

# Near Infra-Red Spectroscopy Predicts Crude Protein in Hemp Grain

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**Abstract**

Lorem ipsum dolor sit amet, consectetur adipiscing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat. Duis aute irure dolor in reprehenderit in voluptate velit esse cillum dolore eu fugiat nulla pariatur. Excepteur sint occaecat cupidatat non proident, sunt in culpa qui officia deserunt mollit anim id est laborum.

**Plain Language Summary**

Earthquake data for the island of La Palma from the September 2021 eruption is found ...

**incomplete: may contain errors, run-ons, half-thoughts, etc.**

**0.1 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 20-30% protein (Bárta et al., 2024; Callaway, 2004; 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 simple (Hayes, 2020).

Near-infrared spectroscopy (NIRS) technology is rapid, non-destructive, and cheap. It consists of the measurement of NIR radiation reflected and absorbed from a sample (the spectra) and the relation of the spectra 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). PLSR calculates components that maximize covariance between predictor and response variables. PLSR uses some number of components, often selected via cross-validation, in order to fit the regression model and is commonly used in spectroscopy because it tends to work well with highly-correlated, noisy spectral data (Wold et al., 2001).

A NIRS-scanned sample of undamaged grain may used for other purposes besides its scan or it may planted as a seed. In wheat and corn, grain protein content has been shown to be heritable (Geyer et al., 2022; Giancaspro et al., 2019). This suggests (at least potentially) that NIRS technology could serve as resource to rapidly identify high CP hemp germplasm, enabling the screening of more germplasm as grain, before planting to the field, and thus enabling the more efficient development of high CP hemp grain cultivars.

For this study, a benchtop NIR spectrometer was used to develop a model to predict CP content based on a data set of hemp grain representing multiple years, locations, and cultivars from grain and dual-purpose hemp types using PLSR.

**0.2 MATERIALS AND METHODS**

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### 0.2.1 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) (NA 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.

### 0.2.2 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. A 1/4 rectangular sample cup (5.7 cm  $\times$  4.6 cm) was used.

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.

### 0.2.3 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 as described above, but the remaining samples were not aggregated.

### 0.2.4 Model Development

Calibration and validations sets were created by dividing the laboratory CP values into tertiles according to their percent CP in order to ensure that the range of CP values was present in both calibration and testing sets. Within each tertile, 75% of the samples were randomly assigned to the calibration set and the remaining 25% were assigned to the testing set. For each calibration set, models were developed in the caret package using PLSR. In fitting the model, the number of components was optimized over a grid search from 1-20. Model performance was evaluated with 25 iterations of bootstrapping and minimized root mean squared error (RMSE) in selecting the number of components in the final model .

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Initially a number of common spectral preprocessing methods were tested by creating 100 calibration and testing sets, as described above. Spectral data from those data sets were transformed by each of the following methods: 1) first derivative, 2) Savitzky-Golay (SG) using the first derivative, third order polynomial, and a window of size 5, 3) gap-segment derivative using the first derivative, a gap of eleven, and a segment size of 5, 4) standard normal variate (SNV), 5) standard normal variate fol-

lowing Savitzky-Golay (SNV-SG) (same SG parameters as above), 6) SNV-detrend with second order polynomial, and 7) multiplicative scatter correction. As a comparison, models were also developed using untransformed spectra.

For each of these preprocessing methods, models were fit and predictions were made on the corresponding validation set (since there were 8 preprocessing methods, 8 separate models were fit for each of the 100 sets. The relationship between the predicted and actual values of the testing set were calculated via RMSE,  $R^2$  and Ratio of Performance to InterQuartile distance (RPIQ), three common model assessment metrics. Larger  $R^2$  and RPIQ, and smaller RMSE values are superior. Analyses of variance (ANOVA) were performed for each of these metrics in order to compare the preprocessing methods. For each ANOVA, each data set was considered as a subject and different variances were allowed for each preprocessing method.

