ELSEVIER

Industrial Crops and Products 12 (2000) 193–202

INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL

www.elsevier.com/locate/indcrop

# Capillary electrophoresis: novel tool for simmondsins analysis and its application to jojoba breeding

Paul P. Kolodziejczyk [a,*], Wanli Lu [a], Ricardo Ayerza [b], Marie-Anne de Larminat [c]

[a] *POS Pilot Plant Corporation, 118 Veterinary Road, Saskatoon, SK, Canada S7N 2R4*
[b] *Bioresources Research Facility, Office of Arid Lands, University of Arizona, 250 E. Valencia Rd., Tucson, AZ 85706, USA*
[c] *Biolink Inc., 3124 Caen Street, Saskatoon, SK, Canada S7M 3N5*

Accepted 12 May 2000

**Abstract**

Jojoba seed, a rich source of liquid wax, contains also a group of closely related glycosides: simmondsin (SM), demethylsimmondsin and didemethylsimmondsin (DDS). Four ferulates derivatives of SM and demethylsimmondsin have been identified and isolated. Growing interest and potential for application of SMs as feed additives calls for efficient and quick analytical methods both for quantitative and qualitative analyses. The HPLC method is commonly used for SMs analysis; however, the presence of stereosomers of ferulates may require more precise, faster and less expensive analytical method, especially for animal studies. In this report, it is shown that capillary electrophoresis (CE) is a powerful separation and quantitation technique that provides high resolving power, short analysis time, and low operational cost for the analysis of SM derivatives. The CE method for analysis of SMs was developed, scrutinized in interlaboratory ring test, and applied to monitoring the breeding studies. Although CE is not commonly used for analysis of plant material or agriculture products, it has shown great potential for the analysis substances of interest in biological matrices or profiling metabolites of physiologically important molecules. The exposure of simmondsin ferulates (SSFs) to light causes their isomerization, and this effect may be easily quantified by CE. The CE method is also applicable to quality control analysis for processing and purification of SMs used as animal feed additive. 

*Keywords:* Simmondsin; Simmondsin ferulates; Isomers; Jojoba; Capillary electrophoresis; Breeding; Analysis

## 1. Introduction

The jojoba plant (*Simmondsia chinensis*) is a naturally occurring plant in arid regions of Mexico and southern US. Jojoba is successfully grown in other part of the world, for example, Israel and Argentina. The interest in jojoba seed is due to

* Corresponding author. Tel.: +1-306-9782853; fax: +1-306-9783766.
*E-mail address:* paulk@pos.ca (P.P. Kolodziejczyk).

0926-6690/00/$ - see front matter 
PII: S0926-6690(00)00071-6

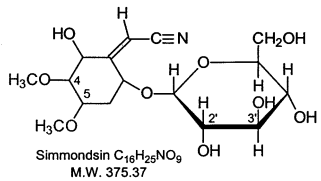
the unique oil present in its seeds. Unlikely the majority of other seed, the content of triglycerides in jojoba seed oil is relatively low. However, high levels of wax esters make this oil a valuable product for cosmetics market.

Simmondsins (SM), anti-nutritional components present in jojoba meal, have attracted the attention of several research groups, and their possible application as animal feed has been studied by Flo et al. (1998).

Analysis of SMs level is of interest to plant breeders, jojoba processors, animal feed industry and researchers conducting animal nutrition studies. SMs represent a group of closely related glycosides: SM, demethylsimmondsin and didemethylsimmondsins (DDS), along with four ferulates: SM-2′-ferulate, SM-3′-ferulate, 5-demethylsimmondsin-2′-ferulate and 4-demethylsimmondsin-2′-ferulate (Van Boven et al., 1994a,b, 1995, 1996) (Fig. 1).

Growing interest and potential for application of SMs as feed additives calls for efficient and quick analytical methods both for quantitative and qualitative analyses. The HPLC is commonly used for SMs analysis; however, the presence of isomeric ferulates may require more precise analysis, especially for animal studies. The action of isomers is an important issue, especially in nutritional studies, because stereochemistry may have a significant effect on the biological activity of given components.

Isomerization might occur during processing or storage of bioactive material, or be a result of altered biosynthesis pathways in plant due to environmental stress or genetic selection and/or manipulation. Therefore, separation and identification of the desired isomer is essential during the discovery of bioactive natural products, and also to support a product through development, registration and production (quality assurance).




Simmondsin $C_{16}H_{25}NO_9$
M.W. 375.37

| C-4 | C-5 | C-2' | C-3' | Compound |
|---|---|---|---|---|
| $OCH_3$ | $OCH_3$ | OH | OH | Simmondsin (SM) |
| OH | $OCH_3$ | OH | OH | 4-demethylsimmondsin |
| OH | OH | OH | OH | Didemethyl-SM (DDS) |
| $OCH_3$ | $OCH_3$ | O-Ferulate | OH | S-2'-ferulate (SF) |
| $OCH_3$ | $OCH_3$ | OH | O-Ferulate | S-3'-ferulate (SF) |
| $OCH_3$ | OH | O-Ferulate | OH | S-5-demethyl-2'-ferulate |
| OH | $OCH_3$ | O-Ferulate | OH | S-4-demethyl 2'-ferulate |

Fig. 1. Structure of simmondsin (SM) and SM analogues present in jojoba meal.

