Optimization of total polar compounds quantification in frying oils by low-field nuclear magnetic resonance

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Abstract

To improve the accuracy of total polar compounds (TPC) quantification in frying oils by low-field nuclear magnetic resonance (LF-NMR), an optimized statistical method was proposed. The method uses a specially designed sequence to detect NMR signal in frying oils and establishes the TPC prediction model by partial least squares (PLS) regression on relaxation properties extracted from the NMR signal. Compared with inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) sequences, the designed sequence provides more relaxation information. Experimental result shows that the proposed method is more accurate than reported methods that based on longitudinal and transverse relaxation times in TPC quantification of frying oils.

**Keywords:** Total polar compounds; LF-NMR; frying oil; combined sequence
Introduction

Frying is one of the most common food processing methods. During the frying of oil, a series of complex chemical reactions such as oxidation, polymerization and hydrolysis will occur, leading to degradation of oil quality and producing substances including polar compounds which are harmful to human body.\textsuperscript{1-6} Total polar compounds (TPC) content of edible oil in frying process is considered as the most reliable single indicator of the degradation process.\textsuperscript{7} The most reliable method for TPC determination is chromatographic method, which separates the polar and nonpolar parts from the sample and then quantifies the polar parts, such as column chromatography, high performance liquid chromatography-size exclusion chromatography (HPLC-SEC or HPSEC)\textsuperscript{8} and high performance thin layer chromatography coupled with densitometry (HPTLC-D).\textsuperscript{9} But chromatographic method is time consuming, imprecision, needs various dangerous chemicals and requires analytical expertise,\textsuperscript{10,11} and thus not applicable for TPC monitoring of frying oils.

With its advantages of rapid and no need for other reagents, low-field nuclear magnetic resonance (LF-NMR) has shown its potential in rapid TPC quantification in frying oils.\textsuperscript{12-15} Both the longitudinal relaxation time ($\textit{T}_1$) and transverse relaxation time ($\textit{T}_2$) were found to have good linear relationship with the TPC content in frying oils.\textsuperscript{12,13} To improve the accuracy of TPC quantification by LF-NMR, Wang et al.\textsuperscript{14} analyzed the detailed transverse relaxation properties extracted by inverse Laplace transformation (ILT) from transverse relaxation signal of frying oil and established a more accurate prediction model by the second peak of transverse relaxation distribution. However, due to the high singularity of ILT on the multi-exponential decay data, it is only possible to obtain a smoothed estimation of the relaxation distribution from relaxation signal,\textsuperscript{16} thus the relaxation distribution could be very different for different frying process.\textsuperscript{15}
Another way to improve the accuracy of TPC quantification in frying oil by LF-NMR might be the using of more NMR properties of samples. To involve more NMR properties, combined sequences were designed and employed to detect the complex relaxation signals.\textsuperscript{17-19} The core idea of combined sequences is to construct a new sequence with two or more sequences of different relaxation properties, such that the obtained NMR signal contains more than one kind of information at the same time. Signal obtained from combined sequence cannot be analyzed in terms of classic relaxation time analysis but are often analyzed using statistical methods.\textsuperscript{19} However, these statistical methods are based on signal intensity which is volume sensitive, and thus are not suitable for TPC prediction unless the volume could be accurately controlled.

In this study, a combined sequence method was proposed for TPC quantification by LF-NMR in frying oils. Firstly, a combined sequence was designed for frying oils. Secondly, the sequence parameters were optimized according to the relaxation properties of frying oil. Finally, the TPC prediction model was established by partial least squares (PLS) regression on the extracted relaxation properties from the combined relaxation signal and the accuracy of proposed method was compared with reported methods.

**Experimental**

**Materials**

Edible oil sample (Luhua, Soybean oil) was purchased from the online store (Jingdong). Frying oils were prepared in laboratory by heating the soybean oil in a frying pan at 190 °C for 96 hours. No food was added during heating. 50 mL samples were taken out every 4 hours and stored in a refrigerating chamber of 4 °C to prevent further degradation. For each sample of the prepared frying oils, 0.20 mL was taken out into a sample tube of outer diameter of 7.5 mm for relaxation signal measurement.
**Apparatus**

A commercial automate purification chromatography equipment (CHEETAH, MP200) was used to perform the column chromatography analysis. NMR signal measurement was performed on a LF-NMR apparatus (Bruker, the minispec MQ60).

