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Determination of the lipid content of organic waste using Time-Domain Nuclear Magnetic Resonance

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1. Abstract

Time-Domain Nuclear Magnetic Resonance (TD-NMR) was used to quantify the lipid contents of 48 different organic waste substrates. Results obtained from TD-NMR were compared to those from Soxhlet extraction, currently the prevalent method for organic waste characterization, especially in the field of anaerobic digestion. Two calibration methods were tested. The first was a self-calibration process using pure oils (NMR1) which showed good repeatability compared to Soxhlet extraction with a better coefficient of variation (5%). Analyses of volatile fatty acids (VFA) and long-chain fatty acids (LCFA) by chromatography were carried out to understand why the NMR1 method produced underestimations for some samples. Statistical analysis showed that the presence of saturated fatty acids had a significant effect on differences between the Soxhlet and NMR1 methods. The second calibration method applied chemometrics to TD-NMR raw data (NMR2), taking Soxhlet extraction values as references. It provided a good prediction of lipid content and avoided the lengthy

calibration procedure usually required for this type of study. Last, the NMR2 method was shown to be highly suited to the quantification of lipids in organic waste, demonstrating better repeatability than the classic Soxhlet method.

Keywords: Anaerobic digestion, chemometrics, LCFA, fats, NMR, Soxhlet, waste

2. Introduction

Biological treatment and recovery processes provide a way to deal with the environmental challenges of organic waste management. One such process, anaerobic digestion, enables the conversion of the organic matter contained in the waste to carbon dioxide and methane, this last being an energy source. Livestock manures are appropriate substrates for such treatment because they contain the macro and micronutrients necessary for the development of micro-organisms. They also have a buffering capacity that can stabilize the process. However, the low dry-matter content of such substrates (< 10% DM), combined with the limited biodegradability of their organic matter, results in quite a low methane potential relative to their volume. Consequently, anaerobic digestion facilities treating livestock manure must add substrates with higher methane potential to achieve profitability (Marañón et al., 2012)

Among these substrates, fats from the food industry show particular promise due to their high methanogenic carbon content and biodegradability. However, the addition of fats can inhibit the process of digestion (Cirne et al., 2007) by lipid hydrolysis that involves the generation of long chain fatty acids (LCFA). If these LCFAs are not consumed as soon as they are produced, they can become adsorbed on bacterial membranes, limiting the exchanges between the micro-organisms and the external solution which in its turn significantly limits the efficiency of micro-organisms (Pereira et al., 2005). Thus, knowledge of the lipid content of

53 waste appears essential, both to ensure a good methane yield and to prevent the risk of
54 inhibition.
55 The literature describes a number of methods for the quantification of lipids. The most
56 common of these, the Soxhlet method, was developed in 1879 to determine the fat content in
57 milk (Soxhlet, 1879). Subsequently, Soxhlet extraction combined with qualitative gas
58 chromatography (GC) and gas chromatography mass spectroscopy (GC–MS) fatty acid
59 analysis (Casado et al., 1998) became widespread and has become a reference method (NF
60 EN ISO 659:2009). Such a method has disadvantages, not just because it uses a toxic solvent
61 but also because it takes several hours for extraction and evaporation after extraction to be
62 completed. An alternative technique for lipid analysis is offered by Time-Domain Nuclear
63 Magnetic Resonance (TD-NMR). This is widely employed in food characterization to
64 quantify the solid fat, water and oil contents of foodstuffs based on well-known international
65 standard methods (see references in Todt et al., 2001). TD-NMR works by applying a
66 sequence of radiofrequency fields to a sample placed in a magnet to excite the molecule
67 nuclei's magnetic moments and record their return to equilibrium after the radiofrequency
68 pulse is switched off. The process allows a decaying signal known as the Free Induction
69 Decay (FID) to be recorded in which the decaying kinetic energy depends on two intrinsic
70 parameters: longitudinal relaxation time (T1) and transverse relaxation time (T2). The NMR-
71 signal amplitudes or the differences in these relaxation constants are correlated to the physico-
72 chemical properties of the samples. For example, the relaxation behaviors of liquid and solid
73 fat components differ, allowing their respective content values to be calculated (Todt et al.,
74 2001). The main advantages of the TD-NMR technique over Soxhlet extraction lie in the
75 former's speed and solvent-free nature. Additional benefits are its affordability and the
76 possibility of reusing samples (non-destructive method). Comparison of TD-NMR and
77 conventional methods using chemical extraction has already been used to estimate the lipid

content of meat, fish muscles, baked cakes and butter (Casey and Miles, 1974; Le Grand et al. , 2007; Roger et al., 2020; Toussaint et al., 2002). For fish muscles, the NMR method was based on a calibration step using pure oil (Toussaint et al., 2002). The authors concluded that, despite the greater stress of the drying step compared with physical methods of fresh sample analysis, this technique is more accurate and should be adapted to calibrate other physical methods. Moreover, careful analysis of T2 relaxation times and measurements of their relative intensity were shown to be very efficient for quantitative analyses undertaking a standard calibration method. A different strategy, based on T1 relaxation time measurements and multi-block chemometric methods has been shown to be useful for an accurate estimation of the fat content in complex products (Roger et al., 2020). This work also demonstrates that chemometric methods offer alternative approaches to the analysis of raw NMR data that avoid the need for lengthy preprocessing methods. In the field of lipids in waste, the use of TD-NMR has received little attention compared to high-field NMR. Indeed, ^{13}C NMR has been used for the characterization of organic matter, especially humic acids, and of changes occurring in materials during composting (Albrecht et al., 2008; Caricasole et al., 2010; Simpson et al., 2011). In 2010, Willson et al. demonstrated the value of TD-NMR as a tool for the quantification of lipids in olive mill waste and municipal wastewater sludge. Their calibration method was based on a linear cross-correlation curve obtained on reference oil analyzed using the Soxhlet method (Willson et al., 2010). A further approach has been to combine T2 data acquired in TD-NMR and PLS regression to determine the water and oil contents in sludge (Jin et al., 2013; Zheng et al., 2013). This work demonstrated that TD-NMR combined with chemometrics provided a speedy alternative (under 5 min) to more time-consuming laboratory methods which in addition required lengthy sample pretreatment. However, the demonstration was performed on only one substrate, sludge, which contained relatively high levels of lipids (> 10% wb).

