Solvent-based strategy improves the direct determination of key parameters in edible fats and oils by ^{1}H NMR

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Running title: Analysis of edible fats and oils by ^{1}H NMR

Abstract

BACKGROUND: Edible fats and oils are very important in nutrition and as main source of energy and also essential nutrients. There are several methods for the analysis of edible fats and oils, but nowadays Nuclear Magnetic Resonance (NMR) is emerging as a powerful tool (albeit complex and high-tech demanding) to identify, quantify and differentiate many types of food including fats and oils. In this sense, the challenges of this technique are the simplification of methodology and taking advantage of a 400MHz NMR equipment.

RESULTS: Through an adequate mixture of solvents, we have developed a methodology to quantify essential parameters in edible fats and oils, including 1,2-DAG, 1,3-DAG and 1-MAG, by using a single experiment and without the need for matrix derivatization.

CONCLUSION: This methodology has been successfully applied to the analysis of olive, sunflower, corn, sesame and peanut oils, as well as butter, walnut, salmon and spicy pork sausage. Moreover, the evolution of thermal oxidation and lipolysis of virgin olive oil and sunflower has been analyzed.

KEYWORDS: ^{1}H NMR, edible fats, oil, sausage, acylglycerols

INTRODUCTION

The production of vegetable oils and, in particular, olive oil, has increased in recent years because of their economic importance in diverse fields.[1] On the other hand, fats from animal origin are widely consumed and their physical and chemical properties can affect food quality[2] and consumer health[3] and should be properly evaluated. Edible fats and oils comprise acylglycerols (Figure 1), mainly triacylglycerols (TAG) and in a minor quantity diacylglycerols (DAG), monoacylglycerols (MAG) and other minority components.[4,5] DAG are found as 1,2- or 1,3-diacylglycerols (1,2-DAG and 1,3-DAG) and it has been shown that these compounds are good indicators of thermal stress and/or aging in extra virgin olive oil,[6] and, also, quality of dry-cured ham can be evaluated according to DAG profile.[7] Manufacturing and storage conditions of edible fats and oils produce additional changes in DAG composition, mainly due to the isomerization of 1,2-DAG to 1,3-DAG. [5,8,9]

MAG are present in vegetable oils at trace levels and there are two isomeric forms of MAG depending on the position of the ester group in the glycerol; 1-MAG and 2-MAG.[10] The formation of both DAG and MAG depends on oilseeds storage conditions,[11] temperature and humidity and their control could be an indicator of origin and quality of the oil.

In addition to the esterification degree of glycerol core, the other fundamental part of this type of molecules are the fatty acid (FA) chains. The relationships between saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), including the palmitoleic or oleic acids, and polyunsaturated fatty acids (PUFA) are relevant information from the nutritional point of view. In particular, the study of PUFA

is of great interest, including essential FA or their metabolites as linoleic acid (LA, omega-6 or n-6 fatty acid type) and α-linolenic acid (ALA, omega-3 or n-3 fatty acid type). Other important PUFA are arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) regarded as "conditionally essential" FA.[12] These acyl chains can be bonded to the glycerol moiety or appear as free fatty acids (FFA) due to lipolysis processes.[13] These FFA are responsible for the free acidity of the edible fat or oil, a crucial factor of the quality as well as the economic value of these products (acid value).

For these reasons, various methods of analysis have been developed and applied to analyze different fats[2] or determine individual components of edible fats and oils.[14] In this context, Nuclear Magnetic Resonance (NMR) has emerged as a useful tool for the determination of important parameters in food[15] and beverages[16–18] and more specifically in edible oils and fats.[19] Many of these studies have been focused on the analysis of vegetable oils, not only for the geographical assignment, but also for the determination, quality assessment and quantification of the major and minor components of oils such as DAG and MAG.[20–23] NMR methods have also been developed for the analysis of PUFA in different matrices.[24,25] Recent reviews have reported the suitability of ^{1}H NMR to carry out studies on hydrolysis and oxidation during lipid digestion under gastrointestinal digestive conditions, proving that this spectroscopic method is a very powerful and simple tool in lipid studies. This technique provides very valuable qualitative and quantitative information about the different types of protons present in food lipids.[26–28]

Proton (^{1}H) NMR has demonstrated its usefulness in oil analysis when 500 MHz or bigger magnetic field is used and also when derivatization strategies are applied.[29-31] Other NMR nucleus different from proton have been successfully used in the characterization studies, such as carbon-13 (^{13}C) NMR,[32,33] albeit the long experimental time is discouraging. However, derivatization with phosphorus compounds has shown to be a very convenient methodology in DAG and MAG analysis by NMR, even suitable with 300 or 400 MHz NMR equipment through phosphorous-31 (^{31}P) NMR analysis[34] and has been successfully used in the determination of DAG.[35–38] In this context, many efforts are being carried out to use lower-field, cheaper and lower-maintenance NMR equipment.[39–41]

For these reasons, nowadays, one the main objectives is the development of fast and simple methods for

the complete analysis of edible fats and oil putting aside derivatization steps or expensive high field equipment. However, the overlapping of signals, when an equipment smaller than 600 MHz is used, makes difficult the analysis of minor compounds, such as DAG, MAG, PUFA and FFA.

It is well established that the use of different solvents, or mixtures of them, affects the chemical shift of signals.[42] Considering previous studies on the use of solvents such as $CDCl_3$/DMSO in the analysis of oils by NMR,[43] the aim of this work is to study and find solvents or solvent mixtures that allow the identification of minor compounds in edible fats and oils and their subsequent analysis and quantification. In addition, this work aims to analyze for the first time using ^{1}H NMR, the fat portion of spicy pork sausage named *chorizo* in Spain. In this respect, and to the best of our knowledge, there is only an unique study using ^{1}H HR-MAS NMR of salami-type sausage (*salchichón*).[44]

MATERIALS AND METHODS

Samples preparation

Samples of olive, sunflower, corn, sesame and peanut oils, as well as butter, walnut, salmon and spicy pork sausage were obtained from commercial sources. Oils were analyzed without a further treatment. For the rest of food types, fat fraction was extracted in advance.

Fat extraction: A representative sample of 150 g of butter, walnut, salmon or dry sausage was homogenized with a kitchen mincer (Moulinex, type D55, 750 W) to obtain an homogenate with a particle size of less than 2 mm. Then, 100 mL of chloroform was added to an aliquot of 10 g of the homogenized product and it was stirred for 2 min using a kitchen hand blender (Braun, type 4191, 600 W). The solids were filtered and a portion of anhydrous Na_2SO_4 was added to the filtrate to dry the solvent. The liquid was filtered again and the solvent was removed in vacuum.