Once the most promising preprocessing method was identified, 1000 more data sets were created and analyzed via that method and performance on the testing sets was summarized with RMSE,  $R^2$ , and RPIQ.

### 0.2.5 Additional software used

We used R version 4.3.3 (R Core Team, 2024) and the following R packages: caret v. 6.0.94 (Kuhn & Max, 2008), data.table v. 1.15.2 (Barrett et al., 2024), emmeans v. 1.10.0 (Lenth, 2024), nlme v. 3.1.163 (J. Pinheiro et al., 2023; J. C. Pinheiro & Bates, 2000), pls v. 2.8.3 (Liland et al., 2023), prospectr v. 0.2.7 (Stevens & Ramirez-Lopez, 2024), skimr v. 2.1.5 (Waring et al., 2022), tidymodels v. 1.1.1 (Kuhn & Wickham, 2020), tidyverse v. 2.0.0 (Wickham et al., 2019).

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## 0.3 RESULTS AND DISCUSSION

### 0.3.1 Laboratory assay CP values

Laboratory assay percent CP values are summarized in the following table. These are similar to the range of CP values observed in the literature, indicating an reasonable basis for a chemometric model. The CP values are left-skewed and two thirds of the samples contained more than 25% CP.

Table 1: Summary of Laboratory Assayed CP Values (Percent Dry Matter)

Mean	Sd	Minimum	First Quartile	Median	Third Quartile	Maximum
26.1	2.5	20.8	23.9	26.4	28.2	30.8

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### 0.3.2 Preprocessing methods comparison

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All preprocessing methods outperformed untransformed spectral data Table 2. Averaged together, all preprocessed spectra were superior to untransformed spectra, with

lower RMSE, and higher  $R^2$  and RPIQ values (significant at  $\alpha$  level  $<0.001$ ). Pre-processing methods had -11.6 % lower RMSE, and had 3.1% higher  $R^2$  7.4% higher RPIQ than unprocessed spectra.

The SNV-SG method had the lowest RMSE, highest  $R^2$ , and highest RPIQ averaging over all iterations. SNV-SG RMSE averaged 1.4% lower, while  $R^2$  and RPIQ averaged 0.4% and 2.4% higher respectively than the next best preprocessing method (SG in both cases), but the difference between the best and second best method by metric were only statistically significant at  $\alpha < 0.05$  for RPIQ. RPIQ was devised to accurately reflect the spread of data in skewed populations (Bellon-Maurel et al., 2010) and thus offers a robust metric for model assessment in this context, where the CP data are skewed. Therefore the superiority of SNV-SG as measured via RPIQ made it the best choice for the final model.

Table 2: Evaluation of Preprocessing Methods by Metric  $\pm$  Standard Error

Preprocessing Method	RMSE	$R^2$	RPIQ
Standard Normal Variate following Savitzky-Golay	1.02 $\pm$ 0.012	0.84 $\pm$ 0.004	3.97 $\pm$ 0.076
Savitzky-Golay	1.03 $\pm$ 0.012	0.83 $\pm$ 0.004	3.88 $\pm$ 0.072
First Derivative	1.07 $\pm$ 0.013	0.82 $\pm$ 0.004	3.77 $\pm$ 0.075
Standard Normal Variate	1.12 $\pm$ 0.016	0.80 $\pm$ 0.005	3.61 $\pm$ 0.081
Gap-segment Derivative	1.12 $\pm$ 0.018	0.81 $\pm$ 0.006	3.60 $\pm$ 0.086
Standard Normal Variate-Detrend	1.13 $\pm$ 0.015	0.80 $\pm$ 0.005	3.55 $\pm$ 0.079
Multiplicative Scatter Correction	1.17 $\pm$ 0.016	0.79 $\pm$ 0.006	3.47 $\pm$ 0.080
Untransformed Spectra	1.22 $\pm$ 0.044	0.79 $\pm$ 0.009	3.42 $\pm$ 0.105

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These results are not surprising. SNV and SNV-detrend both correct light scatter, which is often a function of differences in particle size and sample packing density, although SNV-detrend is often used for densely-packed, powdered samples (Barnes et al., 1989). Here, hemp grain was neither powdered nor densely packed SG is a smoothing filter that regresses on the signal over a series of windows, removing noise while preserving the signal’s shape and features.(Li et al., 2020; Luo et al., 2005). Derivatives remove noise, but not necessarily light scatter

**cite:** Barnes RJ, Dhanoa MS, Lister SJ. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied spectroscopy*, 43(5): 772-777.

