

Study on Relationship between Polar Compounds and LF-NMR Properties in Fried Camellia Seed Oil

Jinying Wang^{1,2,*}, Chaosheng Liu², Dale Sun²

¹State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, P R China

²College of Agriculture and Animal Husbandry, Qinghai University, Xining, P R China

*Corresponding author: wangjinying0128@126.com

Abstract The correlation between classic column chromatography and LF-NMR to determine the total polar compounds were investigated in this study. Results revealed that the content of polar compounds in fried camellia seed oil were keeping elevated during deep frying and reach to reject point up to 24 h, the content level were 27.21%. The significant correlation was existed between TPC and S21, LF-NMR could replace the classic column chromatography as the rapid detection way to determine the TPC in fried oils.

Keywords: *fried camellia seed oil, LF-NMR, polar compounds*

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1. Introduction

Camellia oleifera Abel., an evergreen tree, grows only in middle and southern regions of China, such as Hunan, Jiangxi, Fujian, Anhui, Guangdong provinces, etc. [1]. The world's total plant area of camellia is around 3.3 million ha, of which approximately 3.0 million ha in China standing about 90% of the global total. In addition to Southeast Asia, Japan and other State-owned very little distribution [2,3]. Camellia oleifera is considered to be nutritious and healthy, owing to its fatty acids composition and the presence of bioactive compounds, such as tea polyphenol, tea saponin and squalene, etc. [4]. Camellia oil (CO) is obtained from Camellia oleifera seeds, is characterized by the high proportion of oleic acid (74-89%) [5]. CO has been used as a cooking oil in China for 2300 years [9]. It is characterized by a high proportion of oleic acid (74-89%) [10] and antioxidant compounds, such as sterols, triterpenes [11], tocopherol, squalene [12] and phenolics [13,14].

Polar compounds were resulted from oxidation, polymerization, splitting decomposition and hydrolization during thermal process of edible oils, which exhibits higher polarity than normal vegetable oil molecules [15]. Because of total polar compounds represent the all decomposition product exist in samples, it can be used as good index of degradation of fried oils [16]. Polar compounds had detrimental effect to the man's health. Ames experiment and bone marrow micronucleus rate test conducted by Liu et al. [17] demonstrate that fried oils and polar compounds showed mutagenic effects in some extent. Therefore, in order to control the oil quality applied in the manufacturing industry and experiment process, many countries set the maximum limit for total

polar compounds level in fried oils. Such as, Australia and China were 27%, Belgium, France and Spain were 25%, Germany were 24%.

The determination of polar compounds including two aspects, namely determine the levels of total polar compounds and analysis the constitution of them. As the most reliable and widely used method to determine the total polar compounds in fried oils, column chromatography was the standard method approved by IUPAC [18] and AOCS [19]. It is also the standard method in China. Economic and effective micro-column chromatography [20] and Solid-phase extraction (SPE) [21] were derived from classic column chromatography. After separate the polar compounds by method to determine the total polar compounds and then introduce the fraction onto the high-performance size exclusion (HPSEC). As result of the combined separation criteria used, firstly based on polarity and secondly on molecular size or weight, five groups of compounds are neatly separated and quantified: TG oligomers (TGO), TG dimers (TGD), oxidized TG monomers (ox-TGM), diacylglycerols (DG), and free fatty acids (FFA), in this order of elution [20] [22].

In recent years, the environmental and nondestructive spectrometry method had overtaken the absorption chromatography become the main measure to determine the polar compounds in fried oil rapidly. LF-NMR (Low-field nuclear magnetic resonance) was one of them. LF-NMR, a relaxation spectrum analysis technology based on nuclear magnetic [23], which could detect the physical and chemical information and unique fingerprint database of proton or nucleon of sample simultaneously [24]. As a new type of rapid detection technology, LF-NMR was play an import role in the analysis of fried oil quality [25]. According to Wang et al. [26], there was a significant correlation between S21 in transverse relaxation spectrum and content level of total polar compounds. This theory

was combined with LF-NMR and applied in predict the level of total polar compounds in fried oils by Yang et al. [27]. A number of study focused on the oil quality evaluation by LF-NMR indicate that LF-NMR no only used for oil adulteration [28,29], meanwhile, applied in reflect the quality of fried oils effectively and were not affected by antioxidant [30,31,32].

