Use of near-infrared spectroscopy to predict energy content of Hemp flour (*Cannabis sativa L.*)

ABSTRACT

Near-infrared spectroscopy is used for the rapid evaluation of numerous macronutrients. However, there is limited published data on its effectiveness in assessing the gross energy digestibility (GE) of foods and not much information regarding hemp for animal feed.
The chemical composition of 56 hemp flour samples was determined following the official protocols of the Association of Analytical Chemists and chemometric readings were conducted. GE, gross energy digestibility (GEd) and digestible energy (DE) were estimated using the equations proposed by INRA. A statistical analysis was performed to evaluate the potential use of NIR data to predict the energy content of hemp flour.
Data from laboratory and NIR assessments were 22.54 versus 20.44 for GE (MJ/kg DM), 90.72 versus 90.21 for GEd (MJ/kg DM), and 19.73 versus 20.13, respectively for the loss (%). The results indicated the feasibility of energy value prediction, although further studies are needed to refine the technique. NIR expands the calibration set, allowing increasingly accurate determinations, in the study of the chemical-nutritional characteristics of hemp sativa, even if further investigations are necessary**.**

Aims: The aim of this study was to evaluate the utility of NIR_S for predicting the energy content through the chemical characterization of the flour obtained after the cold pressing of *Cannabis sativa L. seeds*, as well as the possibility of predicting their energy content starting from the data obtained through the NIRs technique.

Study design: The chemical composition of 56 hemp flour samples was determined following the official protocols of the Association of Analytical Chemists and chemometric readings were conducted. GE, gross energy digestibility (GEd) and digestible energy (DE) were estimated using the equations proposed by INRA. A statistical analysis was performed to evaluate the potential use of NIR data to predict the energy content of hemp flour.

Results: Data from laboratory and NIR assessments were 22.54 versus 20.44 for GE (MJ/kg DM), 90.72 versus 90.21 for GEd (MJ/kg DM), and 19.73 versus 20.13, respectively for the loss (%). The results indicated the feasibility of energy value prediction, although further studies are needed to refine the technique. NIR expands the calibration set, allowing increasingly accurate determinations, in the study of the chemical-nutritional characteristics of hemp sativa, even if further investigations are necessary.

Conclusion: The study provides comprehensive insights into the chemical composition of hemp flour, explores its comparison with other seeds, evaluates different analysis methods, and establishes reliable prediction models for energy content.

Keywords: gross energy, gross energy digestibility, digestible energy, Hemp flour, near-infrared spectroscopy

1. INTRODUCTION

Animal nutritionists have long recognized the importance of measuring the nutritive value of feed provided to livestock. The nutritional value of hemp seeds and their processed products, such as oil and flour, has been studied in recent years to understand the nutritional quality of this food matrix [1, 2]. Hemp, traditionally used as a source of fiber, is now increasingly considered for animal feed due to its favorable nutritional characteristics.
Hemp seed production data for 2020 are available from five countries in the FAO database [3]. In Italy, the cultivation of industrial hemp has been permitted through law no. 242/2016, along with the subsequent ministerial circular published in 2017, which outlines the conditions for hemp production, marketing, and use [4].
The increased use of near-infrared reflectance spectroscopy (NIRS) as an alternative to traditional analytical methods for evaluating the energy content of feedstuffs and diets has led to an expansion of knowledge in the field of chemometrics. NIRS is a non-destructive, fast, accurate, and less expensive technique for estimating the chemical composition of feedstuffs [5]. Additionally, NIRS offers advantages over conventional laboratory analytical methods, such as no reagent use and simultaneous determination of multiple parameters (e.g., crude proteins, ether extract, acid detergent fiber, neutral detergent fiber, etc.).
Like classic methods, drying and grinding procedures are fundamental for the NIRS technique. Water strongly absorbs NIR light, and particle size influences the shape of the spectrum. NIRS spectra are also affected by laboratory conditions (e.g., environmental dampness and temperature), which should be as uniform as possible, particularly with respect to temperature [6].
A lack of comprehensive data on the availability of various nutrients in feedstuffs and feeds has hindered the use of NIRS for estimating nutrient content for many animal species and estimating energy content for ruminants [7, 8]. However, few studies have been published on the use of NIRS to assess the composition and nutritive value of food, possibly due to the difficulty in obtaining in vivo data for a robust calibration.
Hemp flour, obtained after pressing oil from the seeds, is an exceptional raw material for producing products with a high nutritional profile. Its value lies in its nutritional composition, characterized by a high content of protein, fiber, and fats, along with vita-mins E, B1, and B2, mineral salts, and phytosterols. Importantly, it does not contain gluten, making hemp flour ideal for preparing products suitable for people with celiac dis-ease. Additionally, it is used in animal nutrition as a protein source, replacing flours from more common oil seeds (soybean, rapeseed, and sunflower).
For animal feed purposes, the high content of NDF provides the flour with a quantity of digestible principles not exceeding 40%, resulting in reduced digestibility. Despite the lower digestibility, hemp flours have a moderate content of digestible protein (about 80%), making them suitable for the feeding of some animal species, such as sheep, goats, and horses, and less suitable for feeding pigs. Some studies report the benefits of using hemp cake for feeding laying hens due to the presence of omega-3 and omega-6 fatty ac-ids in the eggs produced and as feed for farmed fish [9].
The aim of this study was to evaluate the utility of NIRS for predicting the energy content through the chemical characterization of the flour obtained after the cold pressing of *Cannabis sativa L.* seeds, as well as the possibility of predicting their energy content starting from the data obtained through the NIRs technique.