**Procedure**

*Detection of total polar compounds in flying oil.* The standardized preparative flash column chromatography method in Chinese standard (GB 5009.202-2016) was used to determine the TPC content in frying oil. Each frying oil sample to be analyzed (1 g) was dissolved in petroleum ether and placed onto the column. Mixture of petroleum ether and diethyl ether (87:13, v/v) was used as eluent for nonpolar components with flow rate of 25 mL/min and the eluate fraction within 11 min was collected. The polar components were determined by the difference between oil sample and the eluate fraction after evaporation. All of the degradation products except the components (unaltered triglycerides) eluted by the nonpolar eluant were identified here as total polar compounds,\(^{20}\) including polymeric triacylglycerols, oxidized-triacylglycerols, oxidized-monoacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids.\(^{21}\)

*Combined sequence for frying oils.* Inspired by the work of Rudi *et al.*,\(^{22}\) a combined sequence was designed to measure the combined relaxation signals of frying oils, as shown in Fig. 1. Compared with Rudi’s work, the main promotion of this combined sequence is the consideration of self-diffusion of oil molecules besides longitudinal and transverse NMR relaxation properties. In detail, the nuclear magnetization is saturated by a $\pi/2$ pulse, then a $\pi/2$ pulse is applied after an exponential increasing relax time, and then the
Carr-Purcell-Meiboom-Gill (CPMG) train is obtained by applying a series of \( \pi \) pulse. To involve the self-diffusion property, these CPMG trains are measured within the same fixed total time but with different echo times.

**Relaxation signal measurement.** The combined sequence was employed to detect the combined relaxation signal, with optimized sequence parameters, first recovery delay \((D_0)\) of 10 ms, last recovery delay \((D_n)\) of 1500 ms, relax points \((n)\) of 20, first echo time \((E_0)\) of 0.5 ms, last echo time \((E_n)\) of 2 ms, total echo train time \((T_E)\) of 250 ms, recycle delay of 2 s and scans of 4.

**Relaxation properties extraction from combined sequence.** Since the combined relaxation signal can’t be directly processed with conventional relaxation process method, such as exponential fitting and ILT, the combined relaxation signal was firstly separated into independent CPMG trains at each recovery time, thus 20 CPMG trains could be obtained for each combined relaxation signal obtained with 20 recovery times. Then for each CPMG train, mono-exponential fitting was employed to extract the transverses relaxation times. Finally, the first points of all the CPMG trains was treated as saturation recovery (SR) sequence data and processed by mono-exponential fitting to extract the longitudinal relaxation time. Thus, for each combined relaxation signal, 21 relaxation properties could be extracted in total.

**Partial least squares regression analysis for total polar compounds prediction.** PLS regression was employed on the extracted relaxation properties of combined relaxation signal. A PLS package named libPLS (Version 1.2)\(^{23}\) was used for PLS regression on MATLAB (Version R2018b, The MathWorks Inc., Natick, MA). During PLS regression with multivariate, number of the latent variables was determined by the number where root-mean-squared error of
leave-one-out cross-validation (RMSECV) could not be reduced anymore.

To demonstrate the advantage of proposed method, reported methods based upon longitudinal and transverse relaxation signal were performed. Inversion recovery (IR) sequence was employed to detect the longitudinal relaxation signal, with first recovery time of 5 ms, last recovery time of 2000 ms, relax points of 20 and recycle delay of 10 s. CPMG sequence was employed to detect the transverse relaxation signal, with echo time of 0.5 ms, echo number of 1500 and recycle delay of 2 s. PLS was employed on the longitudinal and transverse relaxation time extracted from the longitudinal and transverse relaxation signal by mono-exponential fitting respectively.

Results and Discussion

Parameter determination of the combined sequence

Before the combined relaxation experiments, parameters of the combined sequence were optimized according to the relaxation properties of the frying oils.

