ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2022, *67*(3), 151–158
DOI: 10.33224/rrch.2022.67.3.02

Dedicated to the memory of Professor Sorin Roşca (1938–2021)

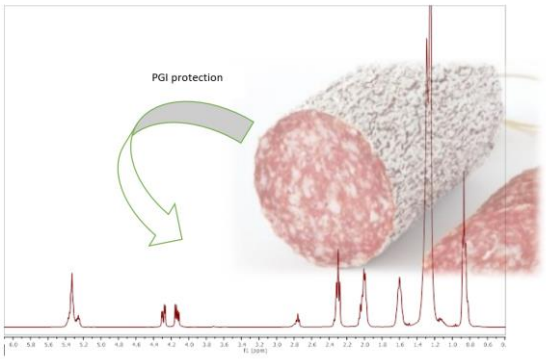
^{1}H-NMR SPECTROSCOPY A USEFUL TOOL FOR THE SIBIU SALAMI GEOGRAPHICAL INDICATION PROTECTION

Maria-Cristina TODASCĂ,[a*] Mihaela TOCIU[a] and Fulvia-Ancuța MANOLACHE[b*]

[a] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Gh. Polizu St., 011061, Bucharest, Roumania
[b] National Research & Development Institute for Food Bioresources IBA Bucharest, 6th Dinu Vintila St., 021102, Bucharest, Roumania

Received February 28, 2022

Development of a rapid method for quality control and authentication of Sibiu Salami, which holds a PGI classification under EU law, was the main goal of this work. To reach this objective the fatty acid profiling (FAP) of triglycerides extracted from dry cured salami and other meat products of its class, were investigated by means of ^{1}H-NMR spectroscopy combined with chemometric analysis of spectral data. Samples of the traditional Sibiu dry-cured salami from the main manufacturers on the Romanian market have been selected. A set of chemometric equations using ^{1}H-NMR data have been used for computation of FAP composition, such as the amount of poly-unsaturated (PUFA), di-unsaturated, monounsaturated (MUFA) and saturated fatty acids (SFA). The results were similar when compared against the standard gas chromatographic method.
The Sibiu dry-cured salami was assessed against other raw dry salami from his category produced in Roumania, investigating the influence of maturation process (specific to Sibiu dry-cured salami) on the FAP.



INTRODUCTION

Sibiu Salami is a traditional Roumanian dry-cured salami which holds a protected geographical indication (PGI) label, based on the legal framework provided by EU Regulation No. 1151/2012[1] of the European Parliament. This dry-cured salami is made from pork meat (Mangalita breed) and pork back fat, in a specific way that implies the use of microorganisms (*Penicillium nalgiovense*) in a long maturation process (more than 60 days). This maturation step offers a unique taste and a specific composition of the product and made it highly appreciated by the consumers.

The EU quality labels on food products encourages diverse agricultural production, local traditional products protection and help consumers to make their decision (sometimes above language barriers) and protect product names from misuse and imitation.[2] The renewed consumers interest in traditional foods make the quality labels an important aid for consumer decision-making and at the same time a challenge for the authorities, which needs reliable and fast analytical methods

* Corresponding authors: cristina.todasca@upb.ro or fulvia.manolache@bioresurse.ro

for authentication and quality control that will be able to protect the labelled products. Many reports have been made regarding approaches for official or newly developed methods to assess the characteristics of food products which holds an EU quality label. For instance, the sensory control methods are evaluated as official tool for protected designation of origin food products and wines, but there are some important barriers that needs to be surpass for the harmonization of the specific technical criteria to meet the high diversity and variability of PDO products. [3]

Nuclear magnetic resonance (NMR) spectroscopy is a technique widely used in chemistry because it offers the most comprehensive structural information on organic compounds. The resulting signals are interpreted from NMR spectra and contain exactly the information required for good structural results. It is a fast and efficient technique that allows direct analysis on triglycerides without prior sample preparation.[4,5] In the recent years, the researchers have made great efforts in establishing new methods for rapid and accurate quantification of acyl chain of triglycerides. In this respect the spectroscopic methods show theirs advantages by comparison with classical methods in terms of rapidity, reproducibility and repeatability.[6,7]

In the quality studies made on fat extracted from back fat and pork meat, sausages and chicken meat, a number of spectroscopic methods have been used, such as: ^{1}H-NMR,[8-11] ^{31}P-NMR,[12] GC-MS.[13] The results obtained by classical analysis and spectral methods (DHS technique coupled with GC-MS) were used in statistical analysis (PCA and LDA) to differentiate between two typical Italian dry-sausages products.[14] NIR method was used for determining the fatty acid composition of the intramuscular fat from lumbar pig (Iberian breed)[15] and PCR analysis was used for animal species identification.[16] At the same time studies have been undertaken for the identification and differentiation of various meat products.[17,18]

