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Profile and relative concentrations of fatty acids in corn and soybean seeds from transgenic and isogenic crops

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ABSTRACT

In this work 44 fatty acids, which were analyzed as methyl esters by GC/MS in scan mode, have been determined in genetically modified corn and soybean seeds. Their relative concentrations have been compared with those of isogenic lines grown in the same conditions. Studied compounds comprised saturated and unsaturated fatty acids, including cis/trans isomers and minor fatty acids. A classical soxhlet extraction and an accelerated solvent extraction have been assayed to extract the fatty compounds from seeds and the GC separation has been carried out on a biscyanopropylpolysiloxane chromatographic column. Soxhlet extraction was selected as the most convenient and applied to compare the samples. Specific compounds, which could denote the origin of the crop have not been observed, but for some sample pairs, significant differences have been found in relation to the percentage of certain acids; the highest differences for major acids were 4.1% in corn and 4.8% in soybean. The concentrations of long chain acids such as 24:0, 26:0 and 28:0 were higher in some isogenic lines whereas the concentrations of short chain acids such as 6:0, 8:0, 9:0, 10:0 and 12:0 were higher in their transgenic counterparts.

1. Introduction

The use of profiling methods in food safety assessment has been suggested as an additional approach that might be in a future a complement to the targeted approach for the detection of compositional differences between genetically modified crops and the parent crops. Compositional analysis serves to assess whether the modified crop has a similar composition compared with the parent in order to know if the genetic modification has changed the level of, among others, key nutrients which are usually present in the traditional cultivar [1]. The development of reference data is therefore of fundamental importance for the usefulness of profiling techniques in order to provide a basis for compositional equivalence and subsequent safety assessment [2]. Among the key components fatty acid pattern has always been considered due to its implication in nutritional and safety assessment [3], for this reason there are several works devoted to the analysis of fatty acids in different transgenic crops, and to compare the content of fatty acids in these cultivars with the content in their isogenic lines, which were cultivated, when it was possible, in similar field conditions. In those works the fatty acid pattern for corn (*Zea mays* L.) seeds is almost limited to the most abundant compounds: 18:1 and 18:2, with values slightly lower in the transgenic seeds, nevertheless other works give information about the presence of fatty acids such as 16:1, 18:0 and 18:3, including in some cases others like 14:0, 20:0 and 20:1 [4–13]. For soybean (*Glycine max* L.) seeds where the fatty acid content is higher, the information is very similar in relation to the acids evaluated, although in this matrix a transgenic line seems to have a little major content in those acids [3].

As data are necessary to know the composition as complete as possible we have focused the work on the basis of achieving great information about fatty acid composition by using new chromatographic columns which are able to enhance the separation of fatty acid isomers. It should be noted that in this work is paid a special attention to some aspects not considered in the previous scientific literature until now on those matrices: the composition of minor acids and the separation, at least tentative, of isomers of fatty acids, mainly cis and trans.

To carry out this study, we have selected several pairs of isogenic–transgenic corn seeds and one pair of soybean seeds; all of them were cultivated in the same field to avoid influence of genetic background and environment onto seed composition [14]. For the extraction of the fatty acids we have compared the classical soxhlet extraction with a pressurized liquid extraction to compare the selectivity of both extractions. The separation of the fatty acid methyl esters is carried out on a highly polar biscyanopropylpolysiloxane chromatographic column [15,16]. Finally the obtained results are compared in terms of detected major and minor fatty acids.

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Table 1
Studied samples.

Crop	Variety	Isogenic	Transgenic
Corn	MON 810	Aristis	Aristis Bt
Corn	MON 810	11 Tietar	5DKC
Corn	MON 810	28Pr33P66	28Pr33P67
Corn	NK 603	ISO VER	287ASDIS
Soybean	Roundup ready	Near-iso	837ASDIS

2. Experimental

2.1. Materials and reagents

Residue analysis grade hexane, methanol and diethyl ether were supplied by Labscan (Dublin, Ireland). Ultrapure water was obtained from a Milli-Ro 6 plus apparatus (Millipore, Milford, MA, USA). Potassium hydroxide 1 M in methanol and boron trifluoride 14% in methanol were purchased from Panreac (Barcelona, Spain) and Sigma–Aldrich (Steinheim, Germany), respectively. Fatty acids methyl ester standards (references 47885-u, 47791 and 47792) were obtained from Supelco (Bellefonte, PA, USA). A rotary evaporator was supplied by Buchi (Flawil, Switzerland), a Micro-Mill grinder by Fisher Scientific (Pittsburg, PA, USA) and an ultrasonic water bath by Selecta (Barcelona, Spain).

2.2. Samples

Eight samples of corn and two samples of soybean have been analyzed. All samples were kindly donated by Dr. A. Cifuentes from Institute of Industrial Fermentations (CSIC, Spain). Each pair (transgenic plus non-transgenic) was grown under the same cultivation conditions. Trade names are shown in Table 1.

Genetically modified events Aristis Bt, 5DKC and 28PR33P67 are derived from Monsanto's MON 810 corn. They contain a *Bacillus thuringiensis* gene (Cry1ab) to provide resistance against insects, especially the lepidopteran *Ostrinia nubilalis* and *Sesamia nonagrioides*, commonly called corn borer.

