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Variation in the alkaloid content of different subspecies of *Chamaecytisus proliferus* from the Canary Islands

M. Muzquiz[a,*], L.M. Robredo[a], C. Burbano[a], C. Cuadrado[a], G. Ayet[a], P. Mendez[b]

[a]*Area de Tecnología de Alimentos, SGIT-INIA, Aptdo 8111, 28080 Madrid, Spain*
[b]*Centro de Investigación y Tecnología Agrarias, Aptdo 60, 38200 La Laguna, Tenerife, Spain*

Abstract

The composition and content of alkaloids in different subspecies of *Chamaecytisus proliferus* from diverse locations in the Canary Islands, was studied using thin-layer chromatography and capillary gas chromatography–mass spectrometry. The results obtained showed that sparteine was the main alkaloid in all samples studied. The sparteine content ranged from 0.06% in the ssp *proliferus* var. *palmensis* to 0.77% in the ssp *meridionalis*. Therefore, by breeding, the subspecies with the lower alkaloid content, could produce plants more suitable for use as animal food, whilst those with a high alkaloid content could be used as a natural source of sparteine.

1. Introduction

Chamaecytisus proliferus (L.fil) Link (Fabaceae:Genisteae) forms a taxonomic complex which is endemic in the Canary Islands. Different ecological, morphological and cytological studies, as well as comparison of several isozyme systems and phenolic composition, indicate that this complex is formed by seven taxa: *C. proliferus* ssp *proliferus* var. *palmensis*, var. *proliferus*, var. *hierrensis*, var. *calderae*, var. *canariae*; ssp *angustifolius* and ssp *meridionalis* [1,2].

Within this group the ssp *proliferus* var. *palmensis*, commonly know as *tagasaste*, is the most important foraging crop in the Canary Islands, which currently represents 5000 Ha [3]. The cultivation of this plant has also acquired importance in some areas of New Zealand and Australia since its introduction there at the end of the nineteenth century [4].

The other subspecies and varieties included in the complex are called *escobones*, which are directly exploited by natural populations. The only exception is the ssp *proliferus* var. *canariae*, which is cultivated in a specific zone in the N.W. of Gran Canaria Island.

The presence of alkaloids in *Chamaecytisus proliferus* ssp *proliferus* var. *palmensis*, specifically sparteine in the aerial part and calycotomine in the seeds, has been known for a long time [5]. This fact could be a disadvantage for the use of this plant as animal food. Although in practice the use of *tagasaste* did not present problems in ruminants, even if it was the sole feed for long periods [6], there are reports of negative effects in horses [7]. However, the alkaloid level in the other subspecies and varieties of the complex is unknown.

Although this plant has a lower nutritional

* Corresponding author.

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value than alfalfa, its high mineral content together with its other agronomical characteristics make *Chamaecytisus proliferus* a potentially important forage.

In the present work, a study of the alkaloid content in the seven subspecies and varieties of the complex from diverse locations in the Canary Islands was carried out using different chromatographic techniques.

2. Experimental

Seeds from natural populations of the following subspecies of *Chamaecytisus proliferus* were collected: ssp *proliferus* var. *palmensis*, ssp *proliferus* var. *proliferus*, ssp *meridionalis*, ssp *proliferus* var. *hierrensis*, ssp *proliferus* var. *calderae*, ssp *proliferus* var. *canariae*, ssp *angustifolius*. Next the seeds were sown in seedbeds and when the seedlings had reached a suitable size they were transplanted to an experimental plot in the Centro de Investigación y Tecnología Agraria, where they are maintained as a collection. There, all the subspecies were grown under the same edaphic and climatic conditions. Samples were taken from that collection, in different periods of the year, according to the following procedure: a section of a complete branch was cut into pieces, which were dried to constant weight in a fan-assisted oven at 60°C (usually up to 2 days). Then the samples were ground and sieved through a 0.5-mm sieve.

