When detection of dairy food fraud fails: An alternative approach through proton nuclear magnetic resonance spectroscopy

Anamaria Hanganu^{1,2} and Nicoleta-Aurelia Chira^{3*}

¹Faculty of Chemistry, Department of Organic Chemistry, Biochemistry and Catalysis, Research Centre of Applied Organic Chemistry, University of Bucharest, 90-92 Panduri Street, RO-050663 Bucharest, Romania

²Institute of Organic Chemistry “C.D. Nenitescu” of the Romanian Academy, 202B Spl. Independentei, 060023 Bucharest, Romania

³Faculty of Applied Chemistry and Materials Science, “C. Nenitescu” Organic Chemistry Department, University “Politehnica” of Bucharest, 1-7 Polizu Str., 011061 Bucharest, Romania

ABSTRACT

This paper investigated the limits of the current approach for the determination of the fatty acids profile of milk fats from proton nuclear magnetic resonance data based on the hypothesis that the signal at 0.96 ppm, currently assigned in the literature as a marker for the “short chain fatty acids,” is generated only by the butyric moiety (not by all of the short-chain fatty acids, which also include C6:0—caproic acid). The hypothesis was tested and experimentally confirmed. Moreover, the triplet at 0.96 ppm can also be due to n-3 fatty acids such as linolenic acid (C18:3); therefore, a previously reported methodology for the fatty acids profiling of dairy products—considered as general in the literature—cannot be used in fraud-detection approaches because it allows linolenic acid to be mistaken for butyric acid, consequently leading to misclassification of adulterated samples as nonadulterated. To support our opinion, we have applied the current literature approach for the determination of the fatty acids composition of 3 synthetic nondairy fat blends and have obtained fatty acid compositions similar to milk fats, allowing for their misclassification as genuine milk fats. However, in reality, the blends had very different compositions, as confirmed by gas chromatography. Consequently, we have highlighted the weaknesses of the existing methodology for the detection of dairy food adulteration. In return, new proton nuclear magnetic resonance descriptors based on various integral ratios of signals associated with CH₂ moiety versus signals associated with butyric and n-3 fatty acids were proposed to detect adulterations.

Key words: milk fat, fatty acids profile, proton nuclear magnetic resonance descriptors, butyric acid, detection of adulteration

INTRODUCTION

Milk fat (MF) is one of the most expensive food products; therefore, it may be subjected to fraud (replacement with cheaper fats or oils; Pereira et al., 2019; Leite et al., 2019). In some countries (particularly the developing countries), adulteration of dairy products is recognized as a serious issue (Handford et al., 2016). For example, in Romania, the state institutions are aware of the practice of counterfeiting dairy products on a considerable scale; therefore, an agreement between the National Authority for Consumers Protection and the Producers' Association of Romanian Milk Industry was established on January 21, 2020, aiming at the collaboration of the 2 parties to reduce the counterfeiting practice on dairy products (ANPC, 2020). The agreement proves that fraud in the dairy industry is a problem acknowledged by the authorities; consequently, developing efficient methods for the detection of fake/adulterated products is necessary.

Milk fat is distinguished from other fats and oils by the short- and medium-chain fatty acids (FA) content. The standard method for the accurate determination of the fatty acids profile (FAP) of milk and dairy products, GC (Månsson 2008; Laučienė et al., 2019), requires harmful chemicals and is time-consuming (almost 2 h/sample with an experienced operator). Therefore, faster alternative methods are welcome. Recently, spectroscopic methods coupled with multivariate data analysis have received attention, as they are considerably faster and more convenient from the viewpoint of the practical procedure. For example, modified partial least square regression applied on near infrared spectroscopic data allowed quantification of 19 FA in cheese (from C8:0 to C20:0, including saturated and unsaturated species) with individual confidence levels of up to 96% (González-Martin et al., 2020). The capabilities, advantages, limitations, and drawbacks of other chromatographic (GC-MS, HPLC, LC-MS) and spectral [fluorescence, Fourier-transform infrared,

Received November 9, 2020.

Accepted February 27, 2021.

*Corresponding author: nicoleta.chira@chimie.upb.ro

near infrared, mid infrared, nuclear magnetic resonance (NMR)] techniques combined with multivariate data analysis to detect adulteration of dairy products were systematically discussed in excellent reviews (Kamal and Karoui 2015; Alexandri et al. 2017).

The standard method for the specific determination of the butyric moiety of MF or MF-containing fat blends is based on the saponification of fat with KOH. The corresponding FA are liberated from the resulting soaps by acidification with H_3PO_4 , before the separation of the water-insoluble and water-soluble FA. Butyric acid is subsequently directly determined by GLC in the presence of valeric acid as an internal standard (Dieffenbacher and Pocklington, 1992). Although accurate, the method is laborious, and reagent- and time-consuming. Sacchi et al. (2018) recently developed a faster method for the determination of butyric acid in MF blends with lard and vegetable margarine through ^{13}C -NMR spectroscopy, based on the distinct C_1 and C_2 resonances of the butyryl backbone, used as markers of milk triacylglycerols. A modern approach based on Raman spectroscopy coupled with chemometrics (Gómez-Mascaraque et al., 2020) has allowed quantification of 28 FA from butter, including C4:0, as influenced by the cow feeding systems.

A recent paper (Tociu et al., 2018) aiming at the determination of the FAP of MF through chemometric equations on ^1H -NMR data reported a rapid quantification of the FA on 4 classes: short-chain, polyunsaturated, monounsaturated, and long-chain saturated FA. The equations resulted from the integrals of the ^1H -NMR signals of MF, based on the contribution of specific protons from each FA class. For example, the short-chain FA were determined from the integral of the triplet at 0.96 ppm (assigned to the terminal methyl of C4-C8 acyl chains) weighted against all methyl terminal protons of all fatty acyl chains. The method was further applied for the differentiation of cheese substitutes (made of vegetable ingredients) from genuine bovine milk cheeses and the detection of adulteration of cheeses with “exogeneous” fats (mainly palm oil) on the basis of the ratio of long-chain:short-chain FA, which is typically lower than 6.89 for bovine MF (Tociu et al., 2018). The adulteration of dairy products with other fats and oils will result in an increase of the above-mentioned ratio, considering the only source of short-chain FA is MF. The major drawback of this approach is that n-3 FA (such as linolenic acid) also have a distinctive ^1H -NMR resonance (also triplet) at 0.96 ppm (Brescia et al., 2004), overlapping the triplet from the short-chain FA; therefore, these 2 species cannot be determined independently. Moreover, only C4:0 contributes to the 0.96 ppm triplet; the methyl from C6:0 resonates at 0.85 ppm together with the rest of

the fatty acyl chains. According to the proposed chemometric equations, linolenic acid may be counted as a short-chain FA, falsely increasing the short-chain FA content of MF blends with other fats/oils. The issue becomes serious because the reported method plays a key role in the detection of adulteration.

