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# Vibrational spectroscopy to predict *in vitro* digestibility and the maturity index of different forage crops during the growing cycle and after freeze- or oven-drying treatment

S. Tassone[a], G. Masoero[a,b], P.G. Peiretti[c,*]

[a] *Department of Agriculture, Forestry and Food Sciences, University of Torino, Grugliasco, Italy*
[b] *Accademia di Agricoltura di Torino, Torino, Italy*
[c] *Institute of Sciences of Food Production, National Research Council, Grugliasco, Italy*

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ABSTRACT

The aims of the study were to utilize vibrational spectroscopy as a rapid predictive tool of forage quality; to compare two preparation methods, freeze- (FD) *vs.* oven-dried (OD); to focus on the progression of intra- and inter-family maturity by adopting a multivariate crop maturity index (CMI) based on composition, digestibility and tillage traits. A panel of forages ($n = 158$) composed of 12 crops (borage, chia, false flax, flax, galega, hemp, perilla, quinoa, ravizzone, safflower, sunflower and white lupin) derived from 8 botanic families, sampled at different vegetative stages, and which were FD or OD, were examined. Two spectrometers were used at different spectral regions: a Perkin Elmer IdentiCheck™ (PE, B-band, 714–1025 nm; C-band, 1026–2500 nm, D-band, 2501–3333 nm) and a portable Analytical Spectral Device (ASD, A-band, 350–713 nm, UV–Vis; B-band, as above). The absorption spectra were constantly higher in the OD samples and showed very high discriminability. The average prediction response (RPD, defined as the performance-deviation ratio) was better with the PE instrument, because of its enhanced band capabilities. However, the response over the spectral regions differed on the basis of which instrument was used and according to the preparations. The ASD instrument was more efficient in the B-band, for the OD preparation and better than PE in the pooled calibration (RPD:1.63 *vs.* 1.20; P=0.0005). A significant superiority in the NIR C-band for the FD preparation was observed (RPD: 2.46 *vs.* 1.95; P=0.004), while, unexpectedly, the MIR D-band was 25% more performing (RPD: 2.78 *vs.* 2.21; P=0.0005). The ash, the neutral detergent fiber (NDFom) and its indigestible part (INDF) were placed at the highest prediction rank in both instruments, albeit at different precision levels, caused by the different instrumental capabilities, with an overall

*Abbreviations:* ADFom, acid detergent fiber expressed exclusive of residual ash; ASD, Analytical Spectral Device; CMI, crop maturity index; CP, crude protein; *D*, days after seeding; DM, dry matter; DNDF, digestible neutral detergent fiber; EE, ether extract; FD, freeze-dried; GE, gross energy; INDF, indigestible neutral detergent fiber; IVTD, *in vitro* true digestibility; MIR, medium infra-red; MSE, mean square error; NDFD, *in vitro* neutral detergent fiber digestibility; NDFom, neutral detergent fiber expressed exclusive of residual ash; NIRS, near infrared spectroscopy; OD, oven-dried; OM, organic matter; PE, Perkin Elmer; RPD, ratio-performance deviation; $r^2$v, *r*-square of reciprocal validation; SD, standard deviation; SECV, standard error in cross-validation; VC, variation coefficient; 1-VR, *r*-square of internal cross-validation.

* Corresponding author. Tel.: +39 011 6709230; fax: +39 011 6709297.
*E-mail address:* piergiorgio.peiretti@ispa.cnr.it (P.G. Peiretti).

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1-VR avg. of 0.81 (1-VR, defined as the *r*-square of internal cross-validation). In a composite FD-OD equation, the best prediction was made by the INDF (1-VR of 0.91 and 0.88 for the PE and ASD instruments, respectively). The worst performances were observed for the digestible neutral detergent fiber (DNDF) prediction. The CMI was influenced by the INDF ($R^2$ 0.91) and was accurately predicted by vibrational spectroscopy (RPD 5.2 and 2.9 for PE and ASD, respectively). CMI was able to summarize the botanical differences and highlight a rank between the eight families from the less mature pole: *Boraginaceae* and *Chenopodiaceae* < *Lamiaceae* < *Asteraceae* and *Fabaceae* < *Cannabaceae* < *Brassicaceae* < *Linaceae* (the most mature type). Four particular wavelengths have been identified as they are related to the CMI, namely: 701 (red), 905, 2451 and 2799 nm, respectively, in the A, B, C and D bands.