The samples analysed were identified in a way in which the first numbers indicate the different natural populations of the same subspecies and variety (in letters) and the last numbers refer to the year in which they were collected. These samples are: ssp *proliferus* var. *palmensis*: 24 PA-92, 3 PA-92, 24 PA-93, 11 PA-93; ssp *proliferus* var. *proliferus*: 156 PR-92, 128 PR-92, 128 PR-93, 146 PR-93; ssp *meridionalis*: 80 PE-92, 63 PE-92, 63 PE-93, 95 PE-93; ssp *proliferus* var. *hierrensis*: 179 H-VI-92, 179 H-IX-92, 183 H-93; ssp *proliferus* var. *calderae*: 18 TB-92, 16 TB-92, 16 TB-93; ssp *proliferus* var. *canariae*: 40 C-94, 181 C-94, 193 C-94; ssp *angustifolius*: 138 A-94, 139 A-94, 144 A-94.

2.1. Chemicals

The chemicals, all of analytical or reagent grade, were supplied by Prolabo (Paris, France), Merck (Darmstadt, Germany), or Fluka (Buchs, Switzerland).

2.2. Extraction

The extraction of the milled material was performed as described in the method of Muzquiz and co-workers [8,9]. Finely ground *Chamaecytisus proliferus* samples (0.5 g) were homogenized in 5% trichloroacetic acid (3×5 ml) with an Ultra-Turrax homogenizer and centrifugated at 700 *g* for 10 min. After centrifugation, the supernatants were alkalinized with 1 ml of 10 *M* NaOH and the alkaloids were extracted with 3×10 ml of dichloromethane. The dichloromethane extract was evaporated to dryness and the alkaloids were dissolved in 1 ml of methanol. A 0.5-ml volume of the extract was added to 0.5 ml of a solution of codeine in methanol (2 mg ml^{-1}) to provide an internal standard. The samples with an alkaloid content above the linear range of the calibration curve were diluted beforehand.

2.3. TLC analysis

The qualitative study of the alkaloids was performed by thin layer chromatography. A 5-μl aliquot of the methanol extract above mentioned was applied to Whatman silica gel plates (LK6DF, 20×20 cm, 250 μm) and developed with chloroform–cyclohexane–diethylamine (6:4:1, v/v). For visualization, UV light and Dragendorff's reagent were used, and when the plates were dried they were sprayed with Bouchardat's reagent.

2.4. GLC analysis

The chromatographic instrument used was a Perkin-Elmer Autosystem (Norwalk, CT, USA) equipped with a PND (phosphorus–nitrogen detector), an automatic injection sampler and a Turbochrom programme. The capillary column

used was SPB-1 (30 m × 0.25 mm I.D.) (Tekno kroma, Bellefonte, PA, USA), helium was the carrier gas. The temperatures of the injector and detector were 240°C and 300°C, respectively. The oven temperature was 150°C, increased at 5°C min^{-1} to 235°C and finally held at that temperature for 15 min.

A calibration curve was prepared with the alkaloid standard sparteine (Sigma, St. Louis, MO, USA). Response was linear over the range 0–0.312 mg ml^{-1} and the coefficient of determination was >0.99.

2.5. GLC–MS analysis

A Hewlett-Packard gas chromatograph 5890 was coupled with a mass selective detector 5971 that was combined with the G1034B Software for MS ChemStation data system (Waldbronn, Germany). For capillary GLC–MS the same capillary column and conditions as above were used.

2.6. Statistical analysis

The data were analysed for variance using the BMDP-7D ANOVA programme [10] and the mean values compared using Duncan's multiple range test.

3. Results and discussion

Fig. 1 shows the presence of sparteine in all samples analysed. The highest sparteine content appeared in three samples of the subspecies *meridionalis*.

The identity of this alkaloid was unequivocally confirmed by its mass spectral degradation pattern and comparison with literature (MS: *m/z* 234 $[M]^+$ (17), 137 (100), 98 (94), 193 (25), 110 (19), 84 (15), 122 (12)) [11] (Fig. 2). Other authors indicate the presence of other alkaloids such as lupanine, in higher concentration than sparteine [12], and calycotomine [13] in the genus *Chamaecytisus*. However, our GLC–MS results indicate that none of these alkaloids was detected in the subspecies studied. The alkaloid cytisine, which is reported to appear in the genus *Cytisus* [14], was also not detected.