The present work aimed to discuss the limits of the ^1H -NMR spectroscopy for the determination of the FAP of dairy products, with emphasis on adulteration scenarios. From the hypothesis that only the methyl terminal protons from butyric moiety resonate at 0.96 ppm, the short-chain FA cannot be determined as a class. Moreover, because the marker signals of butyric and linolenic moieties overlap at 0.96 ppm, these species cannot be determined independently, and thus may be misleading in fraud-detection approaches. Hence, the novelty of our study was such: (1) to evaluate the previously reported ^1H -NMR methods of determining the FAP of dairy fats, pointing out their limitations and possibly misleading aspects in authenticity issues, (2) to showcase the weaknesses of a previously reported method by applying it on synthetic nondairy fat blends simulating the butter fat (BF) composition, leading to sample misclassification as genuine dairy fat, and (3) to propose new ^1H -NMR fat descriptors based on various integral ratios of signals associated with CH_2 moiety versus signals associated with butyric and n-3 FA, which can be useful in authenticity approaches. Therefore, our reported results are useful in the food control area to assess dairy product authenticity and to detect fraud.

MATERIALS AND METHODS

Reagents and Samples

Supelco 37 Component FAME Mix was purchased from Supelco; methanol, CH_2Cl_2 (HPLC purity), and anhydrous $MgSO_4$ were purchased from Sigma Aldrich; tributyrin (97%) and caproic acid (p.a.) were purchased from Merck; and $BF_3 \cdot MeOH$ (10–14%) complex was purchased from Alpha-Aesar. Sodium methoxide was freshly prepared from Na (metallic) and methanol. Butter samples of certified bovine origin ($n = 35$) were obtained from Romanian ISO 22000:2018 licensed dairy companies (Napolact and Covalact). Butter fat was extracted from butter samples with CH_2Cl_2 , dried on anhydrous $MgSO_4$, and followed by evaporation of the solvent (Kontson et al., 1973). Linseed and *Lallemantia iberica* seeds were obtained from the National Agricultural Research and Development Institute of Fundulea, Romania. Oil was extracted from seeds according to standard Soxhlet protocol (Shahidi, 2001). Beef and sheep tallow were extracted according to Shahidi (2001),

Table 1. Fatty acid profiles¹ of fats and oils (mean values²)

Oil or fat	But	n-3 (Ln)	n-6 (Di)	MUFA	SFA	Reference
Butter fat ³ (from bovine milk)	4.62	0.73	1.88	26.79	65.98	n = 35, Månsson (2008); Laucčienė et al. (2019)
Goat milk	2.49	0.55	2.68	22.32	71.96	Yurchenko et al. (2018)
Sheep milk	2.57	1.31	2.32	29.75	64.05	Markiewicz-Kęszycka et al. (2013)
Coconut oil ³	0	0	1.85	7.16	90.99	n = 4
Corn oil	0	0.76	48.97	33.67	16.60	Dorni et al. (2018)
Cotton seeds oil	0	0.35	51.81	19.66	28.18	Dorni et al. (2018)
Groundnut oil	0	0	26.96	53.77	19.27	Dorni et al. (2018)
Mustard oil	0	0	0	83.69	16.31	Jamwal et al. (2020)
Brazil nut kernel oil	0	0.01	30.86	37.22	31.92	Cornelio-Santiago et al. (2019)
Palm olein	0	0.3	11.23	43.62	44.85	Dorni et al. (2018)
Safflower oil	0	0.13	76.58	14.21	9.08	Dorni et al. (2018)
High oleic safflower oil	0	0.25	12.78	79.69	7.28	Knothe and Kenar (2004)
Mid oleic sunflower oil	0	0.44	33.44	57.55	8.57	Knothe and Kenar (2004)
Soybean	0	5.16	54.16	24.77	15.91	Dorni et al. (2018)
Sunflower	0	0	62.69	25.92	11.39	Dorni et al. (2018)
Rapeseed oil	0	9.29	18.97	63.17	8.57	Dorni et al. (2018)
Beef tallow ³	0	0.34	2.46	40.41	56.79	n = 5
Pork lard	0	0.56	12.98	45.21	41.25	Indrasti et al. (2010)
Sheep tallow ³	0	0.39	6.25	43.78	49.58	n = 4, Aali et al. (2016)
Chicken fat	0	0	17.30	56.20	26.50	Mohammed et al. (2020)
Palm stearin ³	0	0	5.10	24.84	70.06	n = 5, Liu et al. (2019)
Palm kernel oil ³	0	0	2.58	16.87	80.55	n = 6, Naksuk et al. (2009)
<i>Lallemantia iberica</i> ³	0	68.45	9.09	11.64	10.82	n = 3, Amini et al. (2020)
Linseed ³	0	60.22	16.21	16.03	7.54	n = 5, Anastasiu et al. (2016), Xie et al. (2020)

¹Expressed as g/100 g. But = butyric acid; n-3 = total amount of n-3 fatty acids (mainly linolenic acid, Ln); n-6 = total amount of n-6 fatty acids (mainly di-unsaturated such as linoleic acid, Di); MUFA = total amount of monounsaturated fatty acids; SFA = total amount of saturated fatty acids, except for butyric acid.

²Data were processed from the indicated references.

³Determined in our laboratory (n = number of analyzed samples; all samples were analyzed in triplicate) and in agreement with the indicated references.

with small modifications: the subcutaneous adipose tissue was homogenized with quartz sand before Soxhlet extraction with CH₂Cl₂, dried on anhydrous MgSO₄, and followed by evaporation of the solvent. Coconut oil was purchased from Trio Verde S.R.L. (distributor), and palm stearin and palm kernel oil were purchased from Scintilla Silk (distributor).

Preparation of the Methyl Caproate

Methyl caproate was prepared from caproic acid and methanol, using H₂SO₄ as a catalyst (Li and Watkins, 2001). The ¹H-NMR signals were assigned as follows: 0.85 ppm (triplet, -CH₃, 3H), 1.25 ppm (multiplet, -CH₂-CH₂-CH₃, 4H), 1.64 ppm (quintet, -OOC-CH₂-CH₂-, 2H), 2.26 ppm (triplet, -OOC-CH₂-CH₂-, 2H), 3.60 ppm (singlet, H₃C-OOC-, 3H), where hydrogen atoms that contributed to the referred signals are in italics.

