Evaluation of saponin content in alfalfa (*Medicago sativa L.)* by near infrared spectroscopy

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Introduction

Saponins are a large group of secondary metabolites detected in several plant families and formed by triterpenoids, steroids and steroidal alkaloids (aglycone moiety or sapogenin) glycosylated with one or more sugar chains. These compounds display a broad spectrum of biological properties, such as fungicidal, antibacterial and antiviral activities.[1] It is likely that saponins have a role in plant defence mechanisms against infections.[2] Due to their chemical, physical and physiological characteristics, commercial products containing plant saponins are already available and used in the pharmaceutical, cosmetic and food industries.[3-4] The genus Medicago includes over 80 species,[5] among which lucerne (*Medicago sativa* L.) is the most economically-important, being a major worldwide forage species. The chemical structures of saponins have been determined in different species within the genus Medicago, revealing complex mixtures of high-molecular weight triterpene glycosides in which various molecules represent the aglycone moiety i.e. the sapogenin.[1] Given the economic prominence of lucerne, a number of studies have dealt with lucerne saponins and their biological activity, including their biosynthesis[6] and possible antinutritional effects in animal diets.[7-8] No definite information is available on lucerne saponin levels which can cause reductions in nutrient utilisation and feed conversion efficiency. Furthermore, different animal species have been shown to react differently to saponin-containing diets. Germplasm with high and low levels of saponins was empirically defined mostly on the basis of responses in biological assays, but lucerne saponin content can vary with several factors such as genotype, plant organ, stage of growth or age.[9] The biological activity of *Medicago* saponins is strongly dependent on their constituent sapogenins[1] and their evaluation in the plant material is of importance for a correct use of lucerne for animal feeding.

Chemical analysis of saponins is not simple due to their 'soapy' properties. The presence of these substances can be evaluated by biological tests involving their toxic haemolytic, fungicidal and insecticidal properties. Chemical methods have been also used, such as TLC, HPLC, GC and GC/MS, the latter technique being used to analyse and quantify only the aglycone moieties. Capillary electrophoresis and LC/MS methods have also been employed for the identification and quantification of saponins in the plant extracts.[1]

A rapid predictive method to measure these nutritional quality parameters using near infrared spectroscopy would be valuable, in particular for breeding selection where a large number of samples must be analysed. A feasibility study was performed.

Materials and Methods

Samples

Alfalfa (*M. sativa*) varieties (n=52) widely cultivated in Northern Italy were studied in this investigation. Plant samples were oven dried at 60°C to a constant weight and ground in a mill (Cyclotec, 1 mm sieve). Saponin content was determined[10] and expressed as mg/g dry matter of total sapogenins. Sapogenins were released from the saponin mixture by acid hydrolysis and quantified by GC by using an internal standard method. Alfalfa flour samples were measured in reflectance mode using a monochromator instrument (NIRSystems 6500, Foss Italia) in the VIS-NIR range (400-2098 nm) every 2 nm. Samples were measured twice and the resulting spectra averaged before the analysis of the data.

Data analysis

Calibrations were developed to quantify both the content of specific sapogenins (medicagenic acid, zanhic acid) and the total amount of saponins using the software WinISI III (Intrasoft International). Averaged spectra were divided into two separate sets for the calibration and validation and pre-treated using a first derivative mathematical treatment (1,4,4,1).

Results and Discussion

The alfalfa flour belonged to different varieties, so they have some differences in their chemical composition and colour which are emphasised in the spectral shapes (Figure 1). The principal differences were clearly in

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the visible range below 700nm where the colour effect was dominant on the absorption. To minimise light scattering effects, a first derivative pre-treatment was applied (Figure 2).

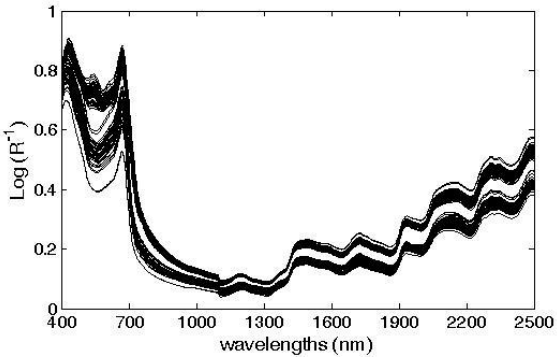

Figure 1. Original alfalfa flour sample spectra.

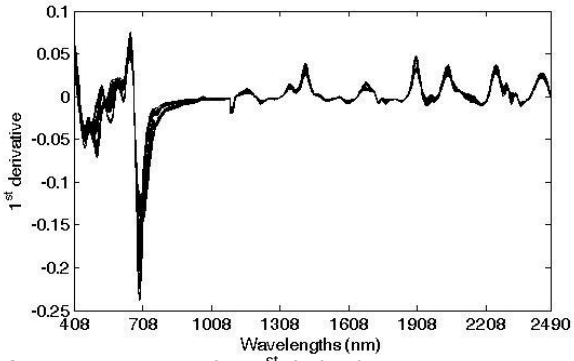

Figure 2. Spectra after 1st derivative pre-treatment.

The saponin content of the samples is summarised in Table 1. The content of medicagenic acid is a little higher than the zanhic acid value with mean contents of 1.42 and 1.10 mg/g respectively. The standard deviation (SD) of the medicagenic acid amount measured is also higher that that for zanhic acid.