The objective of the present work was to evaluate TD-NMR as a technique for quantification of lipids in more complex and heterogeneous organic waste that may contain oils from 2 up to 90% (wet basis). Two methods were used to calibrate the TD-NMR data for the analysis of previously-dried waste from 48 different sources and these were compared to the commonly-used Soxhlet gravimetric method.

3. Material and methods

3.1. Description and conventional characterizations of substrates

48 substrates were selected from the waste and organic residues most commonly used in anaerobic digestion in France (Girault et al., 2010) and as a function of their anticipated lipid content. These substrates derive from agriculture, the municipal and industrial waste and wastewater treatment sectors and the distribution sector. Additionally, 4 references, *i.e.* (1) oleic acid, (2) oleic acid (50%), starch (25%) and casein (25%), (3) trioleic acid and (4) trioleic acid (50%), starch (25%) and casein (25%), were included, resulting in a final total of 52 substrates. The reference samples were prepared using chemicals, namely oleic acid (CAS 112801, Fluka), commercial triolein (CAS 122327, Fluka), casein (CAS 9000719, VWR), starch (CAS 9055258, VWR).

Total solids (TS) were determined following the standard methods in EN 12879 and EN 12880 (AFNOR NF EN 12879:2000; AFNOR NF EN 12880:2000).

Lipid content was determined for each substrate using the Soxhlet extraction method to provide a reference value. Before analysis, all substrates were dried at 105°C until constant weight was achieved. Then, dried products were ground manually with a mortar or using a ball mill to obtain small particles (< 1 mm) and the solids obtained were then stored at 60°C until analysis. For the lipid extraction, a dedicated Soxhlet device was used. Approximately 5

127 g of dry matter was placed in the cartridge and a 5 hours extraction was carried out using 200
128 mL of hexane/isopropanol (3/2) solvent at a temperature above 85°C (Mahesar et al., 2010).
129 Last, the solvent was removed by evaporation and drying and the remaining product was
130 weighed. This remaining fraction, described as hexane extractable material (HEM),
131 corresponded to the lipids contained in the 5 g initial substrate.

132 The wide range of substrates studied allowed total solid contents to be obtained ranging from
133 37 to 949 $g.kg^{-1}$ (wet weight). The lipid content described as HEM and determined by the
134 Soxhlet method ranged from 0.1% to 94.1%, confirming previous observations of the
135 variability of the biochemical composition of waste and exhibiting the anticipated wide range
136 of represented values (Fisgativa et al., 2018).

137 Free long chain fatty acids (Free LCFA) were determined for several substrates. For this
138 purpose, after the samples had been dried at 105°C, LCFA were extracted using the Soxhlet
139 method in hexane / isopropanol (3/2) as described above. Following filtration at 45 µm, the
140 extracts were analyzed using a high-performance liquid chromatograph (HPLC) equipped
141 with a PLRP-S column consisting of styrene/divinylbenzene copolymer and using a gradient
142 elution with a mixture of tetrahydrofuran (THF, CAS109999), 60 mM acetic acid (AA) (CAS
143 64197) and acetonitrile (ACN) (CAS 750508) varying from 60/35 to 90/0 over 100 minutes.
144 The flow rate of the eluent was 0.5 mL/min and the temperature of the oven containing the
145 column was 20°C. Detection was performed using a light scattering rotary evaporator
146 (Varian) with a fixed temperature of 80°C. The temperature of the nebulizer was set at 55°C
147 and the nitrogen flow rate was set at 1.0 L/min. Standard LCFAs with a purity greater than
148 99% were used, *i.e*, palmitic, palmitoleic, linoleic, oleic and stearic acids (Sigma Aldrich)
149 (Sousa et al., 2009), for calibration. Individual free LCFAs were then divided into groups of
150 unsaturated (oleic, linoleic and palmitoleic) and saturated (stearic and palmitic) fatty acids.

151 3.2. NMR analysis

152 NMR measurements were performed at 60°C with a low-field TD-NMR spectrometer
153 operating at a frequency of 10 MHz (Minispec Bruker mq10). The dried samples were placed
154 in a previously weighed glass tube of 30 mm diameter, to a height of approximately 10 ± 2
155 mm. For each sample, two or three tubes were prepared.

156 The determination of lipids by TD-NMR was based on two methods. The first of these
157 (NMR1) has been developed by Toussaint et al. (2002) and was self-calibrated with oil
158 references. For the oleic acid and trioleic acid reference samples, the appropriate volume was
159 directly introduced into the tube to a height of 10 ± 2 mm. The NMR instrument calibration
160 was performed using four tubes filled to heights of between 1 and 10 mm with rapeseed oil
161 (CAS 8002139) composed of 58% oleic acid and 21% acid linoleic corresponding to 79%
162 unsaturated fatty acids and 11% saturated fatty acids. For each sample and each reference
163 tube, a free induction decay (FID) signal was measured with a dwell time of 0.4 µs, during
164 approximately 45 s using a relaxation delay of 5 s and 9 scans. The FID relaxation curves
165 consisted of two distinct parts: the first part lasted less than 70 µs and was attributed to dry
166 macromolecule protons in dry samples while the second part, which had a longer relaxation
167 time, was assigned to liquid-state lipid protons at 60°C (Figure 1). Each FID signal was fitted
168 between 70 and 150 µs using linear regression to obtain a new signal intensity value at 70 µs
169 that was less affected by the signal/noise ratio of the initial signal. The lipid content was
170 calculated using a simple mono-linear calibration equation, as described by Toussaint et al.
171 (Toussaint et al., 2002).

