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Simmondsins quantification by spectrophotometry UV–vis in press cake and in jojoba seeds (*Simmondsia chinensis* (Link) Schneider), from Argentina

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ABSTRACT

In this paper, we describe a simple, fast, and efficient method for the quantification of simmondsins and simmondsin's ferulates in jojoba seeds and residual cakes, without using expensive equipment or laborious techniques. Spectrophotometric measurements (UV–vis) were used for the quantification of the content of simmondsins, total ferulates and total simmondsins in jojoba seeds and residual cakes from the provinces of Catamarca and La Rioja, Argentina. The average values of such components found in seeds, in 100 g of dry and defatted sample, were 11.54 ± 0.91 and 2.24 ± 0.28 g of simmondsins and of simmondsin's ferulates, respectively, whereas the respective values in residual cakes were 10.08 ± 0.31 and 2.93 ± 0.64 g, respectively. A great diversity between the simmondsin contents and their ferulates in seed samples of different origins as well as in different clones was observed. The residual cakes did not present differences respect to the simmondsin contents; however, there were some difference with the simmondsin ferulates. These variations are due to the genetic antecedents of plants and the environmental conditions in which they grow.

1. Introduction

Argentina has become a leader in jojoba (*Simmondsia chinensis*) seed production, with 43% of the world's production (Ayerza, 1990; Ladux and Ortiz, 1999). Jojoba seeds and/or residual cakes, which originate after seeds have been pressed for oil extraction, represent a potential amendment for animal feeds. Once defatted, the major constituents are proteins (from 28 to 30%), and carbohydrates (50% are pentose); there is a smaller amount of minerals, vitamins, and a series of anti nutritional components related to 2-(cianomethylen)-3-hidroxy-4,5-metoxyciclohexyl,β-D-glucoside, commonly denominated simmondsins (Holser and Abbott, 1999; Kolodziejczyk et al., 2000).

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At the moment seven simmondsin derivates have been identified and quantified, these include: simmondsins, 5-demethylsimmondsins, 4-demethylsimmondsins, didemethylsimmondsins, simmondsin 2′-*trans*-ferulates, 5-demethylsimmondsins 2′-*trans*-ferulates, and 4-demethylsimmondsins 2′-*trans*-ferulates (van Boven et al., 1994a,b; Kolodziejczyk et al., 2000; Benzioni et al., 2005). When experimental monogastric animals are fed with food containing simmondsin, a decreased appetite is observed and when they are given higher doses for a long time they die of starvation (Verbiscar and Banigan, 1978; Verbiscar et al., 1980; Flo et al., 1997; Kolodziejczyk et al., 2000). It has been established that only simmondsin and simmondsin 2′-ferulate produce reduction in food intake and can thus serve as a dietary modifier (Cokelaere et al., 1996; Benzioni et al., 2005), for this reason these compounds are considered as anti-nutritional components. The objective of the present paper was to develop a simple method for the quantification of simmondsin contents (SMs), total ferulates (Fs) and total simmondsins (SMT) in residual cakes and jojoba seeds. The research was carried out with samples taken from the provinces of Catamarca and La Rioja, Argentina.

2. Experimental

2.1. General experimental procedures

UV spectra were obtained in a DU-7000 spectrophotometer, a 1-cm-width optic-passage cell was utilized. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 (^{1}H) and 50.32 (^{13}C) MHz. Chemical shifts are given in ppm (δ) downfield from TMS internal standard. The solvent used was $CDCl_3$:CD_3OD (95:05). Chemical shifts and multiplicity determinations were obtained by using standard Bruker software.

Chromatographic separations were performed by column chromatography on Si gel 60 (0.063–0.04 mm) and preparative TLC on Si gel 60 F_{254} (0.2 mm thick) plates.

2.2. Plant material

The seeds used for the characterization of Fs and SMs by RMN were obtained during the 2002 campaign in the Agrinsa Agro Industrial S.A. farm, located in Bañado de los Pantanos, Aimogasta, La Rioja. This region is located at 67°14′W longitude, 28°36′S latitude, and at 865 m above the sea level. The seeds were ground with a blender until a material of homogenous size was obtained.

