ORIGINAL PAPER

UDK: 613.21:355.12

DOI: 10.5937/hralsh2302034P

Near-infrared spectroscopy analysis of proteins and fats in food

Marina Perunicic Lucic, Sinisa Masic
Medical Military Academy, Belgrade, Serbia

Corresponding author:
Marina Perunicic Lucic
Medical Military Academy,
Crnotravska 17, 11000 Belgrade, Serbia
Phone: +381 63 85 86 872
E-mail address: marina.perunicic@gmail.com

Received 19. 11. 2024.
Revised 02. 12. 2024.
Accepted 10. 12. 2024.

Abstract

Spectroscopy is a chemical method that applies the interaction of optical energy with a sample to analyze its formation and which covers wavelengths invisible to the eye from 780 nm to 2500 nm. We analyze proteins and fats in homogenized triple daily meals without bread for the army. This is important because of the energy of the whole meal and this is in correlation to activity of soldiers. We analyzed this food by near-infrared spectroscopy over eight years from 2017 to 2024 and compared our results to classic techniques: Kjedahl–volumetric method for analysis of proteins and Gerber-acidobutirometric method for analysis of fats. We used samples with different concentrations of protein and fat and created their spectra for calibration curves with spectral range from 4000 to 12800 cm^{-1}. Our findings confirm that the results are more similar from year to year because we had more and more new spectra on the calibration curve for both proteins and fats in these kinds of samples. For example, in the first year, we had 60% of our results for proteins different in the range of 5-10% or less from Kjedahl method, and then in 2024, that percentage was 95%. It was a really good comparison. For fats, 50% of our results were different in the range of 5-10% or less from standard Gerber method, and then in 2024. that percentage was 95%. The main advantage of spectroscopy is there is no preparation of samples or use of any reagents and also you can have results in less than 5 minutes. We have compared more than one hundred samples and developed a good method for fast and precise quantifications of proteins and fats in triple daily meals for the army.

***Keywords**: NIR spectroscopy; proteins; fats; triple daily meal for the army.*

INTRODUCTION

The military in Serbia has strict rules both in discipline and rules in nutrition, especially regarding proteins and fats. The military has a triple daily meal in relation to the official military act of the Ministry of Defense [1].

Fatty acids are important because of the energy they provide to the body, as well as for the preservation of cardiovascular functions in the body, and they also participate in the maintenance of hormonal balance. Their transport role and their role in building cells and immunity are not insignificant either. They are found in many foods such as dairy products and meat [2]. In 2023, the World Health Organization confirmed that fat intake should be up to 30% at most, as it is today [3].

Proteins are composed of amino acids, and the main ingredient in their structure is nitrogen. Their functions in the body are multiple. For example, structural, catalytic, protective, transport, regulatory, and many others [4]. The recommended daily intake of protein depends on many factors such as gender, age, body weight, lifestyle, and more. The recommended daily intake according to the Food and Drug Administration (FDA) is 50 g per day. Most international recommendations for the intake are 0.8 g per kilogram of body weight [5].

We analyzed energy value of meal and provided our opinion on whether there is a deficit or a surplus of any of the parameters that affect the energy value. We also determined protein content using the classic Kjedahl–volumetric method and fat content using the Gerber-acidobutirometric method. The principle of the Kjeldahl method is the conversion of nitrogen-containing organic matter from the sample into an ammonium ion that reacts with sulfuric acid in the presence of a catalyst, so ammonium sulfate is formed as a result.

The Kjeldahl method for protein analysis in milk is conceded by the world organization Codex Alimentarius as standard. This was confirmed by the Food and Drug Organization in 2017. The first step is digestion in the presence of concentrated sulfuric acid, then distillation in the presence of a base, and finally titration. All of these steps are a reason why the method is not fast [6,7]. The classic Gerber fat determination method was developed in Switzerland in the 19th century by Dr. Gerber, after whom it was named. The principle of the method is based on the separation of fat in the presence of sulfuric acid of a certain density and amyl alcohol. After centrifugation, the percentage of fat is read on a particular butyrometer [8].