172 The second method (NMR2) used a chemometric calibration approach applied to the raw FID
173 signals. The samples were sorted in ascending order of lipid content. Measurements from one
174 in every two samples (1, 3, 5, etc.) of this vector were included in the test set used to validate
175 the calibration models. The other individual samples were allocated to the calibration set. This

procedure resulted in 71 calibration samples and 73 test samples with similar lipid content distributions. The raw NMR spectra were smoothed, using the Savitsky & Golay method (Savitzky and Golay, 1964), with a window width of 107, a polynomial order of 2, and a degree of derivation of 0. Principal Component Analysis (PCA) was applied to the spectra in the calibration dataset in order to detect clusters (Wold et al., 1987). A Partial Least Squares (PLS) model was then calibrated and optimised using the calibration set, cluster by cluster (Wold et al., 2001). Next, each sample from the test set was projected into the PCA space in order to determine the cluster it belonged to, and was inputted to the corresponding PLS model. This step concluded with the creation of a vector based on all the predicted values.

Last, in order to obtain an estimate of lipid content for all samples, a cross-validation of the above procedure was performed. Here, the spectra for each sample were used in turn to provide the test set for a model calibrated on the other samples.

For both methods NMR1 and NMR2, the following statistical information was calculated between the reference sample mean values and the predicted values: r, SEP, Bias. In addition, since replications had been performed, a standard deviation of repeatability $\boldsymbol{\sigma_{rep}}$was calculated on the predicted values.

3.3. Statistics

Statistical analysis was performed using Statgraphics Centurion XVI to compare the two techniques used for lipid quantification (Soxhlet and NMR1). The tests were carried out in random order. The result chosen for analysis was the measured HEM % and two factors have been analyzed, the method and the individual sample (matrix type). The procedure tested the significant differences between two samples of data collected as pairs. The test t (assuming Gaussian distribution) was realized with a reliability level of 95%. A second statistical analysis was performed to reveal whether or not the saturation index of fatty acids influenced

201 the difference observed between the two methods. A simple regression with variance analysis
202 was also performed.

203

204 **4. Results and discussion**

205 4.1. Comparison between NMR1 and Soxhlet extraction

206 Forty-eight waste substrates of different origins were analyzed by TD-NMR and results were
207 compared to those of the commonly used Soxhlet gravimetric method. The NMR1 method
208 included a pre-calibration step using pure oils. The lipid contents determined by NMR1
209 ranged from 1.84 ± 0.20% to 89.71 ± 1.36%, excluding the commercial reference substances
210 (Table 1). The determined lipid contents of the two reference oils, oleic acid (S49) and trioleic
211 acid (S51), were slightly higher than 100% while mixtures of these oils with starch and casein
212 (S50 and S52) gave values of around 52%, consistent with the expected 50% lipid content.

213 Figure 2 sets out the values of total lipids estimated by the NMR1 method as a function of
214 those measured by Soxhlet. A good linear correlation can be noted between the two methods
215 as attested by the r of 0.96. The linear regression slope is below 1 (0.897) due to the
216 underestimation that occurs using the NMR1 method for samples where lipid content is given
217 between 20 and 80% HEM. However, the lipid content estimation is very good for very low
218 (< 20%) and high (> 90%) lipid contents.

219 The NMR1 method showed a tendency to slightly overestimate lipid content compared to the
220 Soxhlet method for sixteen (outlier) waste samples relative to the selected linear regression
221 (S6, S7, S12, S13, S15, S19, S24, S25, S27, S31, S42, S44, S49, S50, S51, S52). It also
222 underestimated the lipid contents for samples S22, S23, S26 and S28. However, when HEM
223 was less than 20% (S17, S32 to S34, S47 and S48 for instance), the NMR1 method showed
224 better results than the Soxhlet method. The Soxhlet method's limit of quantification (LOQ)

225 value was determined at 2% HEM, explaining a reverse trend for low lipid contents. Half
226 concentrations below 20% HEM produced a difference greater than 40%. Hexane extraction
227 may be less effective when the fat bonds to complex molecules. When the lipid content
228 determined by NMR1 is lower than that obtained by the Soxhlet method, the underestimation
229 may be influenced by a non-selective Soxhlet extraction that could be caused by a too low
230 heating temperature or variations in the saturation levels of fats in different products. Indeed,
231 if the waste fraction contains large quantities of saturated fats then the determination of lipid
232 content by NMR1 is closely linked to the lipid's state. Overall, the NMR1 method exhibits a
233 lower repeatability error, estimated to be 35% lower than the Soxhlet method.

234 In order to find an explanation for the differences between the two methods, the influence of
235 the proportion of saturated fatty acids and of the degree to which the fat is hydrolyzed on the
236 NMR1 results was studied (table 2). Figure 3 shows the linear regression between the quantity
237 of saturated free LCFAs (in $mg.gTS^{-1}$) and the absolute difference between the NMR1 and
238 Soxhlet lipid contents. The correlation coefficient in the linear model was R^2 0.76, indicating
239 a moderately strong relationship between the variables. In samples where results using the
240 NMR1 method came within 10% of those produced by Soxhlet extraction, the concentrations
241 in oleic acid were the highest with 75% unsaturated fatty acids as against 25% saturated fatty
242 acids. Of the 23 substrates investigated, two thirds contained free fatty acids with unsaturated
243 long chains. The differences in the results produced by the two methods were generally
244 greater when the LCFAs analyzed by HPLC were predominantly saturated fatty acids, an
245 exception being sample S47 (beach algae) in which the HEM % content was very low (< 1%
246 HEM), falling below the limit of quantification (2%).