The samples used for the quantification of SMs, Fs and SMT by spectroscopy UV–vis were classified according to type and origin in residual cakes (collected in the wax extraction process), *clone (**clone*: seeds from plant obtained through vegetative reproduction of the original plant adapted to the geographic zone studied (Ayerza, 1990)) seeds (clones 310; 206; Marcelo and Dora) and set of clone seeds (LR and C), as listed bellow:

- *RC laboratory*: residual cakes obtained from seeds of the 2003 harvest on Agrinsa Agro Industrial S.A. farm, La Rioja, processed through cold pressing with a hydraulic press made of stainless steel at laboratory scale.
- *RC Expeller 2003*: residual cakes obtained from seeds of the 2003 harvest on Agrinsa Agro Industrial S.A. farm, La Rioja, processed in an expeller-like press at industrial scale.
- *RC Expeller 2002*: residual cakes obtained from seeds of the 2002 harvest on Agrinsa Agro Industrial S.A., farm, La Rioja, processed in an expeller-like press at industrial scale.
- *LR 2003*: set of clones of jojoba seeds of the 2003 crop from Agrinsa Agro Industrial S. A. farm, La Rioja.
- *C 2003*: set of clones of jojoba seeds from the 2003 crop on Jocat S.A. farm, Catamarca.
- *Clone 310 LR 2003*: clone of jojoba seeds, from 6000 plants, from the 2003 crop on Agrinsa Agro Industrial S.A. farm, La Rioja.
- *Clone 206 LR 2003*: clone of jojoba seeds, from 6000 plants, from the 2003 crop on Agrinsa Agro Industrial S.A. farm, La Rioja.
- *Clone Marcelo C 2003*: clone of jojoba seeds, from 8400 plants, from the 2003 crop on Jocat S.A. farm, Catamarca.
- *Clone Dora C 2003*: clone of jojoba seeds, from 8400 plants, from the 2003 crop on Jocat S.A. farm, Catamarca.

2.3. Preparation of the pattern sample of simmondsins and derivates

As pattern sample, we used a mixture of SMs and Fs extracted and purified from 10 g of defatted jojoba seeds taken randomly from the 2002 harvest, according to Kolodziejczyk et al. (2000) procedure; 1.3 g were obtained from the mixture of SMs and Fs with purity higher than 95% established by nuclear magnetic resonance.

2.4. Extraction of SMT from vegetal material

In order to perform the extraction of SMs and Fs, the samples (50 g) were processed according to Abbott et al. (1999), i.e., ground, sifted (mesh no. 40), defatted (through Soxhlet with *n*-hexane during 12 h) and extracted with methanol (3× 50 mL) through shaking at room temperature during 30 min. The resulting extracts were concentrated at reduced pressure (40–50 °C), dissolved with methanol HPLC grade and taken to a final volume of 1 mL.

2.5. Spectrophotometric measurement UV–vis

The stock solution (0.022 mg/mL) was prepared from the pattern sample of SMs and Fs in methanol. Different aliquots of stock solution were used to obtain six calibration standards in concentration range from 0.008 to 0.020 mg/mL. The calibration curves were performed at two wavelengths, 220 and 323 nm.

2.6. Statistical analyses

Determinations were performed in triplicate. Data were statistically analyzed with Infostat program (2002). Statistical differences between the samples were determined through ANOVA test at the 5% level ($\alpha \leq 0.05$) of significance for all

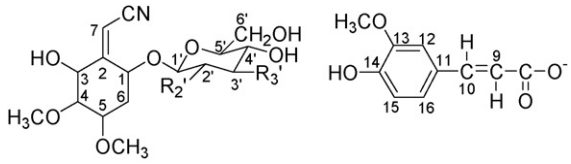

Fig. 1 – Structure of simmondsin and ferulate.

parameters evaluated. Whenever ANOVA indicated significant difference, comparison of means by least significant difference (LSD) was carried out. Correlation analysis was performed employing Pearson's test. The SMs content was calculated by the difference between SMT and Fs. The correlation equations were performed by minimal-square linear regression.