Several liquid chromatography (LC) and gas chromatography (GC) methods have been reported for isomers however; these methods generally require specialized and expensive columns. Recently capillary electrophoresis (CE) was recognized as a complementary technique to LC and GC for the separation of biologically active substances including compounds. CE is a sensitive and versatile technique, which has emerged over the past decade into the forefront of analytical methodology. Its applicability is enhanced by short separation times, facile and rapid methods of development and requirement for very small amounts of analyte.

The potential of CE in the determination of positional isomers of organic compounds could prove this technique very useful for analysis of biologically active substances. This paper describes an application of CE for analysis of SMs in jojoba meal and its analogues.

## 2. Materials and methods

### 2.1. Instrumentation

CE was performed with a Hewlett Packard $HP^{3D}$ instrument equipped with autosampler and photodiode array detector (DAD). HP ChemStation software version A.04.01 was used for instrument control, data analysis and report generation. Non-coated fused-silica capillary 50 μm × 80.5 cm (efficient length 72 cm) was used. The buffer applied consisted of 150 mM boric acid, 75 mM sodium dodecylsulphate (SDS), 15 mM dimethyl-β-cyclodextrin, adjusted with sodium hydroxide to pH 8.5. The temperature of the capillary was maintained at 35°C by the instrument thermostating system. Samples were injected by applying a pressure of 50 mbar for 3.5 s. Detection was performed at 220 nm, and the resulting signal was

Table 1
General characteristics of the two locations where jojoba clones were planted

| Location | Pampa de Villacuri, Peru | Catamarca, Argentina |
|---|---|---|
| Ecosystem | Atacama Desert | Arid Chaco |
| Latitude (South) | 14° 00′ | 28° 28′ |
| Elevation (m) | 398 | 500 |
| Rainfall (mm/year) | 1 | 379 |
| Irrigation (mm/year) | 1670 | 500 |
| Mean temperature (°C) | 21.1 | 20.2 |

fed to the computer for storage and real-time display of the electropherogram. A voltage of 30 kV was applied.

NMR analysis was performed using a 300 MHz Bruker spectrometer. Samples for analysis were dissolved in $CD_3OD$.

Mass spectrometry (MS) analysis was done using positive ion electrospray (ESP) with a Fisions Trio 2000 instrument. The samples were dissolved in water–acetonitrile 1:1 v/v mixture at concentration approx. 1 μg/μl). Ten microlitres of sample was injected using loop injection. Mobile phase was 50% acetonitrile: water containing 1% formic acid flowing at rate of 20 μl/min. Spectra were recorded by scanning from 100 to 1400 Da every 5 s.

For isomerization experiment a light source used was Plant Grow n' Show 75 W lamp (General Electric). The sample was dissolved in water at concentration of 1 mg/ml and placed in stirred glass vial at distance of 20 cm from the light source. Aliquots were withdrawn at 0, 60 and 120 min for analysis. No attempts to optimize the isomerisation process have been made in this study.

### 2.2. Materials

Floratech International, AZ provided the defatted jojoba meal used for method development.

Boric acid, SDS, dimethyl-β-cyclodextrin, and gallic acid were purchased from Sigma (St. Louis, MO).

For routine analyses, seeds from ten jojoba clones growing in commercial plantations of Argentina and Peru, five from each location, were harvested in 1996 (both locations) and in 1997 (Argentina). Peru plants were from Pampa de Villacuri (Atacama Desert region), and the Argentine plants were from the Arid Chaco Ecosystem (Table 1). Four plants from each clone were selected at random, and the mature seeds were collected from the ground, by hand. They were cleaned, dried in the shade and weighed after drying. The seeds were stored at ambient temperature until September 1997, when the content of liquid wax and SM content were determined. The International Flora Technologies Laboratory (Gilbert, AZ) determined the liquid wax content analysis (following AOCS Official Method Ac 3-44). After extraction of the liquid wax, the residue, jojoba meal, was sent to POS Laboratory for SM and its derivatives determination according to procedure described below. The experimental design used was completely randomized, with four replications. Each variable was compared by analysis of variance. When the $F$ value was significant, the means were separated with Duncan's New Multiple Range Test. Correlation coefficients were determinated between liquid wax content and, percentage of total SM, percentage of DDS and percentage of SSF.

## 3. Results and discussion

### 3.1. Preparation of standards

As pure standards of SM and its derivatives were not available, the isolation of SM, simmondsin ferulate (SSF) and DDS was carried out in the laboratory. Three standards SM, SF and DDS were prepared by means of column chromatography on silica according to the procedure of Van Boven et al. (1994a,b, 1996).

