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Study on the NIR Spectroscopy to Predict Energy Content of Hemp Flour (*Cannabis sativa L.*)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FS, GP, GA, GG and RP conceptualization, formal analysis, data curation, investigated the work and wrote and prepared the original draft of the manuscript. Author FS did the writing—review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

Aims: 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.

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.

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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.

The literature indicates that hemp holds promise as a viable alternative to soybeans. This is attributed to hemp sharing essential characteristics with soybeans as a rotational crop, including profitability, potential as an energy crop, and the ability to maintain soil fertility. The findings reveal observable patterns of both complementary and substitution relationships in the case of hemp–wheat and hemp–soybean pairings, respectively. Moreover, the results suggest the potential for hemp monoculture to exhibit a positive response, enduring for multiple years, in reaction to self-positive shocks affecting hemp acreage [3].

Hemp flour, and specifically hemp seeds, contain high levels of omega-3 and omega-6 fatty acids. If hemp were incorporated into the diet of livestock, it could enhance the fatty acid content in the final products derived from these animals. Consequently, the consumption of these enriched products may also yield health benefits for humans.

The conducted research has revealed that the use of hemp seeds or their by-products as a supplement in the diet of dairy ruminants promotes an improvement in the fatty acid profile of the milk they produce. Particularly in dairy cows, an increase in urea concentration has been observed, attributed to the rise in the concentration of raw proteins in the diet, along with a decrease in fat and protein content in the milk. Unfortunately, this study did not maintain an equivalent concentration of proteins and fats in the various diets used to feed the cows, and there was no assessment of the acidic profile of their milk [4].

Experiments on digestibility of hemp seed flour have also been conducted in both sheep and cows, indicating that the flour is as digestible as canola flour [5]. As expected, the seeds of the plant represent the most nutritious fraction, with an average crude protein value of around 21.77% of dry matter and an average lipid content of 23.5% of dry matter [4].

Hemp seed production data for 2020 are available from five countries in the FAO database [6]. 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 [7].

There are various microscopic techniques used for sample analysis, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), and infrared spectrophotometry. In scanning electron microscopy (SEM), the emitted electronic beam is controlled to perform a television-type scan, exploring the surface of the object under examination.

The transmission electron microscope (TEM) shares a schematic structure with an optical microscope, replacing the light source with an electron source and using electromagnetic lenses instead of optical ones. The interior of the electron microscope operates under vacuum conditions. The electron source generates a beam of electrons with uniform velocity, concentrated onto a thin film of the sample being observed. After passing through the sample, the electron beam encounters the magnetic fields of the objective and projector, reach a fluorescent screen to produce a visible image or a photographic plate.

The infrared spectrophotometry, instead, involves recording interactions between materials and infrared radiation, inducing molecular vibrations associated with different functional groups. By interpreting spectroscopic signals acquired through spectrum acquisition, it becomes possible to identify substances or families of substances that generated the "infrared spectrum."

Infrared imaging has considerable advantages over conventional mapping experiments, namely short measuring times and improved spatial resolution. As with other microscopic techniques, the output of the measurements is readily comprehensible to non-spectroscopists.

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 [8]. 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 [9].

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 [10,11]. 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 vitamins 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 acids in the eggs produced and as feed for farmed fish [12].

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.

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. MATERIALS 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). After collection, the samples were ground using a 1 mm sieve with a knife mill and subjected to chemical composition analysis, including dry matter (DM), crude protein (CP), ethereal extract (EE), and ash. The analysis was conducted according to the procedures outlined by [11], with the respective identification numbers 2001.12, 978.04, 920.39 and 930.05 assigned to DM, CP, EE and ash. Furthermore, the neutral detergent fiber, acidic detergent fiber and acidic detergent lignin of the free ash were determined, following the guidelines provided by [13,14].

From the rough analysis, gross energy (GE), gross energy digestibility coefficient (GEd) and digestible energy (DE) were estimated using the equations proposed by [15] and researchers from [16].