The preprocessing methods examined represent a portion of those available. As well, preprocessing methods tend to have a number of user-adjustable parameters whose various permutations were not tested. This subset of preprocessing methods and parameters nonetheless contained substantial variations in model quality, demonstrating the importance of the selection of an appropriate preprocessing method.

### 0.3.3 Final model development and summary

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The model improved most rapidly as the number of principal components increased from 1 to 7, with the inclusion of each additional PC being associated with a decrease in RMSE of 5-12%. From 8 to 12 PCs, model performance continued to improve, although gains were more modest (decrease in RMSE of 0.7-3%). With 13 or more PCs, performance gains were minimal and the relative ranks of the models tended to be stable Figure 1.

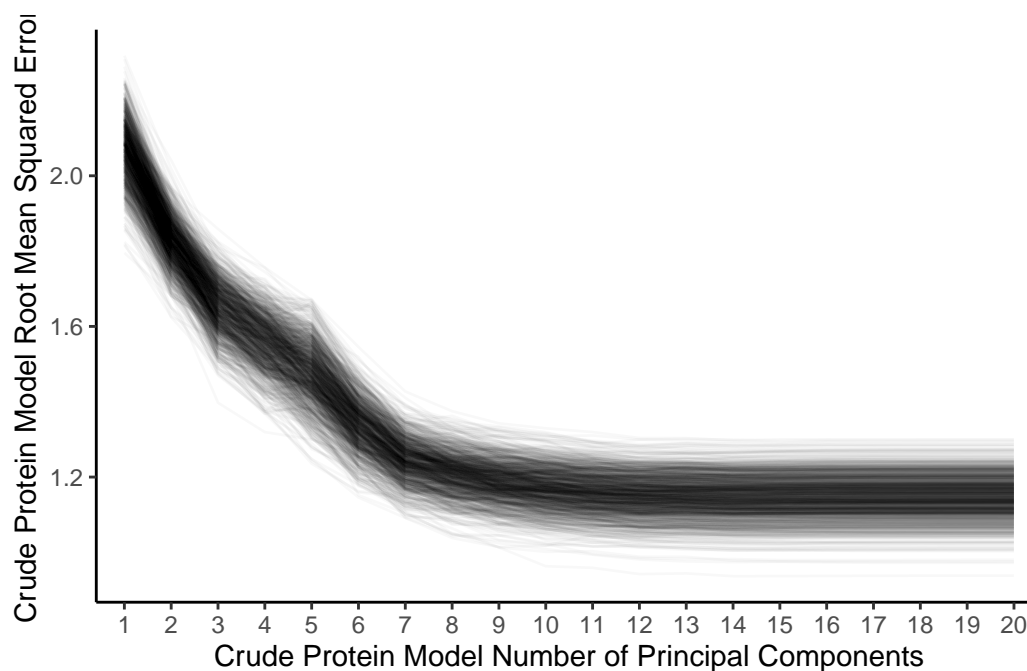


Figure 1: Decreasing RMSE with increasing number of PCs

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Final model performance was similar, but not identical to, that obtained during the initial comparison of preprocessing methods. The final models' mean RMSE was 1.03,  $R^2$  was 0.83, and RPIQ was 3.89 (all calculated on the test sets). Despite the generally good model performance, a subset of poor models can be seen. For example, Figure 2 shows twenty-one models with  $R^2$  below 0.7. **more comment on poor models?**