We also analyzed these parameters by spectroscopy in the near-infrared field. In the 18th century, Frederick W.H. identified the first non-visible part of the absorption spectrum. Although the technique faced challenges throughout history, today it is a well-established method for food analysis [9]. Spectroscopy is a section of science that studies interactions of electromagnetic radiation and matter. Absorption of infrared radiation by molecules is quantized, and the molecule can only absorb those wavelengths that correspond to vibrational energies of that molecule. Vibrational frequencies are characteristic of certain functional groups. These characteristic vibrations are used for material identification and structure determination of an unknown compound [10]. Along with infrared (IR) and Raman spectroscopy, Near-infrared spectroscopy (NIR) belongs to the field of molecular vibrational spectroscopy, which examines molecular vibrations using electromagnetic radiation and covers wavelengths invisible to the eye [11,12].

The research revealed 17 standard analysis methods that include different raw materials and products. It found 16 different documents with analysis of legumes, cereal grains, flour, and oil seeds as well as two formal techniques for the determination of fats in food products. There are some other approved methods for different kinds of food [13].

NIR spectroscopy is an ideal analytical method for processing an enormous number of samples, allowing control authorities to confirm high capacity and capability while preventing fraud and verifying food authenticity. We have now developed a fast method that requires no reagents for determining fats and proteins in the military's triple daily meals.

MATERIAL AND METHODS

There have been a lot of analyses of food by near-infrared spectroscopy and Raman spectroscopy over the years. NIR spectroscopic quantitative analysis that characterizes the nutritional and commercial value of cereals is widespread. Typically, a quantity of sample components such as oil, protein, starch, and water is information from interest and can be determined by NIR spectroscopy. It was also used in the analysis of one kind of walnut that was contaminated by insects [14], as well as to separate healthy and defective hazelnuts with the help of differences in their spectra [15]. Using this technique, the salmon storage process was studied in relation to the oxidative changes in the oil [16]. Fats and carbohydrates were analyzed in pumpkin seeds by this method and the researchers were satisfied with the results [17]. Proteins are analyzed in protein powder mix products and the results were very good in comparasion to the Dumas method [18].

Our laboratory analyzed 124 samples of triple meals for military over eight years. We conducted all analyses by classic methods and compared the results obtained by near-infrared spectroscopy.

Proteins are determined in the presence of a catalyst and with heating, so organic matter is oxidized in the presence of sulfuric acid. Nitrogen, which is released in the form of ammonia, makes ammonium sulfate with sulfuric acid. Then, as a result of the action of bases on the created ammonium sulfate, ammonia is released and distilled into an acid of known molarity. The final titration determines the amount of remaining acid, which is in correlation to the pre-distilled amount of ammonia, and the amount of nitrogen in the tested sample is obtained. The amount of nitrogen and the correction factor determine the total amount of crude protein in the sample.

Fats are examined by Gerber method in the presence of sumporic acid with density of 1.74 and amyl alcohol in a heated bath at 65 °C and in a certified butyrometer. Then, it was centrifuged, and the percentage of fats was read.

Also, in the near-infrared field, we made a lot of spectra of proteins and fats necessary for calibration during these years. Primarily, we had 32 scans of background in front of each sample and then 32 scans for every single sample. This system includes resolution 8.000, sample gain 1.0, optical velocity 1.2659, aperture 95.00, detector InGaAs 2.6 um, beamsplitter CaF2, and source was white light. The results were many different spectra for different concentrations of proteins and fats. In every spectrum, we had absorbance in interaction with wavenumbers. In correlation with that, we made a calibration curve and determined these parameters. Sample preparation was easy, requiring only homogenization, without the use of any reagents. A small amount of sample was scanned, and we had results within a few minutes.

RESULTS AND DISCUSSION

We analyzed 123 triple meals for the army. Protein content ranged from 2,8% to 6,0% and fat content

ranged from 2,7% to 6,5% over eight years. Our laboratory compared the results of NIR to classic techniques: Kjedahl–volumetric method for analysis of proteins and Gerber-acidobutirometric method for analysis of fats. Of course, every year we had many new spectra for these meals as regards better comparison for both parameters presented in **Table 1**.

