

Seed compositional studies of some species of Papilionoideae (Leguminosae) native to Argentina

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Abstract: Seeds of 17 wild leguminous species belonging to the Papilionoideae subfamily were analysed for their proximate, fatty acid and sterol compositions. *Centrosema virginianum*, *Tipuana tipu*, *Adesmia volckmanni* and some species of *Desmodium* contained high amounts (>300 g kg⁻¹) of protein. *Geoffroea decorticans* and *Clitoria cordobensis* were noteworthy for their high oil content (>350 g kg⁻¹). The seed lipids had a high proportion of unsaturated (oleic and linoleic mainly) fatty acids. Linolenic acid had the highest value in *Adesmia volckmanni* (25.4% of total fatty acids). β -Sitosterol was the major component of the sterol fraction. Chemical analyses indicated that all species studied are suitable sources of animal feed.

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Keywords: proximate composition; fatty acids; sterols; seeds; feed; Papilionoideae

INTRODUCTION

The Leguminosae are widely cultivated and are usually recognised as one of the most important crop families. A variety of leguminous seeds from wild species feature quite prominently in the diet of rural communities in many developing countries.

Among the leguminous plants growing in Argentina, certain species of the subfamily Papilionoideae are important vegetation elements in arid and semiarid regions, where many of them have provided food for man and his animals since ancient times. In particular, some species from the genera *Geoffroea*, *Tipuana*, *Indigofera*, *Desmodium*, *Galactia*, *Centrosema*, *Clitoria*, *Aeschynomene*, *Stylosanthes*, *Adesmia*, *Lathyrus* and *Crotalaria* have leaves, fruits and seeds that are considered to be good feed for ruminant animals. However, little is known about their chemical composition.^{1–3}

In evaluating the nutritional quality of seeds, protein and oil contents as well as fatty acid and sterol compositions of the oil occupy a special place. To date, there have been no studies of the presence of these seed components in Argentinean species of Papilionoideae. The broad objective of our programme is to obtain new chemical information for characterising the

native species of Leguminosae used as feed for ruminants. This paper reports the results of proximate and oil analyses of some species from the genera mentioned above. Since these wild plants grow abundantly in poor, developing areas, they could possibly become a good, low-cost food for domestic animals.

MATERIALS AND METHODS

Plant materials

Mature seeds from 17 species of the Papilionoideae (Leguminosae) subfamily were used in this study. Three seed samples (10 g each approximately) from each species were analysed. Details of plant materials are given in Table 1. Voucher specimens are deposited at the Herbarium of the Instituto de Recursos Biológicos, INTA, Castelar (BAB).

Proximate analysis

Moisture, fat, nitrogen and ash contents were determined according to AOAC methods.⁴ Protein content was calculated as mg % N \times 6.25.⁵ Carbohydrate content was calculated by difference.⁶

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Table 1. Collection data from studied species

Species	Code	Population site
<i>Geoffroea decorticans</i> (Hook et Arn) Burkart	GD	Córdoba: Túneles de San Juan
<i>Tipuana tipu</i> (Benth) Kuntze	TT	Córdoba: Capital
<i>Indigofera parodiana</i> Burkart	IP	Córdoba: Río Tercero
<i>Indigofera suffruticosa</i> Mill	IS	Bolivia: 5 km from Coroico
<i>Desmodium cuneatum</i> Hook et Arn	DC	Córdoba: Villa la Bolsa
<i>Desmodium tortuosum</i> (Sw) DC	DT	Córdoba: Parador El Condor
<i>Desmodium neo-mexicanum</i> A Gray	DN	Córdoba: Mina Clavero
<i>Desmodium subsericeum</i> Malme	DS	Salta: Quebrada del Toro
<i>Galactia latisiliqua</i>	GL	Córdoba: Capital
<i>Centrosema virginianum</i>	CV	Córdoba: Copina
<i>Clitoria cordobensis</i> Burkart	CC	Córdoba: Agua de Oro
<i>Aeschynomene rudis</i> Benth	AR	Bolivia: La Paz
<i>Stylosanthes montevidensis</i> Vogel	SM	Córdoba: Camino a Altas Cumbres
<i>Stylosanthes gracilis</i> Kunth	SG	Córdoba: Río Tercero
<i>Adesmia volckmanni</i> Phil	AV	Neuquén: Junín de los Andes
<i>Lathyrus magellanicus</i> Lam var <i>glaucescens</i> Speg	LM	Tierra del Fuego
<i>Crotalaria anagyroides</i> Kunth	CA	Salta: Rosario de Lerma

Oil extraction

Approximately 5 g of seeds from each sample were extracted with *n*-hexane in a Soxhlet apparatus. The extracted lipids were dried over anhydrous sodium sulphate and the solvent was removed by vacuum distillation at 40°C.