In other studies, the fatty acid composition of meat products is influenced by breed[19] and the type and composition of animal feed as well as the breeding grounds.[20] Thus, the fat from pigs, fed on fishmeal, is rich in poly-unsaturated fatty acids (C22:5 and C22:6).[21] The animal's diet rich in hydrogenated fats produces a decrease in linoleic acid content. Dry Salami fabricated with fat and meat from pigs fed on diets with partial replacement of maize by rice bran, shows some enrichment in poly-unsaturated fatty acids.[22] Similar results have been observed by addition of different types of fat[23] and dietary supplementation containing vegetable oil.[24]

Studies concerning various methods designed to improve the technological process of manufacturing have been done.[25-31] Researchers from Spain, Italy and France proved that using different types of starter cultures in the process of obtaining dry salami, generates a safety improvement and higher preservation of the product.[32,33] Visessanguan and co. have been studying changes in the fatty acid profile induced by the maturation process, respectively the autoxidative phenomenon that contributes to the taste and species characteristics ham.[34]

Another important aspect is the development and evaluation of properties of meat products and increasing the bioactive compounds content [35-37] and their use as functional foods.[38]

The main goal of this paper is to investigate the Sibiu dry cured salami authenticity compared with other dry salami of his class (Sinaia, Banatean and Dacia) using ^{1}H-NMR spectral information.

RESULTS AND DISCUSSION

A total of 22 samples of different types of dry cured salami produced in Romania and commercialised under 8 commercial labels (marked C1 - C8) were investigated. The ^{1}H-NMR analysis was performed directly on the triglycerides (the fat extracted from the salami) without prior sample preparation.

Figure 1 presents the typical 5.5-0.5 ppm area of a ^{1}H-NMR fat extracted Sibiu salami spectra. The integral values of signal A-J are used in the chemometrical computations for FAPs according with their spectral attribution at 0.85 ppm (signal A - C**H$_3$** groups of all fatty acids, except linolenic acid), 0.96 ppm (signal B - C**H$_3$** groups of linolenic acid), 1.20 ppm (signal C - all -(C**H$_2$**)n- groups), 1.60 ppm (signal D - β alkyl groups -C**H$_2$**-CH_2-COOR), 2.02 ppm (signal E - allylic protons -C**H$_2$**-CH=CH-), 2.20 ppm (signal F - the α alkyl groups -C**H$_2$**-COOR), 2.76 ppm (signal G - bis-allylic proton -CH=CH-C**H$_2$**-CH=CH-), 4.19 ppm (signal H -**CH$_2$**-O-COR Glycerol, α position), 5.15 ppm (signal I -CH-O-COR Glycerol β position), 5.29 ppm (signal J -CH=CH - All unsaturated fatty acids). The assignment of signals was based on previously reported assignments on model fatty acids esters.[39]

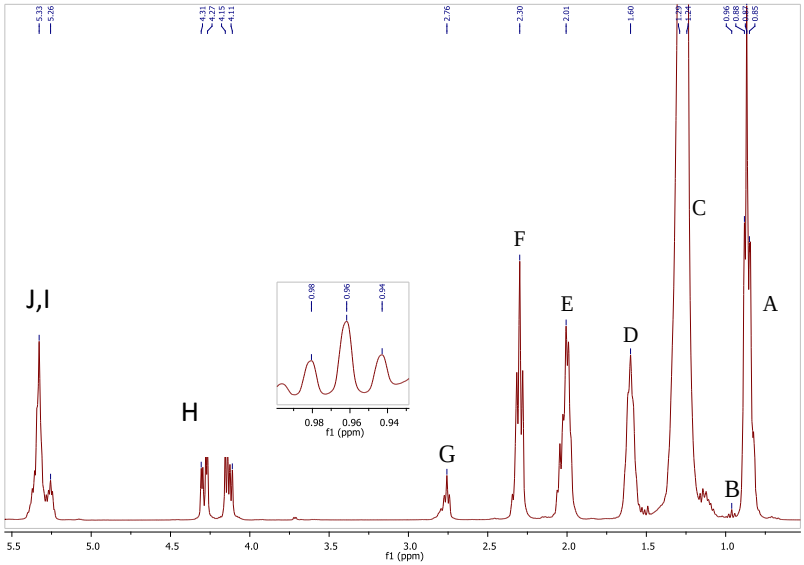


Fig. 1 – Area 5.5 - 0.5 ppm of a ^{1}H-NMR spectrum of fat extracted from dry-cured Sibiu salami.