The 287ASDIS corn and 837ASDIS soybean events are glyphosate-tolerant transgenic crops. The resistance to the herbicide arises from the insertion of gene cassettes that express some synthetases from *Agrobacterium* species.

Whole seeds were milled to fine powder using a grinder, 99% passed through a 1 mm screen mesh. The samples were stored in sealed plastic bags at 4 °C until analysis.

2.3. Soxhlet extraction

The fatty compounds were extracted from a powdered sample amount of 3.00 ± 0.01 g in a 250 mL soxhlet system using diethyl ether as extractant for 3 h. Then, the liquid phase was taken and evaporated in a rotary evaporator at 25 °C under a gentle vacuum. The residue was dissolved in 5 mL of *n*-hexane with the help of an ultrasonic bath to prepare the fatty acid methyl esters.

2.4. Pressurized liquid extraction

Extractions were carried out in a Dionex accelerated solvent extractor system ASE200 equipped with a solvent controller unit from Dionex (Sunnyvale, CA). Two sequential extractions were performed with methanol or *n*-hexane as solvents at the following temperatures: 50, 75, 100, 125 and 175 °C. Previous to each experiment an extraction cell heat-up was applied for a given time, which changed according to extraction temperature (the heat-up time is automatically fixed by the equipment). Namely, 5 min heat-up was used when extraction temperature was set at 50, 75 and 100 °C, 7 min at 125 °C and 9 min at 175 °C. Likewise, all the extractions were performed in 11 mL extraction cells containing 750 mg of milled sample. Cell was filled with solvent up to a pressure of 1500 psi to carry out the extraction for 15 min. After that, cell was rinsed with 60% cell volume using extraction solvent and solvent was purged from cell with nitrogen gas. Between extractions, a rinse of the complete system was made in order to overcome any extract carry-over.

Finally, the collected extract was concentrated with the help of a rotary evaporator and 5 mL were taken with *n*-hexane.

2.5. Hydrolysis and methylation

A volume of 2 mL of *n*-hexane extract was mixed with 2 mL of 1 M KOH in methanol. Then, the hydrolysis was carried out in closed 15-mL vials at 90 °C for 20 min. After that, 3 mL of BF_3 in methanol at 14% were added to the mixture, which was heated again at 90 °C for 20 min. Finally, 6 mL of water were added and the vial was manually shaken for 15 s. Afterwards, the aqueous phase was removed and the organic layer was further washed with 6 mL of water; the methylation conditions were adapted from a previous work [17]. The extract in *n*-hexane was ready for injection in GC except for soybean samples, for which a 1:10 dilution with *n*-hexane was made.

2.6. GC/MS analysis

An Agilent Technologies 7890A gas chromatograph (Wilmington, DE, USA) was directly coupled to an Agilent Technologies 5975C mass spectrometer. The chromatograph was fitted with a 100 m × 0.25 mm × 0.20 μm (biscyanopropylsiloxane) SP2560 column from Supelco (Bellefonte, PA, USA). The oven temperature was kept at 50 °C for 1 min and programmed at 6 °C/min to 205 °C, held for 17 min, and then programmed at 6 °C/min to 250 °C, held there for 20 min. The carrier gas (helium) flow was kept constant at 1 mL/min (equivalent to a pressure of 180 kPa at 50 °C). Pulsed splitless injection (1 μL) was performed with an Agilent HP7683B automatic sampler at an injection port temperature of 230 °C; the pressure pulse was 123 kPa for 0.75 min and the purge valve was on at 0.75 min; the transfer line temperature was 250 °C. The MS temperatures were as follows: ion source: 230 °C, quadrupole: 150 °C. Electron multiplier voltage was maintained at 200 V above autotune value. Electron impact spectra were recorded in the scan range 40–500 u.

The biscyanopropylsiloxane capillary columns have been devised and recommended for the separation of fatty acid isomers. In this way, we fitted a 100 m column to enhance the separation of fatty acids contained in the isogenic and transgenic seeds, which had not been considered in the scientific literature until now in our knowledge. The oven temperature program was studied to achieve the better separation; in particular, it was paid attention to the separation of the cis/trans isomers of the 18:2 and 18:3 acids, the 205 °C isotherm temperature for 17 min allowed a good separation of the four 18:2 isomers and a partial separation of the eight 18:3 cis/trans isomers.

The identification of the methyl esters of fatty acids in extracts was performed by comparison of retention times and spectra with those of standards and by using commercially available spectral libraries. Table 2 shows the fatty acids identified as methyl esters in samples and the repetitivity of the analyses estimated on Aristis Bt corn. The comparison of the most abundant acids was carried out by studying the peak areas recorded in the total ion chromatogram, for minor compounds the comparison was done by using the ion chromatograms of selective *m*/*z* ratios (see Table 2).

Quantification was based on the normalization method: peak area of each fatty acid was compared to the total area of all fatty acid peaks. Analyses were carried out by quintuplicate.

3. Results and discussion

3.1. Fatty acid extraction

Two different extraction procedures were initially assayed to study the fatty acid composition of the samples in order to compare the extraction selectivity and increase the information obtained from the samples: a pressurized liquid extraction with methanol or *n*-hexane and a soxhlet extraction with diethyl ether.

In general terms, similar chromatograms were obtained with both procedures, nevertheless, with the pressurized system the extracted fatty acid content was usually lower, maybe, due to the small cell volume (and sample amount). It should be also remarked that peak areas were about 45% higher after the soxhlet extrac-

Table 2
Acids identified as methyl esters in samples, *m*/*z* ratio followed to quantify minor acids and RSD of the analyses ($n = 5$).