247 Saturated fatty acid content in S22, S23, S26, and S28 (effluent waste from processing
248 industries) was, respectively, 95.9, 179.5, 654.2, 157.4 $mg.gTS^{-1}$, while unsaturated acid
249 content was 10.4, 37.9, 136.1, 0.9 $mg.gTS^{-1}$ respectively. Saturated and unsaturated fat

250 contents in S27 and S29 (fats from industrial wastewater treatment), where the values were
251 closer for the two methods, were 202.6 and 153.2 mg.gTS^{-1} respectively, as against 222.9 and
252 67.2 mg.gTS^{-1}, respectively. These samples contained under 3 mg.gTS^{-1} stearic acid but
253 displayed a higher oleic acid content. On the other hand, it was found that, in samples where
254 unsatisfactory deviations in fat content levels were produced using NMR1 compared with the
255 Soxhlet method (S22, S23, S26, S28), there were high concentrations of saturated fatty acids
256 (palmitic and stearic acids) (Table 3). Reminder: the NMR calibration was carried out using
257 sunflower oil, composed mainly of unsaturated oleic acid.

258 The saturation levels of fatty acids do not fully explain the NMR1 underevaluation of some
259 samples. At 60°C, the NMR signal at 70 µs should be generated by lipid protons alone.
260 Although it is possible that the relaxation time of water protons may interfer with that of lipid
261 protons, this can be discounted in the present case as all samples were dried before being
262 analyzed. We do know, though, that the proton density of saturated fats is slightly higher
263 (1.216) than that found in non-saturated fats (about 1.210), which means that NMR1
264 estimation is not precise for lipids since it considers all lipids to contain the same proton
265 density. Additionally, saturated fatty acids are known to have higher melting points than
266 unsaturated lipids. For example the melting points of saturated palmitic and stearic acids are
267 63°C and 69°C respectively, as compared to 13°C for oleic acid, which is unsaturated. In this
268 instance, the temperature set during the TD-NMR analyses was insufficient for these kinds of
269 lipid. In light of the results, the TD-NMR equipment was tested on palmitic acid at 60°C and
270 66°C. No signal was detected by TD-NMR at 60°C whereas a measurement could be obtained
271 at 66°C, demonstrating that an appropriate temperature setting for NMR measurements is
272 essential to the accurate quantification of lipids in complex samples such as organic waste.
273 Statistical data analysis shows that the differences between the Soxhlet and NMR1 results did
274 not follow a normal distribution, with a significant sign test showing a P probability of

0.0019. Values from NMR1 showed better repeatability (coefficient of variation = 5%) than those from the Soxhlet method (coefficient of variation = 8%). The greatest differences were observed for samples with very low HEM concentrations (S17, S18, S32-S36, S47, S48). When the HEM content was above 3% (S22, S23, S26 and S28 for instance), NMR1 method values were 16% lower than Soxhlet extraction values and displayed very high variability between samples. The extended storage of S22, S23, S26 and S28 may have led to the degradation of fats by hydrolysis (depolymerization of organic compounds), decreasing the length of LCFA aliphatic chains. Indeed, with each lipid molecule oxidation loop, two carbons leave the aliphatic chain (Sousa et al., 2009). This process results in free fatty acids with less carbon. Therefore, the significant difference between the two methods may be related to the state of hydrolysis of these fats. The Soxhlet method is likely to have measured the fatty acid contents of medium chains while these would not have been analyzed by TD-NMR.

In order to better identify the impact of the outliers on the statistical test, these data were removed. This resulted in a normal distribution of differences between the Soxhlet and NMR1 methods with a P probability of 0.16. Therefore, without the outliers, the difference between both methods was not significant. The results indicated that NMR1 tends to underestimate lipid content levels with an average difference of 23% for contents ranging from 0 to 20% HEM. Distribution in the waste sourced from local authorities (S43 to S48) was calculated to be normal, with no significant differences (P = 0.99). For this category, two classes were distinguished: when HEM was above 10%, the NMR1 method was superior to the Soxhlet method for some very low-fat products in this category, (ie. S47 and S48).

4.2. Comparison between NMR2 and Soxhlet methods

299 In order to obtain maximum relevant chemical information from the waste studied,
300 chemometrics were applied to the raw TD-NMR data, providing an efficient and rapid method
301 (here called NMR2) of determining the TD-NMR signal information contents (Rutledge et al.,
302 1997; Rutledge et al., 1999). Recently, the potential use of various chemometric methods in
303 the analysis of raw TD-NMR signals has been demonstrated in a number of studies, offering
304 an alternative to the controversial and time-consuming pre-processing techniques usually
305 performed for this purpose (ie. NNLS or ILT methods) (Roger et al., 2020; Mas Silvia et al.,
306 2021). Their application here to the TD-NMR raw data thus provided a way to avoid the time-
307 consuming calibration procedure developed by Toussaint et al. (2002).

308 A PLS model was calibrated on half of the samples and tested on the other half, following the
309 procedure described in the Materials and Methods section, and was then cross validated on all
310 samples to provide the same number of NMR2 predictions as the Soxhlet method. The lipid
311 contents determined by NMR2 extend over a wider range than the NMR1 results, from 0.94 ±
312 0.06% to 96.45 ± 3.23% (Table 1). The lipid contents determined for the two reference oils,
313 oleic acid (S49) and trioleic acid (S51), were slightly lower than 100% while mixtures of
314 these oils with starch and casein (S50 and S52) gave values around 59%, slightly higher than
315 the expected 50% lipid content. Figure 4 shows the values of total lipids estimated by the
316 NMR2 method as a function of those measured by the Soxhlet method. As for NMR1, the
317 lipid contents determined by NMR2 are linearly correlated to those measured by Soxhlet
318 extraction (r of 0.98). Due to the cross validation, the linear regression slope is close to 1
319 (0.988). The test procedure on half of the samples (results not shown) produced a slope very
320 close to 1 (1.03), indicating good learning by the PLS model. The NMR2 method showed
321 greater accuracy than Soxhlet extraction, with a 5% coefficient of variation for fat content
322 exceeding 20% HEM, compared to the 8% achieved by the Soxhlet method. The standard
323 deviation of repeatability was calculated from the Soxhlet values and from the values