In short, defatted jojoba meal was extracted with methanol, evaporated under reduced pressure to dryness. The residue was dissolved in a chloroform–methanol mixture 93:7 (v/v) and loaded on a silica column. SMs were eluted from the column first with a mixture of chloroform–

methanol 93:7 (v/v), then with a chloroform–methanol 85:15 (v/v) mixture, and finally, a more polar mixture consisting of chloroform–methanol–water 50:50:2 (v/v) was applied.

Thin layer chromatography (TLC) and CE were used for monitoring the fractions content and their homogeneity. Fractions containing SM, SF and DDS were combined and evaporated under reduced pressure. The residue was dried over $P_2O_5$ in vacuum overnight. Fraction containing DDS was re-chromatographed on the silica using chloroform–methanol–water 50:50:2 (v/v) system. All components were homogenous upon TLC analysis.

TLC was performed on silicagel glass plates (Merck) using the solvent system chloroform–methanol (60/40 v/v). The presence of substances was localized with short-UV light and by spraying the plates with 1-naphtol reagent (2% 1-naphtol in 95% ethanol containing 10% sulfuric acid. After spraying, the plates were heated at 110°C in an oven for 5 min.

The identity of isolated substances was confirmed using NMR and MS methods.

The finding were in full agreement with the NMR data previously published elsewhere (Van Boven et al., 1994a,b, 1995, 1996.

MS revealed intense molecular ions plus 23 U (sodium) and molecular ions plus proton. Some fragmentation ions were also present, for example, ion 214 corresponds to the elimination of glucose and oxygen from the SM molecule.

Simmondsin (SM)
MS: $m/z$ 398 (M + Na), 276 (M + 1), 214.
Simmondsin 2′-ferulate (SF)
MS: $m/z$ 574 (M + Na), 552 (M + 1), 339
Didemethylsimmondsin (DDS)
MS: $m/z$ 370 (M + Na), 348 (M + 1), 217, 186.

### 3.2. CE analysis of isolated standards

Isolated standards of simmondsins (SM, SF and DDS) were analyzed by CE using conditions as described above. The electropherograms of prepared standards are presented in Fig. 2(2.1, 2.2, and 2.3), respectively.

The advantage of the photo-diode array detector permits to record the UV-VIS spectra of analyzed components directly in capillary. Spectra of SM, SF and DDS recorded during analysis experiment are depicted in Fig. 2(2.1a, 2.2a and 2.3a), respectively.

Jojoba seed contains also small amount of demethylsimmondsins however, in this study, demethylsimmondsins have been neither isolated nor quantified.

It is possible that certain minor components might overlap with major peaks in CE determinations of SMs, this feature is not unique to CE, but common in other methods (HPLC, GC). This issue will be definitely solved only when all components present in jojoba seed are identified and appropriate standards are available. At present, good agreement is seen in analysis results from CE and HPLC determinations. However, identification of other components and reporting on their analysis by CE is an interesting challenge and will be undertaken in this laboratory.

### 3.3. Calibration curves for separated SMs

Calibration curves for SM, SF and DDS were constructed by preparing solution of purified jojoba standards, containing gallic acid as an internal standard, SM or analogue standard at known concentrations and analyze them using CE procedure. The results are presented in Fig. 3a–c. Very good and consistent results of CE analysis were observed. Within applied range of concentration (16–2360 μg/ml of SMs) the correlation between amount ratio (standard weight/weight of internal standard) was $r = 0.99999$ for SM, $r = 0.99970$ for SF and $r = 0.9996$ for DDS, respectively.

### 3.4. CE analysis of jojoba meal

The CE method was applied to the analysis of jojoba meal and its derivatives for SMs level.

A typical extraction procedure was as follows:

1. jojoba meal was ground in the coffee grinder
2. about 0.5 g of jojoba meal was weighted into a 50 ml centrifuge tube
3. 1 ml of internal standard containing 5 mg/ml of gallic acid solution was added
4. 25 ml of methanol was added to the above mixture