2. MATERIAL AND METHODS

A set of 56 hemp samples, specifically the Futura75 variety, was obtained from two different farms located in the Campania Region (Southern Italy). Following collection, the samples were ground using a 1-mm screen with a knife mill, and subjected to chemical composition

analysis, including dry matter (DM), crude protein (CP), ether extract (EE), and ash. The analysis was conducted in accordance with the procedures outlined by [10], with the respective identification numbers 2001.12, 978.04, 920.39, and 930.05 assigned for DM, CP, EE, and ash. Additionally, neutral detergent fiber, acid detergent fiber, and free ash acid detergent lignin were determined, following the guidelines provided by [11].
From the proximate analysis, gross energy (GE), the coefficient of digestibility of gross energy (GEd), and digestible energy (DE) were estimated using the equations pro-posed by [12] and the researchers of [13].
Fresh food samples were milled through a 1-mm sieve and scanned twice in reflectance mode in the spectrophotometer using a Büchi Instruments (Inc model NIRFlex N-500). The instrument has silicon and InGaAs diode arrays and an intense broadband light source, allowing measurement of reflectance from a large area of the sample surface (in a container of approximately 10 cm in diameter). The diodes were centered at 10 nm intervals, but software was used to spline-interpolate spectra to a data interval of 5 nm. The two spectral ranges of the instrument are spliced at 950 nm to cover a range from 800 to 2500 nm, chosen because many characteristic absorptions of amines fall into the same regions of alcohols as the N-H and O-H bonds are similar. The instrument acquisition time averages 30 spectra s^{-1}, and a spectral scan was defined as the average spectrum generated after 1 s of acquisition.
Chemical composition and measured GE, GEd, and DE of compound foods used for calibration are presented in Tables 1 and 2.
All statistical methods for the evaluation of data were carried out to determine the possible use of NIRs data for predicting the energy content of hemp flour extraction and relationships among all considered data using [14].

3. RESULTS AND DISCUSSION

In Table 1, it is observed that the flour is rich in protein using both methods. The NDF represents a high proportion in hemp flour. As can be seen, the protein content of the sample is comparable to that reported in the literature for hemp flour using both methods. Table 1 shows lower protein content and higher structural carbohydrate content com-pared to the literature [9, 15, 2]. These differences could be attributed to the oil extraction method, which may have influenced the chemical composition of the analyzed samples. Similar results were reported by [15], who tested hempseed cake in cattle nutrition. The table also shows a high concentration of NDF in the cold-pressed hemp seed cake. The fat content may be justified by the pressing process, which removes approximately 63% of the fat from the whole seed, as indicated by [16].
If we compare the protein content obtained in our analyses, the data show that hemp has a higher protein content than seeds such as rapeseed and sunflower but lower than soy. In fact, soybeans are considered the main source of vegetable proteins, with a composition very close to that of animal-origin food. It should be noted that while soybeans contain anti-nutritional factors, such as trypsin inhibitors requiring thermal treatment for elimination, hemp contains a smaller amount, making its proteins more digestible [17].
The significant differences shown in Table 1 are related to the different methods used for determination. In particular, the NIRs technique involves more reading replications of a single sample compared to the duplication that takes place in the laboratory. Even though the reliability of the results in NIRs depends a lot on the calibration curves that need continuous updating, the differences, except for the ash content compared to NFE, are not significant enough to deem spectroscopy readings unreliable.