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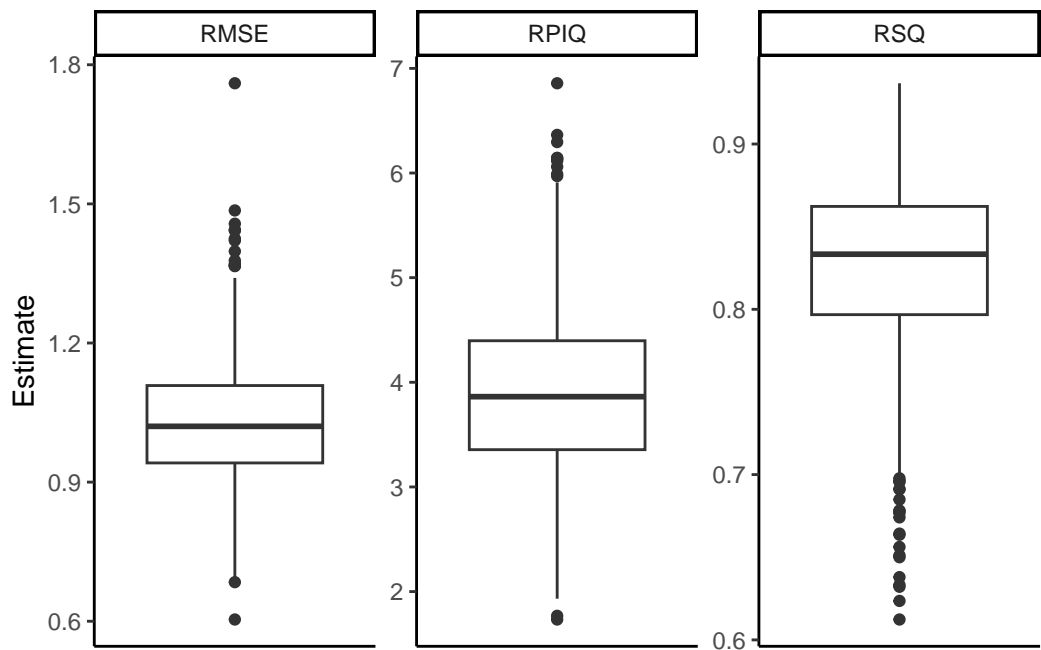


Figure 2: Final model validation set performance (1000 iterations)

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```
Call:
lm(formula = difference ~ adj_cp, data = temp_dat)
```