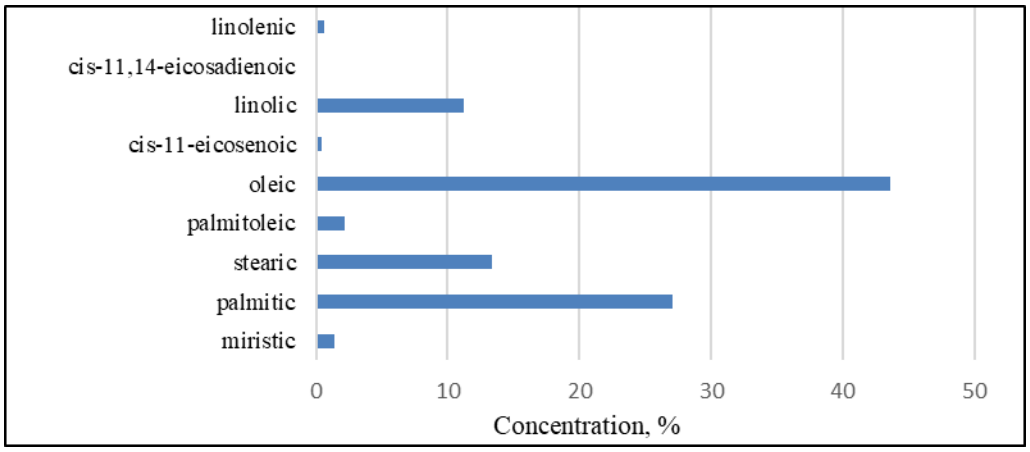


Fig. 2 – The mean values of the fatty acids within the Sibiu dry salami, based on GC-FID analyses.

The relevant region for the ^{1}H-NMR analysis of a dry salami extracted fat is the area 0-3 ppm of the spectra. The integral values of the corresponding signals have been marked with I_A, I_B, I_C, I_D, I_E, I_F, I_G and their numerical values are used in the computation. Interpretation of spectra results were achieved using chemometric methods which were developed into the research group on previous study of the vegetable oils.[40] In the previous study there were 5 methods (5 equation systems based on NMR data) investigated for establishing the fatty acid profile on 4 classes of fatty acids (saturated, monounsaturated, di-unsaturated and poly-unsaturated fatty acids) for vegetable oils. Based on the GC-FID results the best fit for fatty acid profiling was obtained using method 5. Similar results were confirmed in the literature when studies on pork meat products from Calabria Italy were investigated.[8]

To confirm the NMR results the Sibiu salami fat was analysed as well by standard GC-FID method, the samples were subjected to transesterification procedure beforehand. The FAP was obtained using a standard 37 methyl esters of fatty acids known, as describe in material and methods.

From the chromatograms obtained in the GC-FID analysis, it was determined that samples of Sibiu dry salami show the specific signal corresponding to cis-11,14-eicosadienoic fatty acid. This result correlates with compositional analysis made on pork back fat. Cis-11,14-eicosadienoic fatty acid is absent in fat obtained from other type of meat, thus, the presence or absence of this fatty acid may be an indicator in determine that recipes for producing Sibiu dry salami is complied.

Using the experience obtained previously on vegetable oils, in the current study we investigated the equations systems applied on the NMR data for

establishing FAP of the fat extracted from Sibiu Salami. Table 1 presents the results of the computation that led to the determination of saturated and unsaturated fatty acids of the fat extracted from samples of Sibiu salami, based on ^{1}H-NMR spectral data. These data have been correlated with the results of GC-FID analysis and the values in Table 1 are presented respectively:

- SFA: myristic acid, palmitic acid and stearic acid, heptadecanoic acid;
- MUFA: palmitoleic acid, oleic acid, cis-10-heptadecanoic acid and 11-eicosenoic acid;
- PUFA: di-unsaturated acids (linoleic acid and cis-11,14-eicosadienoic acid) and poly-unsaturated acid (linolenic acid).