## 1. Introduction

Digestibility is the most common nutritive parameter used in feeding standards for ruminants (Coleman and Moore, 2003; NRC, 2001), and is the basal unit when evaluating the nutritive value of forage (Jancik et al., 2011; Wang et al., 2009). In fact, the accurate estimation of forage digestibility is a prerequisite for diet formulation, the economic evaluation of forages and the prediction of animal responses (Ricci et al., 2009). Digestibility can be estimated through several techniques, whose results can differ considerably (Huhtanen et al., 2006). Forage digestibility can be studied *in vivo*, *in situ* and *in vitro* (Cone et al., 1999). Chemical composition parameters have also been used to estimate the digestibility of forages, since it is well known that the structure and thus the components of the plant, vary as the stage of maturity advances. Given that *in vivo* determinations of digestibility are laborious, expensive and difficult to standardize, *in situ* and *in vitro* techniques have been developed (Gosselink et al., 2004; Stern et al., 1997). Much of this work has been done on ruminant species, and has provided estimates that are closely correlated to *in vivo* digestibility values (Earing et al., 2010; Goldman et al., 1987; Stern et al., 1997). Over the years, various procedures have been developed and modified to determine digestibility. Recently, an efficient alternative to the traditional *in vitro* method (Tilley and Terry, 1963) has been developed by Ankom Technology (Fairport, NY, USA). This *in vitro* filter bag technique, which uses Daisy$^{II}$, is a reliable and simple technique that is easier and less time-consuming than the conventional *in vitro* technique (Damiran et al., 2008; Holden, 1999; Mabjeesh et al., 2000; Truijllo et al., 2010). It has been shown to increase labor efficiency and precision. However, the technique involves the *inoculum* of rumen fluid, which is the main factor that can introduce errors into neutral detergent fiber digestibility assays (Goeser and Combs, 2009).

A valid alternative method is the physical, rapid and non-destructive vibrational spectroscopy technique, which represents a radical departure from conventional chemical methods, in that the whole matrix derived from crops is characterized in terms of its absorption properties. In fact, all organic molecules constantly vibrate, and continue to absorb energy from incoming photons to increase their vibrations. Vibrational spectroscopy, based on the medium infra-red regions (MIR, 2500–25,000 nm) deals directly with fundamental vibrations and specific absorptions; the overtones that rebound in the near infra-red (NIR), the visible (Vis) and in the UV regions (2500–350 nm) are instead gained from powerful chemometrics to deconvolute intrinsic chemical information. The technique offers the advantages of simplicity, speed, no chemical waste and more cost-effective prediction, even though it requires laborious calibration procedures and the choice of the data treatment is complex. NIRS has revolutionized the nutritional characterization of animal feeds (Coleman and Moore, 2003; Givens and Deaville, 1999), and has been shown to be able to predict *in vivo* digestibility (Deaville et al., 2009; Decruyenaere et al., 2009; Landau et al., 2006). The neighboring spectral regions, such as UV, Vis, or MIR, are instead rarely used in animal nutrition studies.

The main objective of this study was to evaluate the potential of vibrational spectroscopy applied to the available electromagnetic radiation (UV–MIR) to predict the chemical composition, and *in vitro* digestibility assessed by the Daisy$^{II}$ system, adopting two different instruments and two sample preparation methods (oven-dried, or freeze-dried). Because of the availability of a wide range of botanically variable grass species, the second aim of the study was to focus on inter-species and family variations. Thus, a linear multivariate CMI, which is able to synthesize information from all the available parameters, has been formulated for the forages; the CMI could also be predicted by means of vibrational spectroscopy.

## 2. Materials and methods

### 2.1. Plant material and chemical analyses

Twelve sets of field data of borage (*Borago officinalis* L.), galega (*Galega officinalis* L.), false flax (*Camelina sativa* L.), flax (*Linum usitatissimum* L.), hemp (*Cannabis sativa* L.), chia (*Salvia hispanica* L.), safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.), white lupin (*Lupinus albus* L.), perilla (*Perilla frutescens* L.), ravizzone (*Brassica campestris* L. *var. Oleifera*) and quinoa (*Chenopodium quinoa* Willd.), collected in various studies from 2002 to 2010, have been used in this experiment. Overall, 158 samples of these green crops were collected at progressive morphological stages, up to sub-maturity, in order