Up to date, there is no report on the use of LF-NMR as a rapid detection technology for analysis of Camellia seed oils. Therefore, the objective of this study was to investigate the correlation between chemical process (classic column chromatography) and spectrometry method (Low-field nuclear magnetic resonance, LF-NMR) to determine the level of total polar compounds.

2. Materials and Methods

2.1. Materials

The crude CO were collected in Dong Lan where located in Guang Xi Province in China. The refined CO were purchased from local market. The n-hexane, Petroleum ether (b.p. 30-60°C), diethyl ether, silica gel - particle size 0.063-0.200 mm (70-230 mesh) and sea sand were all acquired from Sinopharm Chemical Reagent Co., Ltd in ShangHai.

2.2. Frying Protocol

Frying experiments were carried out using 8 L capacity domestic deep-fat electric fryer (HY-81, GangYang electromechanical equipment company) equipped with a removable stainless steel wire basket and a thermostat. Fresh potato was shelled shortly before frying and cut into chips (approximately 40-50×10×10 mm), which needed rinsed with cold water and drained for a while prior to being submerged into the 4 L sample G. To avoid temperature losses in the oil during frying, small amounts of potatoes (125 g) were fried each time. A batch of potato chips was fried for 4 min at 11 min intervals for a period of 6 h per day with 24 batches fried per day for 7 consecutive days. Frying oil sample (50 g) was withdrawn in amber bottles at 2 h intervals and after cooling to room temperature stored at -20°C until further analysis. During the frying period, the fryer was left uncovered. After each frying operation, the fryer was switched off and the temperature was allowed to drop to 60°C until the next session. No fresh oil was added for replacement of the oil removed during sampling. The fryers were then cupped with their lids and the remaining oil was allowed to cool overnight. For the first batch of each day frying test, the oil was needed conditioned by heating to 185 ± 50°C and held for 30 min.

2.3. Determination of Total Polar Compounds

According to the standard method of China [15] and AOCS [20], 2.5±0.1 g of used frying oil or fat dissolved in the 10 mL elution solvent is introduced onto a glass column filled with a slurry of silica gel and elution solvent. A chromatographic glass column of (2 cm× 40 cm), and

25 g of silica gel (70-230 mesh) - adjusted to a water content of 5% are used. The elution solvent is a mixture of light petroleum (b.p. 30-60°C) and diethyl ether 87:13 (V/V). Elution of non-polar compounds, including mostly unoxidized TG, is carried out with 150 mL of the elution solvent and then the polar compound fraction is eluted with 150 mL of diethyl ether. A dropping funnel is used and the flow rate is adjusted to about 2.5 mL/min. Fractions are collected in round bottom flasks and solvents are removed with the aid of a rotary evaporator. Fractions can be dried to constant weight using a stream of nitrogen. The mass fractions of non-polar compounds (w_{np}) and the polar compounds (w_p) are given by the formula:

$$W_{np} \% = \frac{m_{np}}{m} \times 100 \quad (1)$$

$$W_p \% = \frac{m_p}{m} \times 100 \quad (2)$$

where m_{np} is the mass (in g) of the nonpolar fraction 1, m_p is the mass (in g) of the polar fraction 2, and m is the mass (in g) of the test portion added to the column

The efficacy of the separation is assessed by thin-layer chromatography (TLC). Glass plates coated with silica gel, 0.25 mm layer, and a mixture of light petroleum, diethyl ether and acetic acid 70:30:2 (V/V/V) as developing solvent are used. After plate development and solvent evaporation, the plate is sprayed with a phosphomolybdic acid solution and heated at 120-130°C to visible spots. The efficacy of the separation of polar compounds and non-polar compounds were estimated by retardation factor (Rf).