3. Results and discussion

The RMN-^{1}H spectrum of the pattern sample showed the characteristic profile for an analog mixture of SMs with purity higher than 95%. In such mixture there are two major components, simmondsin and simmondsin ferulates, with a 2:1 relation. The most representative signals that allowed us to determine the general composition are based on signals observed at δ 5.72 *d* (2.2 Hz) and δ 5.74 *d* (1.8 Hz), assigned to H-7, and the signals at δ 4.39 *d* (8.0 Hz), and δ 4.58 *d* (7.3 Hz) assigned to H-1′. Both main components are metoxylated by the signals observed at δ 3.42 *s*, 3.44 *s*, 3.47 *s*, and 3.49 *s*, assigned to metoxyl groups at positions C-4 and C-5, respectively (Fig. 1). The presence of the ferulic acid was determined according to the signals observed at δ 6.33 *d* (15.7 Hz), δ 7.64 *d* (15.7 Hz), δ 6.89 *d* (8.4 Hz), and δ 3.92 *s* assigned to H-9, H-10, H-15, and OCH_3 in position C-13, respectively, and two signals overlapped centered at 7.07 ppm assigned to H-12 and H-16, (Fig. 2). The RMN-^{13}C spectrum was in good agreement with the mixture composition mentioned above.

The data obtained from the analysis of the RMN-^{1}H and ^{13}C spectra were identical with the published data for SMs and Fs (van Boven et al., 1994a,b; Lein et al., 2002).

The calibration curves were obtained at 220 and 323 nm, corresponding to the maximum absorption wavelengths for the components to be quantified (Kolodziejczyk et al., 2000). Since Fs absorb at both wavelengths, and SMs only absorb at 220 nm, through the difference between the values recorded at 220 nm and 323 nm, SMs quantities were determined.

$$\text{SMT} \quad \text{Abs}_{220} = 40.86X_{(g/l)} \quad R^2 = 0.986$$

$$\text{Fs} \quad \text{Abs}_{323} = 24.43X_{(g/l)} \quad R^2 = 0.994$$

SMs and SMT values obtained from residual cake and jojoba seed extracts, through the technique described before, are the detailed in Table 1. As we can observe in this table, there are differences between the SMs, Fs and SMT contents of the analyzed samples, which are statistically significant. Residual cakes presented differences among them regarding to the Fs and SMT contents but not to the SMs. When analyzing "the sets of seed clones", significant differences were found not only among SMs, Fs and SMT contents but also among the places of origin, La Rioja presented the highest values for each variable.

