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Quantitative analysis of chlorophyll and protein in alfalfa leaves using fibre-optic NIR spectroscopy and PLS regression

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The performance of near-infrared (NIR) spectroscopy as a rapid technique for the estimation of chlorophyll and protein contents in alfalfa (*Medicago sativa*, L.) was investigated. Leaf samples from 33 cultivars were taken from three plant positions (apical, middle and basal) in two different harvests made in an experimental plot located in the province of Salamanca (Spain). A fibre-optic probe was employed directly on fresh leaves to measure near infrared spectra between 1100-2200 nm. Chemical analyses were performed using analytical reference methods; chlorophyll was measured by spectrophotometry of dimethyl sulphoxide extracts and protein content was quantified using the Kjeldahl method. Partial least squares (PLS) regression models were developed with a calibration set of 120 samples spanning a concentration range of 0.39-4.60 mg g⁻¹ (fresh weight) for chlorophyll and 9.92-45.32% (dry matter) for protein content. The models obtained were validated with independent test samples. The statistics of validation provided the best accuracy using a first derivative transformation of the data in both parameters. Thus, we achieved standard errors of prediction of 0.24 mg g⁻¹ and 3.27% for chlorophyll and protein contents, respectively. These results encourage the use of NIRS equipped with a fibre-optic probe to monitor and assess the composition and quality of forages in a non-destructive way.