Fatty acid and sterol analyses

The fatty acid methyl esters (FAMEs) of oils were prepared and analysed by gas chromatography according to Maestri *et al.*⁷ The identification of the compounds was carried out by CG-MS⁸ and by comparison of the retention times with those of reference compounds. The oils and FAMEs were examined for the presence of hydroxy and epoxy fatty acids by IR. The IR spectra were obtained in KBr discs.⁸ Iodine values (IVs) of oils were calculated from fatty acid percentages.⁹

The sterols were separated from the unsaponifiable material of the oils, purified and fractionated accord-

ing to Maestri and Guzmán¹⁰ and Lamarque *et al.*¹¹ The identity of each sterol was confirmed by GC and GC-MS operating as described previously.¹¹

RESULTS AND DISCUSSION

The seed proximate composition is presented in Table 2. Total protein contents varied between 159 and 446 g kg⁻¹, with a mean value of 304 g kg⁻¹. *Tipuana tipu*, *Centrosema virginianum* and *Adesmia volckmanni* had the highest protein contents. The smallest values were observed in *Stylosanthes montevidensis* and *S gracilis*; values were similar to protein contents in other Leguminosae species.^{5,12,13} Total ash contents ranged between 23 g kg⁻¹ in *Clitoria cordobensis* and 49 g kg⁻¹ in *A volckmanni* and fell within the range reported for other leguminous seeds.^{5,8,14} As usual for the proximate composition in plants, there was a wide variation in oil content (62–472 g kg⁻¹). High oil contents were found in seeds of *Geoffroea decorticans*,

Species code	Moisture	Oil	Protein	Ash	Carbohydrate
GD	82±2.0	472±5.4	216±1.5	29±1.0	201
TT	93±4.0	291±4.2	427±9.1	31±2.0	158
IP	107±3.1	68±2.1	242±1.2	41±4.1	542
IS	131±2.1	126±7.7	252±6.5	32±1.0	459
DC	86±3.3	110±2.2	347±4.4	42±2.2	415
DT	94±1.1	82±1.5	329±9.0	39±1.9	456
DN	112±5.2	126±9.2	295±2.0	34±0.8	433
DS	114±8.4	99±3.2	332±7.3	35±0.9	420
GL	105±2.2	62±3.1	335±5.1	32±1.2	466
CV	37±2.0	70±3.0	425±8.1	37±1.1	431
CC	141±5.2	363±4.0	304±5.3	23±1.9	169
AR	119±3.5	163±3.0	300±8.1	39±2.5	379
SM	109±7.2	144±2.6	159±9.8	39±2.8	549
SG	92±3.1	151±4.3	199±8.7	38±2.6	520
AV	85±3.4	178±7.4	446±9.5	49±3.7	242
LM	26±1.1	150±8.5	244±7.5	32±2.1	548
CA	136±5.1	92±5.0	317±2.4	32±1.3	423

Table 2. Seed proximate composition (g kg⁻¹ dry matter) from studied species (mean value±SD, n=3)

T tipu and *C cordobensis*. These species can be classified as high-yielding and promise to be a good oil source from a quantitative point of view.

To our knowledge the present paper is the first report on the fatty acid composition of seed oils from the studied species. The FAME composition of seed oils is shown in Table 3. Twelve fatty acids were identified and their concentrations were in the range of those described in the literature for other Leguminosae seed oils.^{8,11,15-17} There were no unusual fatty acids, as indicated by IR and GC-MS analyses. Typically, high proportions of linoleic (C18:2) and oleic (C18:1) acids were observed in most species. The highest linoleic acid concentrations (as percentage of total fatty acids) were found in *T tipu*, *Indigofera parodiana* and *Lathyrus magellanicus*, whereas *G decorticans*, *C virginianum* and *C cordobensis* had the largest amounts of oleic acid. Present results show that the FA composition of the last three species is different from those reported for most Leguminosae seeds, which show lower oleic/linoleic (O/L) acid ratios.^{8,16,18} Linolenic acid (C18:3) had high values (>10%) in *Desmodium subsericeum*, *A volckmanni* and species of the tribe Phaseoleae (*Galactia latisiliqua* and *C virginianum*). Saturated acids were mainly composed of palmitic (C16:0) (7.2–20.6%) and stearic (C18:0) (1.3–6.5%) acids. Furthermore, some species contained substantial amounts of behenic (C22:0) acid (*D subsericeum*, *S montevidensis* and *S gracilis*). The presence of large amounts of long-chain saturated fatty acids is unusual for commonly used vegetable oils. Only peanut oil is known to contain up to 6% long-chain saturated fatty acids.^{19,20}

The studied species varied considerably in the iodine value of their oils (from 94.3 in *C virginianum*

to 157.4 in *A volckmanni*). Most of these oils can be placed into the semi-drying class.

