFT_04g027330 [*Sorghum bicolor*]	gi|242065900	6.79/24,208	6.34/29,131	80	6	1	8.9×10^{-4}	-2.23
69	Pathogenesis-related protein 2 [*Zea mays*]	gi|105990543	4.70/17,101	4.2/15,571	77	5	1	0.0012	4.03
70	Hypothetical protein SORBIDRAFT_04g027330 [*Sorghum bicolor*]	gi|242065900	6.79/24,208	4.03/16,155	300	6	1	8.9×10^{-4}	2.23
80	TPA: heat shock protein 70 [*Zea mays*]	gi|414868557	5.13/71,447	4.83/17,210	113	17	9	0.0016	-3.23
89	Superoxide dismutase [Cu-Zn] 4A [*Zea mays*]	gi|162462586	5.46/15,079	5.81/17,329	66	8	1	0.0013	-2.70
90	Putative glutathione peroxidase [*Zea mays*]	gi|226501294	6.59/18,619	5.76/16,531	102	20	3	0.0015	-3.30
94	Lactoylglutathione lyase [*Zea mays*]	gi|226500150	5.49/15,084	5.31/15,411	60	10	1	8.9×10^{-4}	-4.20
Functional category: energy									
37	Glyceraldehyde-3-phosphate dehydrogenase [*Zea mays*]	gi|293889	6.25/26,486	6.66/33,022	92	10	2	8.9×10^{-4}	-3.00
38	Malate dehydrogenase [*Zea mays*]	gi|195628708	7.63/35,669	6.87/33,217	75	10	3	7.7×10^{-4}	3.20
Functional category: metabolism									
39	Hypothetical protein precursor [*Zea mays*]	gi|226491894	7.11/34,443	6.96/27,909	197	47	12	1.7×10^{-3}	2.50
40	Hypothetical protein precursor [*Zea mays*]	gi|226491894	7.11/34,443	6.80/27,738	477	54	14	1.2×10^{-3}	2.14
Functional category: intracellular traffic									
21	Hypothetical protein ZEAMMB73_176599 [*Zea mays*]	gi|413945376	5.48/50,339	5.75/45,291	55	22	9	1.2×10^{-3}	2.26
Functional category: signal transduction									
25	14–3-3-like protein GF14–6 [*Zea mays*]	gi|195635799	4.84/30,985	4.46/29,131	393	50	12	7.0×10^{-4}	2.90
Functional category: protein synthesis									
28	Elongation factor 1-beta [*Zea mays*]	gi|195618244	4.55/23,440	4.29/27,134	77	24	4	1.2×10^{-3}	3.00
36	Ribosome-inactivating protein [*Zea mays*]	gi|58803213	6.02/33,401	6.17/31,753	105	18	6	9.3×10^{-4}	-2.97
63	Eukaryotic translation initiation factor 5A [*Zea mays*]	gi|162458009	5.61/17,714	3.51/17,630	86	26	3	9.3×10^{-4}	3.14
Functional category: cell structure									
74	Profilin-5 [*Zea mays*]	gi|162461296	4.59/14,219	4.14/13,195	99	24	2	7.0×10^{-4}	-3.75
76	Profilin-5 [*Zea mays*]	gi|162461296	4.59/14,219	4.05/12,718	55	24	2	1.2×10^{-3}	3.33
Functional category: protein destination and storage									
93	Pectinesterase inhibitor domain containing protein precursor [*Zea mays*]	gi|259490412	5.78/18,783	5.63/16,940	198	49	7	1.5×10^{-3}	-4.19
Functional category: cell growth/division									
26	TPA: rab28 protein [*Zea mays*]	gi|414864900	4.54/28,391	3.99/30,653	383	55	9	9.5×10^{-4}	2.22
34	Embryonic protein DC-8 precursor [*Zea mays*]	gi|226497424	6.16/33,552	6.35/30,653	74	20	5	1.7×10^{-3}	-2.60
Functional category: unclear classification									
22	Unknown [*Zea mays*]	gi|219885633	5.10/71,517	5.83/41,527	121	15	8	9.5×10^{-4}	2.29
91	Unknown [*Zea mays*]	gi|194696816	6.30/16,531	6.12/15,824	76	10	1	7.0×10^{-4}	4.49
95	Uncharacterized protein LOC100279055 [*Zea mays*]	gi|226530955	5.42/18,806	5.39/15,989	209	34	4	1.7×10^{-3}	-4.49
96	Unknown [*Zea mays*]	gi|194696816	6.30/16,531	6.70/14,500	97	32	4	7.0×10^{-4}	-4.61

[a] Score is a measure of the statistical significance of a match.
[b] Percentage of predicted protein sequence covered by matched peptides.
[c] A positive or negative average ratio represents a relative increase or decrease, respectively, in the abundance of protein species from T compared to NT maize flour.

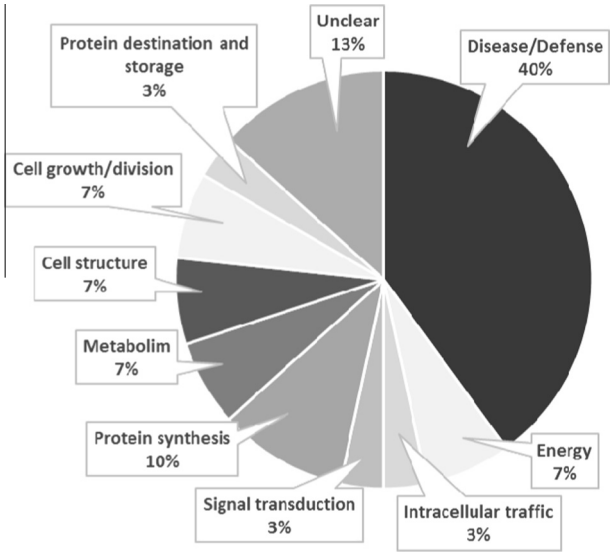

Fig. 2. Graphic representing the functional distribution of protein species (in %) identified in the differentially abundant proteins between T and NT maize flour.

unclear classification, which may suggest that the consumed products are not equivalent; however, the identified differences are not dangerous to health, and the food can be considered safe. No toxins or allergenic proteins were found, such as lipid transfer protein (LTP) (Nakajima, Teshima, Takagi, Okunuki, & Sawada, 2007; Pastorello, Farioli, et al., 2000; Pastorello, Pompei, et al., 2003), thierodoxin (Weichel et al., 2006) or 50 kDa protein (Pasini et al., 2002). Moreover, in agreement with previous transcriptomics and metabolomics results, the differences were all variety-specific and could therefore not be directly attributed to the MON810 transgenic character (Ioset et al., 2007).

The potential unintended effects identified herein could very well fall within the natural variability that exists among maize lines (which was beyond the scope of this study), such as differences between landraces, or more between diverse locations and climates. For this reason, this study was focussed on the final product. The investigation of quality performance across different products is an important requirement of both industrial use and consumers (Pompa et al., 2013).

The presented data would serve as an exploratory study into the use of proteomic techniques for the determination of safety or even for a simple comparison between different maize flours commercialized in Brazil. The use of proteomic technology for this purpose should be considered on a case-by-case basis, due to the complexity and duration of the assays involved and the importance in relation to GM food.

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