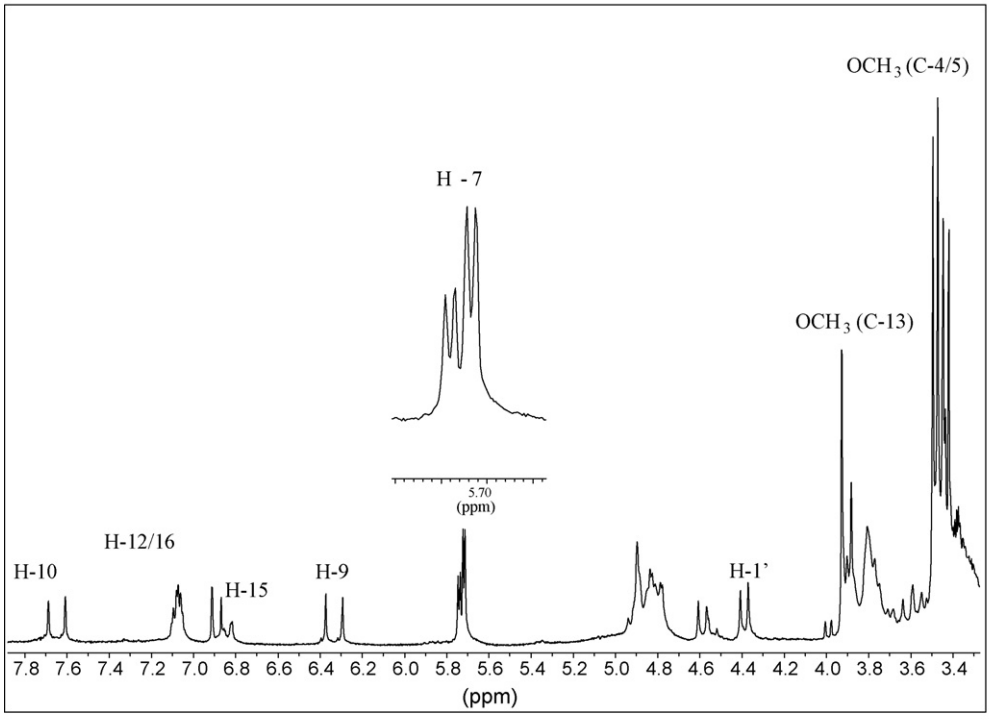

Fig. 2 – RMN-^{1}H spectrum of pattern sample of simmondsins and derivates in 3–8 ppm region.

Table 1 – Simmondsins (SMs), ferulates (Fs) and total simmondsins (SMT) content in residual cakes and jojoba seeds (values expressed as g/100 g of defatted dry sample)

Type of sample	Samples	SMs	Fs	SMT
Residual cakes	RC Laboratory 2003	9.51 ± 0.45a	3.50 ± 0.13b	13.01 ± 0.32ab
	RC Expeller 2003	9.51 ± 0.45a	4.53 ± 0.14c	15.04 ± 0.21b
	RC Expeller 2002	9.51 ± 0.45a	1.00 ± 0.01a	10.96 ± 0.76a
ANOVA		n.s.	$P \leq 0.001$	$P \leq 0.05$
Set of clones	LR 2003	13.35 ± 0.27b	2.37 ± 0.06b	15.72 ± 0.22b
	C 2003	9.55 ± 0.04a	2.04 ± 0.03a	11.59 ± 0.07a
ANOVA		$P \leq 0.01$	$P \leq 0.05$	$P \leq 0.01$
Clones	Clone 310 LR 2003	17.21 ± 0.11d	0.96 ± 0.09a	18.17 ± 0.02b
	Clone 206 LR 2003	14.38 ± 0.93c	1.44 ± 0.33a	15.82 ± 1.26b
	Clone Marcelo C 2003	10.00 ± 0.47b	2.60 ± 0.38b	12.60 ± 0.09a
	Clone Dora C 2003	7.11 ± 0.09a	4.31 ± 0.04c	11.42 ± 0.05a
ANOVA		$P \leq 0.001$	$P \leq 0.01$	$P \leq 0.01$

Reference: medium value ± standard error ($n = 3$). n.s.: not significant. LR: samples from the province of La Rioja. C: samples from the province of Catamarca. For each type of sample: different letters within the same column indicate significant differences.

Clones also presented significant differences among the analyzed variables, the highest SMs content was found in clone 310 LR 2003 from La Rioja; regarding the Fs, lowest values were observed in the clones from La Rioja, on the other hand, clones from Catamarca presented significant differences among them. As far as SMT is concerned, clones from La Rioja presented the highest values.

The variance analysis results among the variables analyzed for all the samples indicated significant differences ($P \leq 0.001$).

A correlation analysis was performed among the variables of the studied clones, a negative correlation between the simmondsin contents and the simmondsin ferulates was found ($P \leq 0.05$), and these results are in agreement with the ones obtained by Kolodziejczyk et al. (2000). The authors mentioned above analyzed and compared seed clones from Perú and Argentina, the average values obtained and expressed as g/100 g of defatted sample were 12.33 ± 1.02 for SMs, and 1.51 ± 0.20 for Fs in the ones from Argentina. Similar results were found in the present work for clones from Argentina (12.14 ± 1.48 for SMs and 2.36 ± 0.50 for Fs) and higher than the ones found by Lein et al. (2002) (SMs 8.54 g/100 g of sample A and 7.49 g/100 g of sample B). With respect to Cappillino et al. (2003), they informed the greatest values of SMT (22.7 g/100 g of sample) in seeds from Chile.

We can conclude that the samples present a great diversity in the contents of SMs, Fs, SMT. After analyzing the literature and according to the results obtained in the present work, we can assert that the variation in such contents among the different clones is due to the genetic antecedents of the plants, the climatic conditions, and also to agro management in which they grow (Ayerza, 1996; Kolodziejczyk et al., 2000; Benzioni et al., 2005).