Table 1 - Chemical composition obtained from traditional chemical composition and NIRs data on hemp flour samples.

		Min	Max	Means	Std dev
Weende	DM	91.02	94.32	93.20	0.76
Van	CP	19.82	26.89	23.82**	1.53
Soest	NDF	48.94	62.87	56.18	3.50
	EE	7.10	27.87	12.09**	5.99
	ASH	5.14	8.49	7.03*	0.80
NIRs	DM	88.66	98.38	93.98	2.86
	CP	10.12	24.76	20.19**	3.63
	NDF	34.20	59.62	47.28	7.01
	EE	5.04	19.52	10.68**	2.86
	ASH	2.88	5.86	3.90*	0.75

Table 2 - GE content, apparent digestibility of GE and DE content obtained from traditional chemical composition and NIR data on hemp flour samples.

		Min.	Max	means	std. Dev.
Weende	GE	20.73	28.37	22.54	1.70
	GEd	89.7	93.0	90.7	0.90
	DE	18.76	26.29	19.73	1.49
NIR	GE	19.58	25.22	20.44	1.72
	GEd	88.3	91.8	90.2	1.70
	DE	17.31	23.12	20.13	1.18

The data shown in Table 2 demonstrate good correspondence between the data calculated from chemical determinations (Weende and Van Soest) and those obtained with NIRs determinations. The apparent digestibility of GE values obtained appears high. [17] and [18] report that hemp flour has high digestibility related to a high degree of digestibility of proteins. [17] reports that this good digestibility can be linked to an immediate release of bio accessible amino acids.
In Table 3 we report the correlation matrix among the considered parameters.

Table 3 - correlation matrix among the considered parameters

	Correlations	Sign.
GE *vs* GE_NIR	-0.035	NS
GEd *vs* GEd_NIR	-0.317	0.017
DE *vs* DE_NIR	-0.141	NS

In Table 3, we report the correlation matrix among the considered parameters. No significant correlation was found between GE and DE. However, for all parameters obtained by NIRs techniques, a negative correlation was found. These results are probably related to the different values obtained in the chemical compositions and reported in Table 1.
The data were also analyzed separately for GE, GEd, and DE using multiple linear regressions to evaluate the relationship between energy content and various predictor variables. Stepwise regression was used to eliminate variables that did not influence variation in the model. The adjusted R2 selection method was used to make the final decision about the best models.

From the examination of regressions (Table 4 and 5), it is possible, using CP_NIR, EE_NIR, NDF_NIR, and NFE_NIR as independent variables, to obtain a good prediction model for GE. Tables show that the best predicting model was the last one, as demonstrated by the best R^2_{Adj} and the significant level of independent variables.

Table 4 - Linear regression model summary for GE prediction

Model	R	R^2	R_{2AdJ}	standard error of estimate
1	0.641a	0.410	0.399	1.314
2	0.723b	0.523	0.504	1.194
3	0.755c	0.571	0.545	1.144
4	0.977d	0.955	0.952	0.373

a. *predictors: (constant), CP_NIR*
b. *predictors: (constant), CP_NIR, EE_NIR*
c. *predictors: (constant), CP_NIR, EE_NIR, NDF_NIR*
d. *predictors: (constant), CP_NIR, EE_NIR, NDF_NIR, NFE_NIR*