NMR sample preparation: The resultant extracted fat or the oil (120 mg) was dissolved in the corresponding deuterated solvent or solvent mixture (550 μL). $CDCl_3$, C_6D_6, acetone-d_6, CD_3CN and mixtures of $CDCl_3$/DMSO-d_6, CCl_4/DMSO-d_6, CS_2/DMSO-d_6/$CHCl_3$ were used as deuterated solvents.

Oil degradation process: 150 g of commercial extra virgin olive oil and sunflower oil were weighted in two 250 mL erlenmeyer flasks and placed on a hot plate with stirring (magnetic bar) and temperature control (fuzzy-logic thermostat). The first 24 h of heating, the temperature was set to 160 ºC, and then raised to 190 ºC for the rest of the experiment. Aliquots (1 mL approx.) were removed after 0, 1, 1.5, 2.5, 4.5, 7, 10, 24.5, 26, 28.5, 33.5, 37.5 and 43.5 h and analyzed in triplicate. The NMR sample was prepared as discussed above.

Total acidity

An aliquot of fat sample (1 g of high-hydrolyzed samples or 4 g for high-fresh samples, m) was dissolved by adding 10 mL of ethanol/diethyl ether (1:1). A titration was carried out, using phenolphthalein as an indicator, against a titrated solution of 0.05 M KOH in ethanol. Acid Value = 56.1 × 0.05 × V/m.[45]

NMR Spectroscopy Analysis and Processing

NMR spectra were recorded on a Bruker Avance 400 spectrometer equipped with a 5 mm inverse probe (BBI H-BB Z-GRD). Acquisition of spectra was carried out with TOPSPIN software (version 2.1). Spectra were processed using MNova program (Mestrelab Research, Santiago de Compostela, Spain). The spectrometer transmitter was locked to $CDCl_3$ frequency for the $CDCl_3$ solvent, DMSO-d_6 frequency for a solvent mixture $CDCl_3$/DMSO-d_6, CCl_4/DMSO-d_6 and CS_2/DMSO-d_6/$CHCl_3$. All the spectra were acquired at 298 K.

The ^{1}H NMR spectra were recorded with ^{13}C decoupled pulse sequence (Waltz-16) to eliminate satellite signals that hinder the interpretation of minor compounds. The spectral window was 20 ppm, and data were collected into 32k data points after 64. The relaxation delay (d1) was set to 10 s. The spectra were acquired using TOPSHIM tools and the NMR SampleXpress Lite that allows the automatic analysis of several samples.

Free Induction Decays (FID) files were exported into the MNova program and an exponential window function was applied prior to carry out Fourier Transform, in order to obtain an optimal signal-to-noise

ratio. Spectra phases were manually corrected by selecting the sub-menu “Phase Correction” and baseline were manually adjusted by the “cubic splines” algorithm. Signals integration was manually carried out in ^{1}H NMR spectra. All identified metabolites were assigned by using information previously published (in the case of $CDCl_3$)[43] or by spiking experiments with glyceryl trilinolenate, glyceryl trilinoleate, glyceryl trioleate, glyceryl tristearate, 1,3-dilinoleoyl-*rac*-glycerol, 1,2-dipalmitoyl-*rac*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, 1-myristoylglycerol and 2-lauroylglycerol. These compounds were purchased at Sigma-Aldrich Inc.

RESULTS AND DISCUSSION

As mentioned above,[23] the use of $CDCl_3$ does not allow an accurate analysis of DAG, 1-MAG and FFA due to signal overlapping (Figure 2a). In order to solve this occurrence, more laborious methods of derivatization to phosphorus compounds have been carried out in order to use ^{31}P NMR.[36] Considering previous studies,[46] we decided to test other different solvents to improve the separation of signals.

The use of C_6D_6, being an aromatic compound, could lead to alterations in the chemical shifts of signals. When NMR experiments were carried out under these conditions, an optimum solubility was observed; however, effective separation of signals was not achieved. Although the >C<u>H</u>OH signal corresponding to 1,3-DAG appeared separated from the TAG signals, a new, unexpected overlapping took place and two main signals were merged (C1 methylene and allylic protons).

The next solvent used was deuterated acetone (acetone-d_6), which dissolves fat samples with difficulty. This solvent allowed to separate the TAG and 1,3-DAG signals, but the residual solvent signal overlapped with the allylic proton signals and altered its integration. The last solvent tried was CD_3CN; however, it had to be discarded due to solubility problems.

The improvements obtained encouraged us to continue testing different solvent mixtures. Therefore, we used a mixture of $CDCl_3$ and DMSO-d_6 in a ratio 3:1 (Figure 2b). Under these conditions, the signal corresponding to >C<u>H</u>OH (▲) of the 1,3-DAG did not overlap with other signals. In addition, this solvent mixture allowed to analyze the FFA, which correlated with acid value analysis.

The presence of two different sources of deuterium in the sample hinders lock and shim automation; therefore, mixtures of DMSO-d_6 with other non-deuterated solvent were then tested. When the mixture used was CCl_4/DMSO-d_6 in a ratio of 9:2 (Figure 3c), we achieved better signal separation. Thus, the signals corresponding to the 1,3-DAG glycerol moiety (Δ and▲) do not overlap with C1 methylene of TAG (○) nor 1,2-DAG (□) and can be easily quantified. Moreover, the signals of 1,2-DAG corresponding to >C<u>H</u>OCOR (■) and –C<u>H</u>$_2$OH (■) appear isolated either albeit the related to –C<u>H</u>$_2$OCOR (□) still remains masked under the TAG signals (Figure 2c). A similar situation is observed for 1-MAG: while >C<u>H</u>OH (★) and –C<u>H</u>$_2$OH (★) signals appear isolated, the –C<u>H</u>$_2$OCOR (☆) signal overlaps with –C<u>H</u>$_2$OCOR (Δ) signal of 1,3-DAG. Finally, 2-MAG signals were not found in any of the oils nor fat extracts. In order to analyze the possible overlapping of the 2-MAG signals, a 2-lauroylglycerol spectrum was recorder under the same conditions, observing that one of the signals corresponding to the C2 proton appears isolated from the rest of the signals, allowing its determination.