Kjedahl method, and then in 2024, that percentage was 95%. For fats, 50% of our results were different in the range of 5-10% or less from the standard Gerber method, and then in 2024, that percentage was 95%, as illustrated in **Figure 3**. Low signal sensitivity can be a problem when determining components with low concentrations with this technology, which is the main drawback [19]. So our conclusion was that military foods can be used for method development while diet must be excluded. We considered that the difference in results between two completely different methods up to 10% is quite acceptable. As the years passed, we had more and more spectra in the database and the results we concluded are more similar, as illustrated in **Figure 4**.

Table 1. The difference in protein and fat concentrations determined by NIR and classical methods over 8 years.

Percent of difference	Year	2017.	2018.	2019.	2020.	2021.	2022.	2023.	2024.
0-10%	Proteins	60,0	79,9	93,3	92,9	81,0	92,8	93,4	95,0
	Fats	50,0	73,3	73,4	87,5	73,4	85,7	93,3	95,0
10-20%	Proteins	33,3	13,4	6,7	7,1	19,0	7,2	6,6	5,0
	Fats	31,2	13,3	13,3	12,5	20,0	7,1	6,7	-
20-30%	Proteins	-	6,7	-	-	-	-	-	-
	Fats	18,8	13,4	13,3	-	6,6	7,2	-	5,0
>30%	Proteins	6,7	-	-	-	-	-	-	-
	Fats	-	-	-	-	-	-	-	-

Absorption measured in the near-infrared field (800–2500 nm or 12500-4000 cm^{-1}) of the electromagnetic spectrum is measured between the visible part of the spectrum and the middle infrared part invisible to the naked eye. Molecules with a hydroxyl, amino, carboxyl, or sulfhydryl group have the strongest absorption in this area [18]. Spectrum of our samples can be seen below in **Figure 1** and **Figure 2**.

In the first year, we had 60% of our results for proteins differing in the range of 5-10% or less from the

Finally, pretreatment of the food is not required, which is a great advantage of this method, as is the use of any reagents. It is also very important for us as members of the Ministry of Defense, to provide a fast, efficient, accurate, and precise method for determining the energy value of this type of food for soldiers.

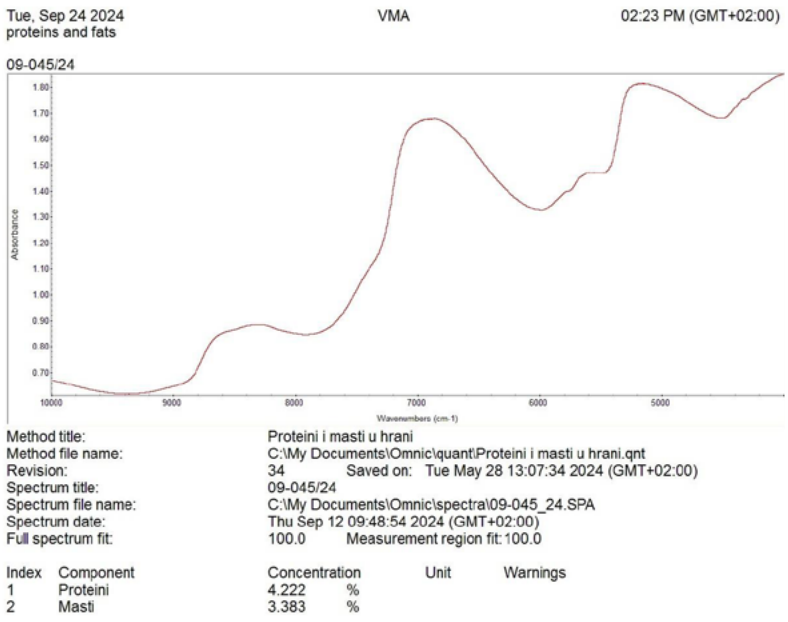

Figure 1. IR spectrum of one of samples-triple daily mail for the army. This is one of the reports and there are results of protein and fat in that sample.