Table 5 - ANOVA for the regression models d

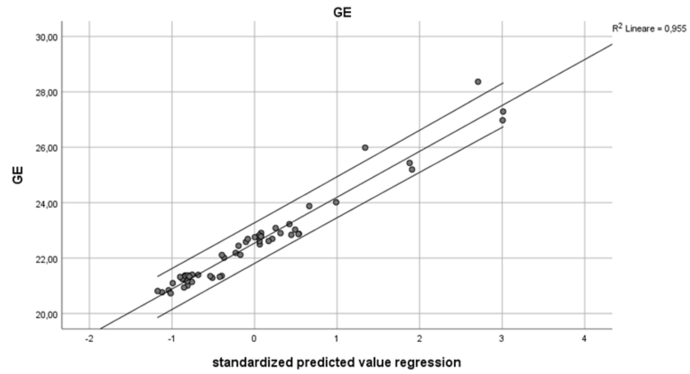
Model	sum of squares	df	Mean Square	F	Sign.
Regression	145.51	4	36.38	261.60	0.0001
Residual	6.81	49	0.14		
Total	152.32	53	36.38		

Examination of the table shows the goodness of the forecast model chosen, as highlighted by the low value of the residue. The predictive model summary for evaluating GE shows high R2 and Adjusted R2 values.
GE may be predicted with the following equation:

$$Y= - 0.14 – 0.377(CP\_NIR) + 0.744 (EE\_NIR) + 0.323 (NDF\_NIR) + 0.328 (NFE\_NIR) \pm \varepsilon$$

In Figure 1, we report the relationship between observed and predicted GE with the confidence interval at 95% obtained with the linear regression model. The distribution of the standardized residuals of the predicted regression value confirms the correctness of the model applied to our hemp flour sample.

Figure 1 - The relationship between GE observed and GE predicted for the statistical model.



Tables 6 and 7 show the linear regression model summary for GEd prediction and the ANOVA for the regression models.

Table 6 - Linear regression model summary for GEd prediction

Model	R	R^2	R^2_{AdJ}	standard error of estimate
1	0.660a	0.436	0.426	0.73402
2	0.894b	0.798	0.791	0.44305
3	0.919c	0.845	0.836	0.39172

a. predictors: (constant), CP_NIR
b. predictors: (constant), CP_NIR, EE_NIR
c. predictors: (constant), CP_NIR, EE_NIR, ASH_NIR

The predictive model summary for evaluating GEd shown high R^2 and R^2_{AdJ} values.

Table 7 - ANOVA for the regression models c

Model	sum of squares	df	Mean Square	F	Sign.
Regression	43.627	3	14.542	94.772	0.001
Residual	7.979	52	0.153		
Total	51.606	55			

Examination of the table shows the goodness of the forecast model chosen, as highlighted by the low value of the residue.

GED may be predicted with the following equation:

$$Y = 92.65 - 0.352(CP\_NIR) + 0.348 (EE\_NIR) + 0.375(Ash\_NIR) \pm \varepsilon$$

In Figure 2, we report the relationship between observed and predicted GEd with the confidence interval at 95% obtained with the linear regression model. The distribution of the standardized residuals of the predicted regression value confirms the correctness of the model applied to our hemp flour sample.

Figure 2 - The relationship between GEd observed and GEd predicted for the statistical model.

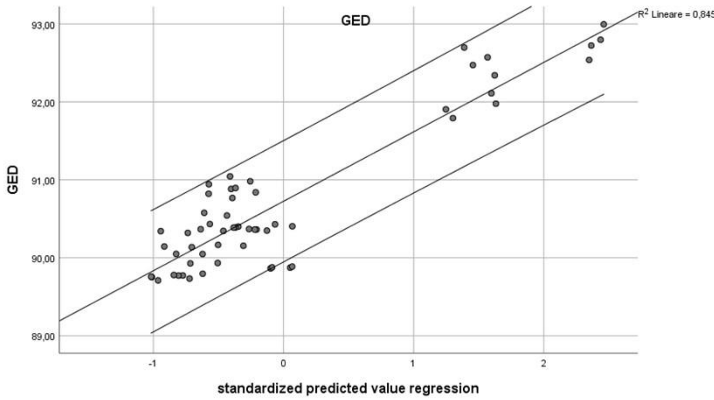


Table 8 and 9 shows linear regression model summary for DE prediction and the ANOVA for the regression models.