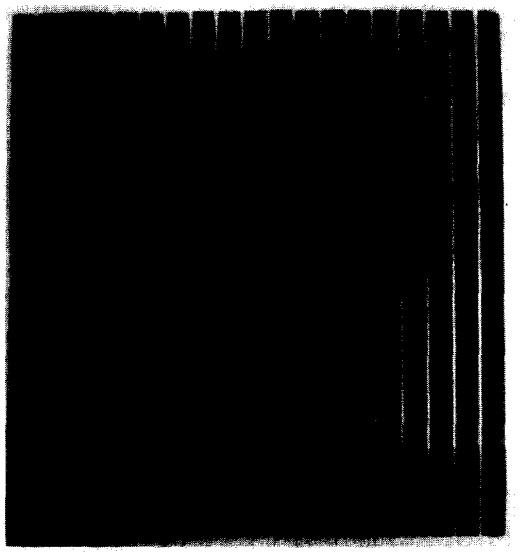

Fig. 1. Separation of alkaloid extracts from *Chamaecytisus proliferus* by thin-layer chromatography. Whatman silica gel plates (LK6DF, 20 × 20 cm, 250 μm), developed with chloroform–cyclohexane–diethylamine (6:4:1, v/v). Standard: 1 = sparteine. Samples: 2 = 24 PA-92; 3 = 3 PA-92; 4 = 11 PA-93; 5 = 156 PR-92; 6 = 128 PR-93; 7 = 146 PR-93; 8 = 80 PE-92; 9 = 63 PE-93; 10 = 95 PE-93; 11 = 179 H-VI-92; 12 = 183 H-93; 13 = 18 TB-92; 14 = 16 TB-92; 15 = 40 C-94; 16 = 181 C-94; 17 = 193 C-94; 18 = 139 A-94; 19 = 144 A-94.

The results obtained show that sparteine is the main alkaloid in all samples (Fig. 3), representing about 90% of the total alkaloid content in the majority of the samples, except in the ssp *proliferus* var. *palmensis* and in the sample 63 PE of the ssp. *meridionalis* collected in September 1992, in which sparteine represents around 75%.

Analysis of the GLC results shows that large differences exist in sparteine and the total content of alkaloids between the samples (Table 1). Statistical analysis shows a clear difference in sparteine and total alkaloid content of the ssp. *meridionalis* from the others. On the other hand, although significant differences exist between the other subspecies and varieties of ssp *proliferus* in relation to the sparteine content, the total alkaloid content does not vary substantially between them.