Sample Derivatization. The FAME of vegetable oils (linseed, coconut, *Lallemantia iberica*, palm stearin, and palm kernel oil) were transesterified with methanol, using BF₃-MeOH complex as a catalyst, according to the standard protocol (Li and Watkins, 2001). Methylation was carried out under alkaline conditions in methanol

with MeONa as a catalyst because isomerization may occur to FA containing conjugated double bonds (such as in dairy and ruminant meat products, which may contain conjugated linoleic acids) in the case of animal fats (BF, beef and sheep tallow) under acidic conditions and high temperature (Li and Watkins, 2001).

Gas Chromatograms. The gas chromatograms of FAME were recorded on an Agilent Technologies 7890A instrument equipped with autosampler and triple axis MS detector model 5975 C VL MSD. The operating conditions were previously described (Anastasiu et al., 2016). The FAP on 5 categories (i.e. C4:0, n-3, n-6, monounsaturated, and saturated FA) are presented in Table 1.

The ¹H-NMR Spectra

The ¹H-NMR experiments were recorded in a field of 6.9 T using a Bruker Fourier spectrometer operating at ¹H Larmor frequency of 300.18 MHz. The ¹H-NMR spectral acquisition characteristics were as follows: 45° pulse, 5.37-s acquisition time, 6.1-kHz spectral window, 16 scans, 20 K data points, 1-s delay time; all spectra were recorded at 25°C. Fat samples were dissolved in CDCl₃ (isotopic purity 99.8% D, Sigma Aldrich).

Statistical Data Processing

All samples were analyzed in triplicate. We used XL-Stat (Addinsoft, 14 d free trial) for data processing and 3-dimensional data visualization.

RESULTS AND DISCUSSION

Comparative ^1H -NMR Spectral Characterization of BF and Other Fats and Oils

Figure 1 presents the superimposed spectra of BF (a), tributyrin (b), linseed oil (c), and methyl caproate (d). The ^1H -NMR spectra of oils and fats are similar

in terms of signals. They usually display 10 signals (illustrated in Figure 1c for the case of linseed oil) that are characteristic to specific structural groups of the fatty acyl chains and glycerol backbone, as described in Table 2 (Knothe and Kenar, 2004). Linseed oil was chosen to illustrate the specific ^1H -NMR signals because it displays all the main signals with high intensity and contains large amounts of C18:3.

It is generally accepted that BF differs from other fats and oils due to its specific FA composition with characteristic short-chain FA moiety (C4:0 and C6:0), as well as important amounts of medium-chain FA (C8:0–C12:0). It appeared that BF had a distinctive signal (triplet B, see Figure 1) that corresponded to

Figure 1. Superimposed proton nuclear magnetic resonance (^1H -NMR) spectra of methyl caproate (a), linseed oil (b), tributyrin (c), and butter fat (d). f1 refers to the chemical shift.

Table 2. Chemical shifts and peak assignment of proton nuclear magnetic resonance spectra of fats and oils (Knothe and Kenar, 2004)

Signal	Chemical shift (ppm)	Proton ¹	Compound
A	0.85	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$	All acids except butyric acid and linolenic acid
B	0.96	$-\text{OOC}-\text{CH}_2-\text{CH}_2-\text{CH}_3$	Butyric acid
B'	0.96	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_3$	Linolenic acid
C	1.24	$-(\text{CH}_2)_n-$	All fatty acids
D	1.64	$-\text{CH}_2-\text{CH}_2-\text{COO}-$	All fatty acids
E	2.02	$-\text{CH}_2-\text{CH}=\text{CH}-$	All UFA
F	2.26	$-\text{CH}_2-\text{COO}-$	All fatty acids
G	2.76	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$	n-6 (linoleic) acid and n-3 (linolenic) acid
H	4.19	$-\text{CH}_2\text{OCOR}$	H in the sn-1/3 position of the glycerol backbone
I	5.15	$-\text{CHOCOR}$	H in the sn-2 position of the glycerol backbone
J	5.29	$-\text{CH}=\text{CH}-$	All UFA

¹The protons contributing to each specific signal are in italics.

the terminal $-\text{CH}_3$ group from butyric acid moiety that was shifted downfield compared with the rest of the FA due to the proximity of the ester group. Caproic acid moiety on the other hand—although considered as a short-chain FA—contributed through its methyl group to signal A (Figure 1d), together with the rest of the FA, except for the linolenic acid, whose terminal methyl ¹H-NMR signal B' (Figure 1c) was also shifted downfield (due to its proximity to the C=C double bond) and overlapped with signal B from butyric acid. Therefore, we disagreed with Tociu et al. (2018) on 2 issues: (1) signal B (0.96 ppm) cannot be assigned in a bulky manner to the short-chain FA class (because it results only from the contribution of C4:0), and (2) signal B cannot be considered as a distinctive signal (marker) for C4:0 (and even less for the short-chain FA class; Tociu et al., 2018) because it completely overlaps the terminal methyl from any n-3 FA (e.g., linolenic). Even if BF contains small amounts of C18:3 (up to 1–2%), its contribution to signal B should not be neglected. Moreover, for the detection of adulteration in dairy products, considering signal B as a marker for dairy fats can be misleading, falsely allowing linolenic acid to account for butyric acid.

On the other hand, it should be noticed that at the same amplification degree (calibrated on signal H, Figure 1a and c), ¹H-NMR spectrum of a genuine BF sample differs from spectra of other oils and fats due to the very weak signal G, corresponding to *bis*-allylic protons from n-3 and n-6 FA. Indeed, BF contains low amounts of linolenic acid (0.19–1.45%) depending on species, diet, lactation stage, or breed (Månsson, 2008; Markiewicz-Kęszycka et al., 2013) and linoleic acid (2–3%), respectively, which explains the very small *bis*-allylic moiety contributing to signal G. In addition, other signals associated with unsaturated FA (i.e., signal J: unsaturated protons $-\text{CH}=\text{CH}-$ and signal E: allylic protons), although present due to the large amount of oleic acid (C18:1), are less intense than in the case

of more unsaturated oils. Therefore, when facing ¹H-NMR spectra of fats from uncertain dairy provenience, before proceeding to calculations, analysts should first investigate possible structural characteristics (such as the ratios of the integral values of signals J, G, B, and A, respectively) that might indicate adulteration with cheaper fats and oils.

Can FAP of Milk and Nonmilk Fat Blends Be Determined from the ¹H-NMR Data?