Table 8 - Linear regression model summary for DE prediction

Model	R	R^2	R^2_{AdJ}	standard error of estimate
1	0.659a	0.434	0.423	1.30956
2	0.760b	0.578	0.561	1.14214
3	0.786c	0.617	0.594	1.09788
4	0.974d	0.948	0.944	0.40926

a. predictors: (constant), CP_NIR
b. predictors: (constant), CP_NIR, EE_NIR
c. predictors: (constant), CP_NIR, EE_NIR, NDF_NIR
d. predictors: (constant), CP_NIR, EE_NIR, NDF_NIR, NFE_NIR

The predictive model summary for evaluating DE shown high R^2 and R^2_{AdJ} values.

Table 9 - ANOVA for the regression models d.

	sum of squares	df	Mean Square	F	Sign.
Regression	149.297	4	37.324	222.841	0.001
Residual	8.207	49	0.167		
Total	157.504	53			

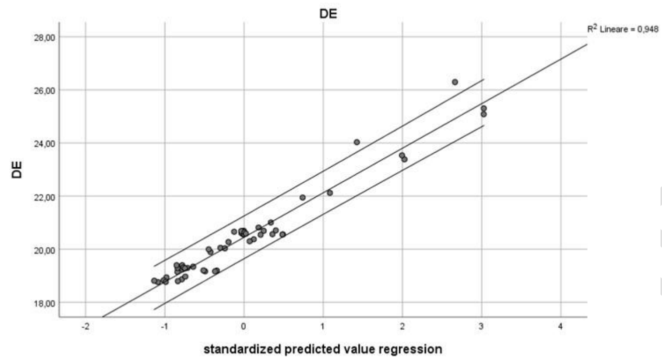
Examination of the table shows the goodness of the prediction model chosen, as highlighted by the low value of the residue.

DE may be predicted with the following equation:

$$Y = 0.10 - 0.436\,(CP\_NIR) + 0.773\,(EE\_NIR) + 0.304(NDF\_NIR) + 0.309(NFE\_NIR) \pm \varepsilon$$

Figure 3 we report the relationship between observed and predicted DE with the confidence interval at 95% obtained with linear regression model. The distribution of the standardized residuals predicted regression value confirms the correctness of the model applied in our hemp flour sample.

Figure 3 - The relationship between DE observed and DE predicted for thestatistical model.



Hemp flour shows richness in protein using both methods, with a notable presence of NDF [19].
Protein content aligns with literature values, but differences in protein and carbohydrate content compared to other studies may be attributed to the oil extraction method [20].
Differences in protein and carbohydrate content, especially lower protein and higher structural carbohydrate content compared to literature values, may be linked to the oil extraction method's influence on sample composition [21].
Protein content in hemp is higher than in rapeseed and sunflower but lower than in soy.
Despite soybeans being a primary source of vegetable proteins, hemp proteins are more digestible due to fewer anti-nutritional factors [22].
Table 1 reveals significant differences attributed to different methods, notably with the NIRs technique involving more reading replications than the laboratory duplication.
Despite differences, the reliability of NIRs results is indicated, especially for parameters except for ash content compared to NFE.
Table 2 demonstrates good correspondence between chemical determinations and NIRs determinations, with apparent high digestibility of GE values.
No significant correlation was found between GE and DE, but negative correlations were observed for all parameters obtained by NIRs techniques.
Multiple linear regressions were conducted, and the best predicting model for GE was identified using CP_NIR, EE_NIR, NDF_NIR, and NFE_NIR as independent variables.
The predictive model for GE includes CP_NIR, EE_NIR, NDF_NIR, and NFE_NIR, demonstrating high R2 and R2Adj values.
GEd and DE are also predicted using similar equations, showing good model correspondence and reliability.
Figures 1, 2, and 3 visually represent the relationship between observed and predicted values, confirming the correctness of the applied models for GE, GEd, and DE.

4. CONCLUSION

The classic measurements of the chemical composition of food content using AOAC methods require time and involve the use of solvents that must then be disposed of as special waste. Near-Infrared (NIR) spectroscopy is rapid and does not produce special toxic waste; therefore, it is being studied as a potential screening method for the analysis of chemical composition. The study provides comprehensive insights into the chemical composition of hemp flour, explores its comparison with other seeds, evaluates different analysis methods, and establishes reliable prediction models for energy content.

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