The sparteine content in some of the varieties

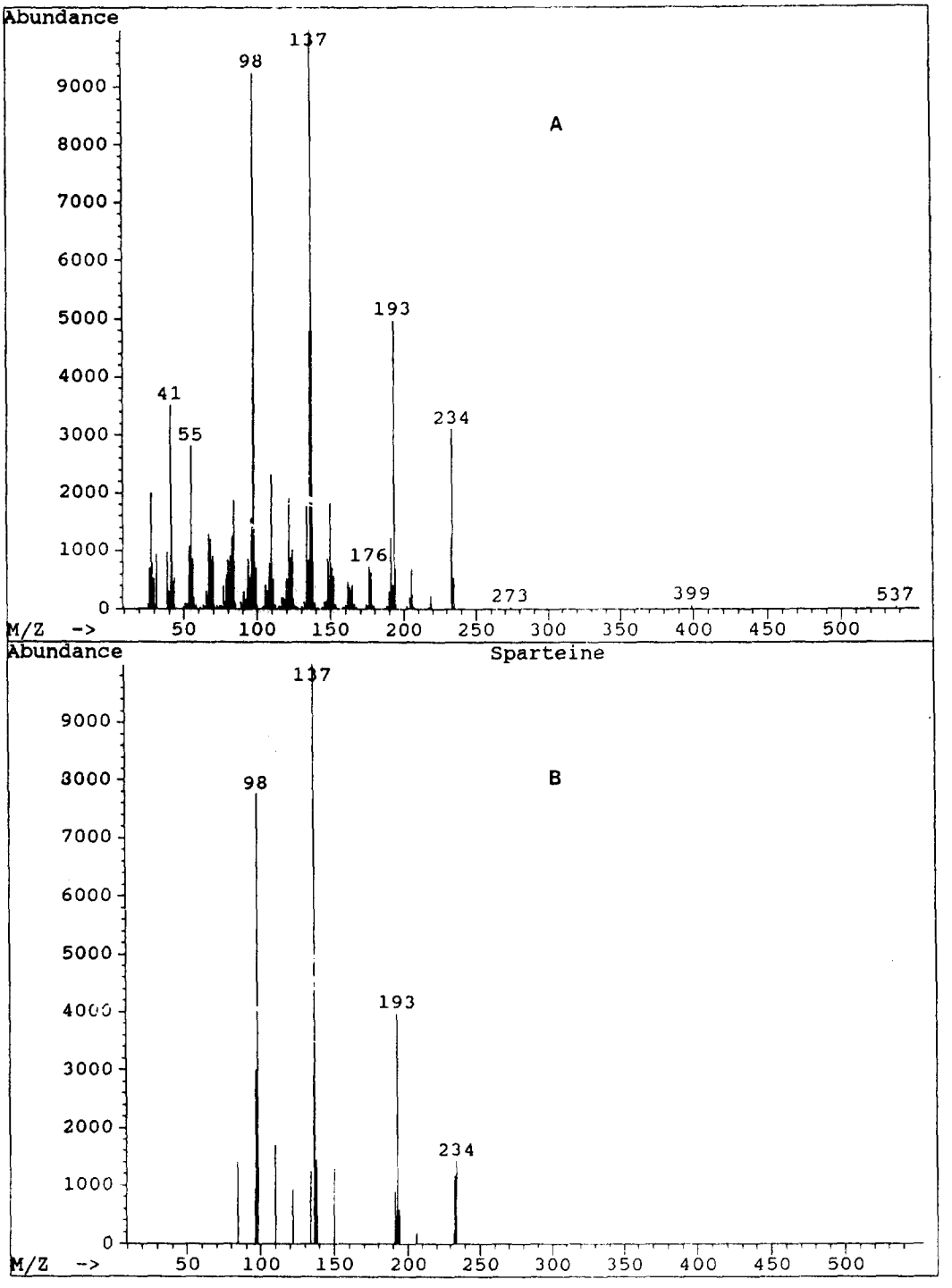

Fig. 2. Mass spectra of sparteine alkaloid obtained from *Chamaecytisus proliferus* sample (A) and sparteine standard (B).