Retention time (min)	Abbreviation	Acid	*m*/*z*	RSD (%)
16.33	4:0	Butyric	74.0	0.4
17.72	5:0	Pentanoic	74.0	0.3
19.17	6:0	Hexanoic	74.0	0.2
20.81	7:0	Heptanoic	74.0	0.3
22.36	8:0	Octanoic	74.0	0.1
24.01	9:0	Nonanoic	74.0	0.3
25.49	10:0	Decanoic	74.0	0.2
28.42	12:0	Dodecanoic	74.0	0.04
29.81	13:0	Tridecanoic	74.0	0.1
31.19	14:0	Tetradecanoic	74.0	0.04
			TIC	6.6[a]
32.58	15:0	Pentadecanoic	74.0	0.05
33.79	15:1	cis 10-Pentadecenoic	222.2	0.09[c]
34.05	16:0	Hexadecanoic	TIC	2.8[a]
35.05	16:1u	xx-Hexadecenoic	74.0	0.07
35.21	16:1c	cis 9-Hexadecenoic	74.0	0.06
35.56	17:0	Heptadecanoic	74.0	0.02
			TIC	7.4[a]
36.70	16:2u	xx-Hexadecadienoic	67.0	0.5
36.82	17:1c	cis 10-Heptadecenoic	250.2	0.1
			TIC	7.5[b]
37.31	18:0u	x-Methylheptadecanoic	74.0	0.6
37.83	18:0	Octadecanoic	TIC	2.2[a]
38.49	18:1u	xx-Octadeceneoic	264.2	0.2
38.68	18:1c	cis 9-Octadeceneoic	TIC	0.6[a]
39.73	19:0	Nonadecenoic	74.0	0.3[c]
40.68	18:2tc	trans 9, cis 12-Octadecadienoic	95.1	0.5
41.00	18:2cc	cis 9, cis 12-Octadecadienoic	TIC	1.3[a]
41.52	20:0	Eicosanoic	74.0	0.2
			TIC	5.8[b]
42.79[d]	18:3ctt 18:3cct	cis 9, trans 12, trans 15-Octadecatrienoic cis 9, cis 12, trans 15-Octadecatrienoic	95.1	0.4
43.20	18:3ctc	cis 9, trans 12, cis 15-Octadecatrienoic	95.1	0.7
43.21	20:1 u	xx-Eicosenoic	292.3	0.1
43.31	20:1c	cis 11-Eicosenoic	292.3	0.08
			TIC	7.5[a]
43.84	18:3ccc	cis 9, cis 12, cis 15-Octadecatrienoic	TIC	2.5[a]
44.20	21:0	Heneicosanoic	74.0	0.2
46.05	20:2cc	cis 11,cis 14-Eicosadienoic acid	81.0	0.1
46.87	22:0	Docosanoic	74.0	0.07
			TIC	6.6[a]
48.47	22:1c	cis 13-Docosenoic	320.3	0.09
48.75	22:1u	xx-Docosenoic	320.3	0.006
49.23	23:0	Tricosanoic	74.0	0.3
51.43	24:0	Tetracosanoic	74.0	0.05
			TIC	8.4[a]
52.82	24:1c	cis 15-tetracosenoic	348.3	0.2
53.52	25:0	Pentacosanoic	74.0	0.2
55.63	26:0	Hexacosanoic	410.4	0.09
57.73	27:0	Heptacosanoic	424.4	0.1
59.89	28:0	Octacosanoic	438.4	0.3

TIC, peak integrated in the total ion chromatogram; u, unknown position and spatial distribution of the double bond.
[a] Major compound, RSD estimated on Aristis Bt sample.
[b] Major compound, RSD estimated on Near-iso sample.
[c] Minor compound, RSD estimated on Near-iso sample.
[d] Coelution of two compounds.

Table 3
Relative percentages of major acids in transgenic and isogenic varieties ($n = 5$).

Acid	Aristis	Aristis Bt	11Tietar	5DKC	28Pr33P66	28Pr33P67	ISO VER	287ASDIS	Near-iso	837ASDIS
14:0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:0	12.6[a]	13.4[a]	14.6	14.3	14.5	14.6	14.7[a]	15.6[a]	12.5	12.1
17:0	–	–	–	–	–	–	–	–	0.1[a]	0.2[a]
17:1c	–	–	–	–	–	–	–	–	0.04[a]	0.1[a]
18:0	5.8	4.0	4.0	3.5	4.1	3.9	4.8[a]	3.2[a]	8.7	8.8
18:1c	32.1	32.0	29.4	28.9	32.6	31.8	28.4[a]	32.5[a]	30.9[a]	26.1[a]
18:2cc	47.5	47.4	48.5	50.0	45.4	46.0	49.0[a]	46.2[a]	39.6[a]	41.9[a]
20:0	–	–	–	–	–	–	–	–	0.8	0.7
20:1c	0.2	0.4	0.5	0.4	0.5	0.5	0.3[a]	0.5[a]	0.3[a]	0.2[a]
18:3ccc	0.5[a]	2.0[a]	2.2	2.0	2.0	2.1	2.0[a]	1.2[a]	6.1[a]	9.0[a]
22:0	0.5	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.7	0.7
24:0	0.6	0.5	0.4	0.5	0.5	0.6	0.4	0.3	0.3[a]	0.1[a]
Total saturated	19.7[a]	18.3[a]	19.4	18.7	19.5	19.6	20.3[a]	19.5[a]	23.2	22.7
Total monounsaturated	32.3	32.4	29.9	29.3	33.1	32.3	28.7[a]	33.0[a]	31.2[a]	26.4[a]
Total polyunsaturated	48.0	49.4	50.7	52	47.4	48.1	51.0[a]	47.4[a]	45.7[a]	50.9[a]

