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Assessment of the effect of drying methods on the chemical composition and *in vitro* gas production of two woody species

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SUMMARY – The effect of the drying method on the forage quality of two browse species (*Arbutus unedo* and *Robinia pseudoacacia*) was investigated at three phenological stages. Hand-picked samples were dried either in oven or under frozen conditions (freeze-drying). The chemical composition of the samples was determined and their nutritive value was assessed using the *in vitro* gas production technique after incubation for 3, 6, 12, 24, 48, 72 and 96 h. The kinetics of fermentation were described using the equation $p = a + b(1 - e^{-ct})$. The crude protein (CP) content was always higher ($P < 0.05$) in *R. pseudoacacia* than in *A. unedo*. As a result of maturation, CP decreased from 23.6 to 14.6% in *R. pseudoacacia* and from 9.2 to 7.2% in *A. unedo*. Neutral detergent fibre (NDF) and lignin content increased with maturity. Freeze-drying the samples had no effect on CP but resulted in lower concentrations ($P < 0.05$) of NDF and lignin. The drying method did not affect gas production from *A. unedo* samples ($P > 0.05$). However, freeze-dried *R. pseudoacacia* samples produced a higher volume of gas at all phenological stages ($P < 0.05$). It was concluded that the drying technique could affect the chemical composition and fermentation of animal feeds. Furthermore, fermentation characteristics and CP content of *R. pseudoacacia* make it a promising fodder species for livestock.

Key words: Browse, *in vitro* gas production, chemical composition, drying methods, quality.

RESUME – *"Effet du mode de séchage sur la composition chimique et la production de gaz de deux espèces ligneuses". L'effet de deux méthodes de séchage sur les caractéristiques nutritionnelles de deux arbustes (*Arbutus unedo *et* Robinia pseudoacacia*) a été étudié pendant trois stages phénologiques. Les échantillons prélevés ont été séchés selon deux méthodes : le séchage à l'étuve (méthode traditionnelle) et le séchage par la congélation. La composition chimique des arbustes a été déterminée et la valeur nutritive a été estimée* in vitro *par la technique de la production de gaz après incubation des échantillons pendant 3, 6, 12, 24, 48, 72 et 96 h. La cinétique de la fermentation a été établie par l'équation* $p = a + b(1 - e^{-ct})$*. Les résultats obtenus montrent que la teneur en matières azotées totales (MAT) a été élevée chez* R. pseudoacacia *par rapport à* A. unedo*, pour le pourcentage de la diminution de MAT, à cause de la maturation plus élevée chez* R. pseudoacacia *que chez* A. unedo*. La teneur en parois totales ou NDF et de la lignine a augmenté en fonction de l'âge de la plante. Le séchage après congélation n'a pas affecté la teneur en MAT, en revanche il a réduit la concentration du NDF. Au contraire, la production gazeuse chez* R. pseudoacacia *a été élevée pendant tous les stages phénologiques. La technique de séchage a pu affecter la composition chimique et la production de gaz. D'ailleurs les résultats suggèrent que* R. pseudoacacia *est une espèce fourragère prometteuse pour les ruminants.*

Mots-clés : *Arbustes, production de gaz* in vitro*, composition chimique, modes de séchage, qualité.*

Introduction

The important role of multipurpose trees and shrubs for nutrition of small ruminants has been especially documented in the Mediterranean zone (Perevolotsky *et al*., 1998). This type of feed is an important supplement under extensive animal production systems, particularly as the availability and quality of herbaceous species change drastically with maturity during the dry season.

Evaluation of forage can be affected by several factors such as how the samples are prepared (e.g. by drying) or the analytical procedure used. Drying temperature may have a significant impact on the nutritive value of browse species. Nastis and Malechek (1988) working with *Quercus gambelli* have reported that oven-drying at 55, 65 or 100°C reduced digestibility of foliage samples more than freeze-drying. On the other hand, Makkar and Singh (1991) have reported no significant difference in the neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin content of *Quercus incana* under different drying conditions.