The sterol composition is listed in Table 4. All seed oils examined contained seven 4-desmethylsterols identified as cholesterol, campesterol, stigmaterol, β -sitosterol, Δ^5 -avenasterol, Δ^7 -stigmastanol and Δ^7 -avenasterol. The GC-MS analyses showed C29 sterols (β -sitosterol and stigmaterol) as the major sterols, a predominance that is usual in plant sterol composition. In all species studied, β -sitosterol predominated and its amount represented 28.2–77.9% of the total sterol content. Cholesterol was present in comparatively small quantities (tr–1.9%), similar to some Leguminosae previously investigated.^{15,18,21}

From the results of the present investigation it appears that seeds from some species analysed are a valuable source of oil and protein, comparable in quantity to those of some edible plant species. Because of their high protein content and taking into account their value as animal feed, the seeds from *C virginianum* and *Desmodium* spp could be used as a protein supplement to low-protein feeds such as cereal grains.

On the other hand, the seeds from *G decorticans* and *C cordobensis* represent a neglected, potential source of oil. The high levels of mono- and di-unsaturation of their oils are similar to that of any other edible oil, such as sunflower, rapeseed and peanut oils.^{20,22} A recent trend to improve the efficiency of animal production is to give ruminants feeds which contain fat supplements.²³ Accordingly, the mentioned species may be a good source of oil and essential unsaturated fatty acids.

Finally, the analytical results discussed in this paper will serve as a basis to perform new experiments in animals fed with these native legume seeds.

Table 3. Fatty acid composition (% of total fatty acids), iodine value (IV) saturated/unsaturated (S/U) and oleic/linoleic (O/L) acid ratios from seed oils of the studied species (mean value \pm SD, n=3)