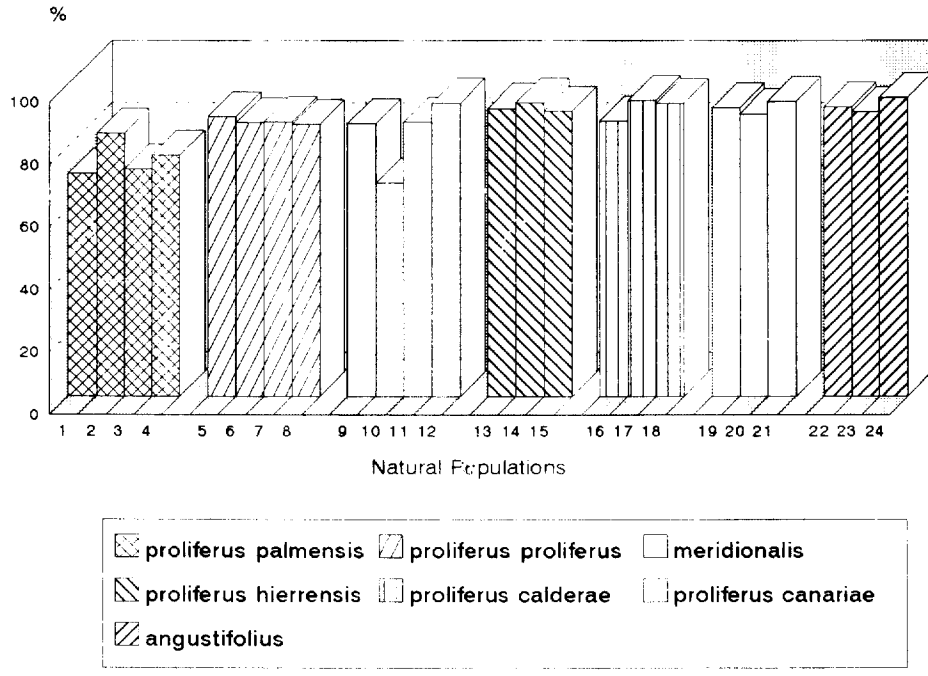

Fig. 3. Ratio of sparteine and the total alkaloid content in different subspecies of *Chamaecytisus proliferus*. Samples: 1 = 24 PA-92; 2 = 3 PA-92; 3 = 24 PA-93; 4 = 11 PA-93; 5 = 156 PR-92; 6 = 128 PR-92; 7 = 128 PR-93; 8 = 146 PR-93; 9 = 80 PE-92; 10 = 63 PE-92; 11 = 63 PE-93; 12 = 95 PE-93; 13 = 179 H-VI-92; 14 = 179 H-IX-92; 15 = 183 H-93; 16 = 18 TB-92; 17 = 16 TB-92; 18 = 16 TB-93; 19 = 40 C-94; 20 = 181 C-94; 21 = 193 C-94; 22 = 138 A-94; 23 = 139 A-94; 24 = 144 A-94.

of the subspecies *proliferus* (var. *hierrensis* and var. *proliferus*) varied with the time of the year in which they were collected, so further studies will be needed before conclusions can be drawn in this respect.

The samples with the smaller content of sparteine were the natural populations 24 PA-92, 24 PA-93 and 11 PA-93 of the ssp. *proliferus* var. *palmensis* and the population 179 H-IX-92 of the ssp. *proliferus* var. *hierrensis*. Within the different leguminosae that are being used in human and animal nutrition, the genus *Lupinus* contains high concentrations of quinolizidine alkaloids [8,15]. Sparteine was found in significant amount in *L. luteus* and *L. mutabilis*, and some authors consider this alkaloid to be one of the more toxic alkaloids of these plants. The safe limit of toxicity has been established between 0.04 and 0.05% for human and animal nutrition, respectively [16]. Some varieties of *C. proliferus* ssp *proliferus* as indicated above have a slightly higher content than this accepted limit. Therefore, these genotypes should be further considered for improvement in breeding programmes.

On the other hand, the samples 80 PE and 63 PE of the ssp. *meridionalisis* contain the highest sparteine content. The pharmacological properties of sparteine used alone or in combination with other compounds provide a wide range of applications as pharmaceutical products. They can be used for cardiac problems as an antiarrhythmic, and also as hypnotic sedative, narcotic analgesic and a labour inducer. Portugal is currently the only producer of sparteine from the raw material *Sarothamnus scoparius*, with the amount of sparteine present in this broom reaching its maximum at the beginning of flowering (0.85%) and falling in autumn to 0.24% [17]. The high sparteine content in the samples mentioned above in the ssp. *meridionalis* indicate that they could be considered as an alternative source for sparteine.

Table 1
Sparteine and total alkaloid content (%, ±S.E.) in different subspecies of *Chamaecytisus proliferus*

Subspecies	Natural population	Sparteine	Total alkaloid
proliferus	24 PA-92	0.056 ± 0.002	0.079 ± 0.003
var. *palmensis*	3 PA-92	0.166 ± 0.005	0.197 ± 0.005
	24 PA-93	0.069 ± 0.006	0.095 ± 0.006
	11 PA-93	0.081 ± 0.002	0.105 ± 0.001
	Mean	0.092 ± 0.025 a[a]	0.119 ± 0.027 b
proliferus	156 PR-92	0.304 ± 0.006	0.339 ± 0.007
var. *proliferus*	128 PR-92	0.307 ± 0.005	0.350 ± 0.005
	128 PR-93	0.395 ± 0.019	0.448 ± 0.019
	146 PR-93	0.329 ± 0.007	0.377 ± 0.008
	Mean	0.333 ± 0.021 b	0.378 ± 0.025 b
meridionalis	80 PE-92	0.759 ± 0.024	0.868 ± 0.028
	63 PE-92	0.772 ± 0.003	1.131 ± 0.004
	63 PE-93	0.754 ± 0.042	0.858 ± 0.052
	95 PE-93	0.342 ± 0.032	0.364 ± 0.035
	Mean	0.656 ± 0.105 c	0.805 ± 0.160 a
proliferus	179 H-VI-92	0.163 ± 0.008	0.177 ± 0.009
var. *hierrensis*	179 H-IX-92	0.059 ± 0.002	0.062 ± 0.002
	183 H-93	0.162 ± 0.002	0.177 ± 0.003
	Mean	0.127 ± 0.035 ab	0.139 ± 0.038 b
proliferus	18 TB-92	0.362 ± 0.011	0.412 ± 0.011
var. *calderae*	16 TB-92	0.261 ± 0.028	0.276 ± 0.029
	16 TB-93	0.255 ± 0.022	0.272 ± 0.024
	Mean	0.292 ± 0.035 ab	0.320 ± 0.046 b
proliferus	40 C-94	0.553 ± 0.012	0.598 ± 0.010
var. *canariae*	181 C-94	0.106 ± 0.008	0.117 ± 0.008
	193 C-94	0.469 ± 0.013	0.498 ± 0.013
	Mean	0.375 ± 0.137 b	0.404 ± 0.147 b
angustifolius	138 A-94	0.106 ± 0.002	0.114 ± 0.002
	139 A-94	0.480 ± 0.008	0.527 ± 0.008
	144 A-94	0.173 ± 0.007	0.181 ± 0.007
	Mean	0.252 ± 0.115 ab	0.274 ± 0.128 b
		(6.40)[b]	(5.97)