Several laboratory methods (chemical and biological) have been used to evaluate the quality of the foliage of woody plants (Tilley and Terry, 1963; Goering and van Soest, 1970; Dowman and Collins, 1982). All methods used in the past were based on gravimetric measurements of feed substrate (Getachew *et al*., 1998). On the other hand, the *in vitro* gas production technique (Menke and Steingass, 1988) has been suggested as a useful technique for measuring both the rate and the extent of fermentation, in addition to the residue of fermentation at different intervals of time (Blümmel and Orskov, 1993).

The objective of the present study was to assess the effect of drying temperature (oven-drying vs freeze-drying) on the chemical composition and the *in vitro* gas production of the foliage of two ligneous species harvested at different phenological stages.

Materials and methods

The experiment was carried out at the Aristotle University's farm (40° 34' E, 23° 43' N, at sea level) in northern Greece. The investigated fodder plants were *Arbutus unedo* (L.), which is an evergreen shrub and *Robinia pseudoacacia* var. *monophylla* (L.), which is a broad-leaved deciduous leguminous tree. For each species, foliage was hand-plucked (i.e. leaves and twigs <2 mm) from three individual plants at three phenological stages. Samples were harvested from different individual plants in spring during the season of rapid growth (immature), in the middle of summer when growth had just terminated (mature) and at the end of summer when growth had long ceased and the woody parts were hard (very mature). The collected samples were divided into two groups. One group was oven-dried at 60°C for 48 h and the other was freeze-dried at –50°C for 72 h. This was followed by chemical analysis for crude protein (CP) (AOAC, 1990), NDF and acid detergent lignin (ADL) (van Soest *et al*., 1991).

For the *in vitro* gas technique, rumen liquor was obtained from three goats fed browse twice per day. Preparation of buffer solutions and rumen liquor-buffer (1:2 v/v) was as described by Menke and Steingass (1988). Samples of 110 ± 5 mg (in duplicates per run) were placed in pre-warmed (40°C) calibrated glass syringes (50 ml). Each syringe was inoculated with 15 ml of the rumen/buffer mixture followed by incubation in a water bath maintained at 39°C. Gas production was recorded after 3, 6, 12, 24, 48, 72 and 96 h. The mean gas volume readings were adjusted to the exponential equation $p = a + b(1 - e^{-ct})$ (Orskov and Mc Donald, 1979), where p is the gas production at time t, $a + b$ is the potential gas production and c denotes the rate of gas production. Data were evaluated by analysis of variance using GLM procedures of SAS. Steel and Torrie (1980) least significant difference test (LSD) was used to detect differences between means.

Results and discussion

At all phenological stages, the CP content of *R. pseudoacacia* was significantly higher than that of *A. unedo* (Table 1). NDF and lignin content increased in both species with maturation. It is well known that mature plants become more fibrous and lignified while their CP content declines (Tsiouvaras and Nastis, 1990; Papachristou and Papanastasis, 1994). Drying temperature had no effect ($P > 0.05$) on the CP content of the foliage of the two tested species. However, oven-drying increased ($P < 0.05$) the NDF and lignin contents of both species at all phenological stages (Table 1). These findings are in agreement with previous studies (Deinum and Maassen, 1994; Papachristou and Nastis, 1994). Differences in NDF content between the drying methods were probably the result of non-enzymatic browning (Maillard products). Heat treatment is known to cause the polymerization of sugar residues with amino acids resulting in a brown complex with physical and chemical properties similar to lignin (artifact lignin) (van Soest, 1994).

Oven-drying or freeze-drying of *A. unedo* samples at the first and second phenological stages had no effect on gas volume; but at the third stage, oven-dried samples of *A. unedo* yielded a higher volume of gas (Fig. 1). On the other hand, freeze-dried samples of *R. pseudoacacia* produced a significantly higher amount of gas than the oven-dried ones at all phenological stages (Fig. 2).

Both oven-drying and freeze-drying are reported to induce physico-chemical changes mainly in the nitrogenous constituents of forage and this is likely to affect degradation (Abdalla *et al*., 1988). Thus,

the lower volume of gas from *R. pseudoacacia* may have been due to the presence of high concentration of protein leading to more intense Maillard reaction when the sample was oven-dried. As the Maillard products do not degrade easily, gas production was depressed with oven-drying. Merkel *et al*. (2000) reported that oven-drying leguminous samples may reduce their feed value. Van Es and van der Meer (1980) recommended freeze-drying for the *in vitro* experiments in order to avoid the formation of insoluble polymers.