Species code	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	IV	S/U	O/L
GD	tr ^a	7.2 \pm 0.2	ND ^b	4.3 \pm 0.1	53.7 \pm 2.0	30.7 \pm 0.9	ND	1.5 \pm 0.02	1.2 \pm 0.01	1.3 \pm 0.03	ND	tr	104.9	0.17	1.75
TT	tr	9.9 \pm 0.3	tr	4.5 \pm 0.2	15.9 \pm 0.5	62.8 \pm 2.1	0.4 \pm 0.02	2.6 \pm 0.05	1.0 \pm 0.04	2.1 \pm 0.09	0.1 \pm 0.01	0.6 \pm 0.03	130.2	0.24	0.25
IP	tr	14.1 \pm 0.5	tr	6.2 \pm 0.2	6.0 \pm 0.3	62.1 \pm 2.2	9.3 \pm 0.5	1.1 \pm 0.01	tr	0.5 \pm 0.02	ND	0.6 \pm 0.04	143.5	0.29	0.10
IS	tr	12.6 \pm 0.4	0.6 \pm 0.02	2.9 \pm 0.1	20.7 \pm 0.6	50.2 \pm 1.8	7.9 \pm 0.4	1.2 \pm 0.01	0.7 \pm 0.03	1.9 \pm 0.09	ND	1.2 \pm 0.09	132.4	0.25	0.41
DC	tr	12.2 \pm 0.3	ND	3.5 \pm 0.2	22.0 \pm 0.6	48.7 \pm 1.5	7.9 \pm 0.3	0.9 \pm 0.01	0.7 \pm 0.03	2.8 \pm 0.1	ND	1.2 \pm 0.1	130.3	0.26	0.45
DT	tr	13.9 \pm 0.4	ND	3.6 \pm 0.2	32.3 \pm 0.8	36.9 \pm 1.4	8.2 \pm 0.4	1.9 \pm 0.02	0.7 \pm 0.02	2.3 \pm 0.1	ND	tr	119.0	0.28	0.87
DN	tr	12.7 \pm 0.3	ND	3.1 \pm 0.2	22.9 \pm 0.5	50.0 \pm 1.6	6.9 \pm 0.2	1.2 \pm 0.02	0.7 \pm 0.02	2.4 \pm 0.09	ND	tr	130.7	0.24	0.46
DS	tr	12.1 \pm 0.3	ND	4.0 \pm 0.3	19.5 \pm 0.4	48.1 \pm 1.7	10.2 \pm 0.7	1.7 \pm 0.03	0.6 \pm 0.01	3.6 \pm 0.2	ND	tr	133.2	0.27	0.40
GL	tr	12.9 \pm 0.3	0.6 \pm 0.05	6.5 \pm 0.5	20.2 \pm 0.6	35.3 \pm 1.3	15.6 \pm 0.9	1.2 \pm 0.02	1.9 \pm 0.09	1.4 \pm 0.1	ND	4.2 \pm 0.3	127.0	0.35	0.57
CV	tr	20.6 \pm 0.6	2.4 \pm 0.1	6.4 \pm 0.5	33.4 \pm 0.9	16.4 \pm 0.8	11.3 \pm 0.7	1.9 \pm 0.04	1.5 \pm 0.04	2.2 \pm 0.1	tr	3.4 \pm 0.3	94.3	0.53	2.03
CC	tr	11.3 \pm 0.2	tr	2.8 \pm 0.2	52.8 \pm 1.8	19.2 \pm 0.8	8.7 \pm 0.5	0.6 \pm 0.01	1.5 \pm 0.05	1.7 \pm 0.09	0.2 \pm 0.02	0.9 \pm 0.08	107.5	0.21	2.75
AR	tr	20.2 \pm 0.5	ND	3.4 \pm 0.2	6.4 \pm 0.7	59.2 \pm 1.7	5.2 \pm 0.4	1.2 \pm 0.03	ND	1.5 \pm 0.07	ND	1.5 \pm 0.09	127.4	0.41	0.11
SM	tr	12.6 \pm 0.2	0.7 \pm 0.05	2.4 \pm 0.1	17.5 \pm 0.9	48.8 \pm 1.2	4.3 \pm 0.2	1.6 \pm 0.04	2.6 \pm 0.08	6.6 \pm 0.5	0.8 \pm 0.04	1.9 \pm 0.1	119.4	0.34	0.36
SG	tr	14.1 \pm 0.3	0.3 \pm 0.02	4.0 \pm 0.3	20.4 \pm 1.2	53.0 \pm 1.9	1.6 \pm 0.1	1.6 \pm 0.05	0.7 \pm 0.02	3.0 \pm 0.2	0.1 \pm 0.01	1.1 \pm 0.09	119.8	0.31	0.38
AV	tr	9.2 \pm 0.1	0.1 \pm 0.01	1.3 \pm 0.1	27.4 \pm 1.4	34.7 \pm 1.0	25.4 \pm 1.3	0.6 \pm 0.02	0.3 \pm 0.01	0.6 \pm 0.02	ND	0.3 \pm 0.03	157.4	0.14	0.79
LM	tr	9.4 \pm 0.2	tr	3.6 \pm 0.3	16.2 \pm 0.6	65.7 \pm 2.1	4.2 \pm 0.4	0.6 \pm 0.01	tr	ND	ND	ND	145.2	0.16	0.25
CA	tr	19.3 \pm 0.4	tr	5.0 \pm 0.4	15.1 \pm 0.5	54.2 \pm 1.9	3.9 \pm 0.2	1.1 \pm 0.04	0.6 \pm 0.04	0.6 \pm 0.01	ND	ND	123.0	0.35	0.28

^a Trace, <0.1%.

^b Not detected.