[a] Means followed by the same letter are not significantly different.
[b] Figures in parenthesis are *F*-values from variance analysis. All values are significant at the 0.1% level.

References

[1] J. Francisco-Ortega, unpublished Ph.D. thesis, University of Birmingham, 1992.
[2] J.R. Acebes-Ginoves and M. del Arco-Aguilar, Vieraea, 20 (1991) 191.
[3] P. Mendez, REUR Technical Series, F.A.O., 28 (1993) 141.
[4] J. Francisco-Ortega, M. Fernandez-Galván and A. Santos-Guerra, N.Z. J. Agric. Res., 34 (1991) 471.
[5] E. White, N.Z. J. Sci. Technol., 25 (1943) 93.
[6] M. Borens, MS thesis, University of Canterbury, Lincoln College (unpublished), 1986.
[7] V. Perez, in J.V. Perez-Ventoso (Editor), Le tagasaste (Cytisus proliferus varietas) fourrage important, Semain Medicale, Paris, 1892.
[8] M. Muzquiz, C. Burbano, C. Cuadrado and C. de la Cuadra, Invest. Agr. Prod. Prot. Veg., 8 (1993) 351.
[9] M. Muzquiz, C. Cuadrado, G. Ayet, C. de la Cuadra, C. Burbano and A. Osagie, J. Agric. Food Chem., 42 (1994) 1447.

[10] W.J. Dixon, BMDP Statistical Software, Software Release, 1988.
[11] C. Meißner and M. Wink, in M. Wink (Editor), GC/MS Analyse von Alkaloiden Nordamerikanischer Lupinen in Lupinen 1991-Forschung, Anbau und Verwertung, Universität Heidelberg, Heidelberg, 1992, p. 91.
[12] A. Daily, N. Kotsev, L. Llieva, H. Dutschewska and N. Mollov, Arch. Pharm., 311 (1978) 889.
[13] E.P. White, N.Z. J. Sci. Technol., 33 (1951) 44.
[14] J.A. Mears and T.J. Mabry, in J.B. Harbone, D. Boulter and B.L. Turner (Editors), Alkaloids in the Leguminosae in Chemotaxonomy of the Leguminosae, Academic Press, London, 1971, p. 73.
[15] M. Muzquiz, C. Burbano, M.J. Gorospe and I. Rodenas, J. Sci. Food Agric., 47 (1989) 205.
[16] R.F. Keeler, in P.R. Cheeke (Editor), Quinolizidine Alkaloids in Range and Grain Lupins in Toxicants of Plant Origin, Vol. I, Alkaloids, CRC Press, Boca Raton, FL, 1989, p. 133.
[17] L. Norton-Brandão, Marketing Alkaloids for the Chemical and Pharmaceutical Industry–The Case for Sparteine in Agriculture. Lupinus mutabilis: Its Adaptation and Production under European Pedoclimatic Conditions, Comission of the European Communities, Luxembourg, 1992, p. 161.