In the case of most oils and fats (of both animal and vegetal origin), ¹H-NMR spectra may be used as raw data for the determination of the FAP on 4 classes: saturated, monounsaturated, di-unsaturated, and tri-unsaturated FA (Knothe and Kenar (2004); Chira et al., 2011). The equations leading to the FA composition derive from the integrals of the ¹H-NMR spectra of oils, based on the contributions of specific protons from each class of FA. The tri-unsaturated FA may be computed assuming this class is mainly constituted of n-3 FA such as the α -linolenic acid, whose terminal methyl group has a distinctive triplet (signal B', Figure 1c) shifted downfield (0.96 ppm) compared with the rest of the FA (signal A, 0.85 ppm). (Knothe and Kenar; 2004). Indeed, α -linolenic acid is the main representative of this class, occurring in considerably larger amounts than its corresponding γ -linolenic acid or other tri-unsaturated n-6 FA, that can therefore be neglected.

In the case of genuine BF, butyric acid has also a distinctive signal (triplet) corresponding to the terminal $-\text{CH}_3$ group, shifted downfield compared with the rest of the FA currently occurring in the MF, including caproic acid. As previously mentioned, the short-chain FA class of BF is composed of butyric acid and caproic acid, of which only butyric acid may be determined independently, based on its distinctive signal (triplet B, Figure 1). Caproic acid, on the other hand, contributes through its methyl group to signal A, together with

the rest of the FA, except for the linolenic acid, whose terminal methyl signal overlaps signal B from butyric acid. In these conditions, the balance of specific proton contribution leads to the following equations:

$$I_A = 3k(Sat + Mono + Di) \Rightarrow Sat + Mono + Di = \frac{I_A}{3k}, \quad [1]$$

$$I_B = 3k(But + Ln) \Rightarrow But + Ln = \frac{I_B}{3k}. \quad [2]$$

Because all fatty acyl chains (including butyric moiety, Figure 1b) have methylene groups contributing to signals F (adjacent to $-\text{COO}$) and D (in β position from $-\text{COO}$),

$$I_D = 2k(But + Ln + Sat + Mono + Di) \Rightarrow But + Ln + Sat + Mono + Di = \frac{I_D}{2k}, \quad [3]$$

$$I_F = 2k(But + Ln + Sat + Mono + Di) \Rightarrow But + Ln + Sat + Mono + Di = \frac{I_F}{2k}, \quad [4]$$

$$I_E = 4k(Mono + Di + Ln) \Rightarrow Mono + Di + Ln = \frac{I_E}{4k}, \quad [5]$$

$$I_G = k(2Di + 4Ln) = 2k(Di + 2Ln) \Rightarrow Di + 2Ln = \frac{I_G}{2k}. \quad [6]$$

Signals I (corresponding to the single proton in the sn-2 position of the glycerol backbone) and J ($-\text{CH}=\text{CH}-$) are partially overlapping each other, and thus cannot be integrated individually; however, their integrals may be computed from signal H (corresponding to 4 protons in sn-1/3 position from glycerol):

$$I_I = k \times I_H / 4, \quad [7]$$

$$I_J = I_{I+J} - k \times I_H / 4 = k(2Mono + 4Di + 6Ln) = 2k(Mono + 2Di + 3Ln) \Rightarrow Mono + 2Di + 3Ln = \frac{I_{I+J} - k \times I_H / 4}{2k}. \quad [8]$$

The sum of all molar fractions equals 1, leading to another equation:

$$But + Ln + Sat + Mono + Di = 1, \quad [9]$$

where $I_A, I_B, \dots, I_J$ are the integrals of A, B, $\dots$ and J signals, respectively; *But*, *Sat*, *Mono*, *Di*, and *Ln* = molar ratios of butyric, SFA (except for butyric acid, MUFA, n-6 FA (di-unsaturated FA, i.e. linoleic acid), and n-3 FA (tri-unsaturated FA, i.e., linolenic acid), respectively.

Additionally, k is a constant that is specific for each spectrometer/software; it is a computed coefficient that correlates the integral with the number of protons that generate the signal. Consequently, k value may be determined based on different signals. For example, replacing equation 9 into equation 3 or 4 leads to

$$k = \frac{I_D}{2} \quad [10]$$

or

$$k = \frac{I_F}{2}. \quad [11]$$

From equation [1] + equation [2], we get the following:

$$k = \frac{I_A + I_B}{3}. \quad [12]$$

Constant k can also be computed as a mean of the values obtained from equations 10 to 12.

Because protons leading to signals A, B, D, and F have different relaxation times (especially longitudinal T1), the corresponding integral value per one proton is slightly different; therefore, k value will differ when computed with equations 10 to 12. From our previous experience working with vegetable oils, the best results (in terms of the best correlation with GC data) were obtained when k was computed according to equation 12 (Chira et al., 2011).

Equations 1 through 9 form a system of 9 equations with 6 unknowns (i.e., *But*, *Ln*, *Sat*, *Mono*, *Di*, and k). However, equations such as (1, 2, and 3) or (5, 6, and 8) are circular, and thus interdependent (e.g., equation 5 + equation 6 = equation 8). Consequently, only equations 1, 2, 5, 6, and 9 are independent, leading to an undetermined system with 5 equations and 6 unknowns that cannot be resolved. Moreover, there are not 2 independent equations with *But* and *Ln* as unknowns from which *But* and *Ln* could be specifically determined.

However, given that pure BF contains low amounts of C18:3, there is temptation to neglect its contribution to signal B as in the approach proposed by Tociu et al.

Figure 2. Expanded spectral window of the *bis*-allylic region: (a) capture from Brescia et al., 2004 (reprinted with permission from the *Journal of the American Oil Chemists' Society*) of buffalo milk fat recorded on a 500-MHz instrument; (b) bovine butter fat, recorded on a 300-MHz instrument.

(2018), allowing for the assessment of the butyric, diunsaturated, monounsaturated, and saturated moieties as molar ratios, respectively: If we replace equation 12 in equation 2:

$$But = \frac{I_B}{I_A + I_B}. \quad [13]$$

If we replace equation 12 in equation 6:

$$Di = \frac{3}{2} \times \frac{I_G}{I_A + I_B}. \quad [14]$$

If we subtract equation 14 from equation 5:

$$Mono = \frac{3}{4} \times \frac{I_E - 2I_G}{I_A + I_B}. \quad [15]$$

Saturated (molar %):

$$Sat = 1 - But - Mono - Di. \quad [16]$$

This method may be useful for the rapid determination of the FAP of genuine dairy products; however, in addition to the inherent lack of accuracy caused by the deliberate neglect of linolenic acid, the approach is risky for authentication matters.