–, without data.
[a] Significant differences ($p < 0.05$).

tion in relation to the use of *n*-hexane as extractant solvent. As the objective was to extract the greatest quantity of fatty acids a soxhlet extraction may result more advantageous without considering the involved time, which can be reduced using multiple soxhlet extractions.

As regards to the pressurized extraction, the performance of the extraction with methanol was logically very low, while the extraction with *n*-hexane was more effective. In both cases, the recoveries of fatty acids were not strongly dependent on the extraction temperature.

The selectivity of the extractions resulted to be similar, even the use of methanol in the pressurized liquid extraction supplied chromatogram profiles equivalent to the other approaches. The only difference seems to be related to the yield of the extraction. After these results, soxhlet extraction with diethyl ether was selected as the most suitable extraction procedure to study the content of major and minor acids in seeds.

3.2. Major compounds

Table 3 shows the most abundant acids found in corn and soybean samples with their relative amounts expressed as area percentage in relation to the total acid area. The quantification of these compounds has been carried out in the total ion chromatogram after five replicates. The relative standard deviation (RSD) of the percentage determination varied between and 1.3% and 8.4%. Soybean extracts were diluted ten times to determine the acid profile because the fat percentage was much higher in relation to corn samples. The fat average amount was about 2% in corn seeds and 15% in soybean.

The most abundant fatty acids in corn were, in decreasing concentration order, 18:2cc, 18:1c, 16:0, 18:0 and 18:3ccc which agrees with the scientific literature data which are commonly obtained by using a flame ionization detector [6,7,13,18]. A *t*-test was applied to establish if there was a significant difference between their means.

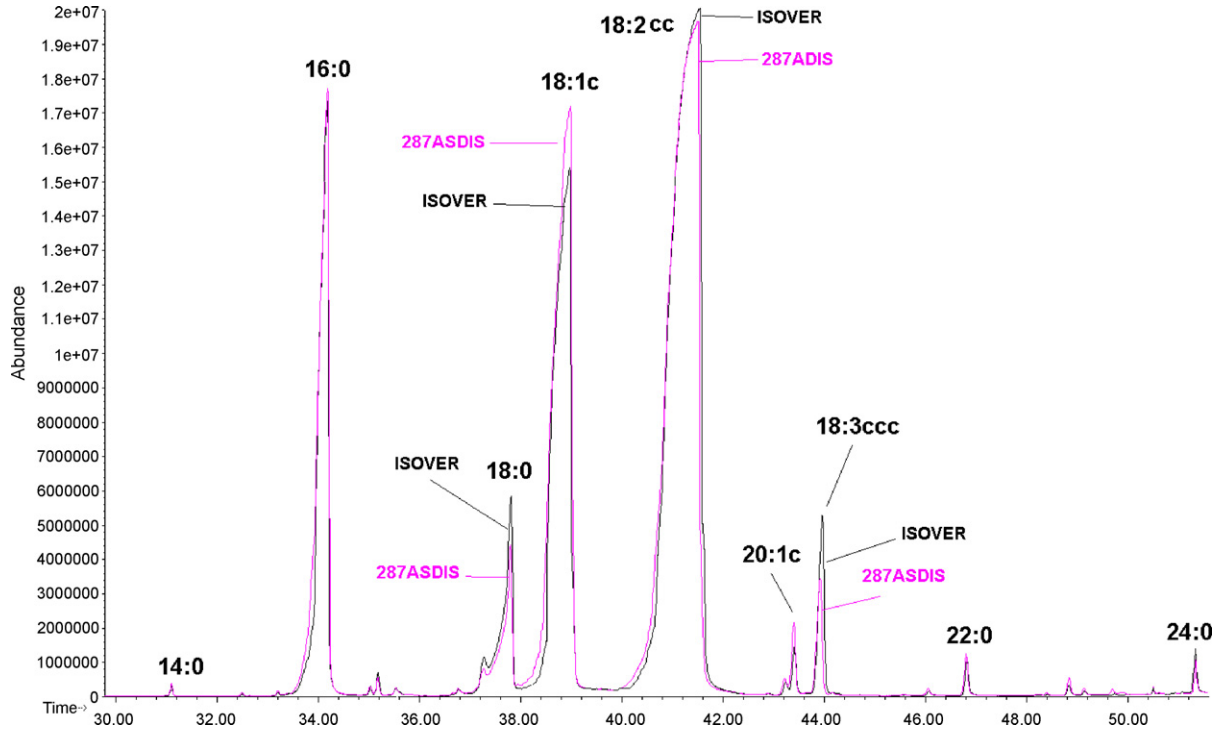

Fig. 1. Comparison of total ion chromatograms obtained for ISOVER and 287ASDIS corn lines.