This solvent mixture also allows a better separation between olefinic proton signals and >C<u>H</u>OCOR (●) signal from TAG glycerol core, making it easier to quantify both signals (Figure 2c). Besides, the solvent effect allows the integration of the carboxylic acid protons as in the protocol proposed by Skiera et al.[43] Additionally, new information can be extracted from this protocol, since protons from methylene C2 in the acyl chain are distinguishable whether they come from esterified fatty acids or free fatty acids.

To avoid CCl_4 as a solvent, which use is discouraged by regulations, we tested its replacement by CS_2. However, in the same proportion as above tried, there are some problems of miscibility. Therefore, a CS_2/DMSO-d_6/$CHCl_3$ mixture is used in an 8:2:1 ratio. This mixture circumvents the use of an excess of expensive deuterated solvent, avoids regulated substances like CCl_4 and achieves a resolution of the DAG and MAG signals like that obtained with the previous mixture (Figure 2d). The use of other solvent combination mixtures (C_6D_6 with CCl_4 or CS_2 and acetone-d_6 with CCl_4 or CS_2) did not improve the separation obtained in the case of CS_2/DMSO-d_6/$CHCl_3$.

In conclusion, the best option for the ^{1}H NMR analysis of TAG, 1,2-DAG, 1,3-DAG, 1-MAG, 2-MAG, FFA and other key parameters in edible fats and oils is the use of CCl_4/DMSO-d_6 or CS_2/DMSO-d_6/$CHCl_3$ as solvent systems, which allow an excellent separation of signals with a minimum cost of deuterated solvents.

Table S1 (see supporting information) shows the assignment of the main signals to the corresponding type of protons, named with letters from **A** to **U** for CS_2/DMSO-d_6/$CHCl_3$ solvent system, which are shown in the spectra of Figure 3 and Figure 4. It is important to note that we could obtain relevant information related to many of the nutritional and sensory parameters of edible oils or fat in a single ^{1}H NMR experiment. As mentioned above,[23,47] for a complete separation of the signals at $CDCl_3$ it was necessary to use high field equipment[21] or additional derivatization experiments.[36]

The signals corresponding to different types of acyl chain protons can be assigned according to their positions within the chain. Thus, the terminal methyl groups provide signals that overlap to give the signal **A**.[48] In the case of n-3 unsaturated alkyl chains, the chemical shift is slightly different and corresponds to the signal **B**.

Signal $\mathbf{D_a}$ corresponds to methylene protons in β-position in relation to the carboxylic group, except those of DHA, which appear as signal $\mathbf{F_a}$, and EPA and ARA acyl groups, which appear as signal $\mathbf{D_b}$. The signals **F** ($\mathbf{F_a}$, $\mathbf{F_b}$ and $\mathbf{F_c}$) correspond to methylene protons in α position from carboxylic group. So, $\mathbf{F_a}$ corresponds to methylene protons in free fatty acids, $\mathbf{F_b}$ to methylene protons except DHA and $\mathbf{F_c}$ to methylene protons in β-position and α-position for DHA. To the best of our knowledge, this is the first time that the $\mathbf{F_a}$ signal appears as a separated peak and this fact is relevant since it allows an easy and direct quantification of FFA (see Figure S7).

The signal **E** corresponds to different types of allylic protons. The **G** and **H** signals correspond to protons of the bis-allylic type, i.e. protons in α-position with respect to two different double bonds. The **G** signal corresponds to diunsaturated chains (such as LA), while the **H** signal corresponds to polyunsaturated chains (such as ALA, DHA, EPA…). The signal **C**, at 1.17–1.38 ppm, is due to methylene

protons not assigned previously. Finally, the signal **U** corresponds to olefinic protons. The chemical shift of the *cis* and *trans* protons attached to double bonds differs slightly, although they overlap.

On the other hand, we can also distinguish the signals corresponding to the glycerol moiety. The most intense signals **P** correspond to methylene protons at positions C1 and C3 of the glycerol core for TAG and overlap with signals **Q** of the protons in the C1 position for 1,2-DAG compounds. The signal **T** is due to the protons in positions C2 of the glycerol core for TAG.

Other minor compounds from lipolysis could be conveniently identified. Thus, the signal **I** corresponds to the protons of the carbon C3 of the glycerol core for 1-MAG. The signal **J c**orresponds to the C1 and C3 protons in 2-MAG. The signal **K** is due to the protons at C3 for 1,2-DAG. The signal **L** corresponds to protons at C2 for 1-MAG. The signal **M** corresponds to C2 protons for 1,3-DAG and the signal **N** corresponds to proton attached to C1 of 1-MAG, and overlap with signal **O**, which is assigned to the protons at position C1 and C3 for 1,3-DAG. The signal **R** is due to the protons at C2 for 2-MAG. The concentration of 2-MAG is always lower than that of 1-MAG due to isomerization by migration of the acyl group.[49] Finally, the signal **S** is due to the protons at C-2 for the 1,2-DAG.

It is important to notice that the change of solvent not only affects to the chemical shift but also the shape of different signals. In general, with the mixture of solvents based on DMSO-d_6, the peaks appear more resolved and sharp. For instance, some signals such as **I** and **O** from diasteorotopic protons of the glycerol moiety are narrower compared to the same signals when $CDCl_3$ is used. Other systems of diastereotopic protons such as the two protons attached to C1 and C3 of glycerol in TAG (-CH_2-OR, signal **P**) are resolved into two signals with a multiplicity of “dd” each one, namely -CHH’-OR and are indicated as signals **P** and **P’**. The same feature occurs with the two protons attached to C1 of 1,2-DAG (signal **N**) and C1 of 1-MAG (signal **Q**) that split into signals **N** and **N’** as well as **Q** and **Q’**, respectively (see Table 1 and spectra of Supporting Information).

With these data, two essential parameters for studying the quality of an edible fat and oil, such as total iodine value (**IV**) and acid value (**AV**), can be quickly analyzed. One of the usual methods to calculate the iodine value is the Wijs method.[50] The iodine value measures the degree of unsaturation of a compound

with several double bonds and could be substituted by direct determination of proportion of olefinic protons, measured by ^{1}H NMR.[51] The correlation of results is very high (Figure S1) and we can obtain the **IV** following this equation:

$\mathbf{IV_{NMR}} = 4.37 + 13.11 \times \mathbf{OP}$, (R = 0.9993)

Where **OP** is the proportion of olefinic protons, which can be calculated dividing the area of the olefinic protons by the total area.