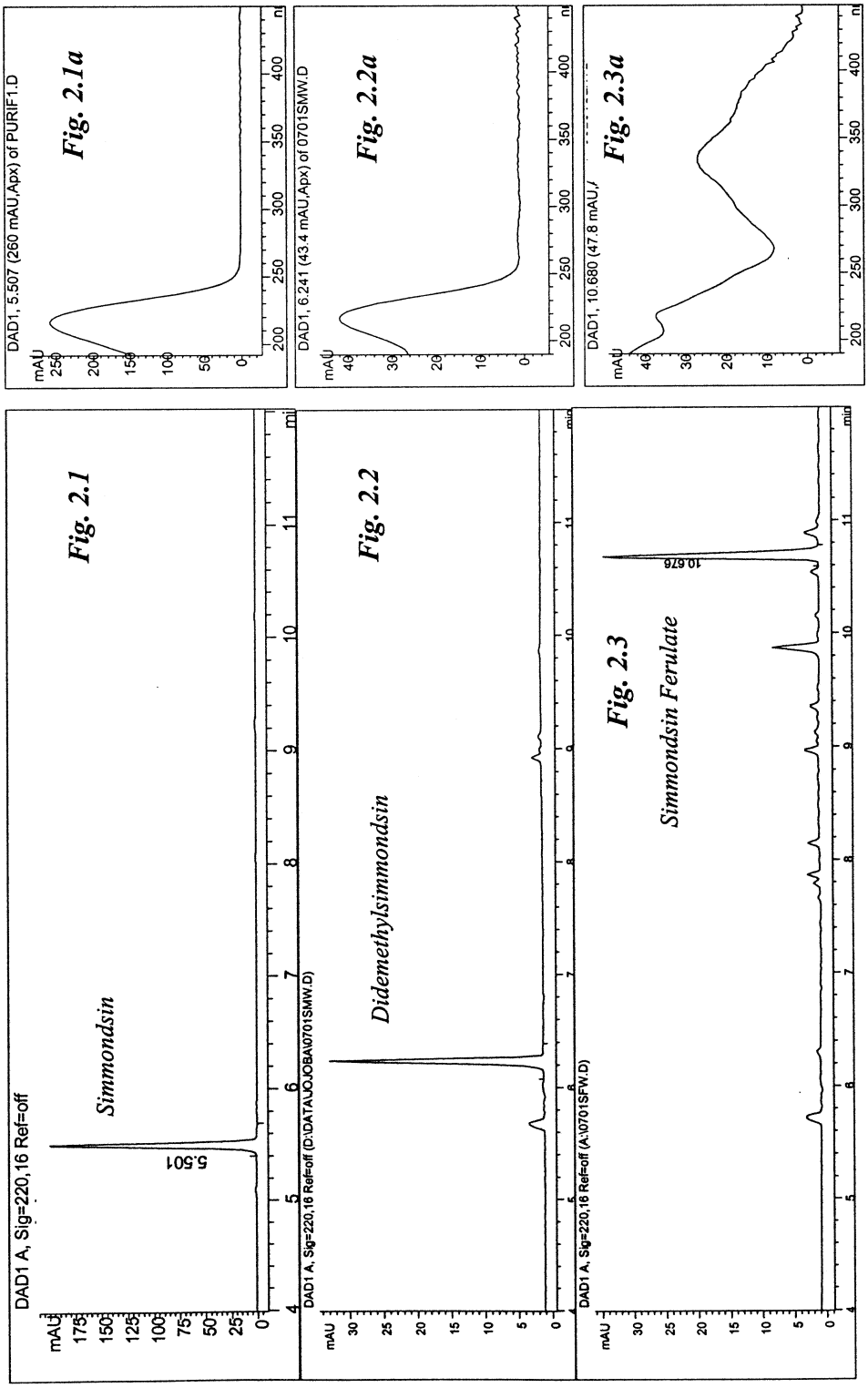



Fig. 2. Capillary electrophoresis (CE) analysis of simmondsin (SM) standards isolated from jojoba meal. 2.1a, 2.2a and 2.3a show UV-VIS spectra corresponding to peaks of SM, simmondsin ferulate (SSF) and didemethylsimmondsin (DDS), respectively. Refer to text for details of analysis.

5. the mixture was sonicated for 30 min
6. then it was centrifuged at 2000 rpm for 10 min
7. the supernatant was transferred into a 125 ml round bottom flask
8. steps 4–7 were repeated twice
9. methanol was evaporated under reduced pressure and operations were performed under dimmed lights or using amber flasks
10. the residue was re-dissolved in double distilled water and diluted to 25 ml using volumetric flask

CE analysis was performed as described in Section 2. Typically, analysis results for SMs in jojoba meal are reported as percentage of oil-free, moisture-free meal weight. Therefore, meal sample was subdivided and evaluated for oil content and moisture using standard analytical methods [AOCS, 1998: Aa 3–38 and Ac 3–44].

The application of this method for jojoba meal analysis is presented in Fig. 4. The main components of jojoba meal are indicated, and their migration times are as follows: SM, 5.7 min; DDS, 6.1 min; SSF, 10.7 min. Gallic acid which is used as an internal standard, exhibits peak at 18.2 min.

The efficiency of SM extraction was tested for both methanol and water. In this procedure, methanol did prove to be a superior extraction solvent to water (data not shown).

The CE analysis method was validated for POS internal use and this laboratory participated in the interlaboratory studies, which compared proficiency of analysis. The results of this study are presented in this issue (Abbott et al., 2000).