Fresh food samples were ground through a 1 mm sieve and scanned twice in reflectance mode in the spectrophotometer using a Büchi instrument (model NIRFlex N-500 Inc). The NIRS spectrometer works in the near infrared spectral region (12500-4000 cm-1). It consists of a halogen lamp as a source and an array of InGaAs diodes and an intense broadband light source, which allows the measurement of reflectance from a large area of the sample surface (in a container of approximately 10 cm in size diameter). The diodes were centered at 10 nm intervals, but software was used to interpolate the spectra over a 5 nm data interval. The instrument's two spectral ranges are joined at 950 nm to cover a range from 800 to 2500 nm, chosen because many absorptions characteristic of amines fall in the same regions as alcohols where the N-H and OH bonds are similar.

The analysis was carried out in reflectance to minimize the effects of the physical shape of the sample.

The acquisition time of the instrument averaged 30 spectra s^{-1} and a spectral scan was defined as the average spectrum generated after 1 s of acquisition.

It should be noted that hydrogen bonds occur at very high frequencies due to the very low mass of this atom. This is why the intensity of the transmitted radiation was measured in the near-infrared (NIR) field rather than in the mid-infrared (MIR).

The chemical composition and measured GE, GEd and DE of the compound feeds used for calibration are presented in Tables 1 and 2.

All statistical methods for data evaluation were performed to determine the possible use of NIR data to predict the energy content of hemp flour extraction and the relationships between all data considered using [17].

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. Table 1 shows lower protein content and higher structural carbohydrate content com-pared to the literature [12,18,2]. 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 [18], 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 [19].

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 [20].

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.

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. [20] and [21] report that hemp flour has high digestibility related to a high degree of digestibility of proteins. [20] 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. 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 R^2_{adj} 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.

In Table 3 we report the correlation matrix among the considered parameters.

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

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

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 R^2 and R^2_{Adj} 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 Fig. 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.

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

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

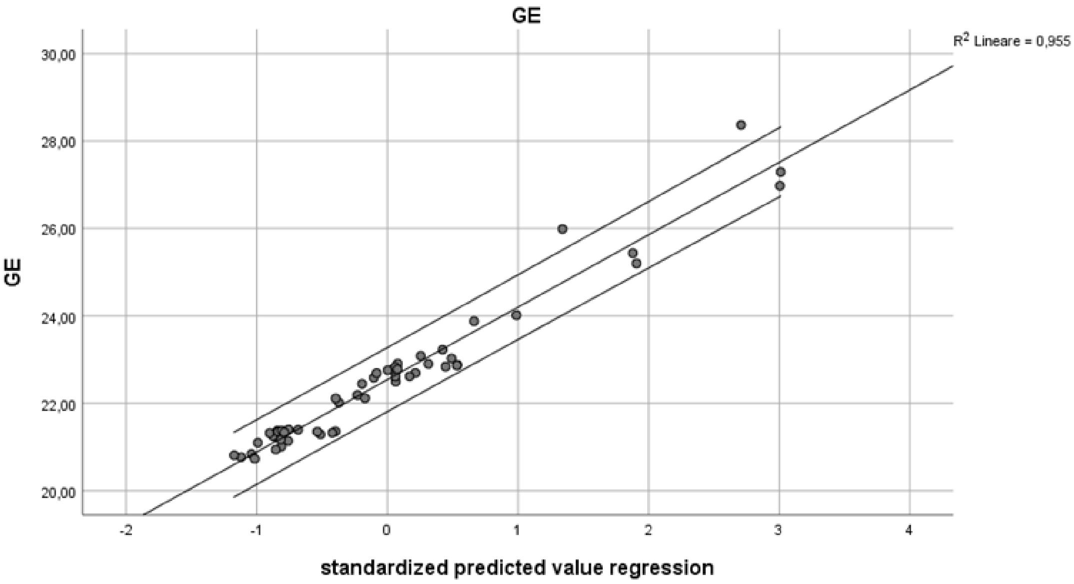


Fig. 1. The relationship between GE observed and GE predicted for the statistical model

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

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) ± ε

In Fig. 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.