On the other hand, if spectra are recorded on instruments operating at frequencies higher than 500 MHz, the indetermination of the original chemometric equation system (i.e. equations 1, 2, 5, 6, and 9) could be resolved. Brescia et al. (2004) suggested a different approach for the determination of C18:3 in buffalo MF from ^1H -NMR data, based on the *bis*-allylic signal G. At frequencies higher than 500 MHz, the 2 triplets

corresponding to the *bis*-allylic moieties from linolenic (signal G₁, shifted downfield at 2.83 ppm) and linoleic acids (signal G₂, shifted upfield at 2.79 ppm) appear almost separated (Figure 2a). Consequently, the authors proposed to separately integrate the 2 triplets, allowing the determination of C18:3 and C18:2 moieties as follows:

$$I_{G_1} = 4k \times Ln \Rightarrow Ln = \frac{I_{G_1}}{4k}, \quad [17]$$

$$I_{G_2} = 2k \times Di \Rightarrow Di = \frac{I_{G_2}}{2k}. \quad [18]$$

Consequently, the rest of the molar fractions (*But*, *Mono*, and *Sat*) may be determined based on equations 2, 5, and 1, respectively.

Compared with the neglect of the C18:3 fraction discussed in equations 13 through 16, this approach allows for the determination of linolenic acid, increasing the FAP accuracy. However, errors in the assessment of the *Ln* and *Di* moieties may still be considerable because the separate integration of signals G₁ and G₂ does not respect the general rule of signals integration (i.e. from baseline to baseline). In addition, on instruments operating at frequencies lower than 500 MHz, the 2 triplets appear partially overlapped (Figure 2b); therefore, their individual integration is not possible. Consequently, butyric acid cannot be unequivocally determined from ^1H -NMR data with acceptable accuracy.

However, the butyric acid moiety may be determined from quantitative ^{13}C -NMR data. Compared with ^1H -NMR spectra, the electronic configuration of the ^{13}C nucleus determines the spreading of the chemical shifts over ~200 ppm frequency range, resulting in minor signal overlapping and, as a consequence, enabling more

refined structural information to determine the butyric fraction. Butyric acid displays 2 distinctive ^{13}C resonances (1 peak at 173.13 ppm, corresponding to sn-1,3 carbonyl carbon, and 1 peak at 35.34 ppm, assigned to the methyl group), which appear well-separated from adjacent signals. These 2 resonances may therefore be integrated separately and represent markers for butyric acid. According to Sacchi et al. (2018), any of these signals may be exploited to assess the amount of butyric acid by referring their areas to the corresponding signals of long-, medium- and short-chain FA (C6–C22). This approach has led to good results in the evaluation of the MF content in fat blends, based on the ^{13}C -NMR analysis of C4:0. However, for quantitative ^{13}C -NMR analysis, when carbons have different relaxation behavior (as in the case of the carbonyl carbons and methylene/methyl carbons of the fatty acyl chains), the longitudinal relaxation times (T1) must be known for all carbons to ensure they are completely relaxed between

2 consecutive pulses. Consequently, q ^{13}C -NMR spectra should be acquired using experimental conditions that allow for complete relaxation of carbon nuclei between 2 subsequent pulses, based on the known T1 values for different acyl carbons (Ng, 2000). Long relaxation times and high number of scans necessary to obtain higher signal/noise ratios for quantitative determinations with acceptable accuracy make q ^{13}C -NMR spectroscopy a time-consuming technique. From time perspective, the method becomes comparable with GC, which, although lengthy, offers an accurate and detailed sample FAP.

Investigating Dairy Product Adulteration Through ^1H -NMR: Traps and Challenges

Food fraud has been intensively studied through several methods. Generally, these methods are based on extensive databases of authentic samples which are analyzed for their composition or other specific features

Figure 3. Superimposed spectra of butter fat (BF; blue) and simulated BF (ternary mixture of *Lallemantia iberica* oil 6% + coconut oil 40% + beef tallow 54%; red).

Table 3. Simulated butter from nonmilk fats

No.	Oil or fat	Amount (%)	Fatty acid profile ¹ (g/100 g, mean value ² ± SD)									
			But		n-3 (Ln)		n-6 (Di)		MUFA		SFA	
			¹ H-NMR	GC	¹ H-NMR	GC	¹ H-NMR	GC	¹ H-NMR	GC	¹ H-NMR	GC
1	<i>Lallemantia iberica</i> oil	6	4.46 ± 0.32	—	—	4.11 ± 0.28	2.78 ± 0.19	3.05 ± 0.21	25.86 ± 1.09	26.91 ± 0.62	66.90 ± 1.87	65.93 ± 0.83
	Coconut oil	40										
	Beef tallow	54										
2	<i>L. iberica</i> oil	6	4.36 ± 0.52	—	—	4.17 ± 0.26	3.05 ± 0.23	3.83 ± 0.19	22.06 ± 0.72	23.11 ± 0.57	70.53 ± 2.10	68.89 ± 0.95
	Sheep tallow	20										
	Palm kernel oil	74										
3	Linseed oil	7.5	4.23 ± 0.29	—	—	4.01 ± 0.23	4.63 ± 0.41	5.07 ± 0.24	20.02 ± 0.92	21.30 ± 0.61	71.12 ± 2.05	69.62 ± 0.93
	Palm kernel oil	46										
	Palm stearin	46.5										

¹The proton nuclear magnetic resonance (¹H-NMR) composition was computed according to the method proposed by Tociu et al. (2018). But = butyric acid; n-3 = total amount of n-3 fatty acids (mainly linolenic acid, Ln); n-6 = total amount of n-6 fatty acids (mainly di-unsaturated such as the linoleic acid, Di); MUFA = total amount of monounsaturated fatty acids; SFA = total amount of saturated fatty acids, except for butyric acid.

²Three replicates.

(e.g. fingerprinting through spectroscopic techniques), leading to mathematical models. In the case of an unknown sample (suspicion of adulteration with cheaper fats/oils), the use of ¹H-NMR to assess its FAP may prove misleading, due to the difficulty of correctly assessing the linolenic acid content and the possibility of confusing linolenic acid for butyric acid.

Tociu et al. (2018) have tested their proposed system of chemometric equations on vegetable cheese produced from palm oil and obtained a 100% correct classification of the investigated samples of vegetable provenience based on the “short-chain fatty acids content,” which was lower than the typical value for bovine MF. In this case, the correct classification was due to the fact that palm oil does not contain linolenic acid, leading to the absence of the triplet at 0.96 ppm, considered by the authors as specific for the short-chain FA.