To extract the relaxation properties of the frying oils, an algorithm of ILT named CONTIN$^{24}$ was employed on longitudinal and transverse relaxation signals. The relationship between the relaxation spectrum and TPC content of frying oils is shown in Fig. 2. As shown in the figure, with the increase of TPC content, the two main peaks of longitudinal relaxation spectrum are broadened and merge into one wide peak eventually, and the expected value as indicated by the red vertical line in the figure decreases gradually. While for the transverse relaxation spectrum, with the increase of TPC content, more and more minor peaks appear and increase in amplitude and the expected value as indicated by the red vertical line in the figure decreases gradually. Therefore, both the longitudinal and transverse relaxation properties could be used to quantify TPC content of frying oil, but the influence of frying to the two kinds of

7
relaxation properties are different and thus combined relaxation signal that contained both longitudinal and transverse relaxation properties is to be more useful for TPC quantification.

The recycle delay of the combined sequence was determined by the maximum relaxation time of the longitudinal relaxation spectrum and should be at least 5 times larger than the maximum longitudinal relaxation time which is about 300 ms. Therefore, 2 s was chosen as the recycle delay of the combined sequence. The recovery time was determined by the span of the longitudinal relaxation spectrum. Since the longitudinal relaxation time spans from about 60 ms to 300 ms, the first recovery time was determined as 10 ms which is less than 5-fold of the minimum longitudinal relaxation time to make sure the minimum relaxation time could be included in the relaxation signal. The last recovery time was determined as 1500 ms which is about 5 times larger than the maximum longitudinal relaxation time to make sure the longitudinal relaxation signal is fully relaxed.

Echo times of the CPMG trains were determined by the transverse relaxation time of the transverse relaxation spectrum. The transverse relaxation time spans from about 4 ms to 600 ms, and thus the minimum echo time was determined as 0.5 ms which is less than 5-fold of the minimum transverse relaxation time. Echo times are also related to the self-diffusion coefficient and the final echo time was determined to 2 ms by experiments to enlarge the differences between the combined relaxation signals of frying oils.

As shown in Fig. 3, with the optimized sequence parameters, combined relaxation signals obtained from soybean oils without frying and with 96 hours frying could be distinguished significantly, which demonstrates the determined parameters a good selection for TPC quantification in frying oils by the combined sequence.

Accuracy of proposed method

To demonstrate the advantage of proposed method, reported TPC quantification methods
based upon longitudinal and transverse relaxation times were performed as reference. Besides, another prediction model based upon both the longitudinal relaxation time and transverse relaxation time was trained by PLS regression. The root-mean-square error of fitting (RMSEF) and coefficient of determination ($R^2$) of each TPC prediction model are shown in Table 1. As shown in the table, with more relaxation properties and self-diffusion property been used, the RMSEF of proposed method (Combined) is about 28% less than the RMSEF of method based on longitudinal relaxation time (IR), about 10% less than the RMSEF of method based on transverse relaxation time (CPMG), and about 5% less than the RMSEF of the model based on both the longitudinal relaxation time and transverse relaxation time (IR+CPMG).

**Conclusions**

An optimized TPC quantification method based on a specially designed combined sequence for frying oils was proposed in this paper. Experimental result shows that, with more relaxation properties and self-diffusion been considered, the proposed method is more accurate than reported methods that based on longitudinal and transverse relaxation times in TPC quantification of frying oils.