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

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

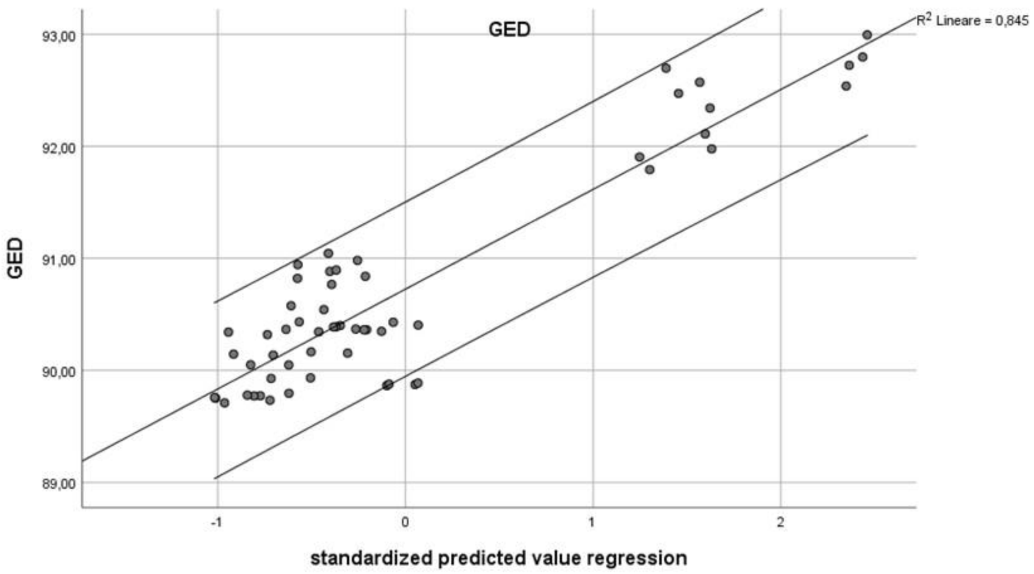


Fig. 2. The relationship between GEd observed and GEd predicted for the statistical model

Table 8. Linear regression model summary for DE prediction

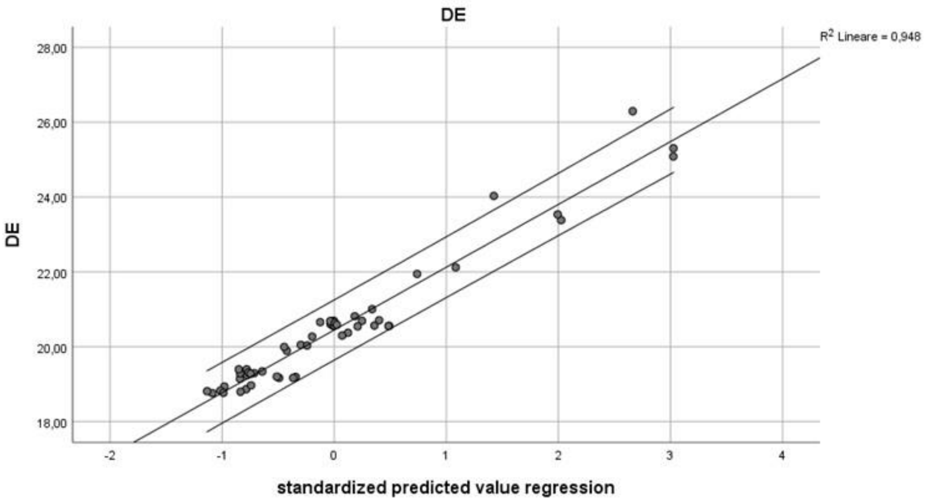
Model	R	R^2	R^2_{AdJ}	standard error of estimate
1	0.659a	0.434	0.423	1.30956
2	0.760b	0.578	0.561	1.14214
3	0.786c	0.617	0.594	1.09788
4	0.974d	0.948	0.944	0.40926

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

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			

Fig. 3. The relationship between DE observed and DE predicted for the statistical model



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) ± ε

Fig. 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.

Hemp flour shows protein richness using both methods, with a notable presence of NDF [21].

The protein content is in line with literature values, but the differences in protein and carbohydrate content compared to other studies can be attributed to the oil extraction method [22].

Differences in protein and carbohydrate content, in particular a higher structural carbohydrate content than literature values, may be linked to the influence of the oil extraction method on the sample composition [23].

The protein content of hemp is higher than that of rapeseed and sunflower, but lower than that of soy.

Although soy is a primary source of plant protein, hemp protein is more digestible due to fewer antinutritional factors [24].

Table 1 reveals significant differences attributed to the different methods, particularly with the NIR technique involving more read replicates than 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.

Figs. 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.

COMPETING INTERESTS

Authors declare that no competing interests exist.

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