predicted by PLS (NMR2), yielding values of 2.93% and 2.19% respectively (Table 1). In fact, the standard deviation of NMR1 repeatability was very small for almost all samples; only a few samples displaying high variability. The standard deviation of NMR2 repeatability was less dependent on individual samples. These values, which are quite large compared to the repeatability obtained in food science or in liquid samples, reflect the heterogeneity of the waste samples used. Nevertheless, once again, the repeatability of these results was better for the prediction based on the TD-NMR method. NMR measurement is indirect and therefore requires calibration using a set of samples with known lipid contents. The standard deviation of repeatability for the NMR measurements, whether NMR1 or NMR2, is therefore derived from a combination of the standard deviation of repeatability for the reference measurement and the stability of the calibration method. NMR1 calibration involves a simple linear regression using gravimetry-based values. NMR2 calibration requires a more complex method using Soxhlet extraction-based values. We can therefore venture the hypothesis that variability in the Soxhlet method caused a degree of instability in the PLS model. Unlike NMR1, NMR2 had very few outliers (S23, S29). This is because the NMR2 calibration method takes into account the biological variability of the samples.

5. Conclusion

In this work, several kinds of waste used in anaerobic digestion were analyzed using the Soxhlet and TD-NMR methods for lipid content determination. The comparative analysis between the Soxhlet method using solvent and the solvent-free NMR1 method showed a better repeatability than the Soxhlet method for lipid content prediction. The degree of hydrolysis in fats resulting from their storage and the degree of saturation in long-chain fatty acids probably influenced lipid measurements, which were overestimated by Soxhlet extraction and underestimated by TD-NMR. It was therefore recommended that TD-NMR

analyses should be carried out at temperatures above 66°C to achieve a good lipid quantification from organic waste containing complex saturated lipids. The application of chemometrics to TD-NMR raw data (NMR2) precludes the application of a long calibration procedure as needed for NMR1 and also allowed lipid content to be predicted with better accuracy and better repeatability than in the Soxhlet method. Despite the need to dry the samples, the TD-NMR technique has a very short acquisition time (< 1 min) compared to Soxhlet extraction, which requires several hours. In conclusion, TD-NMR has the advantage of being accurate, cost effective, faster, non-destructive and solvent-free. Coupled with chemometrics, TD-NMR provides a good alternative method to quantify even low levels of lipid content in heterogeneous organic waste samples, thereby providing a clearer picture of how to set about the recovery of such waste using anaerobic digestion.

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Figures

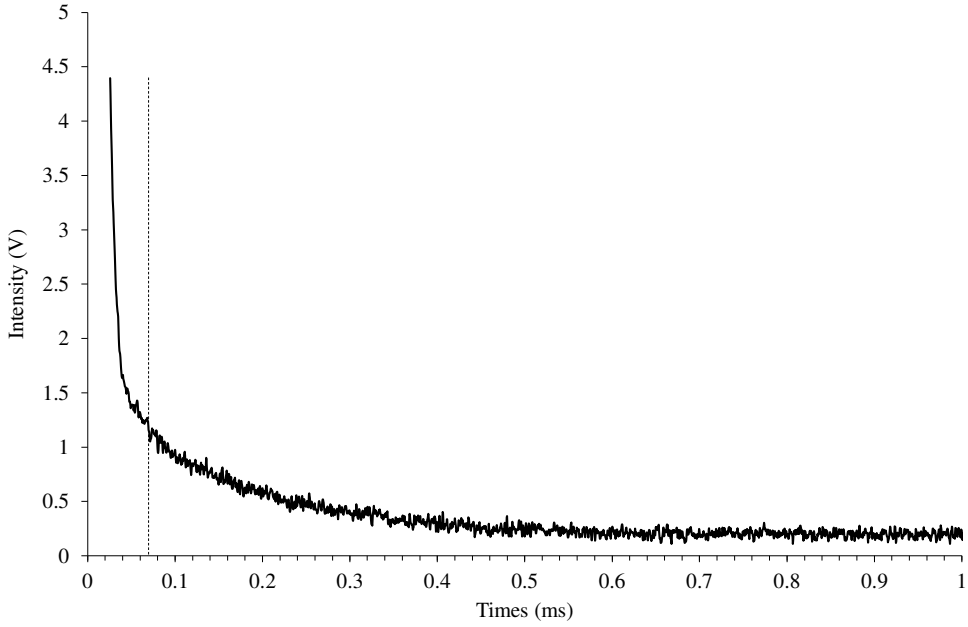

Figure 1: FID signal of cattle manure (S1). The dotted line shows the time limit between the relaxation time of protons corresponding to the dry matter (< 70 µs) and that of lipid protons in liquid-state at 60°C (> 70 µs)