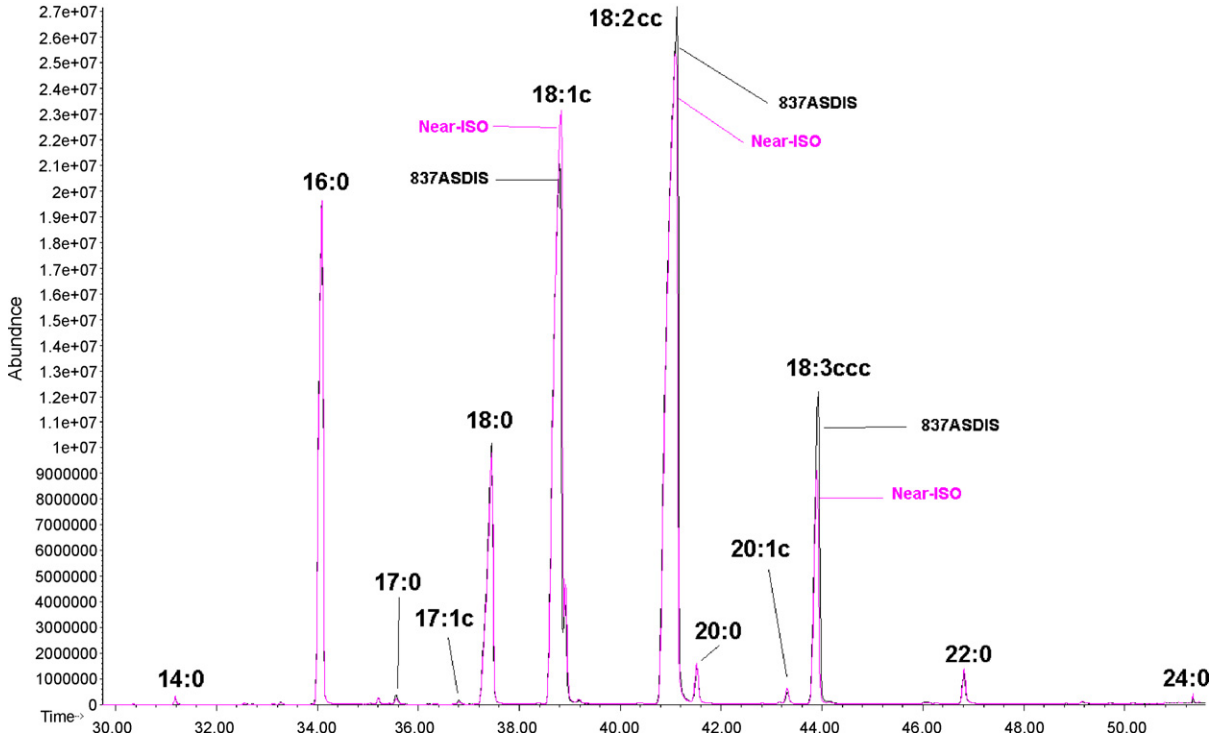

Fig. 2. Comparison of total ion chromatograms obtained for Near-iso and 837ASDIS soybean lines.

There were not observed differences for 11Tietar and 28Pr33P66 with their corresponding transgenic lines ($p < 0.05$) while for Aristis varieties the contents of 16:0 and 18:3ccc were higher in the transgenic line. The higher number of differences was found between ISOVER and 287ASDIS: the percentages of 16:0, 18:1c and 20:1c were higher in 287ASDIS in opposition to 18:0, 18:2cc and 18:3ccc whose concentrations were more abundant in the ISOVER line. Fig. 1 shows a superposition of chromatograms for these samples.

The fatty acid profile obtained for 28PR33P66 and 28PR33P67 hybrids are in consonance with previously published data [8]. On the other hand, significant differences have been found in the fatty acid composition within the NK603 variety.

It was found that the percentage of most abundant fatty acids in non-transgenic soybean seeds decreased in the following order: 18:2cc, 18:1c, 16:0, 18:0 and, finally, 18:3ccc, as the lowest abundant. These acids are considered in some scientific literature and, in this case, our data are not similar. The order of abundance for the first three fatty acids is the same but a presence of 18:3 bigger than that of 18:0 has been reported in several manuscripts [10,19,20]. Only in another study about soybean seeds [21], the abundance of 18:0 and 18:3ccc was also reversed, coinciding with our findings. It could be thought that the hydrolysis type could modify the results [22], but similar alkaline hydrolyses and methylation procedures have been used in the previously cited manuscripts; furthermore, it is also indifferent the use of mass spectrometric or flame ionization detection. As regards to the 837ASDIS transgenic line, it was found that the amounts of 18:0 and 18:3ccc were very close (see Table 3).

In soybean seeds, the relative percentage of fatty acids differs significantly ($p < 0.05$) for many compounds. In the 837ASDIS transgenic variety, the contents of 17:0, 17:1c, 18:2cc and 18:3ccc were higher while the percentages of 18:1c, 20:1c and 24:0 were lower. These major compounds can be seen in the chromatograms from Fig. 2.

It is convenient to point out that the peak area assigned to 18:0 corresponds to the sum of areas of two isomers. This could be better observed in corn samples (Fig. 1), where a small peak attributed to an isomer is eluted on the fronting peak of major 18:0 isomer. Also in these samples, the area of the most prominent acid, 18:2cc, contains the area of 20:0 due to the broadening of 18:2cc peak. In the same way, there is an 18:1 isomer eluted very close to major 18:1c isomer, which can be easily observed in soybean samples (Fig. 2), where their peak areas have been summed.