Species code	Cholest	Campest	Stigmast	β -Sitost	Δ^5 -Aven	Δ^7 -Stigm	Δ^7 -Aven
GD	tr ^a	5.4±0.2	14.7±1.1	75.6±2.3	4.0±0.2	tr	tr
TT	tr	3.2±0.1	15.3±1.2	77.9±3.1	3.5±0.1	tr	tr
IP	tr	6.8±0.4	15.0±1.1	66.5±1.5	9.7±0.4	1.9±0.1	tr
IS	tr	5.9±0.3	14.7±1.4	68.8±1.8	8.6±0.3	1.9±0.2	tr
DC	tr	8.5±0.4	5.4±0.4	76.2±2.1	8.8±0.4	0.9±0.05	tr
DT	tr	8.4±0.5	6.6±0.3	74.9±2.2	7.9±0.3	1.9±0.1	tr
DN	tr	8.3±0.4	6.8±0.4	75.0±2.3	7.6±0.3	2.0±0.2	tr
DS	tr	8.3±0.5	5.7±0.4	75.1±2.2	9.3±0.7	1.5±0.1	tr
GL	tr	15.9±1.1	13.6±1.2	64.4±1.8	5.9±0.4	tr	tr
CV	tr	15.3±0.9	15.8±1.3	65.3±2.1	3.5±0.2	tr	tr
CC	tr	17.3±1.3	19.2±1.4	61.4±1.9	2.0±0.2	tr	tr
AR	1.0±0.01	12.0±0.8	40.8±3.2	41.2±0.7	3.4±0.3	1.4±0.1	tr
SM	1.1±0.01	10.2±0.1	38.8±2.1	47.3±0.8	2.3±0.2	0.2±0.1	tr
SG	1.0±0.01	9.2±0.7	40.6±4.0	46.9±0.5	1.5±0.1	0.7±0.3	tr
AV	1.1±0.01	8.6±0.5	38.4±2.8	49.3±1.0	2.3±0.2	0.2±0.1	tr
LM	0.5±0.03	7.4±0.2	29.9±1.8	61.0±1.1	1.0±0.1	tr	tr
CA	1.9±0.02	27.9±2.0	24.7±2.0	28.2±0.5	7.1±0.4	tr	tr

Table 4. Sterol composition (% of total sterols) from seed oils of the studied species (mean value±SD, n=3)

Cholest, cholesterol; Campest, campesterol; Stigmast, stigmasterol; β -Sitost, β -sitosterol; Δ^5 -Aven, Δ^5 -avenasterol; Δ^7 -Stigm, Δ^7 -stigmasterol; Δ^7 -Aven, Δ^7 -avenasterol.

^a Trace, < 0.1%.

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Species code	Cholest	Campest	Stigmast	β -Sitost	Δ^5 -Aven	Δ^7 -Stigm	Δ^7 -Aven
GD	tr ^a	5.4±0.2	14.7±1.1	75.6±2.3	4.0±0.2	tr	tr
TT	tr	3.2±0.1	15.3±1.2	77.9±3.1	3.5±0.1	tr	tr
IP	tr	6.8±0.4	15.0±1.1	66.5±1.5	9.7±0.4	1.9±0.1	tr
IS	tr	5.9±0.3	14.7±1.4	68.8±1.8	8.6±0.3	1.9±0.2	tr
DC	tr	8.5±0.4	5.4±0.4	76.2±2.1	8.8±0.4	0.9±0.05	tr
DT	tr	8.4±0.5	6.6±0.3	74.9±2.2	7.9±0.3	1.9±0.1	tr
DN	tr	8.3±0.4	6.8±0.4	75.0±2.3	7.6±0.3	2.0±0.2	tr
DS	tr	8.3±0.5	5.7±0.4	75.1±2.2	9.3±0.7	1.5±0.1	tr
GL	tr	15.9±1.1	13.6±1.2	64.4±1.8	5.9±0.4	tr	tr
CV	tr	15.3±0.9	15.8±1.3	65.3±2.1	3.5±0.2	tr	tr
CC	tr	17.3±1.3	19.2±1.4	61.4±1.9	2.0±0.2	tr	tr
AR	1.0±0.01	12.0±0.8	40.8±3.2	41.2±0.7	3.4±0.3	1.4±0.1	tr
SM	1.1±0.01	10.2±0.1	38.8±2.1	47.3±0.8	2.3±0.2	0.2±0.1	tr
SG	1.0±0.01	9.2±0.7	40.6±4.0	46.9±0.5	1.5±0.1	0.7±0.3	tr
AV	1.1±0.01	8.6±0.5	38.4±2.8	49.3±1.0	2.3±0.2	0.2±0.1	tr
LM	0.5±0.03	7.4±0.2	29.9±1.8	61.0±1.1	1.0±0.1	tr	tr
CA	1.9±0.02	27.9±2.0	24.7±2.0	28.2±0.5	7.1±0.4	tr	tr

Table 4. Sterol composition (% of total sterols) from seed oils of the studied species (mean value \pm SD, $n=3$)

Cholest, cholesterol; Campest, campesterol; Stigmast, stigmasterol; β -Sitost, β -sitosterol; Δ^5 -Aven, Δ^5 -avenasterol; Δ^7 -Stigm, Δ^7 -stigmasterol; Δ^7 -Aven, Δ^7 -avenasterol.

^a Trace, < 0.1%.

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