Table 1

FAP of the fat extracted from Sibiu salami measured by ^{1}H-NMR and GC-FID method on samples obtained from the 3 main producers of Sibiu Salami

	SFA, % molare		MUFA, % molare		PUFA, % molare di-unsaturated acids		PUFA, % molare poly-unsaturated acids	
Sample	NMR	GC-FID	NMR	GC-FID	NMR	GC-FID	NMR	GC-FID
C_1	39.57	40.04	43.58	44.20	15.86	14.79	0.99	0.97
C_2	41.78	42.24	46.55	46.19	11.00	10.81	0.66	0.77
C_3	41.54	41.78	45.98	46.09	11.82	11.46	0.66	0.67

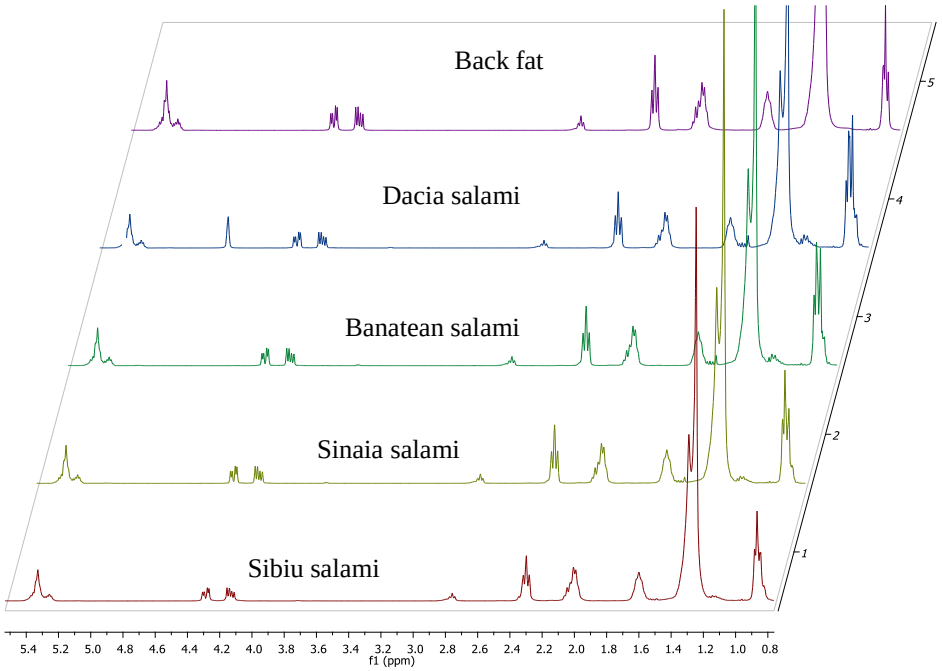


Fig. 3 – ^{1}H-NMR spectra of fat extracted from the studied dry salami samples compared with the pork back fat.

From Table 1 can be noticed that the results obtained by NMR method are similar to those obtained by standard GC-FID method, considered as reference values. The advantage of the NMR method is that the results were obtained in a shorter time (5 minutes), with lower consumption of reagents and without prior samples processing (the NMR analysis was performed directly on the fat extracted from Sibiu Salami).

All ^{1}H-NMR profile of the fat extracted from the studied dry salami samples have a similar shape with the pork back fat, as presented in Figure 3. There are some differences (shape and presence of some signals) between the pork back fat and the fat extracted from all dry salamis due to the changes generated by the processing techniques. Some small differences in the integral values of the signals (for instance the signal at 2.8 ppm) are noticeable too. Therefore, all extracted fats contain similar fatty acids but in different amounts.

In Figure 4 the mean values of the FAPs for the analysed fat is presented. In can been noticed that Sibiu Salami has similar content of SFA and MUFA by comparison with the other dry salami produced in Roumania, however, has the highest content of poly-unsaturated fatty acids.