Table 1. Effect of oven-drying and freeze-drying on the chemical composition (%) of hand-plucked foliage samples at different phenological stages

	Crude protein		NDF		Lignin	
	Oven	Freeze	Oven	Freeze	Oven	Freeze
Arbutus unedo						
Immature	9.2^{d}	9.4^{d}	46.8^{c}	40.8^{e}	17.4^{bc}	14.5^{d}
Mature	6.0^{e}	7.1^{de}	48.2^{b}	44.2^{d}	18.5^{b}	17.0^{c}
Very mature	7.2^{de}	9.1^{d}	50.2^{a}	46.5^{c}	21.1^{a}	17.1^{c}
Robinia pseudoacacia						
Immature	23.6^{a}	23.6^{a}	33.9^{f}	29.6^{g}	5.4^{g}	5.1^{g}
Mature	17.7^{b}	17.6^{b}	35.9^{f}	30.1^{g}	7.5^{e}	6.2^{fg}
Very mature	14.6^{c}	14.6^{c}	39.7^{e}	30.0^{g}	8.1^{e}	6.0^{fg}

^{a,b,c,d,e,f,g}Means for the same component in the same row or column with different letters are significantly different ($P \leq 0.05$).

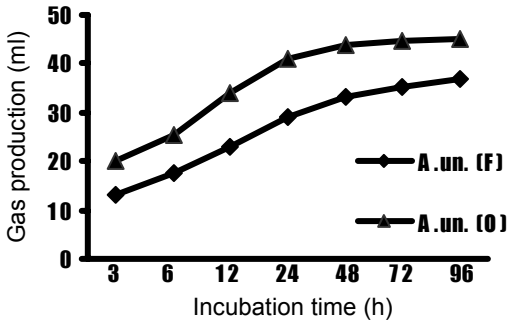

Fig. 1. Gas production (ml) of *A. unedo* at third phenological stage.

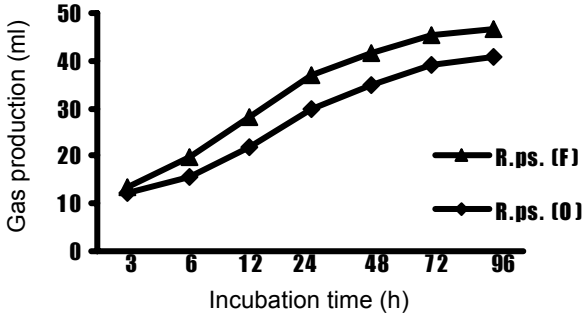

Fig. 2. Average gas production (ml) of *R. pseudoacacia* at three phenological stages.

The two species presented similar rates (c) but different potentials of gas production ($a + b$) (Table 2). This means that freeze-dried *R. pseudoacacia* foliage was more easily degradable than *A. unedo* foliage. This may have been due to a higher lignin content (>14%) in *A. unedo* than in *R. pseudoacacia* (<8%) resulting in depressed degradability (Dzowela *et al*., 1995).

Table 2. Parameters obtained by adjusting the data over a 96 h period *in vitro* incubation of the two samples to the equation $p = a + b(1–e^{-ct})$

	$a + b^{\dagger}$		$c^{\dagger}$	
	Oven	Freeze	Oven	Freeze
A. unedo	43	37	0.0602	0.0581
R. pseudoacacia	40	46	0.0608	0.0633

$^{\dagger}a$, b and c are constants in the exponential equation $p = a + b(1 – e^{-ct})$.

Conclusion

It is suggested that leguminous species with a high content of protein should be dried at low temperature in order to avoid denaturation of protein and prevent depression of fermentation. The high CP and low lignin content observed in *R. pseudoacacia* implies that this species could be considered as potentially valuable for feeding ruminants. However, further research is necessary to validate these findings with animal performance studies.

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