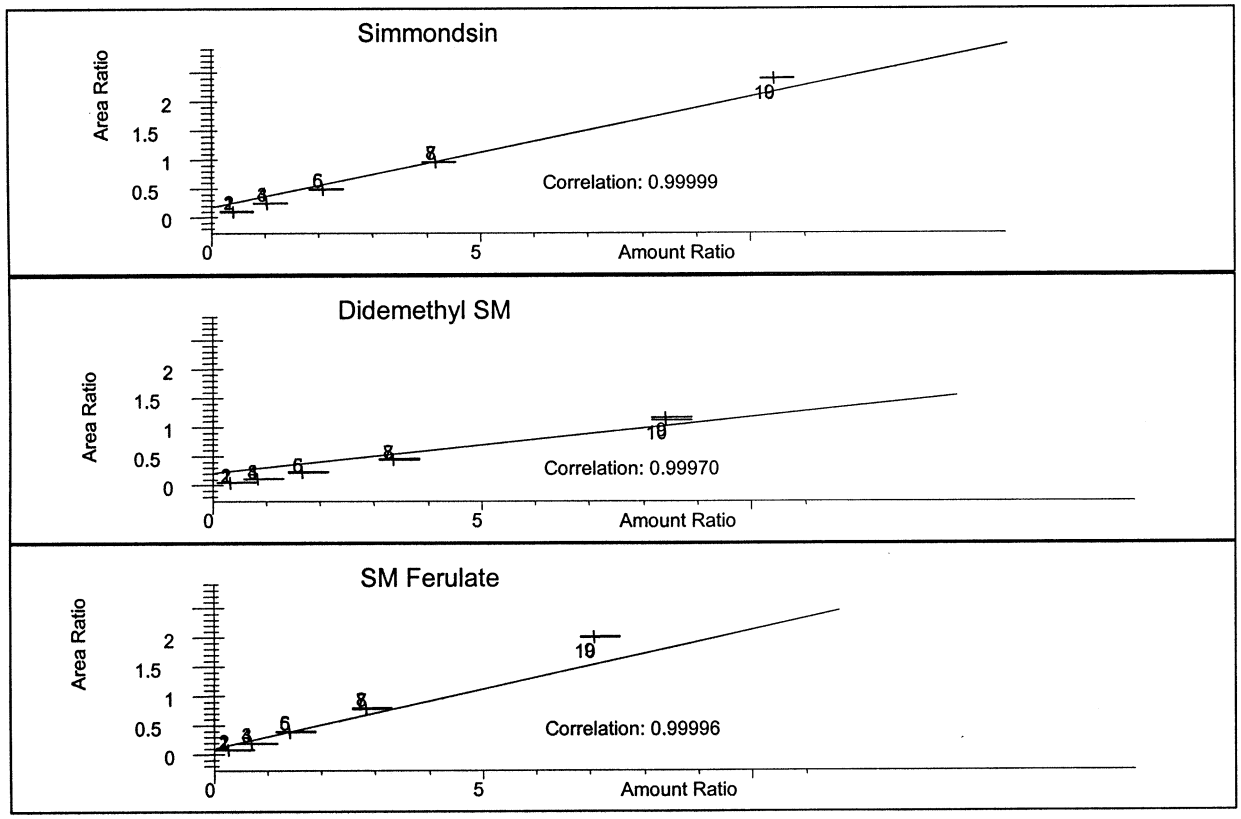



Fig. 3. Calibration curves for simmondsin (SM), didemethylsimmondsin (DDS) and simmondsin ferulate (SF). Plots represent the amount ratio (analyzed component/IS) vs. area under peak corresponding to given component/area under IS peak.