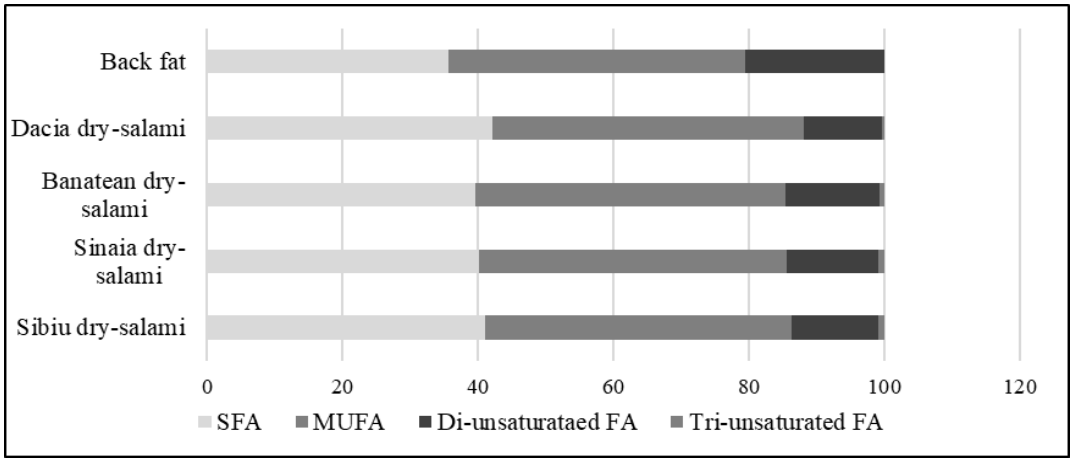


Fig. 4 – The mean values of the FAPs for the analysed fats.

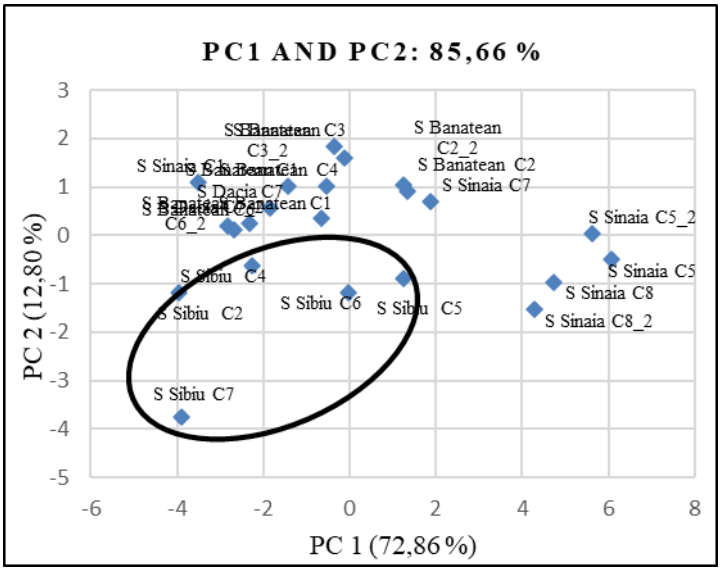


Fig. 5 – The score plot for the first 2 principal components.

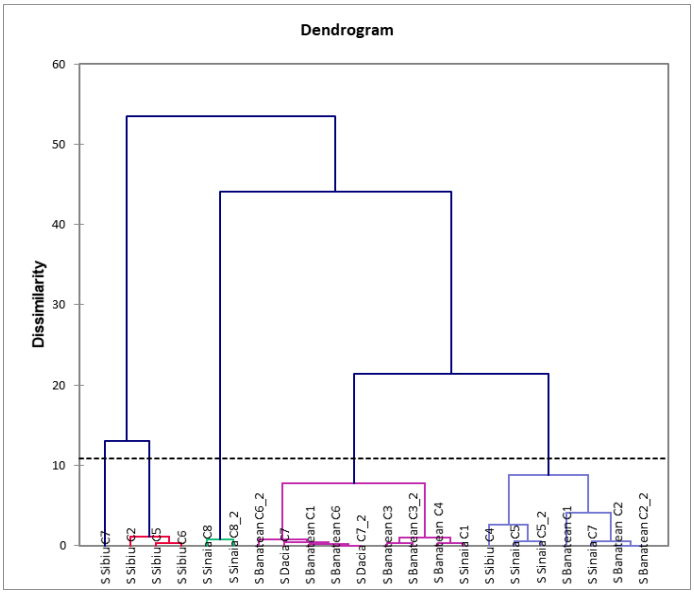


Fig. 6 – The dendrogram obtained for all dry cured salami samples.

The FAP itself it is not sufficient as method for authentication of Sibiu Salami against other types of dry-cured salami of its class. Therefore, we used the NMR information as input data into well established chemometrical methods like Principal Component Analysis (PCA) and Agglomerative hierarchical clustering (AHC). In Figure 5 the score plots for PCA are shown. A good discrimination of the Sibiu Salami samples could be noticed when the used variables are the integral values of the NMR spectra (I_A, I_B, I_D, I_E, I_F, I_G, I_J, I_H, I_I) and the FAP values obtained by computation of NMR spectral data on the 4 classes of fatty acids.

Evaluating the score plot for the main principal components (PC1/PC2), presented in Figure 5 a good separation of all dry-cured salami can be noticed based on the recipe information. Consequently, the Sibiu and Sinaia samples which contain only pork meat/fat are clustered in the lower part of the graph, while the Banatean and Dacia samples which contain pork and beef (as presented in Table 2) are clustering in the upper part of the graph.