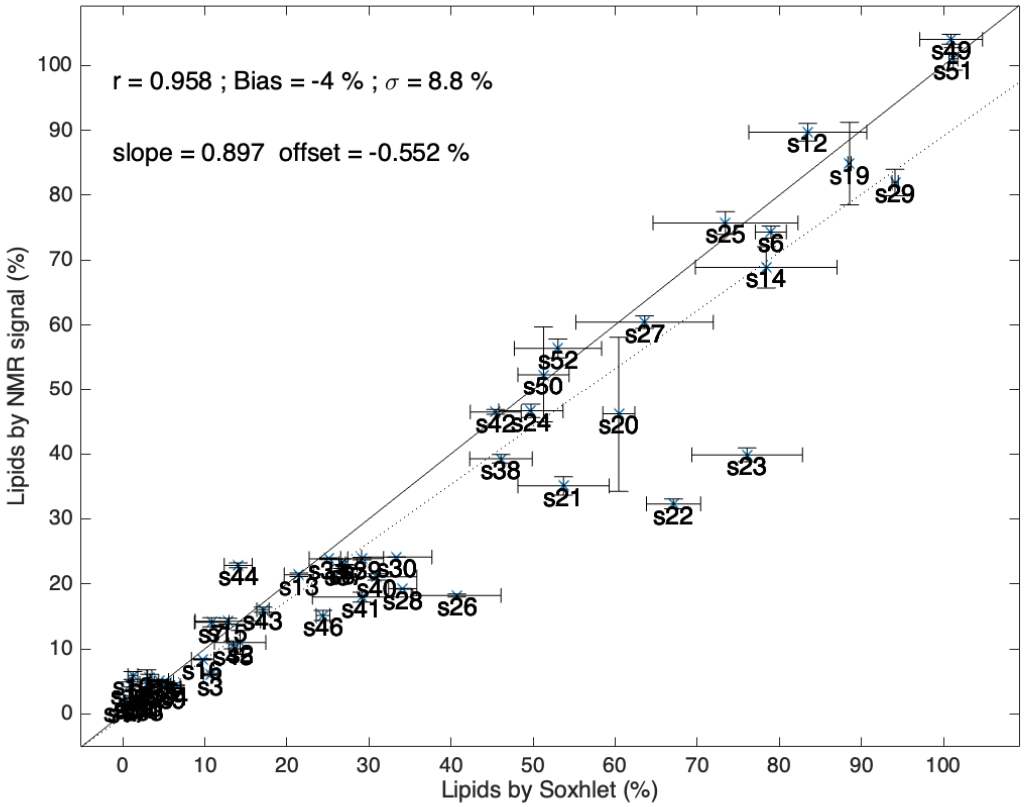

Figure 2: Correlation between lipid content estimated by the NMR1 method and lipid content measured by the Soxhlet method. Horizontal and vertical bars have a length of ± one standard deviation, calculated from repetitions.

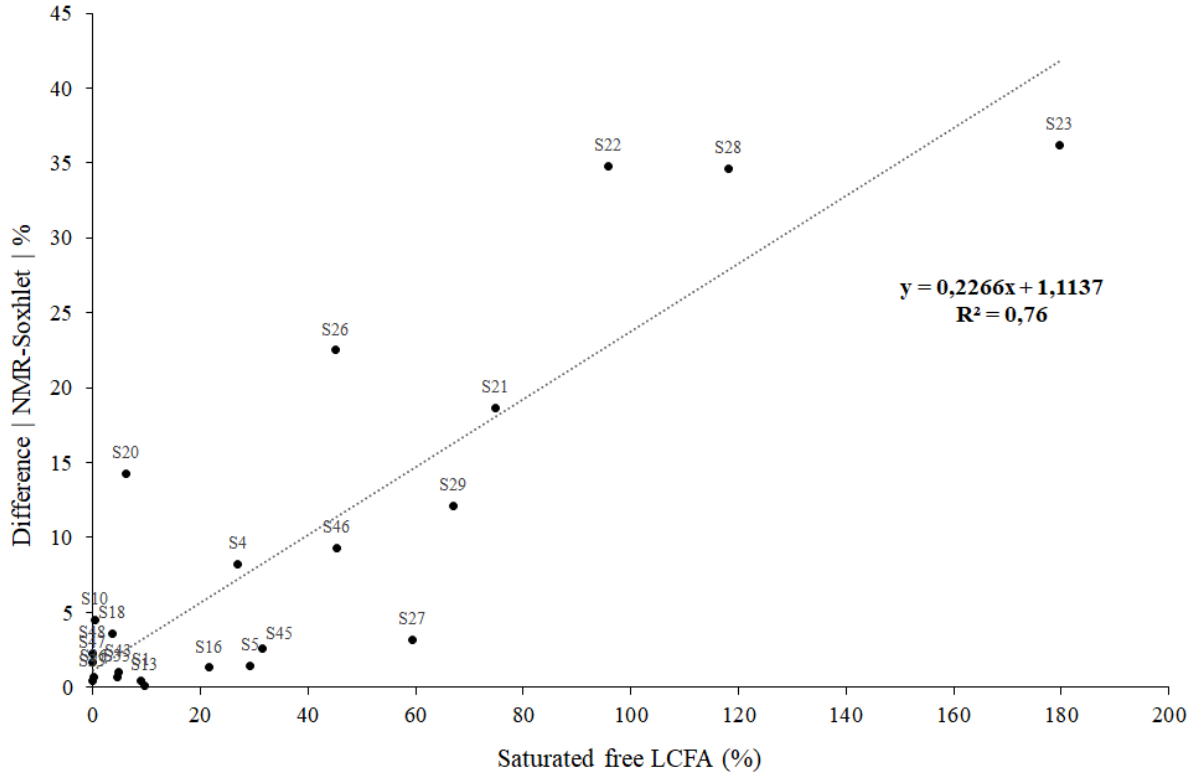

Figure 3: Linear regression between levels of saturated free LCFAs (in $mg.gTS^{-1}$) and the absolute difference between NMR1 and Soxhlet results