The total content in saturated, monounsaturated and polyunsaturated fatty acids was also estimated as it can be seen in Table 3. A *t*-test revealed significant differences in the amounts of saturated acids in Aristis corn varieties, in the unsaturated acid content between Near-iso and 837ASDIS soybean seeds and in the three types of acids for ISOVER and 287ASDIS corn lines. In absolute value, and considering the content in individual acids, the highest concentration differences were 4.1% (28.4–32.5) for 18:1c in ISOVER and 287ASDIS corn lines, and 4.8% (30.9–26.1) for 18:1c also in Near-iso and 837ASDIS soybean lines, which was foreseeable taking into account that genetically modified samples belong to first generation of transgenic seeds.

3.3. Minor compounds

Table 4 shows the percentages of minor acids calculated after recording the peak areas in the ion chromatograms extracted at the *m*/*z* ratios given in Table 2. Some of the least prominent major acids have been also included in this study: they were 14:0, 20:1c, 22:0 and 24:0 for corn samples and 14:0, 17:0, 17:1c, 20:0, 20:1c, 22:0 and 24:0 for soybean samples. Obviously, findings for these latter fatty acids were similar to those abovementioned.

Aristis/Aristis Bt corn pair resulted to have the smaller number (only two) of concentration differences of minor acids according to *t*-test ($p < 0.05$). The fatty acids whose concentrations were significantly different varied for each sample pair as can be seen in Table 4. The RSD of the analyses was comprised between 0.02% and 0.7% ($n = 5$).

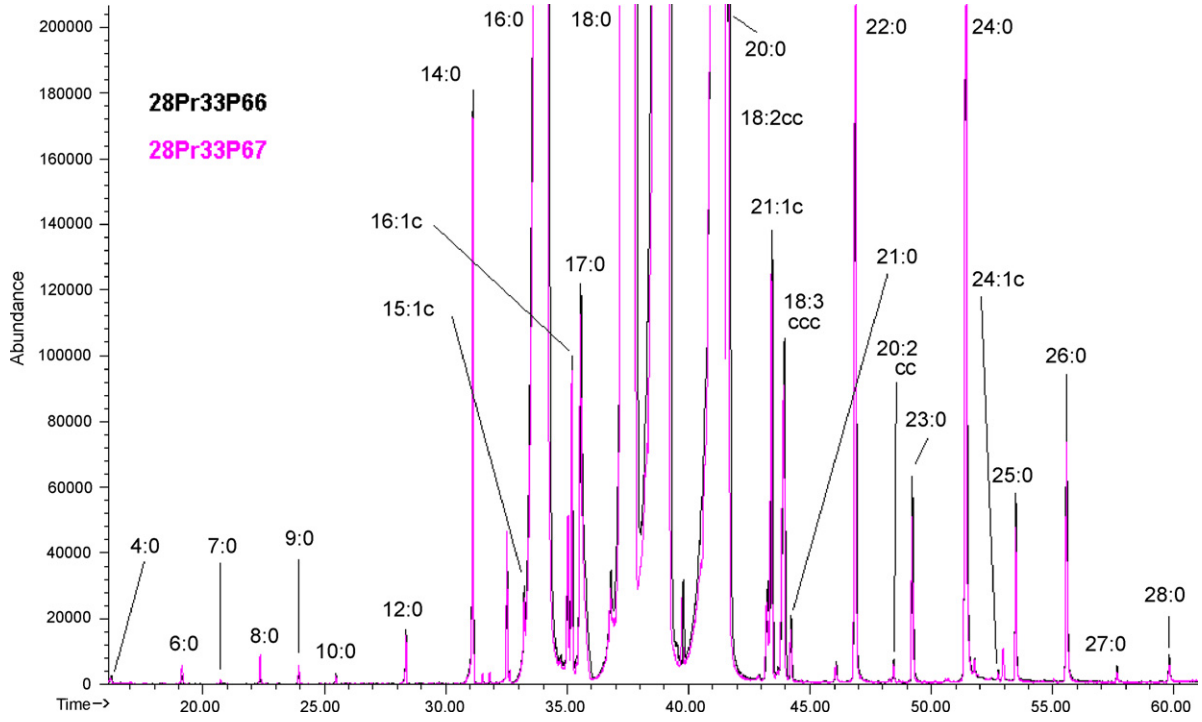

Fig. 3. Comparison of ion chromatograms extracted at *m*/*z* 74.0 for 28Pr66P66 and 28Pr66P67 corn lines.

However, in many cases it has been noted that the concentrations of some acids with short chain length, such as 6:0, 8:0, 9:0, 10:0 and 12:0 seems to be higher in transgenic lines, as happened with corn and soybean seed samples (5DKC, 28Pr33P67, 287ASDIS and 837ASDIS). On the other hand, the concentrations of certain long chain fatty acids, such as 25:0. 26:0, 27:0 and 28:0, were significantly higher in three isogenic lines (28Pr66P66, ISOVER and Near-iso), and 24:0 in Near-iso soybean, too. In any case, these concentration differences were small, not higher than 2.54% in corn and 4.34% in soybean samples. Many differences can be observed in the chromatograms of Figs. 3 and 4, which have been enlarged to emphasize the minor peaks.