The results obtained by Agglomerative hierarchical clustering (AHC) confirms the results from PCA - Figure 6. The Sibiu Salami samples groups in first 2 clusters, the sample differences may occur due to the variation of the pork used as row material.

The chemometrical analysis of NMR spectral data cannot discriminate the samples based on the existence of the maturation step within the processing. The changes made by the microorganisms' development during the maturation are not very important in terms of fat variations but play an important role in taste and conservation.

The chemometrical analysis applied on NMR spectral data show good results when the compliance of the receipt is investigated.

EXPERIMENTAL

Chemicals

Deuterated chloroform ($CDCl_3$) (min. 99.8%) used for the ^{1}H-NMR analysis and the petroleum ether (fraction 40-60^{o}C) used for the Soxhlet extraction of fats were purchased from Merck (Darmstadt, Germany). The substances used for samples derivatizing: methanol p.a. from Sigma - Aldrich (Steinheim, Germany); GC grade methylene chloride from Sigma - Aldrich (Steinheim, Germany); sodium chloride p.a.; hydrochloric acid p.a.; sodium nitrite p.a.; mercuric chloride p.a.

Samples

The analysed samples (22 samples) of dry-cured salami were purchased from the top 4 producers on the Romanian market (covering 98% from the Sibiu Salami production). All samples were analysed in triplicate and the conformity of the Sibiu Salami was established prior to analytical analysis. The Sinaia, Banatean and Dacia dry salami were purchased from the same producers as the one of Sibiu salami. The selection of the dry salami from the same class of products was based on the most similar products in content and technological processing steps, as shown in Table 2. For example: Sinaia dry salami is a raw-dry meat product with a similar recipe, as Sibiu Salami. The technological process for Sinaia salami does not imply the maturation process using the *Penicilium nalgiovense*, mould, as it does the Sibiu Salami.

Classical analysis

The samples were subjected to classical laboratory analysis (water, fat and nitrites content) to check the conformity with the standard requirements for Sibiu Salami. The obtained results are presented in Table 3 where the mean value for each producer is given. Three samples of Sibiu Salami from each producer have been analysed and all samples have been measured in triplicate.

The results for all samples of dry salami have been verified against the standardized values according to ISO 9001:2000. As it can be noticed all the samples were in the limits of the standardized values, although there are some small differences that could be noticed between manufacturers, two of them (C1 and C2) show a lower level of water content in the final product.

GC-FID spectrometric analysis

The standard mixture of 37 fatty acids methyl esters (Supelco™ 37 Component FAME Mix) used for the gas chromatographic analyses was purchased from Supelco.

Fatty acid methyl esters (FAME) were prepared by transesterification of fats with methanol, using BF_3-MeOH complex as catalyst, according to the standard method.

The gas-chromatograms of the fatty acid methyl esters mixtures were recorded on an Agilent Technologies model 7890A instrument. The separation into components was made on a capillary column (Supelco SP^{TM} 2560) with the following characteristics: 30 m length, 0.25 mm inner diameter, 0.25 μm film thickness. The ready for injection solutions were prepared in CH_2Cl_2 of HPLC purity grade.

Fatty acids identification was made by comparing for each peak the retention time with those of a standard mixture of 37 fatty acid methyl esters (Supelco 37 Component FAME Mix).

Both standard mixture and each FAME of the analysed dry salami fats were chromatographically separated under the same conditions, using the same temperature program (oven initial temperature 140 ºC to final temperature 240ºC, heating rate 4ºC/min.), injection volume 1 μL, split rate 100:1, carrier gas He according to the Supelco specifications. Total analysis time for one sample was 55 minutes. The calibration of the signals was made by considering the concentration of each component of the standard mixture, correlated with the detector's response.

^{1}H-NMR spectrometric analysis

The ^{1}H-NMR spectra of the dry salami fats were recorded on a Bruker Avance III 400 spectrometer, using TopSpin 3.2 software, operating at 9.4 Tesla, corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus, equipped with a direct detection broadband probe head and field gradients on z axis. Samples were analysed in 5 mm NMR tubes (Norell 507). The chemical shifts (δ) are reported in ppm (0.00 ppm), using the TMS as internal standard.

