Near Infra-Red Spectroscopy Predicts Crude Protein in Hemp Grain

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Abstract

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## 1 INTRODUCTION

Hemp (Cannabis sativa L.) is an annual crop with potential uses as a source of food or feed from grain, and bast fiber or hurd from the stalk. Hemp cultivars are commonly grown for one or both purposes and a cultivar may be referred to as a grain, fiber, or dual-purpose type. Because of protein’s nutritional importance, the protein content of a grain crop is an prime consideration for researchers, producers, and consumers. Whole hemp grain typically contains approximately 25-30% protein (Bárta et al., 2024; Ely & Fike, 2022). Crude protein (CP) is often used as a proxy for the direct measurement of protein concentration and consists of the multiplication of nitrogen concentration by a conversion factor because measuring nitrogen concentration is relatively easy and cheap via laboratory assay (Hayes, 2020).

Near-infrared spectroscopy (NIRS) technology is rapid, non-destructive, and cheap, and consists of the measurement of NIR radiation reflected from a sample (Roberts et al., 2004). NIR spectra from many samples are related to laboratory values for components such as moisture, protein, fat, or fiber [Roberts et al. (2004)]. NIRS technology has been used since the 1970’s to assess forage CP (Reeves, 2012; Williams, 1975). A NIRS calibration set often consists of samples from one species grown in many environments encompassing the range of expected values from the analyte or analytes (Chadalavada et al., 2022). Partial least squares regression (PLSR) is a typical method used in the agricultural and food sciences to relate spectra to analyte (Roberts et al., 2004).

A NIRS-scanned sample of undamaged grain may subsequently be grown, an important consideration for a plant breeder. In wheat and corn, grain protein content has been shown to be heritable [Giancaspro et al. (2019); Geyer et al. (2022)]. This suggests (at least potentially) that NIRS technology could serve as resource to more rapidly identify high CP hemp germplasm, senabling the delivery of higher CP hemp grain cultivars faster.

For this study, a benchtop NIR spectrometer was used to develop a model to predict CP content based on a data set representing multiple years, locations, and cultivars using PLSR.

## 2 MATERIALS AND METHODS

temp\_id harv\_year loc cultivar type in\_ny  
1: 141 2019 rn041\_gen h-51 dual TRUE  
2: 149 2019 mcg h-51 multistate TRUE  
3: 15 2021 ithaca h-51 multistate TRUE

Source: [Article Notebook](https://rvcrawford.github.io/glowing-system/index.qmd.html)

### 2.1 Hemp Grain Sample Background

Spectral data were obtained from whole (unground) hemp grain samples, harvested at maturity, collected from 2017 - 2021 from 18 cultivar trials in New York (NY) (149 samples). Grain samples were obtained by hand sampling or mechanical harvest and were cleaned of chaff and dried at 30 C for six days in a forced-air dryer. In total, 38 cultivars were represented in the data set. Cultivars were grain or dual-purpose types and included both commercially available and experimental material.

All cultivar trials were planted in randomized complete block design with each cultivar replicated four times. The 2017 data were comprised of samples from the same thirteen cultivars sampled from six NY locations. For those trials, grain was harvested from each plot individually and aggregated by cultivar within each trial. Four subsamples were drawn from each aggregated sample and scanned separately. These spectra were averaged at each 2 nm increment. All remaining samples from 2018-2021 were collected on a per-plot basis. All possible cultivars and possible locations were represented in 2017, but only a selected subset of cultivars and locations were represented in 2018-2021.

### 2.2 Spectral Data Collection and Preprocessing

A benchtop NIR spectrometer (FOSS/ NIR FOSS/ NIR Systems model 5000) was used to obtain the spectra (FOSS North America, Eden Prairie, MN, USA). Spectra were collected every 2 nm from 1100-2498 nm and the logarithm of reciprocal reflectance was recorded.

WINISI software version 1.02A (Infrasoft International, Port Matilda, PA, USA) was used to average the spectra in 2017, as well as to select samples for laboratory assay. Samples were selected according to their spectral distance from their nearest neighbor within the calibration data set with a cutoff of a distance of 0.6 H, where H is approximately equal to the squared Mahalanobis distance divided by the number of principal components used in the calculation (Garrido-Varo et al., 2019). Prior to selection selection, spectra were preprocessed using SNV-detrend with settings 1,4,4,1 for the derivative, gap, smooth, and smooth 2 settings respectively.

### 2.3 Software used:

Additional analysis was performed

### 2.4 Preprocessing

Multiplicative scatter correction (MSC)

standard normal variate (SNV) transformation

The calibration set consisted of

The validation set consisted of

### 2.5 Laboratory Validation

Laboratory assays were performed by Dairy One Forage Laboratory (Ithaca, NY). For those assays, 1mm ground samples were analyzed by combustion using a CN628 or CN928 Carbon/Nitrogen Determinator. Samples from 2017 were aggregated from

Prior to In 2017, an in

## 3 RESULTS AND DISCUSSION

## 4 ACKNOWLEDGMENTS

## 5 SUPPLEMENTAL MATERIAL

## 6 OPTIONAL SECTIONS

## 7 REFERENCES

## 8 FIGURES AND TABLES

Source: [Article Notebook](https://rvcrawford.github.io/glowing-system/index.qmd.html)

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| Figure 1: Timeline of recent earthquakes on La Palma |

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Based on data up to and including 1971, eruptions on La Palma happen every 79.8 years on average.

Studies of the magma systems feeding the volcano, such as (**marrero2019?**), have proposed that there are two main magma reservoirs feeding the Cumbre Vieja volcano; one in the mantle (30-40km depth) which charges and in turn feeds a shallower crustal reservoir (10-20km depth).

Eight eruptions have been recorded since the late 1400s ([Figure 1](#fig-timeline)).

Data and methods are discussed in [Section 9](#sec-data-methods).

Let denote the number of eruptions in a year. Then, can be modeled by a Poisson distribution

where is the rate of eruptions per year. Using [Equation 1](#eq-poisson), the probability of an eruption in the next years can be calculated.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1: Recent historic eruptions on La Palma   | Name | Year | | --- | --- | | Current | 2021 | | Teneguía | 1971 | | Nambroque | 1949 | | El Charco | 1712 | | Volcán San Antonio | 1677 | | Volcán San Martin | 1646 | | Tajuya near El Paso | 1585 | | Montaña Quemada | 1492 | |

[Table 1](#tbl-history) summarises the eruptions recorded since the colonization of the islands by Europeans in the late 1400s.

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| Figure 2: Map of La Palma |

La Palma is one of the west most islands in the Volcanic Archipelago of the Canary Islands ([Figure 2](#fig-map)).

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| Figure 3: Locations of earthquakes on La Palma since 2017 |

Source: [Explore Earthquakes](https://rvcrawford.github.io/glowing-system/notebooks/explore-earthquakes-preview.html#cell-fig-spatial-plot)

[Figure 3](#fig-spatial-plot) shows the location of recent Earthquakes on La Palma.

## 9 Data & Methods

## 10 Conclusion

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