The traditional method for calculating acid value (**AV**) is described above in the section on Material and Methods. We can calculate the same parameter by ^{1}H NMR using the percentage of FFA that indicates the total acidity[43] and is given by the proportion of the signals $\mathbf{F_a}$ and $\mathbf{F_b}$ and also signal $\mathbf{F_c}$ for cases of fats with DHA. The correlation (Figure S2) is validated through the next equation:

$\mathbf{AV_{NMR}} = -0.0648 + 0.6883 \times \mathbf{FFA}$ (R = 0.996)

Where **FFA** is calculated as follows: in samples without DHA: **FFA** (%) = $100 \times (\mathbf{F_a} / \mathbf{F_b})$ and in samples with DHA: **FFA** (%) = $100 \times (\mathbf{F_a} / (\mathbf{F_b} + (\mathbf{F_c} / 2))$.

With these validations, and taking into account the previous bibliography,[25] some parameters of interest could be obtained to evaluate the composition of an edible fat or oil. Thus, the relationship between signal **E** and signal **F** is used to calculate the proportion of saturated and unsaturated alkyl chains as follow:

unsaturated (%) = $100 \times (\mathbf{E} / 2 \times \mathbf{F})$

saturated (%) = $100 \times (1 - \text{unsaturated})$

In most dietary fats the 95% of polyunsaturated fats are due to only two types of fatty acids: LA and ALA. Thanks to this peculiarity, the composition of monounsaturated fat (M), linoleic fat (LA, named omega-6 or n-6 fatty acid) and linolenic fat (ALA, named omega-3 or n-3 fatty acid) can be found as function of several signals of the spectrum. The calculations are as follows:

ALA (%) = $100 \times (\mathbf{B} / (\mathbf{A} + \mathbf{B}))$

LA (%) = $100 \times (\mathbf{G} / \mathbf{F})$ (when signal **G** can be integrated)

LA (%) = $100 \times (((\mathbf{G} + \mathbf{H}) / \mathbf{F})) - 2 \times (\text{ALA}))$ (when signals **G** and **H** overlap)

monounsaturated (%) = unsaturated – LA – ALA

It should be noted that in fats of vegetable origin (including pork, being monogastric animal) the principal diunsaturated fatty acid is the linoleic acid (LA) and the main triunsaturated is the linolenic acid (ALA), allowing to obtain their proportions by this NMR technique. In fats of animal origin (fish, ruminant animals...) this condition does not apply and the equations cannot be simplified. However, we can distinguish the overall n-3 acids, the DHA, and the sum of EPA and ARA.

Therefore, with food, such as fish, which contain significant amount of polyunsaturated fats different from ALA and LA, another calculation method should be used. The chemical shifts of protons -OCO-CH_2-C$\underline{H}_2$- in EPA and ARA appear in the spectra as a separated signal (**D_b**). Similarly, protons -OCO-C$\underline{H}_2$-C$\underline{H}_2$- in the DHA generate another signal (**F_c**). This fact allows the quantification of EPA and DHA, using the following equations:

EPA + ARA (%) = $100 \times (\mathbf{D_b} / (\mathbf{D_a} + \mathbf{D_b}))$

DHA (%) = $100 \times (\mathbf{F_b} / (2 \times \mathbf{F_b} + \mathbf{F_c}))$

unsaturated (%, for fish and ruminant animals) = $100 \times (2 \times \mathbf{E} / (2 \times \mathbf{F_b} + \mathbf{F_c}))$

n-3 (%) = $100 \times (\mathbf{B} / (\mathbf{A} + \mathbf{B}))$

By direct integration, we can calculate the proportion of lipolysis products such as 1,2-DAG, 1,3-DAG and 1-MAG. Signals **K**, **M** and **L**, respectively, do not overlap and suit perfectly for this purpose. It should be noted that the presence of 1,3-DAG is a consequence of the slow isomerization process from 1,2-DAG by the migration of the acyl group from position 3 to position 2.[9,37] In the same way, the signals corresponding to 2-MAG are not detected due to isomerization from 2-MAG to 1-MAG.[51]

In Table 1, we show different parameters of interest to ensure the quality of an edible fat and oil, according to the analysis of olive, sunflower, corn, sesame, and peanut oils, as well as butter, walnut, salmon and two different dry sausages. The parameters obtained are the expected for these types of food. We can appreciate the high content of unsaturated fatty acids in walnuts, the great content of monounsaturated acid (oleic acid) in olive oil, the high proportion of LA in sunflower oil and walnuts and the high percentage of saturated fatty acids in butter and the high proportion of n-3 in salmon. Also noteworthy is

the large proportion of 1,2-DAG in aging dry sausages, which also have the highest level of 1,3-DAG due to high lipolysis activity during long ripening.

Therefore, this methodology provides a robust technique for the analysis of edible oils and fats from different matrices. In order to demonstrate the potential of the methodology to quantify different compounds, including the minor ones such as 1,2-DAG and 1,3-DAG, a thermal degradation experiment,[52] of an extra virgin olive oil and a sunflower oil was carried out. A degradation process takes place through oxidation and lipolysis reactions. With our analysis strategy, we can monitor easily and in one step the evolution of both oxidation and lipolysis processes.

In this context, polyunsaturated fats are susceptible to oxidation at high temperatures and decompose more rapidly than saturated fats. For this study, we used virgin olive oil and sunflower oil heating, first to 160 ºC and then to 190 ºC, as indicated in materials and methods.[53,54] Regarding fatty acids composition, the heat treatment reduces the proportion of unsaturated fats, being remarkable that in sunflower oil the monounsaturated fats remain almost constant. In olive oil, where monounsaturated is the main component, both mono and polyunsaturated fats decay (Figure 5).