However, in the case of dairy products adulterated with cheaper oils/fats containing convenient amounts of linolenic acid, the approach proposed by Tociu et al. (2018) may lead to sample misclassification. It is even possible that in the absence of GC and based only on the ¹H-NMR profile, appropriate vegetable and animal fats/oils compositions (of nonmilk origin) can imitate the ¹H-NMR profiles of MF. Indeed, relatively small amounts (6–7%) of oils rich in linolenic acid such as linseed oil or *Lallemantia iberica* seed oil will ensure the triplet at 0.96 ppm, and the rest up to 100% will be constituted from fats with very low amounts of linoleic acid (n-6) and rich in monounsaturated and saturated FA (such as the coconut oil, beef tallow, palm kernel oil, palm stearin, and mustard oil, Table 1). Such “smart adulterations” can simulate the ¹H-NMR spectra of genuine butter samples. Figure 3 illustrates, as an example, the superimposed ¹H-NMR spectra of a simulated butter of vegetal origin (*Lallemantia iberica* oil 6% + coconut oil 40% + beef tallow 54%) opposite to an authentic BF. As it can be seen, the 2 spectra are similar and easy to mistake in the attempt of determining their FAP from ¹H-NMR data. Indeed, applying the method proposed by Tociu et al. (2018) for the determination of the FA composition of 3 synthetic nondairy fat blends leads to FAP similar to MF, allowing for their misclassification as genuine MF; although, in reality, the blends had very different compositions, as confirmed by GC (Table 3).

How Can ¹H-NMR Data Help in the Detection of “Smart Adulterations”?

Although we have demonstrated that the FA composition of dairy fats cannot be reliably determined from ¹H-NMR data and subsequently used for authentication purposes, it would be wrong to deny the potential of

Table 4. Proton nuclear magnetic resonance (^1H -NMR) descriptors for fats and oils

No.	Oil or fat	n	^1H -NMR descriptor ¹		
			(C+D+E+F+G)/F (descriptor 1)	(C+D+E+F+G)/(I+J) (descriptor 2)	B/(A+B) (descriptor 3)
1	Butter fat	35	11.80 $\pm$ 0.33	10.80 $\pm$ 0.80	0.12 $\pm$ 0.02
2	Sheep tallow	4	14.50 $\pm$ 0.19	11.24 $\pm$ 0.15	0.02 $\pm$ 0.01
3	<i>Lallemantia iberica</i> seed	3	11.21 $\pm$ 0.13	0.99 $\pm$ 0.08	0.59 $\pm$ 0.01
4	Coconut	4	11.09 $\pm$ 0.35	14.13 $\pm$ 0.08	0.01 $\pm$ 0.01
5	Beef tallow	5	13.82 $\pm$ 0.18	12.15 $\pm$ 0.26	0.02 $\pm$ 0.01
6	Palm kernel oil	6	11.50 $\pm$ 0.16	11.05 $\pm$ 0.29	ND ²
7	Linseed oil	5	11.73 $\pm$ 0.15	1.17 $\pm$ 0.17	0.57 $\pm$ 0.02
8	Palm stearin	5	12.69 $\pm$ 0.11	9.97 $\pm$ 0.17	ND
9	Simulated butter 1 ³	3	12.45 $\pm$ 0.18	8.00 $\pm$ 0.23	0.10 $\pm$ 0.02
10	Simulated butter 2 ³	3	11.52 $\pm$ 0.12	8.83 $\pm$ 0.10	0.10 $\pm$ 0.01
11	Simulated butter 3 ³	3	11.91 $\pm$ 0.14	7.97 $\pm$ 0.09	0.09 $\pm$ 0.01

¹Mean values $\pm$ SD, computed based on the integral values of the indicated signals. A, B, C, D, E, F, G, I, and J refer to the integral values of the corresponding signals, according to Table 2.

²ND = not detected (assumed as 0 for graphical data visualization).

³Compositions corresponding to Table 3.

^1H -NMR data in assessing dairy authenticity and fraud detection. An essential characteristic of the milk FAP is its important amount of short- and medium-chain FA. This feature is also reflected in the ^1H -NMR spectra, through signal C, which is generated by $-\text{CH}_2-$ groups that have not been specifically assigned. Therefore,

signal C is correlated with the FA chain lengths. Consequently, key features that needs to be further explored in the detection of dairy products adulteration approaches are the ratios of integrals of signals assigned to $-\text{CH}_2-$ groups (including signal C, allylic, and *bis*-allylic groups) and specific signals related to butyric/n-3 FA

Figure 4. Orthogonal representation of descriptor 1 versus descriptor 2 (analytical formulas corresponding to Table 4). C, D, E, F, G, I, and J refer to the integral values of the corresponding signals, according to Table 2.

and other structural features (such as unsaturation and *bis*-allylic groups). Therefore, we propose the integral ratios $(C+D+E+F+G)/F$, $(C+D+E+F+G)/(I+J)$, and $B/(A+B)$ as 3 new ^1H -NMR fat descriptors (where A, B, C, D, E, F, G, I, and J refer to the integral values of the corresponding signals, according to Table 2). Table 4 presents their mean values for several fats and oils and for the 3 simulated BF compositions described in Table 3.

As it can be seen, fats and oils have specific values of the proposed descriptors, according to their specific compositions. For example, linseed oil and *Lallemantia iberica* seed oil (which are highly unsaturated oils) differ significantly from BF with respect to descriptors 2 and 3, and saturated fats show closer values. On the

other hand, the simulated BF compositions differ from genuine BF with respect to descriptor 2. The orthogonal representation of descriptor 1 versus descriptor 2 (Figure 4) allows for the data visualization. It can be observed that samples cluster according to the species. Not surprisingly, the unsaturated oils group distinctly and away from the BF class, and the more saturated samples group closer to BF. Among these, coconut, beef, and sheep tallow form distinct clusters from BF, and palm kernel oil and palm stearin classes partially overlap BF group. The simulated BF samples (represented in red) place distinctly, but close, to the BF samples. Consequently, the first 2 descriptors are not sufficient for a satisfactory separation of palm kernel oils, palm stearin, and the 3 simulated BF samples from

Figure 5. Three-dimensional orthogonal representation of descriptors 1, 2, and 3 for butter fat, palm kernel oil, palm stearin, and the simulated butter fat samples (analytical formulas corresponding to Table 4). A, B, C, D, E, F, G, I, and J refer to the integral values of the corresponding signals, according to Table 2.

the BF group. In this case, the B:(A+B) ratio (i.e. descriptor 3) can help in the discrimination according to the 3-dimensional orthogonal plot represented in Figure 5.

As it can be observed, palm kernel oils and palm stearin samples group distinctively from the BF samples in the lower side of the plot, and the simulated BF samples place in the upper side, still apart from the BF group, allowing for a clear separation. The separation of fat samples of dairy products that originate from oils and fats of different vegetal or animal origin based on the NMR data was also reported in the literature. For example, according to a recent paper (Guyader et al., 2018), the application of a refocused adiabatic ^{13}C Insensitive Nuclei Enhanced by Polarization Transfer sequence enables discrimination of BF from 30 different oils and fats of animal and vegetal origins (including margarines), proving that butter could clearly be distinguished from plant oil. On the other hand, the separation of butter or margarine into polar (water soluble) and nonpolar fractions (soluble in CDCl_3) and subsequent analysis of these fractions by ^1H -NMR spectroscopy allows for a comprehensive analysis of the fat constituents (i.e., quantification of conjugated linoleic acids, rumenic acid, diglycerides, and linoleic acid) and a clear differentiation of butter samples from margarines (Schripsema, 2008).