```
Residuals:
    Min       1Q   Median       3Q      Max
-2.51794 -0.58132  0.06936  0.50754  2.74745
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.80412     0.17827   4.511 1.31e-05 ***
adj_cp       -0.15334     0.03051  -5.026 1.44e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.9138 on 147 degrees of freedom
Multiple R-squared:  0.1466,    Adjusted R-squared:  0.1408
F-statistic: 25.26 on 1 and 147 DF,  p-value: 1.438e-06
```

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Finally, the pattern of errors was examined on a per-sample basis. Figure 3

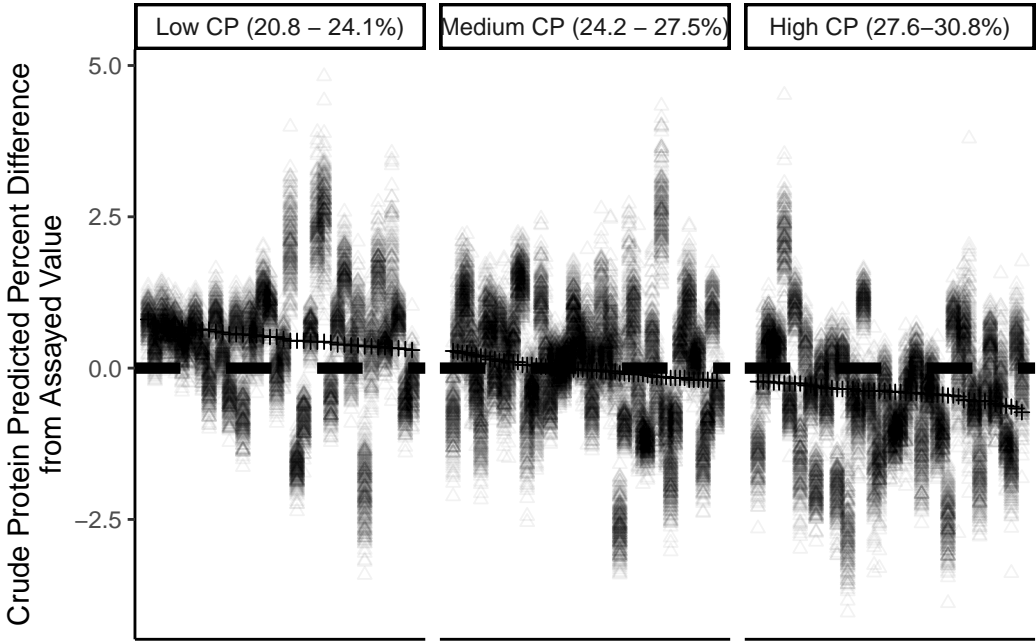


Figure 3: Test set prediction errors on a per-sample basis. Actual sample value set to 0, and samples ranked from least to greatest actual % CP value

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To assess the patterns of errors within the models, a linear model was fit considering the mean estimated error for each sample considering all models where that sample was in the test set when compared to the sample’s actual value. The models overestimated %CP by approximately 0.5 % in the lowest tertile and underestimated %CP by -0.01 % and -0.41 % in the middle and highest tertile, respectively

Key: <loc>

	loc	ith_in_data_set	crude_protein	difference	tmp_ith	adj_cp
	<char>	<int>	<num>	<num>	<int>	<num>
1:	cnoll	4	27.1	-1.826000348	90	6.3
2:	free	91	23.1	-1.608410140	24	2.3
3:	free	100	27.5	0.008386084	100	6.7
4:	free	103	26.0	-0.095290561	64	5.2
5:	freev	66	28.3	0.156805468	117	7.5
6:	freev	69	28.7	0.115415937	128	7.9
7:	freev	70	27.3	0.123941888	93	6.5
8:	ithaca	123	25.9	0.137420230	60	5.1
9:	ithaca	124	23.2	2.325322409	27	2.4
10:	ithaca	125	23.2	2.582685059	25	2.4
11:	ithaca	140	23.5	1.597167816	30	2.7
12:	ithaca	146	28.0	-1.965391942	110	7.2
13:	ithaca	148	26.4	0.145123025	76	5.6
14:	mcg	92	22.7	-0.110406545	20	1.9
15:	mcg	94	28.1	-0.067559276	111	7.3



242	16:	mcg	121	28.3	-2.434565554	115	7.5
243	17:	mg4e	28	25.4	0.034158139	53	4.6
244	18:	mg4e	39	26.1	-0.016921027	66	5.3
245	19:	mg4l	41	26.4	0.118021909	74	5.6
246	20:	rn041_gen	108	27.0	2.600884960	86	6.2
247	21:	rn041_gen	109	23.7	-1.980462540	33	2.9
248	22:	rn041_gen	110	29.0	-2.495337360	135	8.2