Lipolysis reactions will generate diacylglycerols, monoacylglycerols and free fatty acids.[55] The evolution of 1,2-DAG, 1,3-DAG and 1-MAG can be perfectly followed by this method by 400 MHz ^{1}H NMR. It is important to highlight that similar previous studies required derivatization to phosphorous compounds[36] or could not provide information on 1,3-DAG.[56] Lipolysis in olive oil (Figure 6a) occurred faster than in sunflower (Figure 6b). We did not detect free fatty acids (signal **Fa**) during the experiment which is in accordance with observations in other studies.[36]

CONCLUSION

Direct determination of key parameters in edible fats and oils, including 1,2-DAG, 1,3-DAG and 1-MAG, has been achieved through the development and improvement of an easy method based on 400

MHz ^{1}H NMR spectroscopy without the need for derivatization and obtaining the highest information. In this way, and through a single experiment that involves the use of an adequate mixture of solvents, relevant information of fourteen essential parameters for the quality of edible fats and oils has been obtained straightforwardly. For instance, this NMR methodology has been successfully applied to the analysis of olive, sunflower, corn, sesame and peanut oils, as well as butter, walnut, salmon and spicy pork sausage. In addition, the content of free fatty acids in an oil extract can be easily determined because the signal for C2-methylene protons is separated from the others. Finally, the evolution of thermal oxidation and lipolysis of virgin olive oil and sunflower has been analyzed.

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Table 1. Proportions of different key class compounds in edible fat and oil by ^{1}H NMR.

	olive oil	sunflower oil	corn oil	sesame oil	peanut oil	butter	walnuts	salmon	dry sausage	dry sausage (over aged)
% unsaturated	85.11	87.40	76.80	78.46	82.22	25.77	90.11	82.23	51.25	79.02
% saturated	14.89	12.60	23.20	21.54	17.78	74.23	9.89	17.77	48.75	20.98
% monounsaturated	77.67	28.70	35.08	29.96	64.73		20.29		46.78	57.36
% LA	6.11	57.29	39.79	42.63	16.43		56.50		3.09	19.96
% ALA	1.33	1.40	1.93	5.87	1.06		13.31		1.38	1.69
% FFA	0.96	2.08	6.33	3.59	2.01	0.94	0.71	0.86	4.92	29.38
% n-3	-					1.21		17.46		
% EPA and ARA						3.75		4.03		
% DHA						0.40		3.64		
IV	81.09	131.79	109.25	120.67	87.66	31.18	154.85	138.22	51.12	70.97
% 1,2-DAG	0.96	0.64	1.18	1.26	0.94	2.23	1.58	0.21	1.74	4.92
% 1,3-DAG	0.02	0.02	0.03	0.03	0.02	0.01	0.01	0.00	0.07	0.12
% 1-MAG	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.02

Figure captions:

Figure 1. Triacylglycerol, diacylglycerols and monoacylglycerols as well as some fatty acids present in edible fats and oils.

Figure 2. Spectrum region from 3.3 to 5.5 ppm and zoom of the region from 3.3 to 4.3 ppm for a) $CDCl_3$, b) $CDCl_3$/DMSO-d_6 (3:1), c) CCl_4/DMSO-d_6 (9:2) and d) CS_2/DMSO-d_6/$CHCl_3$ (8:2:1) solvent systems. Assignment of TAG, 1,3-DAG, 1,2-DAG and 1-MAG signals revealing their separation.

Figure 3. ^{1}H NMR superposed spectrum of olive oil (blue) and salmon extract (pink) with signal assignment (see table S1). The notation in grey corresponds to signals that should appear in case of sufficient concentration of the compound. For more information concerning the spectra of minor compounds, see Figures S4 and S5 in Supporting Information.

Figure 4. ^{1}H NMR spectra of dry sausage fat (overaged) with signal assignment (see Table S1). The entries in grey correspond to signals that should appear in case of sufficient concentration of the compound. For more information concerning the spectra of minor compounds, see Figures S4 and S5 in Supporting Information.

Figure 5. Evolution of fatty acid degradation in a thermal process for virgin olive oil (a) and sunflower oil (b). The experiments were carried out and processed by triplicate and error bars show the standard deviations.

Figure 6. Evolution of occurrence of diacylglycerols and monoacylglycerols in a thermal process for virgin olive oil (a) and sunflower oil (b). The experiments were carried out and processed by triplicate and error bars show the standard deviations.