CONCLUSIONS

NIR spectroscopy is a fast and precise method that can be used to analyze a large number of samples. Analyses are performed without prior preparation of samples, and no reagents are used. Also, it is simple to operate, and time of analysis is below 5 minutes. The device is cost-effective and keeps up with modern technologies.

The results of our study of protein and fat analysis in triple daily meals for the military showed that we have successfully developed and proved the accuracy and precision of the spectroscopic method compared to the traditional Kjedahl and Gerber methods. The main

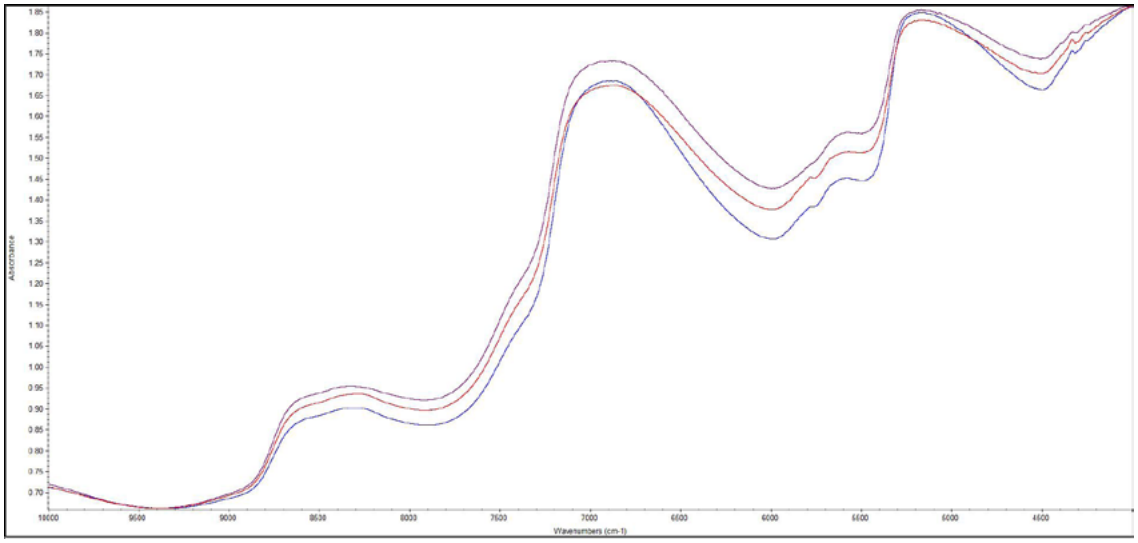

Figure 2. Overlapping NIR- IR spectra of several different triple daily mails for the military. The graph shows the dependence of the absorbance in relation to the wavelength.

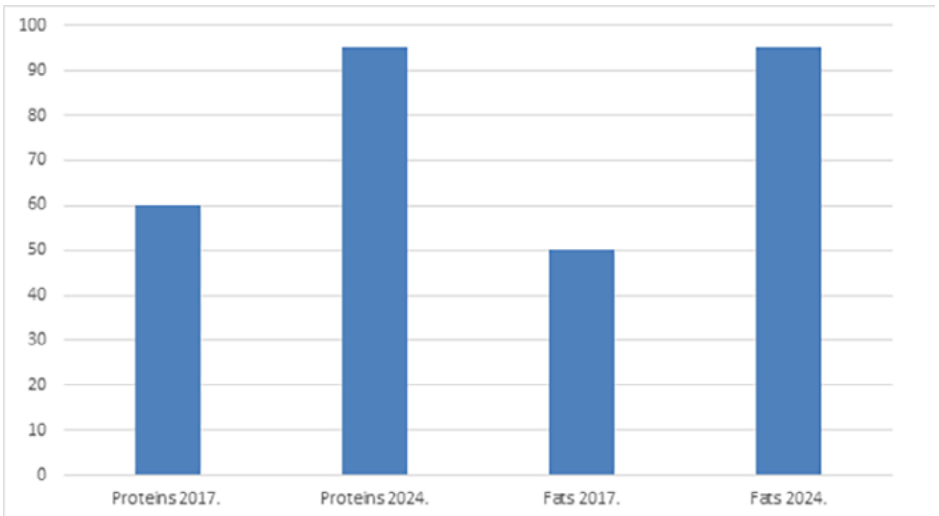

Figure 3. Acceptable difference (0-10%) between the desired values of the first and last year of proteins and fats analysis between the classical and NIR methods.