The total amounts of saturated, monounsaturated and polyunsaturated acids were also calculated (see Table 4). No significant differences ($p < 0.05$) were found; the total percentages were similar in each sample of the pair except for ISO VER and 287ASDIS where the saturated acid content was about 3% higher in ISO VER and the monounsaturated content was about 3% higher in 287

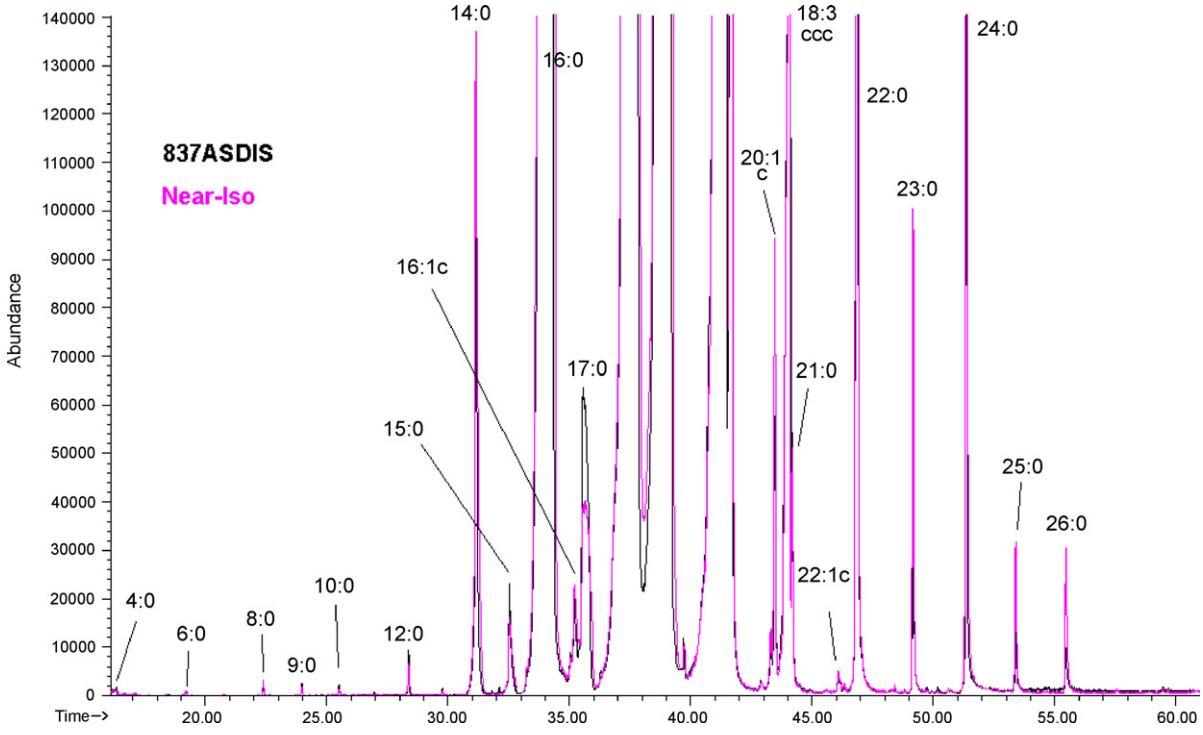

Fig. 4. Comparison of ion chromatograms extracted at *m*/*z* 74.0 for Near-iso and 837ASDIS soybean lines.

Table 4
Relative percentages of minor acids in transgenic and isogenic varieties ($n = 5$).