Fig1

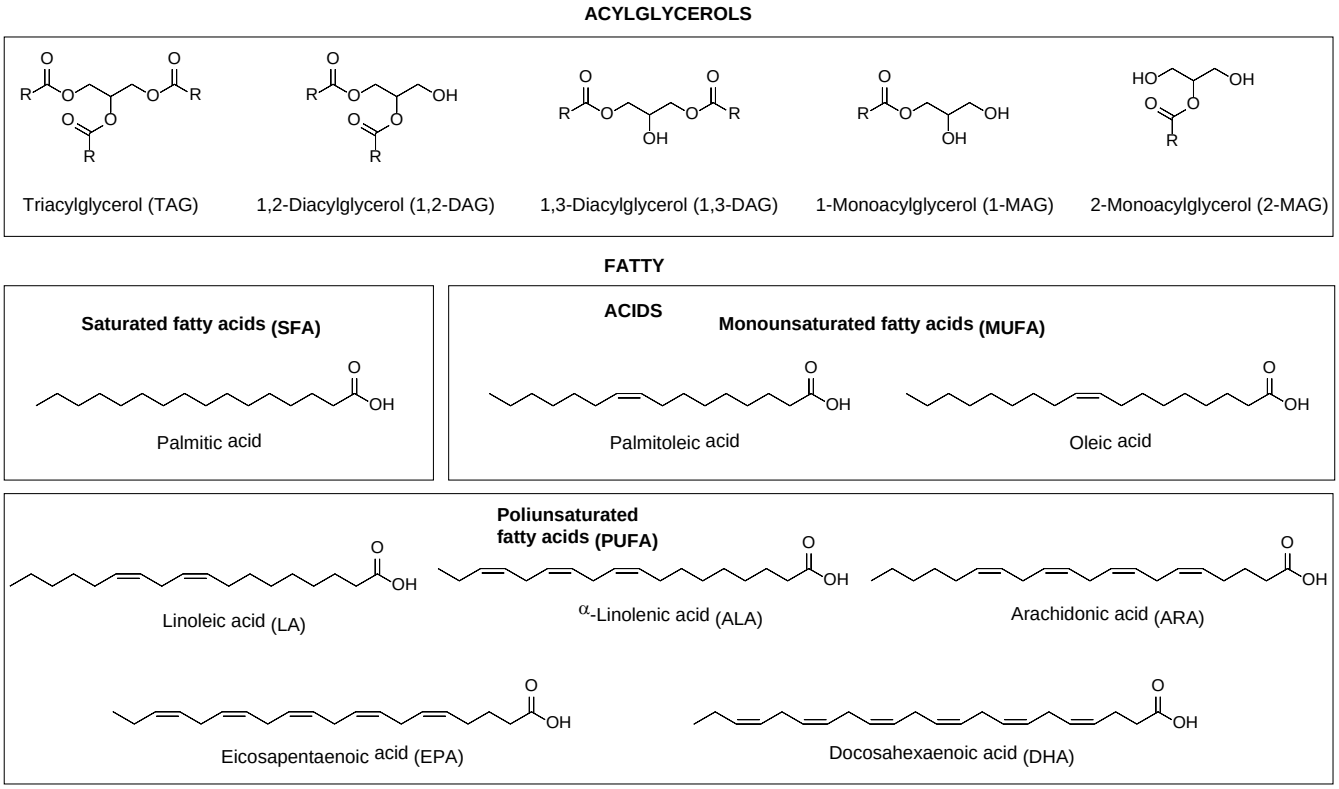

ACYLGLYCEROLS
Triacylglycerol (TAG)
1,2-Diacylglycerol (1,2-DAG)
1,3-Diacylglycerol (1,3-DAG)
1-Monoacylglycerol (1-MAG)
2-Monoacylglycerol (2-MAG)
FATTY
ACIDS
Saturated fatty acids (SFA)
Palmitic acid
Monounsaturated fatty acids (MUFA)
Palmitoleic acid
Oleic acid
Poliunsaturated fatty acids (PUFA)
Linoleic acid (LA)
α-Linolenic acid (ALA)
Arachidonic acid (ARA)
Eicosapentaenoic acid (EPA)
Docosahexaenoic acid (DHA)

Fig2

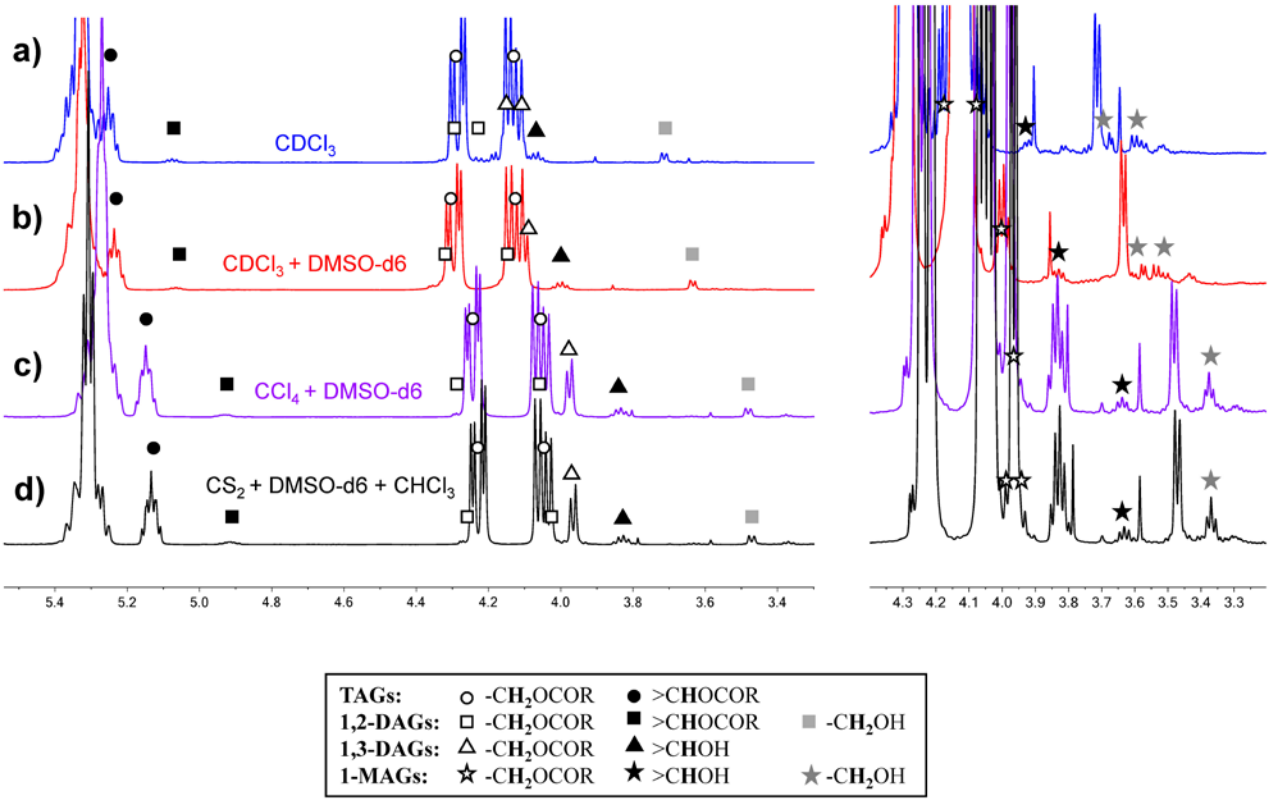
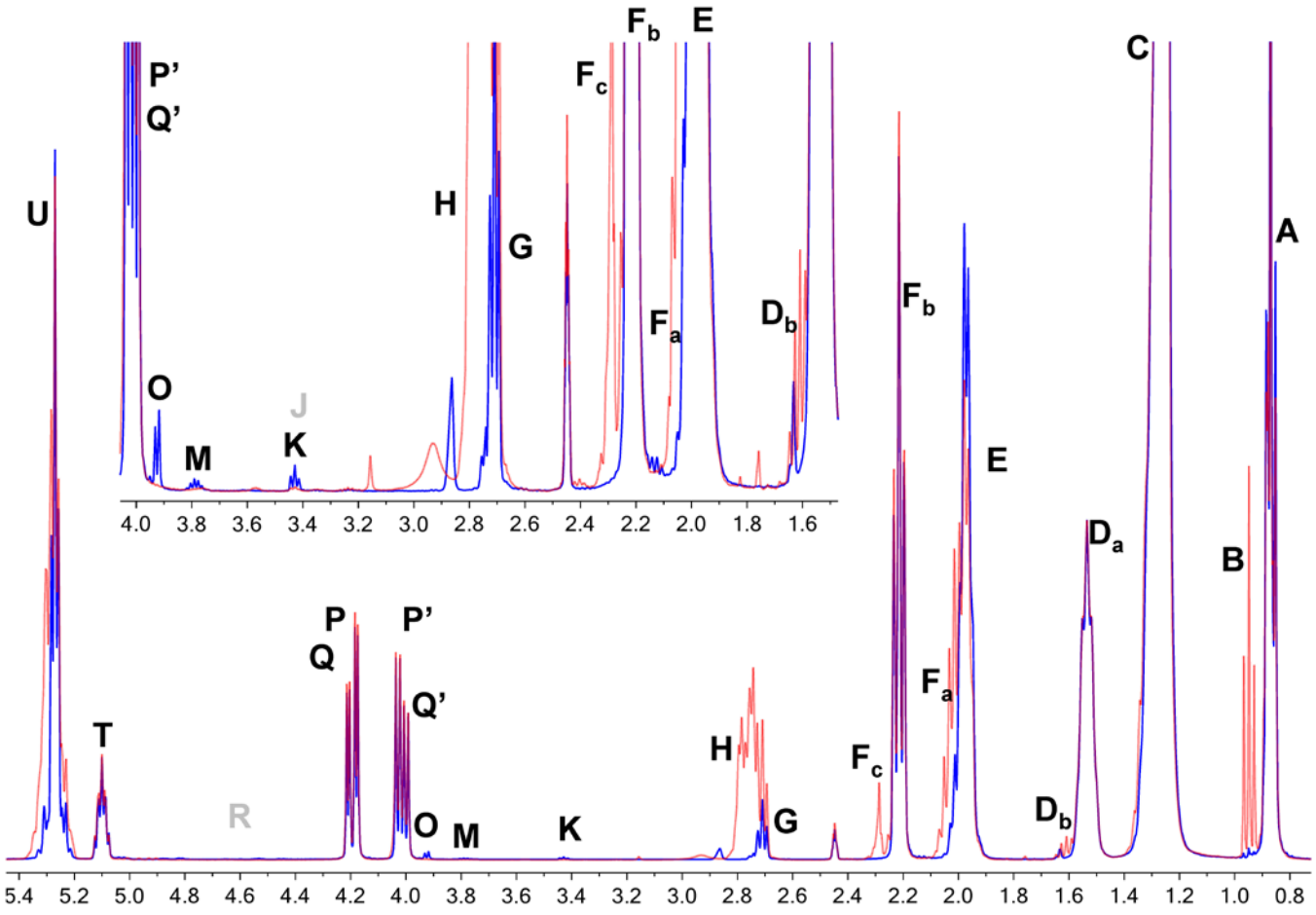