Table 1. Characteristics of alfalfa flour sample sets.

Parameter	Saponins (mg/g)		Medicagenic acid (mg/g)		Zanhic acid (mg/g)	
	Calibration	Validation	Calibration	Validation	Calibration	Validation
Number of samples	42	10	42	10	42	10
Range	2.41-5.71	2.42-5.32	0.80-2.16	0.75-2.36	0.53-2.07	0.61-1.51
Mean	3.76	3.69	1.42	1.33	1.10	1.14
SD	0.69	0.82	0.34	0.49	0.28	0.27

The calibrations developed showed that better results were obtained by quantifying total saponin content rather than that of individual sapogenins (Table 2). Fairly good results were achieved using 5 LVs in the model and the resulting R^2 in calibration (0.72) indicated that it could be useful for screening and approximate prediction. Results obtained quantifying medicagenic and zanhic acids were not acceptable because the very low values found of R^2 cal (0.41 and 0.46) indicated that there is no correlation between the NIR absorbance spectra and these compounds.

Table 2. Statistics of PLS calibration results.

Parameter	Saponins (mg/g)	Medicagenic acid (mg/g)	Zanhic acid (mg/g)
	Calibration	Calibration	Calibration
Number of samples	42	42	42
Outliers	6	7	8
SEC	0.32	0.24	0.15
R^2 C	0.72	0.41	0.46
SECV	0.47	0.26	0.17
Number of terms	5	1	1
RPD c	1.88	1.30	1.36
Segments (LOO)	7	7	7
WL range	400-2098/2	400-2098/2	400-2098/2
Pre-treatment	D1,4,4	D1,4,4	D1,4,4

The validation results of the total saponin model, calculated on a separate sample set having no outliers present, were characterised by R^2 in prediction of 0.651, SEP of 0.468 (lower than the SD of the calibration reference values) and a bias of –0.039. In Figure 3, the scatter plot of prediction vs reference values is shown. The regression line (dashed), with a slope of 0.924 and an intercept of 0.246, confirms that NIR spectroscopy is a valuable tool to measure the total quantity of saponins when analysing nutritional quality parameters of a large number of lucerne samples.

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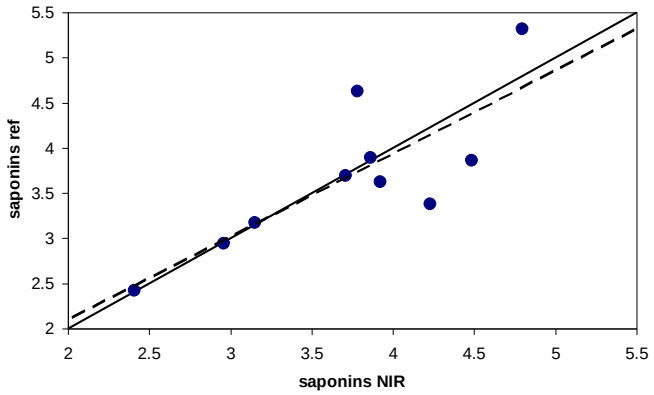

Figure 3. Scatter plot of predicted vs reference values. Solid is a 45° line, dashed is the regression line.

Conclusion

Considering the small sample numbers and the low content of saponins, fairly good results were achieved which could be useful for screening purposes i.e. for selecting samples with low, medium and high saponin content. NIR spectroscopy could give valid information for breeders who have a great interest in the evaluation of plant material in animal feed.

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References

1. A. Tava and P. Avato, *Nat. Prod. Commun.* **1,** 1159-1180 (2006).
2. A. Osbourn, *Phytochemistry* **62**, 1-4 (2003).
3. O. Tanaka, Y. Tamura, H. Masuda and K. Mizutani, "Application of saponins in food and cosmetics: saponins of *Mohave yucca* and *Sapindus mukurossi*." In *Advances in Experimental Medicine and Biology*. *Saponins Used in Food and Agriculture*, Ed by G.R. Waller and K. Yamasaki, Plenum Press, New York, **405,** pp. 1-11 (1996).
4. R. San Martin and R. Briones, *J. Sci. Food Ag.* **88**, 587-605 (2000).
5. K.A. Lesins and I. Lesins, *Genus Medicago (Leguminosae). A Taxogenetic Study*. Dr. W. Junk, The Hague (1979).
6. A. Tava, C. Scotti and P. Avato, *Phytochem. Rev.* **10**, 459-469 (2011).
7. P. R. Cheeke, "Biological effects of feed and forage saponins and their impact on animal production" in *Advances in Experimental Medicine and Biology*. *Saponins Used in Food and Agriculture*, Ed by G.R. Waller and K. Yamasaki, Plenum Press, New York, **405,** pp. 377-385 (1996).
8 S. Sen, H. P. S. Makkar and K. Becker, *J. Agric. Food Chem.* **46**, 131-140 (1998).
9. L. Pecetti, A. Tava, M. Romani, M.G. De Benedetto and P. Corsi, *Eur. J. Agron.* **25**, 187-192 (2006).
10. A. Tava and L. Pecetti, *J. Genet. Breed.* **52**, 33-37 (1998).

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