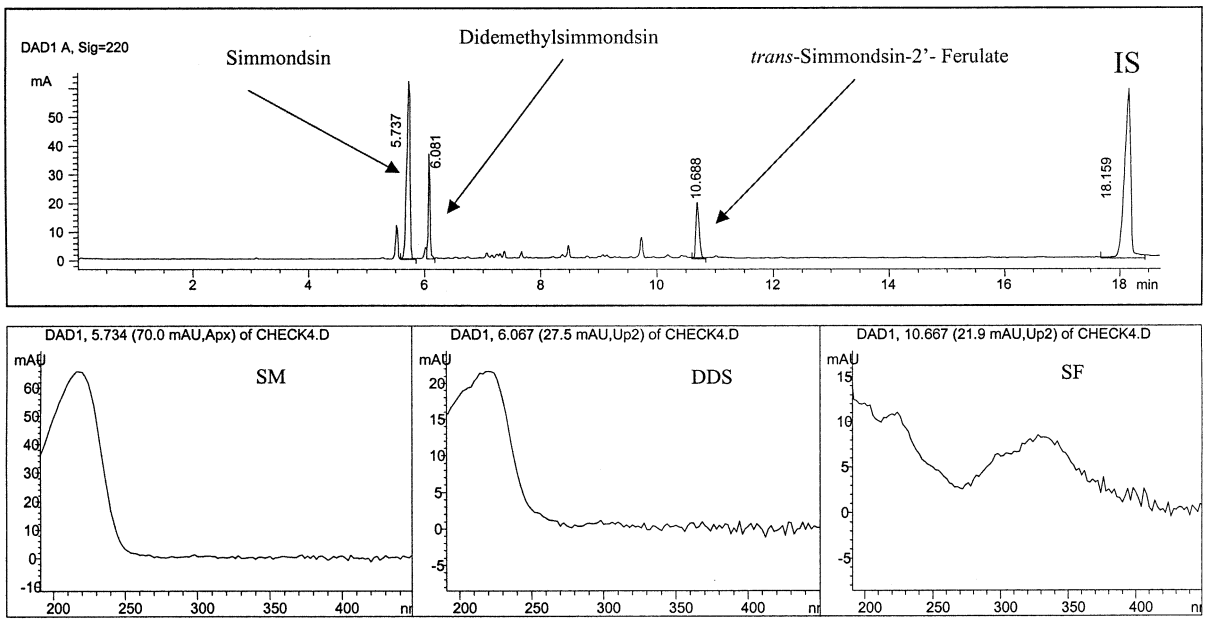



Fig. 4. Analysis of jojoba meal using capillary electrophoresis (CE) method — for details refer to text. Separation was monitored at 220 nm. Lower set of graphs represents UV-VIS spectra recorded at peaks for simmondsin (SM), didemethylsimmondsin (DDS) and simmondsin ferulate (SF).

### 3.5. Photoisomerization

Natural SSFs occur as *trans* isomers. Under exposure to light ferulates may be converted into *cis* isomers. CE might be an ideal tool for detection and differentiation of those isomers. CE method might be valuable for monitoring of SMs isolation and purification processes, as well as for their biological activity studies. A similar method was reported for analysis of sinapin and ferulates in canola seed by Kolodziejczyk et al. (1998), Kolodziejczyk et al., (1999).

The occurrence of photoisomerization is documented in Fig. 5a–c. SSF (concentration 1 mg/ml) was exposed to wide-spectra light (Plant Grow n' Show, 75 W electric bulb). Exposure-time related peak at ca. 10.8 min corresponds to accumulation of SF *cis* isomer. Another ferulate (impurity present in the standard, presumably 3′-SSF) at migration time 9.8 min is also isomerized to corresponding *cis* isomer at 10.7 min.

After 1 h of exposure the level of *cis* isomer has increased to 18.1% and has reached 41.3 after 2 h of exposure. Note that starting material (time = 0) was already partially isomerized (about 9%).

### 3.6. Breeding studies

The CE method was applied to quality control of processed jojoba meal, but another important application is monitoring of jojoba seed quality in breeding programs.

The breeding studies were performed in Peru and Argentina and the produced seed were analyzed for oil (liquid wax) content and for levels of SM and SM analogues.

Liquid wax content was significantly different ($P < 0.05$) between locations and within locations among clones (Table 2). The Peruvian and Argentine clones yielded average wax content of 56.61 and 49.85%, respectively. Because different clones were utilized in the sites tested, two factors: genetic background and ecosystem characteristics could have affected the wax yield.

The wax contents for both locations are higher than the average wax level (48.96%) obtained

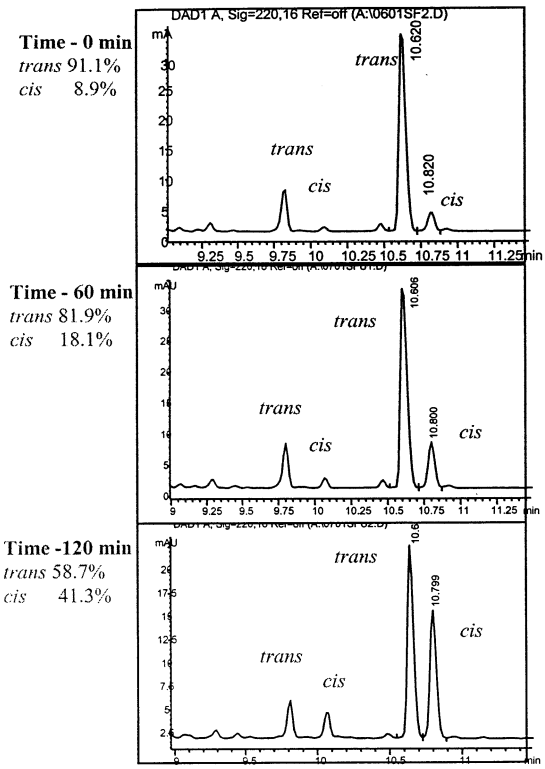



Fig. 5. Transformation of *trans*-simmondsin ferulates (SSF) into their *cis* isomers. Upon exposure to the wide-spectrum light, ferulate simmondsins (at 10.6 and 9.8 min) are transformed into *trans* isomers. New peaks are appearing at 10.8 and 10.1, respectively. Refer to text for details.

from wild plants collected in 20 sites through the Sonora Desert (Ayerza, 1990a). The highest content of wax was 58.75% for seeds from Peruvian clone P-360G, and lowest wax level was found for seeds from Argentine clone A-SF6-245. The clone P-360G was producing higher wax content than the clones from Australia, Chile, and US (Nelson and Palzkill, 1999).