CONCLUSIONS

In this paper, we demonstrated that—contrary to a previously reported method—in the case of the dairy fat samples, the butyric and linolenic moieties cannot be accurately determined from ^1H -NMR spectra due to the overlapping resonances of methyl signals at 0.96 ppm. Moreover, short-chain FA cannot be determined as a class. We have highlighted the weaknesses of the respective method by applying it on 3 synthetic non-dairy fat blends by obtaining FA profiles similar to BF, which lead to their misclassification as genuine dairy provenience. In return, we have proposed 3 new ^1H -NMR fat descriptors based on various integral ratios of signals associated with CH_2 moiety versus signals associated with butyric and n-3 FA that allowed the correct classification of both BF samples and simulated BF compositions as well as other vegetable (linseed, coconut, palm stearin, palm kernel oil, *Lallemantia iberica* seed oil) and animal fats (beef and sheep tallow). The 2-dimensional and 3-dimensional data visualization by means of orthogonal representation of the new descriptors allowed discrimination of MF from the rest of the fat samples. Consequently, ^1H -NMR spectroscopy can be used to detect dairy fraud, but not through FAP determination.

ACKNOWLEDGMENTS

Anamaria Hanganu acknowledges project no. CNFISFDI-2020-0355, “Consolidarea și creșterea competitivității instituționale a Universității din București ca hub de proiecte interdisciplinare și instituție-gazdă pentru cercetători de top din țară și din străinătate,” Bucharest, Romania. The University of Bucharest–UniRem project no. 244 is gratefully acknowledged. The authors thank Constantinescu (Chemont S. A., Mouscron, Belgium) for providing certified *Lallemantia iberica* seed samples. The authors have not stated any conflicts of interest.

REFERENCES

- Aali, M., H. Moradi-Shahrbabak, M. Moradi-Shahrbabak, M. Sadeghi, and H. Kohram. 2016. Polymorphism in the SCD gene is associated with meat quality and fatty acid composition in Iranian fat- and thin-tailed sheep breeds. *Livest. Sci.* 188:81–90. <https://doi.org/10.1016/j.livsci.2016.04.003>.
- Alexandri, E., R. Ahmed, H. Siddiqui, M. Choudhary, C. G. Tsiafoulis, and I. P. Gerothanassis. 2017. High Resolution NMR Spectroscopy as a Structural and Analytical Tool for Unsaturated Lipids in Solution. *Molecules* 22:1663–1735. <https://doi.org/10.3390/molecules22101663>.
- Amini, R., A. Ebrahimi, and A. D. M. Nasab. 2020. Moldavian balm (*Dracocephalum moldavica* L.) essential oil content and composition as affected by sustainable weed management treatments. *Ind. Crops Prod.* 150:112416. <https://doi.org/10.1016/j.indcrop.2020.112416>.
- Anastasiu, A.-E., N.-A. Chira, I. Banu, N. Ionescu, R. Stan, and S.-I. Roșca. 2016. Oil productivity of seven Romanian linseed varieties as affected by weather conditions. *Ind. Crops Prod.* 86:219–230. <https://doi.org/10.1016/j.indcrop.2016.03.051>.
- ANPC. (Autoritatea Nationala pentru Protectia Consumatorilor). 2020. Intalnire de lucru. Accessed Sep. 25, 2020. <https://anpc.ro/articol/1383/intalnire-de-lucru-1>.
- Brescia, M. A., V. Mazzilli, A. Sgarbetta, S. Ghelli, F. P. Fanizzi, and A. Sacco. 2004. ^1H -NMR characterization of milk lipids: A comparison between cow and buffalo milk. *J. Am. Oil Chem. Soc.* 81:431–436. <https://doi.org/10.1007/s11746-004-0918-3>.
- Chira, N. A., M. C. Todasca, A. Nicolescu, A. Rosu, M. Nicolae, and S. I. Rosca. 2011. Evaluation of the computational methods for determining vegetable oils composition using ^1H -NMR spectroscopy. *Rev. Chim.* 62:42–46.
- Cornelio-Santiago, H. P., M. R. Mazalli, C. E. C. Rodrigues, and A. L. de Oliveira. 2019. Extraction of Brazil nut kernel oil using green solvents: Effects of the process variables in the oil yield and composition. *J. Food Process Eng.* 42:e13271. <https://doi.org/10.1111/jfpe.13271>.
- Dieffenbacher, A., and W. D. Pocklington. 1992. Section 2: Oils and Fats. Pages 2.310/1–4 in *Standard Methods for the Analysis of Oils, Fats and Derivatives* – 1st Supplement. 7th ed. Oxford: Blackwell Scientific Publications.
- Dorni, C., P. Sharma, G. Saikia, and T. Longvah. 2018. Fatty acid profile of edible oils and fats consumed in India. *Food Chem.* 238:9–15. <https://doi.org/10.1016/j.foodchem.2017.05.072>.
- Gómez-Masclaque, L. G., K. Kilcawley, D. Hennessy, J. T. Tobin, and T. F. O’Callaghan. 2020. Raman spectroscopy: A rapid method to assess the effects of pasture feeding on the nutritional quality of butter. *J. Dairy Sci.* 103:8721–8731. <https://doi.org/10.3168/jds.2020-18716>.
- González-Martin, M. I., A. M. Vivar-Quintana, I. Revilla, and J. Salvador-Esteban. 2020. The determination of fatty acids in cheeses of variable composition (cow, ewe’s, and goat) by means of near