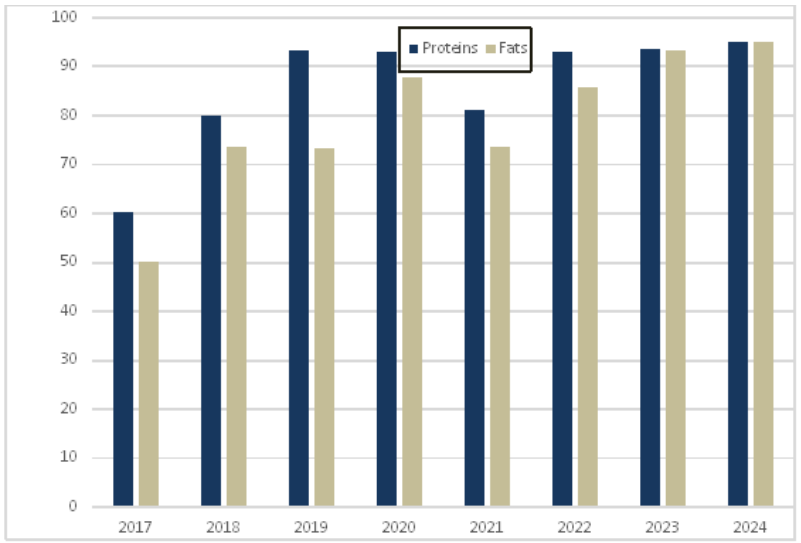

Figure 4. Difference of 5-10% or less in proteins and fats determination by classic methods and NIR over 8 years.

advantages of the method are the speed of determination and no costs associated with buying reagents, as well as no health risks due to evaporation of chemicals or other risks during use. It is necessary to record as many spectra as possible of similar food in order to improve accuracy.

REFERENCES

1. Pravilnik o opštim logističkom potrebama u Ministarstvu odbrane i Vojsci Srbije, Službeni vojni list. 2011;31:511-9.
2. Ann GL, Nikki AF, Frank BH, Kathleen MZ, DariushM, Penny MKE. A healthy approach to dietary fats: understanding the science and taking action to reduce consumer confusion. Am Nutr J. 2017;16(1):53.
3. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food Nutr Pap. 2010;91:1-166.
4. Walia A, Gupta AK, Sharma V. Role of Bioactive Compounds in Human Health. Am Acta Sci. Med. Sci. 2019; 3(9):25-33.
5. Dietary Reference Intakes for Energy, Carbohydrate, Fibre, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients). WHO. Tech Rep Ser 2007:935.
6. Kjeldahl, J. New method for the determination of nitrogen in organic substances. Am J Anal. Chem. 1883;2(1):366-383.
7. Mike B, Harjinder S. Milk proteins. In: A. Goulding, P.F. Fox, J.A. O'Mahony. eds. Milk: An overview. Massachusetts: Academic Press, third edition; 2020:21-98.
8. René B, Thomas B, Rolf K. Densitometric determination of the fat content of milk and milk products. Am Int Dairy J. 2007;17(1):20-23.
9. Da-Wen S. Modern Techniques for Food Authentication Spectroscopic Technique. In: Marena M, Gerard D, Vincent B, eds. Near-infrared (NIR) Spectroscopy. Massachusetts: Academic Press, first edition; 2008:65-99.
10. Krzysztof BB, Christian WH. Breakthrough Potential in Near-Infrared Spectroscopy: Spectra Simulation. A Review of Recent Developments. Am Front Chem. 2019;7(22):48.
11. Krzysztof BB, Justyna G, Christian WH. Near-Infrared Spectroscopy in Bio-Applications. Molecules 2020;25(12):2948.
12. Barton FE II. Theory and principles of near infrared spectroscopy. Spectrosc Eur. 2002;14(1):1012.
13. Giacomo S, Jordi C, Davide DA, Francesco C, José MA. Considerations about the gap between research in Near Infrared spectroscopy and official methods and recommendations of analysis in foods. Current Opinion in Food Science.2024;59:101203.
14. Giovanna Canneddu, Luis Carlos Cunha Junior , Gustavo Henrique de Almeida Teixeira. Quality Evaluation of Shelled and Unshelled Macadamia Nuts by Means of Near-Infrared Spectroscopy (NIR). Am Food Sci.2016; 81(7):C1613-21.
15. Henri L, Navid S, René B, Soeren W, Phat LT, Creydt M, Seifert S, Hackl T, Fischer M. Rapid testing in the food industry: the potential of Fourier transform near-infrared (FT-NIR) spectroscopy and spatially offset Raman spectroscopy (SORS) to detect raw material defects in hazelnuts (Corylus avellana L.). Food Anal Methods. 2024;17:486-97.
16. Nan ZhongYi Peng LiXuan Zong Li, Cheng Xian Gu, Ting Wu. Accurate prediction of salmon storage time using improved Raman spectroscopy. Am J.Food Eng. 2021; 293(2):110378.
17. Ifmalinda, Andasuryani A, Santosa, Putri I. The application of NIRS method for non-destructive measurement of fat and carbohydrates in pumpkin seeds. IOP Conf. Series: Earth and Environmental Science. 2021;922(1):012024.
18. Prashant DI, Roney C, Piyush P, Veronica Z, Erica H, Keith F, Saleem A. Determination of Protein Content by NIR Spectroscopy in Protein Powder Mix Products. J AOAC Int. 2016;99(2):360-3.
19. Karoui R., De Baerdemaeker J. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. Am Food Chem. 2007;102 (3):621–40.