249	23:	rn041_gen	111	29.6	-1.777305991	140	8.8
250	24:	rn041_gen	113	29.5	0.011781362	139	8.7
251	25:	rn041_gen	114	27.7	2.115039075	107	6.9
252	26:	rn00a	14	29.1	-0.003578789	137	8.3
253	27:	rn00a	18	23.1	2.020690041	22	2.3
254	28:	rn00a	19	25.3	-0.110141285	52	4.5
255	29:	rn00a	25	26.6	-2.603177621	78	5.8
256	30:	rn00a	26	28.3	-2.034162792	114	7.5
257	loc ith_in_data_set crude_protein difference tmp_ith adj_cp						
258	grouping		temp_id	harv_year	cultivar		type
259	<char>		<int>	<int>	<char>		<char>
260	1:	worst_predicted_10_pct	32	2017	cfx-1		
261	2:	worst_predicted_10_pct	111	2018	grandi		grain
262	3:	best_predicted_10pct	128	2019	canda		grain
263	4:	best_predicted_10pct	131	2019	nwg-elite		dual
264	5:	best_predicted_10pct	94	2017	picolo		
265	6:	best_predicted_10pct	97	2017	futura 75		
266	7:	best_predicted_10pct	98	2017	cfx-1		
267	8:	best_predicted_10pct	155	2020	nwg-2730		multistate
268	9:	worst_predicted_10_pct	152	2020	futura 75		dual_replant
269	10:	worst_predicted_10_pct	156	2020	bialobrzeskie		multistate
270	11:	worst_predicted_10_pct	9	2021	logan c2		exp.
271	12:	worst_predicted_10_pct	5	2021	crs-1		grain_dual
272	13:	best_predicted_10pct	10	2021	logan x anka c2		exp.
273	14:	best_predicted_10pct	115	2018	nebraska (feral)		dual
274	15:	best_predicted_10pct	113	2018	picolo		grain
275	16:	worst_predicted_10_pct	149	2019	h-51		multistate
276	17:	best_predicted_10pct	56	2017	cfx-2		
277	18:	best_predicted_10pct	67	2017	futura 75		
278	19:	best_predicted_10pct	69	2017	anka		
279	20:	worst_predicted_10_pct	136	2019	earlina 8		grain
280	21:	worst_predicted_10_pct	137	2019	katani		grain
281	22:	worst_predicted_10_pct	138	2019	joey		grain
282	23:	worst_predicted_10_pct	139	2019	futura 75		dual
283	24:	best_predicted_10pct	141	2019	h-51		dual
284	25:	worst_predicted_10_pct	142	2019	hliana		dual
285	26:	best_predicted_10pct	42	2017	tygra		
286	27:	worst_predicted_10_pct	46	2017	katani		
287	28:	best_predicted_10pct	47	2017	cfx-2		
288	29:	worst_predicted_10_pct	53	2017	cfx-1		
289	30:	worst_predicted_10_pct	54	2017	wojko		
290	grouping		temp_id	harv_year	cultivar		type
291	in_ny	loc2					
292	<lgcl>	<char>					
293	1:	TRUE	geneva				
294	2:	TRUE	freeville				
295	3:	TRUE	freeville				
296	4:	TRUE	freeville				