Acid	Aristis	Aristis Bt	11Tietar	5DKC	28Pr33P66	28Pr33P67	ISO VER	287ASDIS	Near-iso	837ASDIS
4:0	0.05	0.06	0.04	0.04	0.06	0.04	0.04	0.03	0.04[a]	0.06[a]
5:0	0.01	0.02	0.01[a]	0.03[a]	0.01	0.01	0.01	0.01	–	–
6:0	0.12	0.14	0.10[a]	0.16[a]	0.07[a]	0.16[a]	0.10[b]	0.15[a]	0.02[a]	0.03[a]
7:0	0.03	0.05	0.04[a]	0.07[a]	0.03	0.04	0.05	0.04	0.01	0.01
8:0	0.16[a]	0.11[a]	0.12[a]	0.17[a]	0.10[a]	0.23[a]	0.15[a]	0.26[a]	0.06	0.05
9:0	0.09	0.14	0.09[a]	0.13[a]	0.06[a]	0.13[a]	0.07[a]	0.16[a]	0.04[a]	0.06[a]
10:0	0.05	0.07	0.04[a]	0.07[a]	0.06	0.05	0.04[a]	0.06[a]	0.04[a]	0.06[a]
12:0	0.30	0.39	0.25[a]	0.40[a]	0.34	0.32	0.30[a]	0.37[a]	0.19[a]	0.25[a]
13:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.04
14:0	4.56	4.42	4.51	4.31	3.99	4.08	4.04	3.84	5.13	5.51
15:0	1.12	1.27	1.08[a]	1.36[a]	1.02	1.25	1.05	1.24	0.14	1.33
15:1	–	–	–	–	–	–	–	–	0.07	0.05
16:1u	0.94	1.23	1.01	1.17	1.20	1.11	1.00	0.98	0.40	0.44
16:1c	2.23	1.96	2.29	1.89	2.21	2.14	2.34[a]	2.08[a]	1.25	1.41
17:0	6.00	5.74	6.07	5.94	6.28	6.11	6.45	6.39	4.68[a]	8.58[a]
16:2u	0.29	0.31	0.24[a]	0.36[a]	0.20[a]	0.37[a]	0.43	0.36	0.65	0.43
17:1c	0.58	0.49	0.62	0.75	0.56	0.47	0.52	0.57	0.48[a]	1.00[a]
18:0u	6.45	5.99	6.28	6.24	6.26	6.44	6.67	6.73	11.69	12.52
18:1u	0.49	0.39	0.37	0.40	0.49	0.35	0.52	0.45	2.38	2.86
19:0[b]	–	–	–	–	–	–	–	–	0.64	0.58
18:2tc	1.14	1.23	1.30	1.20	1.25	1.37	0.95	0.84	1.57	1.72
20:0	30.85	32.14	32.56[a]	31.05[a]	31.19	31.95	31.25	30.26	25.24	26.27
18:3ctt + 18:3cct	0.25	0.29	0.31[a]	0.18[a]	0.34	0.24	0.24	0.35	0.61[a]	0.91[a]
18:3ctc	0.30	0.39	0.40	0.36	0.31	0.26	0.35	0.28	0.92[a]	1.22[a]
20:1 u	9.23	8.89	8.96	9.26	7.74	9.06	8.62	9.50	0.35	0.29
20:1c	6.09	5.86	5.74	5.84	6.90	5.56	4.12[a]	6.66[a]	5.95[a]	3.22[a]
21:0	0.49	0.65	0.52	0.53	0.62	0.48	0.72	0.51	2.42	2.06
20:2cc	1.16	1.03	0.88	0.93	0.89	0.87	0.89	0.88	0.53[a]	0.88[a]
22:0	9.30	9.54	9.45	9.27	9.35	9.75	9.68	9.58	22.00	20.70
22:1c	0.21	0.26	0.18	0.19	0.28	0.24	0.21[a]	0.26[a]	0.05	0.04
22:1u	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.01
23:0	1.66	1.47	1.54	1.84	1.69[a]	1.38[a]	1.74	1.59	2.29	1.83
24:0	11.20	11.19	10.20	10.77	11.65	11.89	12.36	11.74	9.12[a]	4.78[a]
24:1c	0.11	0.16	0.15	0.15	0.17	0.14	0.15	0.16	–	–
25:0	1.34	1.59	1.40	1.60	1.48	1.21	1.68[a]	1.22[a]	0.54[a]	0.42[a]
26:0	2.89[a]	2.16[a]	2.75	2.92	2.57[a]	2.02[a]	2.67[a]	2.12[a]	0.41[a]	0.35[a]
27:0	0.09	0.10	0.24	0.21	0.25[a]	0.09[a]	0.20	0.11	0.02[a]	0.01[a]
28:0	0.19	0.18	0.23	0.19	0.34[a]	0.16[a]	0.35[a]	0.19[a]	0.02[a]	0.01[a]
Total saturated	76.96	77.43	77.53	77.31	77.44	77.80	79.63	76.61	84.77	85.53
Total monounsaturated	19.90	19.26	19.34	19.66	19.57	19.09	17.51	20.68	10.95	9.31
Total polyunsaturated	3.14	3.25	3.13	3.03	2.99	3.11	2.86	2.71	4.28	5.16

–, without data.
[a] Significant differences ($p < 0.05$).
[b] Coeluted with 18:1cc in corn samples.

ASDIS, regarding to the monounsaturated and saturated content, respectively.

With the used biscyanopropylsiloxane column and chromatographic conditions it has been possible to separate and determine some minor isomers and to look for concentration differences. Differences have been found in punctual cases in some sample pairs; for instance, the contents varied for minor acids such as 16:1c, 16:2u, 18:3ctc, 22:1c.

In relation to the cis/trans isomers, it is worth to state that 18:1t, 18:2tt, 18:2ct, 18:3ttt, 18:3tcc, 18:3tct and 18:3tcc were not detected in samples according to the comparison of the obtained retention times and abundance ratios of characteristic ions with those one obtained from available commercial chromatographic standards. In this work, 18:3cct and 18:3ctt co-eluted, so their peak areas could not be distinguished and a joint contribution was considered.

4. Conclusions

Although the selectivity of the soxhlet and pressurized liquid extraction of fatty compounds from corn and soybean seeds is similar, soxhlet extraction results more advisable when minor compounds are to be studied.

The use of new capillary chromatographic columns, more specific and longer, allows the collection of more information about minor fatty acids and their corresponding isomers.

The profile of major and minor fatty acids is similar in the isogenic and its transgenic counterpart; it had not been found a specific isomer (cis/trans, double bond position) or a compound which denotes specifically the genetic origin of the crop. With irrelevant exceptions, trans fatty acids have not been detected in the samples.

The concentration of some acids is significantly different for some transgenic lines; the highest observed differences for major acids were 4.1% in corn and 4.8% in soybean, the variations were relatively small and within tolerable limits. The concentrations of certain long chain acids such as 25:0, 26:0, 27:0 and 28:0 resulted to be higher in some isogenic lines, whereas the concentrations of some short chain acids such as 6:0, 8:0, 9:0, 10:0 and 12:0 were higher in transgenic lines.

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