Bliska infracrvena spektroskopija – analiza proteina i masti u hrani

Marina Perunicic Lucic, Sinisa Masic

Vojno Medicinska Akademija (VMA), Beograd, Srbija

Kratak sadržaj

Spektroskopija je analitička tehnika koja koristi interakciju optičke energije sa uzorkom za analizu njegovog sastava i koja pokriva talasne dužinene vidljive oku od 780 nm do 2500 nm. Vršimo analize proteina i masti u homogenizovanim trostrukim dnevnim obrocima bez hleba za vojsku. Ovo je važno zbog energetske vrednosti celodnevnih obroka i u korespodenciji sa aktivnošću vojnika. Trostruke dnevne vojne obroke smo analizirali bliskom infracrvenom spektroskopijom tokom osam godina od 2017. do 2024. i uporedili naše rezultate sa klasičnim tehnikama: Kjedahl-volumetrijska metoda za analizu proteina i Gerber-acidobutirometrijska metoda za analizu masti. Koristili smo uzorke sa različitim koncentracijama proteina i masti i napravili njihove spektre potrebne za kalibracionu krivu, sa spektralnim opsegom od 4000 do 12800 cm-1. Naši rezultati potvrđuju da su rezultati iz godine u godinu sve sličniji, jer smo imali sve više novih spektra na kalibracionim krivama proteina i masti u datoj vrsti hrane. Na primer, prve godine smo imali 60% proteina koji se razlikuju u rasponu od 5-10% ili manje od Kjedahlove metode, a onda je 2024. taj procenat bio 95%. Bilo je to zaista uspešno poređenje. Za masti 50% naših rezultata se razlikovalo u rasponu 5-10% ili manje od standardne Gerberove metode, a onda je 2024. godine taj procenat bio 95%. Glavna prednost spektroskopije je što nema pripreme uzoraka i upotrebe bilo kakvih reagenasa, a takođe možete dobiti rezultate za manje od 5 minuta. Uporedili smo više od sto uzoraka i napravili dobru metodu za brzu i preciznu kvantifikaciju proteina i masti u trostrukim dnevnim obrocima za vojsku.

Ključne reči: *NIR spektroskopija; proteini; masti; trostruki dnevni obrok za vojsku.*