```

297     5:  TRUE freeville
298     6:  TRUE freeville
299     7:  TRUE freeville
300     8:  TRUE   ithaca
301     9:  TRUE   ithaca
302    10:  TRUE   ithaca
303    11:  TRUE   ithaca
304    12:  TRUE   ithaca
305    13:  TRUE   ithaca
306    14:  TRUE   ithaca
307    15:  TRUE   ithaca
308    16:  TRUE   ithaca
309    17:  TRUE   ithaca
310    18:  TRUE   ithaca
311    19:  TRUE   ithaca
312    20:  TRUE  geneva
313    21:  TRUE  geneva
314    22:  TRUE  geneva
315    23:  TRUE  geneva
316    24:  TRUE  geneva
317    25:  TRUE  geneva
318    26:  TRUE  geneva
319    27:  TRUE  geneva
320    28:  TRUE  geneva
321    29:  TRUE  geneva
322    30:  TRUE  geneva
323      in_ny      loc2

```

324 Source: [Article Notebook](#)

325 This study is limited in that it represents the creation of one model based upon  
326 spectra collected from one machine. NIRS calibrations can be unique to a particular  
327 machine, even if the machines compared are of the same model (Reeves, 2012). As  
328 well, the calibration and validation sets are relatively small.

329 This research showed the promise of the use of NIRS in order to make predictions  
330 concerning %CP in hemp grain using PLS. Promising preprocessing methods were  
331 identified and a model was validated. Further research could refine a CP model by  
332 including more samples, identifying promising spectral regions, or by examining  
333 other predictive methods.

#### 334 0.4 ACKNOWLEDGMENTS

#### 335 0.5 SUPPLEMENTAL MATERIAL

Table 3: Tally of hemp cultivars and locations. Private cultivars are labeled “cultivar1”, “cultivar2”, etc.

cultivar2	chazy	freeville	geneva	ithaca	willsboro	Total
altair				1		1
anka		1	3	5	2	11
bialobrzeskie		1	3	4	1	9
canda		1	1	1		3
cfx-1		1	2	5		8
cfx-2		1	2	4		7
crs-1	1	1	2	5		9
cultivar1		1				1
cultivar2				1		1

Table 3: Tally of hemp cultivars and locations. Private cultivars are labeled “cultivar1”, “cultivar2”, etc.