SM, DDS and SSF content was significantly different ($P < 0.05$) both for clones and locations (Table 2). SM content was significantly higher ($P < 0.05$) for clone A-SF6-245 (Table 2). The lowest values, without significant difference between them were produced by clones P-311G, A-SF-40, A-SF4-31, P-360G, and P-769G, containing 5.39, 5.61, 5.69, 5.76, and 5.81% of SM, respectively.

The highest levels of DDS were found in clones A-SF-40 and P-311G (9.80 and 8.79%, respectively). This finding may be of interest to jojoba breeders, as usually level of SM in jojoba meal is higher then its analogue DDS. It is difficult to speculate how relevant is this phenomenon, as there are no published data available on comparison of SM and DDS level in different genetic material.

Finally, SF levels were also significantly different with highest SF content for P-360G clone (1.91%) and lowest for A-SF-40 (0.82%).

Table 2
Total liquid wax, simmondsin (SM), didemethylsimmondsin (DDS) and simmondsin ferulate (SSF) content

| Clone | Wax (% of seed weight) | SM (% of defatted meal) | DDS (% of defatted meal) | SSF (% of defatted meal) |
|---|---|---|---|---|
| P-360G | 58.75[a] | 5.76 | 3.78 | 1.91 |
| P-749G | 56.75 | 6.69 | 4.96 | 1.23 |
| P-311G | 56.75 | 5.39 | 8.79 | 0.86 |
| P-323G | 55.90 | 7.46 | 7.68 | 1.90 |
| A-SF4-31 | 55.05 | 5.69 | 5.21 | 1.58 |
| P-769G | 54.90 | 5.81 | 5.33 | 1.67 |
| A-SF5-151-3 | 54.30 | 6.31 | 3.54 | 1.46 |
| A-SF5-121 | 50.80 | 9.51 | 7.89 | 0.84 |
| A-SF-40 | 50.70 | 5.61 | 9.80 | 0.82 |
| A-SF6-245 | 50.50 | 10.20 | 4.70 | 0.85 |

[a] Mean within a column lacking a common subscript differ ($P < 0.05$) according to Duncan's Multiple Range Test.

Table 3
Comparison between plants of the same clone: A-SF6-245 and A-SF5-151

| Clone | Wax (% of seed weight) | SM (% of defatted meal) | DDS (% of defatted meal) | SSF (% of defatted meal) |
|---|---|---|---|---|
| *A-SF5-151* | | | | |
| P-5 | 54.8[a] | 5.27 | 3.80 | 1.85 |
| P-1 | 54.3 | 6.31 | 3.56 | 1.46 |
| P-3 | 52.8 | 4.90 | 3.97 | 1.49 |
| P-2 | 52.8 | 5.08 | 3.87 | 1.37 |
| *A-SF6-245* | | | | |
| P-6 | 50.6 | 10.8 | 7.19 | 0.77 |
| P-4 | 50.5 | 10.3 | 7.68 | 0.85 |
| P-7 | 49.2 | 10.5 | 6.52 | 0.82 |
| P-3 | 49.1 | 10.7 | 6.83 | 0.96 |

[a] Mean within a column (between different clones) lacking a common subscript differ ($P<0.05$) according to Duncan's Multiple Range Test.

Table 4
Comparison between years of the same clone: A-SF-40

| Year | Wax (% of seed weight) | SM (% of defatted meal) | DDS (% of defatted meal) | SSF (% of defatted meal) |
|---|---|---|---|---|
| 1996 | 49.50[a] | 5.69 | 8.46 | 0.82 |
| 1997 | 50.70 | 5.62 | 9.80 | 0.67 |

[a] Mean within a column lacking a common subscript differ ($P<0.05$) according to Duncan's Multiple Range Test.

Two Argentine clones: A-SF6-245 and A-SF5-151 were selected for comparison of wax, SM, DDS and SF levels between plants of the same clone. There was no significant difference in wax content, for either clone. However, significant differences in levels of SMs were observed (Table 3). For both clones, SM content was higher than DDS in all tested plants. These data might indicate, that the reverse pattern observed for A-SF-40 and P-311G may be related to genetic differences rather than environmental factors.

No significant differences ($P<0.05$) in wax content were detected between seeds of the same clone, when different growing seasons were compared (Table 4). This lack of change in wax level between years is different from previous findings (Ayerza, 1990b) for plants growing on the slopes of Pocho Mountains, Cordoba, Argentina. As climatic conditions are known to influence oil content of oilseed (Talha and Osman, 1975; Unger, 1980; Ayerza, 1995). Lack of difference in wax content between 1996 and 1997 growing seasons may reflect very similar weather patterns during these 2 years, contrary to previous conditions.

No difference in SM content was detected between seeds from the same clone between years (Table 4). However, DDSP and SFP were significantly different ($P<0.05$) between years. There is not a direct explanation for these results, but the differences within SM compounds relations between years suggest the three SM compounds could be influenced differently by some environmental factors.