a)
CDCl3
b)
CDCl3 + DMSO-d6
c)
CCl4 + DMSO-d6
d)
CS2 + DMSO-d6 + CHCl3
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4
4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3
TAGs: ○ -CH2OCOR ● >CHOCOR
1,2-DAGs: □ -CH2OCOR ■ >CHOCOR ■ -CH2OH
1,3-DAGs: △ -CH2OCOR ▲ >CHOH
1-MAGs: ☆ -CH2OCOR ★ >CHOH ★ -CH2OH

Fig3


U
P'
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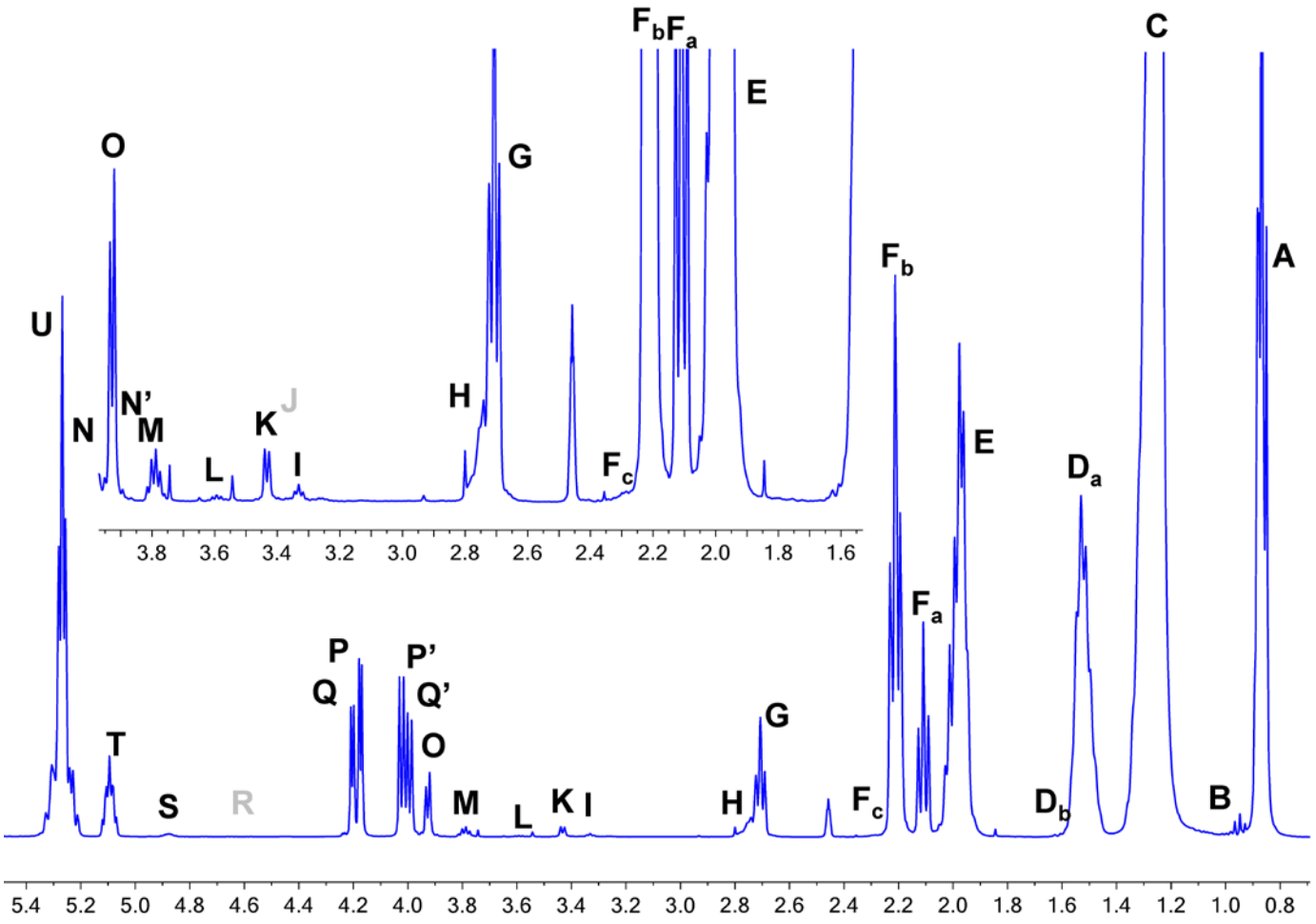
4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8