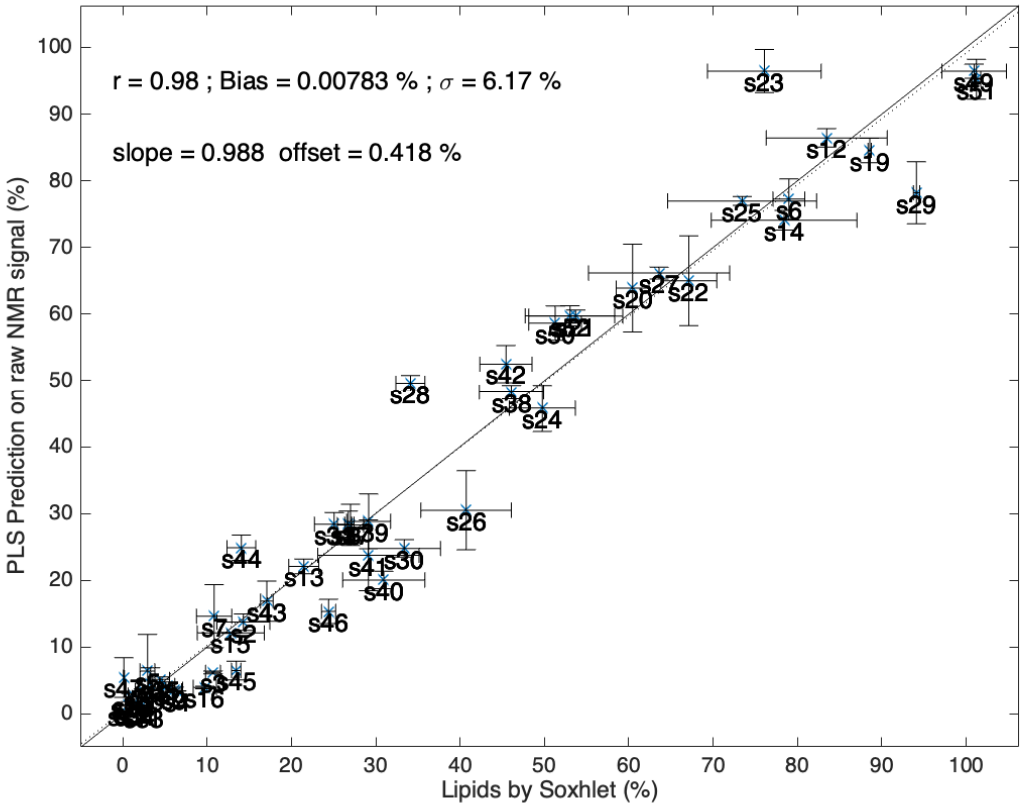

Figure 4: Correlation between lipid content estimated by the NMR2 method and lipid content measured by the Soxhlet method. Horizontal and vertical bars have a width of ± one standard deviation, calculated on the repeated measurements.

494 Table 1. Substrate characteristics (ww: wet weight, TS: Total Solids)

Substrate type	Sample	TS	NMR1	NMR2	MEH
		$g.kg^{-1}$(ww)	% TS	% TS	% TS
Cattle manure	S1	107	4.86 ± 0.13	4.24 ± 0.18	5.27 ± 0.91
Pig manure	S2	39	10.99 ± 1.85	13.74 ± 1.24	14.30 ± 3.13
Pig manure	S3	73	6.05 ± 0.20	6.21 ± 0.22	10.71 ± 0.87
Pig manure	S4	43	4.36 ± 0.10	3.46 ± 0.02	6.42 ± 0.55
Manure	S5	192	4.67 ± 2.14	6.43 ± 5.47	2.94 ± 0.88
Waste from fish slaughterhouse	S6	353	74.32 ± 0.93	77.27 ± 3.03	78.99 ± 1.89
Waste from pig slaughterhouse	S7	n.a.	14.10 ± 0.69	14.62 ± 4.73	10.84 ± 2.10
Waste from pig slaughterhouse	S8	329	23.09 ± 0.71	28.40 ± 1.96	26.74 ± 0.41
Sludge from slaughterhouse wastewater treatment	S9	69	3.96 ± 0.08	3.69 ± 0.32	6.10 ± 1.00
Pig bristle from slaughterhouse	S10	286	5.92 ± 0.61	2.83 ± 0.26	1.24 ± 0.60
Pig bristle from slaughterhouse	S11	n.a.	2.25 ± 0.01	1.33 ± 0.01	2.31 ± 0.01
Fats from slaughterhouse wastewater treatment	S12	557	89.71 ± 1.36	86.44 ± 1.37	83.47 ± 7.18
Pig mucus from slaughterhouse	S13	191	21.41 ± 0.28	22.09 ± 1.09	21.47 ± 1.78
Fats from slaughterhouse wastewater treatment	S14	238	68.88 ± 3.19	74.09 ± 1.48	78.41 ± 8.64
Waste from slaughterhouse	S15	170	14.27 ± 0.45	12.14 ± 0.07	12.82 ± 3.97
Waste from slaughterhouse	S16	166	8.41 ± 0.10	3.98 ± 0.19	9.73 ± 1.37
Bovine blood from slaughterhouse	S17	155	1.84 ± 0.20	1.34 ± 0.03	0.61 ± 0.28
Manure from slaughterhouse	S18	223	4.55 ± 0.38	2.43 ± 0.61	0.96 ± 0.16
Fats from meat industry wastewater treatment	S19	360	84.86 ± 6.36	84.56 ± 1.83	88.56 ± 0.20
Waste from meat industry	S20	330	46.17 ± 11.94	63.92 ± 6.58	60.45 ± 1.95
Fats from industrial wastewater treatment	S21	70	35.08 ± 1.42	59.72 ± 0.91	53.70 ± 5.57
Fats from industrial wastewater treatment	S22	37	32.31 ± 0.75	65.02 ± 6.73	67.11 ± 3.31
Fats from industrial wastewater treatment	S23	114	39.85 ± 1.08	96.45 ± 3.23	76.07 ± 6.74
Fats from industrial wastewater treatment	S24	77	46.62 ± 1.02	45.78 ± 3.50	49.74 ± 3.91
Fats from industrial wastewater treatment	S25	361	75.73 ± 1.75	76.98 ± 0.67	73.44 ± 8.83
Fats from industrial wastewater treatment	S26	37	18.22 ± 0.25	30.49 ± 5.92	40.72 ± 5.37
Fats from industrial wastewater treatment	S27	114	60.43 ± 0.98	66.17 ± 0.88	63.59 ± 8.36
Fats from industrial wastewater treatment	S28	77	19.25 ± 0.07	49.61 ± 1.16	34.12 ± 1.72
Fats from industrial wastewater treatment	S29	91	81.96 ± 2.05	78.21 ± 4.64	94.11 ± 0.38
Waste from food industry	S30	n.a.	24.13 ± 0.02	24.77 ± 1.30	33.38 ± 4.29
Waste from food industry	S31	n.a.	23.87 ± 0.13	28.40 ± 1.75	25.07 ± 2.35
Carrot pulp	S32	66	2.59 ± 0.23	1.64 ± 0.55	1.32 ± 0.40
Onion pulp	S33	241	2.11 ± 0.18	0.94 ± 0.06	2.56 ± 0.35
Shallot pulp	S34	272	2.45 ± 0.10	1.30 ± 0.09	1.48 ± 0.21
Sludge from meat industry wastewater treatment	S35	62	5.14 ± 0.18	5.17 ± 0.37	4.44 ± 1.12
Sludge from dairy wastewater treatment	S36	57	2.71 ± 0.07	3.54 ± 0.24	2.48 ± 0.99
Waste from supermarket	S37	949	22.94 ± 0.05	28.30 ± 3.08	27.00 ± 1.55
Waste from supermarket	S38	356	39.23 ± 0.67	48.24 ± 1.04	46.09 ± 3.82
Waste from supermarket	S39	258	23.90 ± 0.19	28.82 ± 4.11	29.16 ± 2.61
Waste from supermarket	S40	144	21.08 ± 0.49	20.05 ± 1.30	30.95 ± 4.87
Waste from supermarket	S41	144	17.98 ± 0.76	23.74 ± 5.28	29.13 ± 6.02
Waste from supermarket	S42	593	46.44 ± 0.37	52.49 ± 2.78	45.44 ± 3.10