Table 2

Characteristics of the analysed dry salami samples

Dry salami	Meat type	Maturation process
Sibiu	pork	with microorganism *(Penicillium nalgiovense)*
Sinaia	pork	without
Banatean	pork and beef	without
Dacia	pork and beef	with microorganism *(Penicillium nalgiovense)*

Table 3

The medium values of the main parameters of the samples of Sibiu dry-cured salami obtain by the main Romanian manufacturers (C1, C2 and C3)

Classical analysis	Water, % ± SD	Fat, % ± SD	Nitrites, mg/100 g product ± SD
Standard value	max 30	max 46	max 7
C1	22.04±0.02	44.74±0.03	3.85±0.01
C2	24.54±0.02	45.72±0.01	1.9±0.01
C3	26.03±0.02	45.03±0.03	1.26±0.01

Typical parameters for ^{1}H-NMR spectra were: 45° pulse, 2.05 s acquisition time, 6.4 KHz spectral window, 16 scans, 26 K data points, d1=2s (delay). The FID was processed for 0.293 Hz line broadening prior to Fourier transformation. The average acquisition time of the ^{1}H-NMR spectra was approximately 2 minutes. The sample preparation was simply reduced to the dilution 2:8 (V/V) dry salami fat in $CDCl_3$.

CONCLUSION

The direct analyses of fat extracted from dry salami using ^{1}H-NMR is a fast and reliable method for FAPs that can be an important tool for quality control. Furthermore, the uniformity and reliability of data obtained by NMR and GC-FID methods for Sibiu Salami demonstrates that high-resolution NMR spectroscopy could be used as a tool in alternative to standard chromatographic methods for the determination of fatty acid compositions of fat extracted from meat or meat products. The results are important in the enlarged market of the European Union and they can be used to authenticate PGI products such as Sibiu Salami. Comparing Sibiu Salami with other dry salami or dry-cured products obtained in Roumania (Sinaia, Banatean and Dacia salami) slightly different FAPs are obtained. Therefore, methods for authentication of those dry salami can be established based on the FAPs, protecting products of high quality against substitute's products.

At the same time, this research represents a quick solution to ensure quality control, particularly in the case of recipe compliance, where traditional analysis cannot always provide a thorough result and requires substantially more time and resources.

REFERENCES

1. Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs, L 343, **2012**, 1-29.
2. K. G. Grunert and K. Aachmann, *Food Control*, **2016**, *59*, 178-187.
3. F. J. Pérez-Elortondo, R. Symoneaux, I. Etaio, C. Coulon-Leroy and M. Zannoni, *Food Control*, **2018**, *88*, 159-168.
4. J. S. McKenzie, J. A. Donarski, J. C.Wilson and A. J. Charlton, *Prog. Nucl. Magn. Reson. Spectrosc.*, **2011**, *59*, 336-359.
5. W. Jakes, A. Gerdova, M. Defernez, A. D. Watson, C. McCallum, E. Limer, I. J. Colquhoun, D. C. Williamson and E. K. Kemsley, *Food Chem.*, **2015**, *175*, 1-9.
6. A. Hanganu, M. C. Todasca, N. A. Chira, M. Maganu and S. Rosca, *Food Chem.*, **2012,** *134*, 2453-2458.
7. M. Tociu, M.-C. Todască, A. Bratu, M. Mihalache and F. Manolache, *Int. Dairy J.*, **2018**, *83*, 52-57.
8. C. Siciliano, E. Belsito, R. De Marco, M. L. Di Gioia, A. Leggio and A. Liguori, *Food Chem.*, **2013**, *136*, 546-554.
9. Z. Xiao, C. Ge, G. Zhou, W. Zhang and G. Liao, *Food Chem*., **2019**, *274*, 574-582.
10. C. Siciliano, *J. Phys. : Conf. Ser*., **2021**, *1960*, 1-18.
11. F. Tanga , M. Vasasa and E. Hatzakisa, *A. Spyrosb, "Annual Reports on NMR Spectroscopy",* **2019**, *98* **,** 239-306.
12. A. Spyros and P. Dais, *Prog. Nucl. Magn. Reson. Spectrosc*., **2009**, *54*, 195-207.
13. A. M. Ciuciu Simion, J. Carballo and P. Alexe, *J. Agroaliment. Processes Technol.*, **2011**, *17*, 207-213.
14. F. Bianchi, C. Cantoni, M.Careri, L. Chiesa, M. Musci and A. Pinna, *Talanta*, **2007**, *72*, 1552-1563.