- infrared spectroscopy. *Microchem. J.* 156:104854. <https://doi.org/10.1016/j.microc.2020.104854>.
- Guyader, S., F. Thomas, V. Portaluri, E. Jamin, S. Akoka, V. Silvestre, and G. Remaud. 2018. Authentication of edible fats and oils by non-targeted ^{13}C INEPT NMR spectroscopy. *Food Control* 91:216–224. <https://doi.org/10.1016/j.foodcont.2018.03.046>.
- Handford, C. E., K. Campbell, and C. T. Elliott. 2016. Impacts of milk fraud on food safety and nutrition with special emphasis on developing countries. *Compr. Rev. Food Sci. Food Saf.* 15:130–142. <https://doi.org/10.1111/1541-4337.12181>.
- Indrasti, D., Y. B. Che Man, S. Mustafa, and D. M. Hashim. 2010. Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry. *Food Chem.* 122:1273–1277. <https://doi.org/10.1016/j.foodchem.2010.03.082>.
- Jamwal, R., S. Amit, S. Kumari, B. Balan, A. S. Dhaulaniya, S. Kelly, A. Cannavan, and D. K. Singh. 2020. Attenuated total Reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy coupled with chemometrics for rapid detection of argemone oil adulteration in mustard oil. *Lebensm. Wiss. Technol.* 120:108945. <https://doi.org/10.1016/j.lwt.2019.108945>.
- Kamal, M., and R. Karoui. 2015. Analytical methods coupled with chemometric tools for determining the authenticity and detecting the adulteration of dairy products: A review. *Trends Food Sci. Technol.* 46:27–48. <https://doi.org/10.1016/j.tifs.2015.07.007>.
- Knothe, G., and J. A. Kenar. 2004. Determination of the fatty acid profile by ^1H -NMR Spectroscopy. *Eur. J. Lipid Sci. Technol.* 106:88–96. <https://doi.org/10.1002/ejlt.200300880>.
- Kontson, A., A. Tamsma, and F. E. Kurtz. 1973. Method for extracting fat from dry whole milk. *J. Dairy Sci.* 56:144–145. [https://doi.org/10.3168/jds.S0022-0302\(73\)85128-8](https://doi.org/10.3168/jds.S0022-0302(73)85128-8).
- Laučienė, L., V. Andrulevičiute, I. Sinkevičienė, A. Sederevičius, K. Musayeva, and L. Šernienė. 2019. Analysis of fatty acid composition and healthy lipids indices in raw and processed milk. *J. Food Nutr. Res.* 7:386–390. <https://doi.org/10.12691/jfmr-7-5-8>.
- Leite, A. I. N., C. G. Pereira, J. Andrade, N. M. Vicentini, M. J. V. Bell, and V. Anjos. 2019. FTIR-ATR spectroscopy as a tool for the rapid detection of adulterations in butter cheeses. *Lebensm. Wiss. Technol.* 109:63–69. <https://doi.org/10.1016/j.lwt.2019.04.017>.
- Li, Y., and B. A. Watkins. 2001. Unit 1.2: Analysis of Fatty Acids in Food Lipids. Pages D1.2.1–D1.2.15 in *Current Protocols in Food Analytical Chemistry*. J. Whitaker, ed. John Wiley & Sons Inc.
- Liu, C., Z. Meng, X. Chai, X. Liang, M. Piatko, S. Campbell, and Y. Liu. 2019. Comparative analysis of graded blends of palm kernel oil, palm kernel stearin and palm stearin. *Food Chem.* 286:636–643. <https://doi.org/10.1016/j.foodchem.2019.02.067>.
- Månsson, H. L. 2008. Fatty acids in bovine milk fat. *Food Nutr. Res.* 52:1821–1823. <https://doi.org/10.3402/fnr.v52i0.1821>.
- Markiewicz-Kęszycka, M., G. Czyżak-Runowska, P. Lipińska, and J. Wójtowski. 2013. Fatty acid profile of milk - A Review. *Bull. Vet. Inst. Pulawy* 57:135–139. <https://doi.org/10.2478/bvip-2013-0026>.
- Mohammed, H. H. H., G. Jin, M. Ma, I. Khalifa, R. Shukat, A. E. Elkhedir, Q. Zeng, and A. E. Noman. 2020. Comparative characterization of proximate nutritional compositions, microbial quality and safety of camel meat in relation to mutton, beef, and chicken. *Lebensm. Wiss. Technol.* 118:108714. <https://doi.org/10.1016/j.lwt.2019.108714>.
- Naksuk, A., D. A. Sabatini, and C. Tongcumpou. 2009. Microemulsion-based palm kernel oil extraction using mixed surfactant solutions. *Ind. Crops Prod.* 30:194–198. <https://doi.org/10.1016/j.indcrop.2009.03.008>.
- Ng, S. 2000. Quantitative analysis of partial acylglycerols and free fatty acids in palm oil by ^{13}C nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* 77:749–755. <https://doi.org/10.1007/s11746-000-0120-7>.
- Pereira, C. G., A. I. N. Leite, J. Andrade, M. J. V. Bell, V. Anjos, C. A. I. Nunes Leite, J. Andrade, M. J. V. Bell, and V. Anjos. 2019. Evaluation of butter oil adulteration with soybean oil by FT-MIR and FT-NIR spectroscopies and multivariate analyses. *Lebensm. Wiss. Technol.* 107:1–8. <https://doi.org/10.1016/j.lwt.2019.02.072>.
- Sacchi, R., A. Paduano, N. Caporaso, G. Picariello, R. Romano, and F. Addeo. 2018. Assessment of milk fat content in fat blends by ^{13}C -NMR spectroscopy analysis of butyrate. *Food Control* 91:231–236. <https://doi.org/10.1016/j.foodcont.2018.04.011>.
- Schripsema, J. 2008. Comprehensive analysis of polar and apolar constituents of butter and margarine by nuclear magnetic resonance, reflecting quality and production processes. *J. Agric. Food Chem.* 56:2547–2552. <https://doi.org/10.1021/jf072274c>.
- Shahidi, F. 2001. Unit 1.1: Extraction and Measurement of Total Lipids. Pages D1.1.1–D1.1.11 in *Current Protocols in Food Analytical Chemistry*. J. Whitaker, ed. John Wiley & Sons Inc.
- Tociu, M., M.-C. Todasca, A. Bratu, M. Mihalache, and F. Manolache. 2018. Fast approach for fatty acid profiling of dairy products fats using ^1H -NMR spectroscopy. *Int. Dairy J.* 83:52–57. <https://doi.org/10.1016/j.idairyj.2018.03.005>.
- Xie, Y., Z. Yan, Z. Niu, J. A. Coulter, J. Niu, J. Zhang, B. Wang, B. Yan, W. Zhao, and L. Wang. 2020. Yield, oil content, and fatty acid profile of flax (*Linum usitatissimum* L.) as affected by phosphorus rate and seeding rate. *Ind. Crops Prod.* 145:112087. <https://doi.org/10.1016/j.indcrop.2020.112087>.
- Yurchenko, S., A. Sats, V. Tatar, T. Kaart, H. Mootse, and I. Jõudu. 2018. Fatty acid profile of milk from Saanen and Swedish Landrace goats. *Food Chem.* 254:326–332. <https://doi.org/10.1016/j.foodchem.2018.02.041>.

ORCIDS

Anamaria Hanganu <https://orcid.org/0000-0002-0433-752X>
 Nicoleta-Aurelia Chira <https://orcid.org/0000-0002-7786-5649>