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References

1. S. G. Stevenson, M. Vaiseygenser and N. A. M. Eskin, *J. Am. Oil Chem. Soc.*, **1984**, *61*, 1102.
2. S. Paul and G. S. Mittal, *Crit. Rev. Food Sci. Nutr.*, **1997**, *37*, 635.
3. C. Dobarganes, G. Marquez-Ruiz and J. Velasco, *Eur. J. Lipid Sci. Technol.*, **2000**, *102*, 521.
4. F. A. Aladedunye and R. Przybylski, *J. Am. Oil Chem. Soc.*, **2009**, *86*, 149.
5. S. Casal, R. Malheiro, A. Sendas, B. P. Oliveira and J. A. Pereira, *Food Chem. Toxicol.*, **2010**, *48*, 2972.
6. S. Karakaya and S. Simsek, *J. Am. Oil Chem. Soc.*, **2011**, *88*, 1361.
7. C. W. Fritsch, *J. Am. Oil Chem. Soc.*, **1981**, *58*, 272.
8. J. D. Caldwell, B. S. Cooke and M. K. Greer, *J. Am. Oil Chem. Soc.*, **2011**, *88*, 1669.
9. A. C. Correia, E. Dubreucq, S. Ferreira-Dias and J. Lecomte, *Eur. J. Lipid Sci. Technol.*, **2015**, *117*, 311.
10. S. L. Melton, S. Jafar, D. Sykes and M. K. Trigiano, *J. Am. Oil Chem. Soc.*, **1994**, *71*, 1301.
11. X. Q. Xu, *J. Am. Oil Chem. Soc.*, **2000**, *77*, 1083.
12. M. Hein, H. Henning and H. D. Isengard, *Talanta*, **1998**, *47*, 447.
13. X. Sun and R. G. Moreira, *J. Food Process. Preserv.*, **1996**, *20*, 157.
14. C. Wang, G. Su, X. Wang and S. Nie, *J Agric. Food Chem.*, **2019**, *67*, 2361.
15. X. Yang, B. Liu, X. Wang, H. Lu and T. Zhao, *Food Sci.*, **2014**, *35*, 110.
16. J. Butler, J. Reeds and S. Dawson, *SIAM J. Numer. Anal.*, **1981**, *18*, 381.
17. A. Guthausen, G. Zimmer, P. Blumler and B. Blumich, *J. Magn. Reson.*, **1998**, *130*, 1.
18. F. C. Tinsley, G. Z. Taicher and M. L. Heiman, *Obes. Res.*, **2004**, *12*, 150.
19. H. Todt, G. Guthausen, W. Burk, D. Schmalbein and A. Kamlowski, *Food Chem.*, **2006**, *96,*
20. S. Urbancic, M. H. Kolar, D. Dimitrijevic, L. Demsar and R. Vidrih, *Lwt-Food Sci. Technol.*, 2014, 57, 671.

21. I. Ben Hammouda, G. Marquez-Ruiz, F. Holgado, F. Freitas, M. Da Silva and M. Bouaziz, *Eur. Food Res. Technol.*, 2019, 245, 10.

22. T. Rudi, G. Guthausen, W. Burk, C. T. Reh and H. D. Isengard, *Food Chem.*, 2008, 106, 1375.

23. H.-D. Li, Q.-S. Xu and Y.-Z. Liang, *Chemom. Intell. Lab. Syst.*, 2018, 176, 34.

24. S. W. Provencher, *Comput. Phys. Commun.*, 1982, 27, 229.

25. M. D. Hürlimann, *J. Magn. Reson.*, 2001, 148, 367.
### Tables

| Sequence   | RMSE, % (w/w) \(^a\) | \(R^2\) \(^b\) |
|------------|-----------------------|-----------------|
| Combined   | 0.8517                | 0.9983          |
| IR+CPMG    | 0.8980                | 0.9981          |
| CPMG       | 0.9457                | 0.9978          |
| IR         | 1.1780                | 0.9967          |

\(^a\) Root-mean-square error of fitting. Each value is the result of single experiments.

\(^b\) Coefficient of determination.
**Figure Captions**

Fig. 1 Combined sequence for relaxation signal detection of frying oils.

Fig. 2 Relaxation spectra of IR and CPMG experiments of frying soybean oil. \( a \rightarrow c \), frying time \( = (4, 8, 0 \text{ h}) \); \( d \rightarrow y \), frying time \( = (12, 16, 20, \ldots, 96 \text{ h}) \).

Fig. 3 Combined relaxation signal of frying oils with optimized sequence parameters.
Figures

Fig. 1 Combined sequence for relaxation signal detection of frying oils.

\[
D_i = D_0 q^{i-1} \\
E_i = E_0 + (i-1)d \\
m_i = \text{round} \left( \frac{T_E}{2E_i} \right)
\]
Fig. 2  Relaxation spectra of IR and CPMG experiments of frying soybean oil. $a \rightarrow c$, frying time = (4, 8, 0 h); $d \rightarrow y$, frying time = (12, 16, 20, ..., 96 h).
Fig. 3  Combined relaxation signal of frying oils with optimized sequence parameters.
Graphical Index

\[ D_i = D_0 q^{i-1} \]
\[ E_i = E_0 + (i-1)d \quad (i = 1, 2, \ldots, n) \]
\[ m_i = \text{round} \left[ \frac{T \varepsilon}{2E_i} \right] \]