15. I. González-Martin, C. González Pérez, N. García and J. M. González-Cabrera, **2005**, *Meat Sci.*, *69*, 243-248.
16. P. Ulca, H. Balta, İ. Çağın and H. Z. Senyuva, *Meat Sci.*, **2013**, *94*, 280-284.
17. N. Z. Ballin and R. Lametsch, *Meat Sci.*, **2008**, *80*, 151-158.
18. C. Marcos, C. Viegas, A. M. Almeida and M. M. Guerra, *J. Ethn. Foods*, **2016**, *3*, 51-60.
19. E. Nistor, V. Bampidis, M. Pentea, H. Prundeanu and V .Ciolac, *J. Anim. Sci. Biotechnol.*, **2012**, *45*, 386-389.
20. P. Costa, A. F. Costa, P. A. Lopes, C. M. Alfaia and J. A. M. Prates, *J. Food. Compost. Anal.*, **2011,** *24*, 987-994.
21. A. Flatten, E. A. Bryhni, A. Kohler, B. Egeland and T. Isaksson, *Meat Sci.*, **2005**, *69*, 433-440.
22. R. M. L. De Campos, E. Hierro, J. A. Ordonez, T. M. Bertol, N. N. Terra and L. Hoz, *Food Chem.*, **2007**, *103*, 1159-1167.
23. V. Alonso, L. M. Najes, L. Provincial, E. Guillén, M. Gil, P. Roncalés and J. A. Beltrán, *Meat Sci.*, ***2012***, *92*, 366-373.
24. T. M. Bertol, R. M. L. de Campos, J. V. Ludke, N. N. Terra, E. A. P. Figueiredo, A. Coldebella, J. I. dos Santos Filho, V. L. Kawski and N. M. Lehr, *Meat Sci.*, **2013**, *93*, 507-516.
25. F. Gardini, G.Suzzi, A. Lombardi, F. Galgano, M. A. Crudele, C. Andrighetto, M. Schirone and R. Tofalo, *FEMS Yeast Res.*, **2001**, *1*, 161-167.
26. M. A. Del Nobile, A. Conte, A. L. Incoronato, O. Panza, A. Sevi and R. Marino, *Meat Sci.*, **2009**, *81*, 263-269.
27. E. Zanardi, S. Ghidini, M. Conter and A. Ianieri, *Meat Sci.*, **2010,** *86*, 742-747.
28. B. Renaville, A. Prandi, B. Fan, A. Sepulcri, M. F. Rothschild and E. Piasentier, *Meat Sci.*, **2013**, *93*, 495-500.
29. A. Girolami, F. Napolitano, D. Faraone, G. Di Bello and A. Braghieri, *Meat Sci*., **2014**, *96*, 610-616.
30. M. J. Fraqueza, M. Laranjo, S. Alves, M. H. Fernandes, A. C. Agulheiro-Santos, M. J. Fernandes, M. E. Potes and M. Elias, *Foods*, **2020**, *9*, 1-11.
31. A. Teixeira, R. Domínguez, I. Ferreira, E. Pereira, L. Estevinho, S. Rodrigues and J. M. Lorenzo, *Foods*, **2021**, 10, 961.
32. M. Salim Ammor and B. Mayo, *Meat Sci.*, **200**7, *76*, 138-146.
33. R. Talon, S. Leroy, I. Lebert, P. Giammarinaro, J. P. Chacornac, M. Latorre-Moratalla, C. Vidal-Carou, E. Zanardi, M. Conter and A. Lebecque, *Int. J. Food Microbiol*., **2008**, *126*, 227-234.
34. W. Visessanguan, S. Benjakul, S. Riebroy, M. Yarchai and W. Tapingkae, *Food Chem.*, **2006**, 94, 580-588.
35. B. Olmedilla-Alonso, F. Jiménez-Colmenero and F. J. Sánchez-Muniz, *Meat Sci.*, **2013**, *95*, 919-930.
36. W. Zhang, S. Xiao, H. Samaraweera and E. Joo Lee, D.U.Ahn, *Meat S*ci., **2010**, *86*, 15-3.
37. R. Marino, M. Albenzio, A. della Malva, A. Muscio and A. Sevi, *Meat Sci.*, **2015**, *101*, 19-24.
38. E. A. Decker and Y. Park, *Meat Sci.*, **2010**, *86*, 49-55.
39. C. Deleanu, C. Enache, M. T. Caproiu, G. Cornilescu and A. Hirtopeanu, *Rev. Chim.(Bucharest)*, **1994**, *45*, 1046-1052.
40. N. A. Chira, M. C. Todasca, A. Nicolescu, A. Rosu, M. Nicolae and S. I. Rosca, *Rev. Chim.(Bucharest)*, **2011**, *62*, 42-46.