cultivar2	chazy	freeville	geneva	ithaca	willsboro	Total
cultivar3				1		1
cultivar4				1		1
earlina 8			1			1
experimental1				1		1
experimental2				1		1
felina 32		1	2	3		6
futura 75		1	3	4		8
grandi		3	3	4		10
h-51			1	2		3
han-fn-h				1		1
han-nw				1		1
helena		1				1
henola				2		2
hlesia				3		3
hliana			1	1		2
joey		1	1	1		3
katani		2	3	4		9
nebraska (feral)	1			1		2
pewter river		1				1
picolo		1	2	5		8
portugal			1			1
rocky hemp			1			1
sterling gold			1			1
swift	1	1		1		3
tygra		1	3	4		8
uso-31	2	1	2	4		9
wojko		1	3	4		8
x-59		2		1		3
Total	5	24	41	76	3	149

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0.6 OPTIONAL SECTIONS  
0.7 REFERENCES  
0.8 FIGURES AND TABLES

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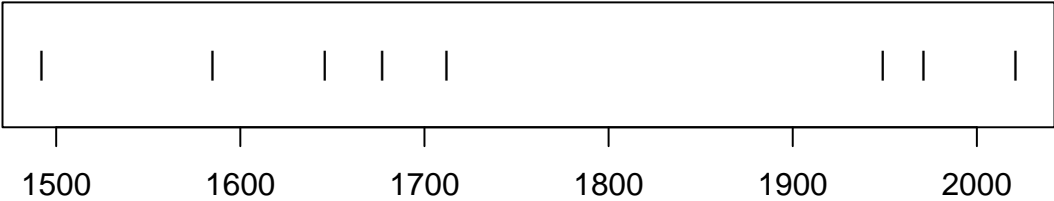


Figure 4: Timeline of recent earthquakes on La Palma

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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 4](#)).

Data and methods are discussed in [Section 0.9](#).

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

$$p(x) = \frac{e^{-\lambda} \lambda^x}{x!} \quad (1)$$

where  $\lambda$  is the rate of eruptions per year. Using [Equation 1](#), the probability of an eruption in the next  $t$  years can be calculated.

Table 4: 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 4](#) summarises the eruptions recorded since the colonization of the islands by Europeans in the late 1400s.

La Palma is one of the west most islands in the Volcanic Archipelago of the Canary Islands ([Figure 5](#)).

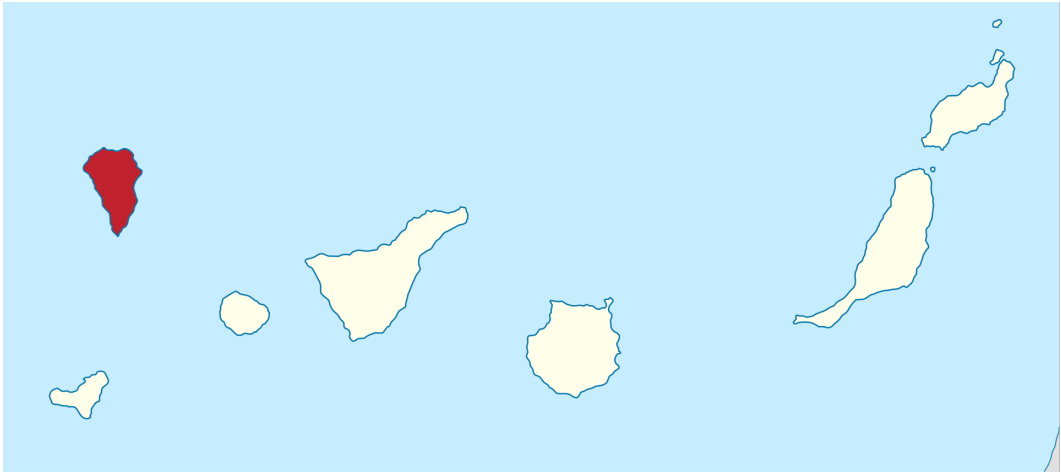


Figure 5: Map of La Palma

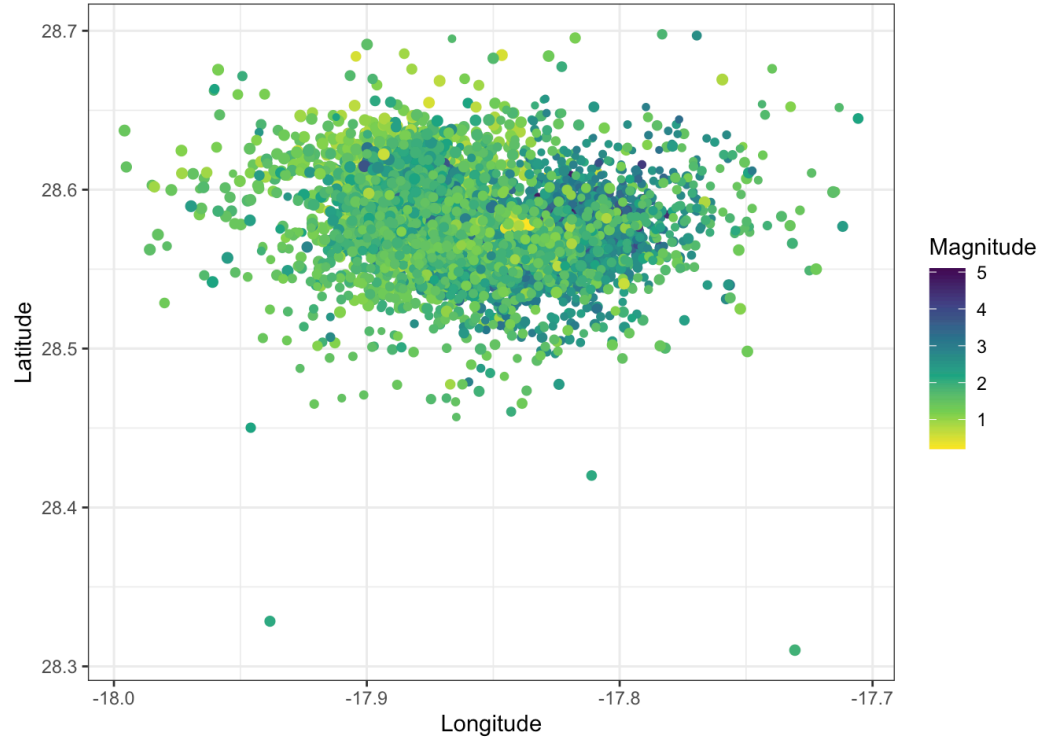


Figure 6: Locations of earthquakes on La Palma since 2017

359 Source: [Explore Earthquakes](#)

360 Figure 6 shows the location of recent Earthquakes on La Palma.

## 0.9 Data & Methods

## 0.10 Conclusion

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