In summary, the wax content was negatively correlated ($P<0.05$) with SM and DDS percent-

Table 5
Correlation coefficients

| Trial pairs | Coefficient[a] |
|---|---|
| Oil vs. % total simmondsin | −0.76 |
| Oil vs. % didemethylsimmondsin | −0.61 |
| Oil vs. % ferulate simmondsin | 0.42 |

[a] Significant at $P<0.05$.

age, but positively correlated with SSF content (Table 5). This indicates the importance of selection specific jojoba clones for SM production, which is a different objective from high levels of wax production in jojoba seed. Additional studies should be conducted to determine if environmental influences on the formation of SMs could be separated from the genetic aspects. The CE method for cost efficient and precise determination of SM and its analogues in jojoba seed may contribute to rational selection of genetic material and explain the influence environmental factors on plant biosynthesis.

## 4. Conclusions

CE is a powerful analytical technique based on physical size and charge of analyzed molecules. CE has recently emerged as a convenient technique for natural products because of its increased separation efficiency compared to HPLC. Major active components in jojoba meal were detected and quantified using CE methodology. Inter-laboratory study, conducted recently (Abbott et al., 2000), proved that this method presents a novel, versatile tool for jojoba breeding programs, quality control in processing of jojoba meal and for meal analysis applied to animal feeding studies.

## References

Abbott, T.P., Flo, G., Frank, L., Holser, R.A., Kolodziejczyk, P.P., York, D., Nelsen, T., 2000. Interlaboratory comparison of simmondsin analysis. Ind. Crops Prod 12, 209–213.

Ayerza, R.H., 1990. Variability of oil content in Jojoba [Simmondsia chinensis L. (Schneider)] seeds harvested in the Sonoran Desert. In: The 8th International Conference on Jojoba and Its Uses, Asuncion, Paraguay, June 17–22. Latin America Jojoba Association, 1990, p. 11.

Ayerza, R.H., 1990. The potential of jojoba [Simmondsia chinensis L. (Scheider)] in the arid Chaco: rooting capacity and seed and wax yield. In: Proceedings of the First International Conference on New Industrial Crops and Products, Riverside, California, USA, Office of Arid Lands Studies, The University of Arizona, pp. 41–46.

Ayerza, R.H., 1995. Oil content and fatty acid composition of chia (Salvia hispanica) from five Northwestern locations in Argentina. J. Am. Oil Chem. 72 (9), 1079–1081.

Flo, G.E., Van Boven, M., Vermayt, S., Decuypere, E., Daenes, P., Cokelaere, M.M., 1998. Effect of simmondsin derivatives on food intake: dose-response curves. J. Agric. Food Chem. 46, 1910–1913.

Kolodziejczyk, P.P., Wang, X., Marianchuk, M., Wang, L., Lu, W., Aramowicz, R., 1998. Phenolics in oilseeds: capillary electrophoresis as an alternative analytical method. In: Sanchez, J., Cerda-Olmedo, E., Martinez-Force, E. (Eds.), Advances in Plant Lipid Research. Proceedings of the 13th International Symposium on Plant Lipids, Sevilla, Spain, July 1998, pp. 31–33.

Kolodziejczyk, P.P., Wang, X., Marianchuk, M., Lu, W., Amarowicz, R., 1999. Phenolics in rapeseed:capillary electrophoresis as a novel analytical method for detection of sinapine, sinapic acid esters and ferulates. In: Lot, L., Pouzet, A. (Eds.), New Horizons for an Old Crop. Proceedings of the 10th International Rapeseed Congress, Canberra, Australia, paper 479.

Nelson, J., Palzkill, D., 1999. Evaluation of Jojoba Clonal Cultivars in Central Arizona. In: 1999 Annual Meeting of the Association for the Advancement of Industrial Crops, Eugene, Oregon, USA, October 17–21, 1999, p. 50.

Official Methods and Recommended Practices of the AOCS, 1998, Champaign.

Talha, M., Osman, F., 1975. Effect of soil water stress on water economy and oil composition in sunflower (*Helianthus annus* L.). J. Agric. Sci. 84, 49–56.

Unger, P.W., 1980. Planting date effects on growth, yield, and oil of irrigated sunflower. Agron. J. 72, 914–916.

Van Boven, M., Toppet, S., Cokelaere, M.M., Daenens, M., 1994a. Isolation and structural identification of a new simmondsin ferulate from jojoba meal. J. Agric. Food Chem. 42, 1118–1121.

Van Boven, M., Daenens, P., Cokelaere, M.M., Jansen, G., 1994b. Isolation and structure elucidation of the major simmondsin analogues in jojoba meal by two-dimentional NMR spectroscopy. J. Agric. Food Chem. 42, 2684–2687.

Van Boven, M., Daenens, P., Cokelaere, M., 1995. New simmondsin 2′-ferulates from jojoba meal. J. Agric. Food Chem. 43, 1193–1197.

Van Boven, M., Daenens, P., Tygat, J., Cokelaere, M., 1996. Determination of simmondsins and simmondsin ferulates in jojoba meal and feed by high-performance liquid chromatography. J. Agric. Food Chem. 44, 2239–2243.