Fig4


U
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D$_b$
B
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5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8

Fig5

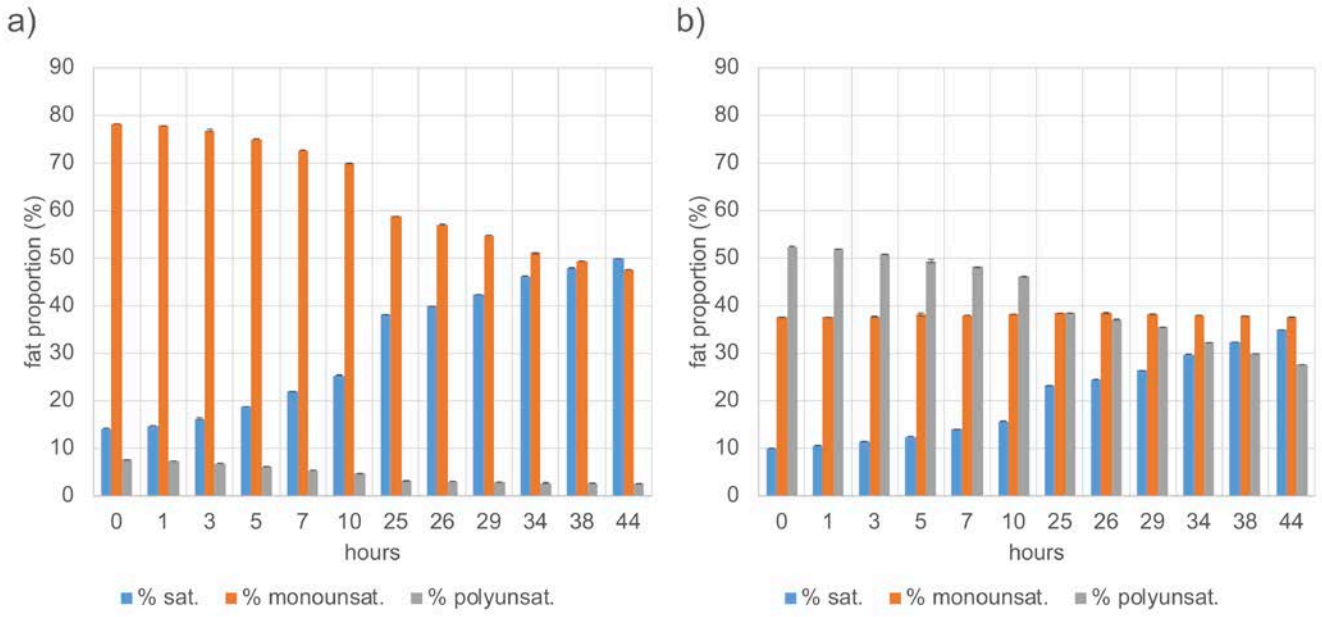
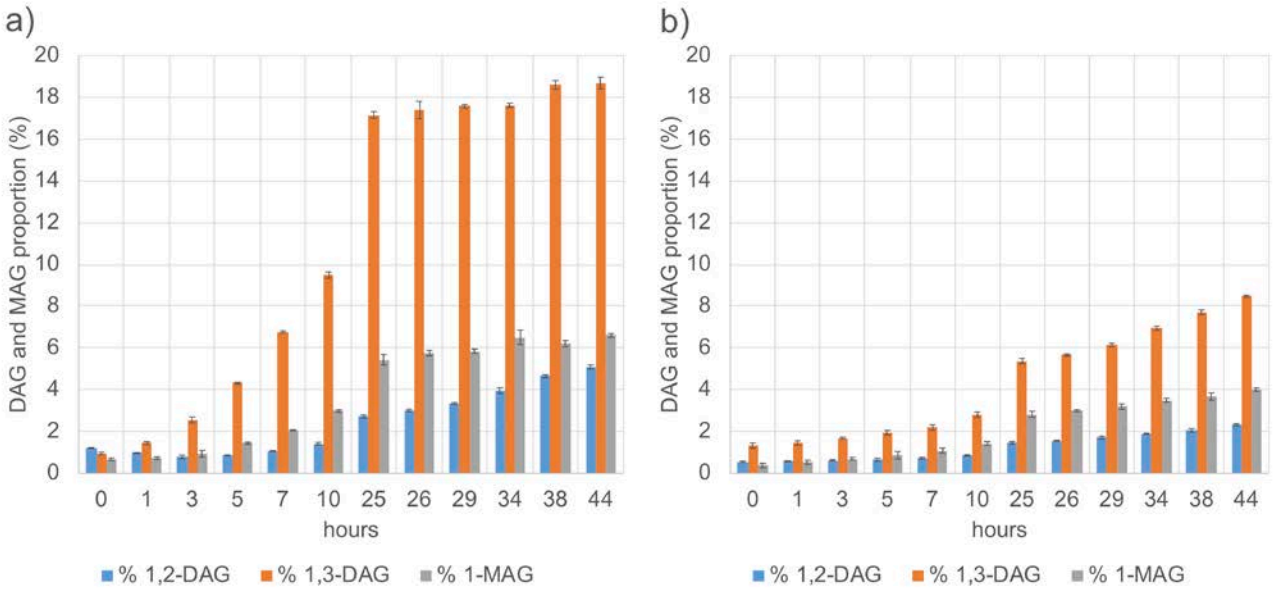

a)
b)
fat proportion (%)
90
80
70
60
50
40
30
20
10
0
0 1 3 5 7 10 25 26 29 34 38 44
hours
% sat.
% monounsat.
% polyunsat.

Fig6


a)
DAG and MAG proportion (%)
20
18
16
14
12
10
8
6
4
2
0
0 1 3 5 7 10 25 26 29 34 38 44
hours
% 1,2-DAG
% 1,3-DAG
% 1-MAG
b)
DAG and MAG proportion (%)
20
18
16
14
12
10
8
6
4
2
0
0 1 3 5 7 10 25 26 29 34 38 44
hours
% 1,2-DAG
% 1,3-DAG
% 1-MAG