Biowaste	S43	924	16.04 ± 0.38	16.89 ± 3.01	17.09 ± 0.75
Biowaste	S44	271	22.86 ± 0.36	24.87 ± 1.90	14.06 ± 1.71
Sludge from municipal wastewater treatment	S45	528	10.63 ± 0.60	6.51 ± 1.40	13.46 ± 0.54
Fats from municipal wastewater treatment	S46	557	15.14 ± 0.78	15.34 ± 1.82	24.41 ± 0.84
Seaweeds	S47	201	1.90 ± 0.40	5.46 ± 2.97	0.14 ± 0.06
Green waste	S48	235	5.59 ± 0.50	4.55 ± 2.38	3.29 ± 0.25
Reference 1: oleic acid	S49	930	104.02 ± 0.76	96.44 ± 1.06	100.93 ± 3.83
Reference 2: oleic acid+starch+casein	S50	1008	52.31 ± 7.39	58.66 ± 2.57	51.27 ± 3.13
Reference 3: trioleic acid	S51	896	101.01 ± 1.73	95.24 ± 2.98	101.21 ± 0.52
Reference 4: trioleic acid+starch+casein	S52	946	56.38 ± 1.45	59.74 ± 1.51	53.02 ± 5.32
Mean value			33.31	29.32	33.32
Standard deviation of repeatability			1.90	2.19	2.93

519 Table 2. Long chain fatty acids

Substrate type	Sample	Saturated free LCFA	Unsaturated free LCFA
		$mg.gTS^{-1}$	$mg.gTS^{-1}$
Cattle manure	S1	8.9	2.6
Pig manure	S4	27	4.6
Manure	S5	29.2	10.2
Pig bristle from slaughterhouse	S10	0.5	1.1
Pig mucus from slaughterhouse	S13	9.7	28.3
Waste from slaughterhouse	S16	21.7	7.6
Manure from slaughterhouse	S18	3.7	0
Waste from meat industry	S20	6.2	10.8
Fats from industrial wastewater treatment	S21	74.8	47.7
Fats from industrial wastewater treatment	S22	95.9	10.4
Fats from industrial wastewater treatment	S23	179.5	37.9
Fats from industrial wastewater treatment	S26	654.2	136.1
Fats from industrial wastewater treatment	S27	202.6	222.9
Fats from industrial wastewater treatment	S28	157.4	0.9
Fats from industrial wastewater treatment	S29	153.2	67.2
Onion pulp	S33	0	3
Sludge from meat industry wastewater treatment	S35	4.6	2.7
Sludge from dairy wastewater treatment	S36	0.2	1.2
Biowaste	S43	4.7	0
Sludge from municipal wastewater treatment	S45	31.6	15.9
Fats from municipal wastewater treatment	S46	45.4	13.8
Seaweeds	S47	0	1.1
Green waste	S48	0	4.7

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533 Table 3. Saturated and unsaturated fatty acid (FA) contents, and NMR and Soxhlet total FA
534 contents

	palmitoleic	**linoleic**	**palmitic**	**oleic**	**stearic**	**NMR**	**Soxlhet**
Sample	mg.gTS^{-1}	mg.gTS^{-1}	mg.gTS^{-1}	mg.gTS^{-1}	mg.gTS^{-1}	% HEM (TS)	% HEM (TS)
S22	8.88	0	58.73	1.54	37.21	66.38	32.31
S23	33.24	0	124.2	4.71	55.29	83.85	39.85
S26	0	104.2	650.4	31.9	3.8	18.22	40.70
S28	0	0	0	0.9	157.4	19.25	53.80
S27	0.1	113.8	201.2	109	1.4	60.43	63.60
S29	0	0	150.9	67.2	2.3	81.96	94.10

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