The proposed method allows SMs, Fs, and SMT quantification but active and inactive derivatives are not distinguished. However, it is well established that the main components of jojoba meal are the biologically active components (Cokelaere et al., 1996). This simple, fast, economic, and reproducible method allows to determine the maximum possible amount of active metabolites. High levels of SMT would lead to its separation to be employed as pharmaceuticals. On the contrary, low SMT concentrations would lead to be used as ingredient protein for animal food.

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REFERENCES

Abbott, T.P., Holser, R.A., Plattner, B.J., Plattner, R.D., Purcell, H.C., 1999. Pilot-scale isolation of simmondsin and related jojoba constituents. Ind. Crops Prod. 10, 65–72.

Ayerza, R. (h), 1990. La Jojoba. Ecología, manejo y utilización. Orientación Gráfica Editora. Buenos Aires, Argentina.

Ayerza, R., 1996. Evaluation of eight jojoba clones for rooting capacity, plant volume, seed yield and wax quality and quantity. In: Princen, L.H., Rossi, C. (Eds.), Proceedings of the Ninth International Conference on Jojoba and its Uses, and of the Third International Conference on New Industrial Crops and Products. 25–30 September 1994, Catamarca, Argentina, pp. 1–3.

Benzioni, A., Mills, D., van Boven, M., Cokelaere, M., 2005. Effect of genotype and environment on the concentration of simmondsin and its derivatives in jojoba seeds and foliage. Ind. Crops Prod. 21, 241–249.

Cappillino, P., Kleiman, R., Botti, C., 2003. Composition of *Chilean jojoba* seeds. Ind. Crops Prod. 17, 177–182.

Cokelaere, M., Flo, G., Daenens, P., Decuypere, E., van Boven, M., Vermaut, S., 1996. Food intake inhibitory activity of

simmondsin and defatted jojoba meal: dose–response curves in rats. In: Janick, J. (Ed.), Progress in New Crops. ASHS Press, Arlington, VA, pp. 377–382.
Flo, G., Vermaut, S., van Boven, M., Daenens, P., Decuypere, E., Pedersen, M., Cokelaere, M., 1997. Growth performance of rats fed jojoba proteins. Possible correlations with trypsin inhibitory activity in jojoba proteins. J. Agric. Food Chem. 45, 4384–4387.
Holser, R., Abbott, T., 1999. Extraction of simmondsins from defatted jojoba meal using aqueous ethanol. Ind. Crops Prod. 10, 41–46.
Infostat, 2002. Facultad de Ciencias Agropecuarias, UNC. Version 1.1.
Kolodziejczyk, P.P., Lu, W., Ayerza, R., Larminat, M.A., 2000. Capillary electrophoresis: novel tool for simmondsins analysis and its applications to jojoba breeding. Ind. Crops Prod. 12, 193–202.
Ladux, J.L., Ortiz, J.M., 1999. El Cultivo de Jojoba en La Rioja y Catamarca. In: CRILaR (Ed.), Resúmenes de la jornada de ciencia y técnica para cultivos de jojoba en La Rioja y Catamarca, 10 de Septiembre de 1999, Anillaco, Argentina. 19.
Lein, S., van Boven, M., Holser, R., Decuypere, E., Flo, G., Liveins, S., Cokelaere, M., 2002. Simultaneous determination of carbohydrates and simmondsins in jojoba seed meal (*Simmondsia chinensis*) by gas chromatography. J. Chromatogr. A 977, 257–264.
van Boven, M., Toppet, S., Cokelaere, M.M., Daenens, P., 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., Janssen, G., 1994b. Isolation and structure elucidation of the major simmondsin analogues in jojoba meal by two dimensional NMR spectroscopy. J. Agric. Food Chem. 42, 2684–2687.
Verbiscar, A.J., Banigan, T.F., 1978. Composition of jojoba seed and foliage. J. Agric. Food Chem. 26, 1456–1459.
Verbiscar, A.J., Banigan, T.F., Weber, C.W., Reid, B.L., Trei, J.E., Nelson, E.A., Raffauf, R.F., Kosersky, D., 1980. Detoxification of jojoba meal. J. Agric. Food Chem. 28, 571–578.