2.4. Determination of Transverse Relaxation Time

Transverse relaxation time (T_2) were determined by CPMG (Carr-Purce II -Meiboom-Gill) impulse sequence, the temperature was set at 32 ± 0.01°C. Weight 2.0 g fried Camellia seed oil accurately put in the nuclear magnetic resonance exclusive test tube (diameter, 15 mm). After maintain constant temperature at 32±0.01°C in Water-bathing 10 min place the tube in the nuclear magnetic resonance probe, stabilize 1 min and then start data collection. When finish the collecting, place the sample in Water-bathing 5 min main constant temperature at 32±0.01°C reassembly so as to next determination. Equipment parameter settings were as follows: Repeating Time (TR) 1500 ms, half-echo time(τ)250 μ s, Echo Count 8000, sampling numbers (TD) 754038, scanning number (NS) 4 times, spectral width (SW) 250 kHz. Each sample repeat sampling 6 times.

2.5. Statistical Analysis

Each experiment was done in triplicate. The results were expressed as mean ± standard deviation and were analyzed by SPSS (version 17.0 SPSS Inc., USA). One-way analysis of variance was performed using ANOVA procedures and regression. Significant differences between the means were determined by Duncan's Multiple Range test. $P < 0.05$ was considered as a level of significance.

3. Results and Discussion

3.1. Optimization of Major LF-NMR Parameters

3.1.1. Repetition Time (TR)

Repetition Time (TR) were the duration of prior sampling to last sampling. Different samples were characterized by different TR, which were related to the energy release speed of them. The magnitude of TR was determined by the longest relaxation time.

As showed in Figure 1, The maximum signal mode of sampling signal was tended to become stable gradually with the enlarge of TR value. It is difficult to maintain a balanced state when the TR value were too small. When TR value reach to 1500 ms the signal modulus value of each sampling was relatively stable, which means that the selected TR value can enable the sample system return to equilibrium state. Along with the further increasing of TR value up to 2 000 ms, the stability of signal collected from sampling were reduced. The greater value of TR were set, the longer waiting time of sampling were acquired and lead to the reduction of experiment effective. Therefore, select the 1500 ms as the optimistic parameter of TR.

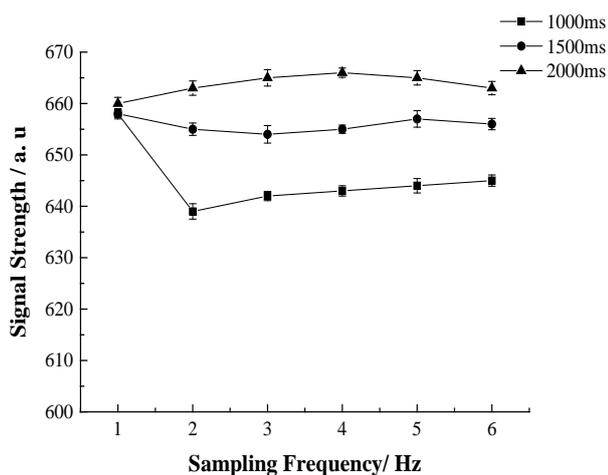


Figure 1. The effect of TR on the stability of LF-NMR detect signal

3.1.2. Half-echo Time (τ)

Half-echo time were the time interval between 90 degree pulse and adjacent 180 degree pulse. The change of τ value may affect the shape of signal, which were resulted from the intra-molecular free diffusion and molecule exchange of sample. Select the minor τ value if there is a need to narrow down the effect of intra-molecular free diffusion on signal, otherwise choose the major one. As show in Table 1, when τ set at 150 μ s, the distributed scope T2 value in acquired curve of CPMG sequence were 0 -1500 ms and signal amplitude in vertical axis were attenuated from maximum to 12. With the increase of τ value the distribution interval of T2 were enlarged gradually. The attenuation in signal amplitude of corresponding echo curve demonstrate the completeness of echo curve. When set the τ value at 250 μ s, signal amplitude was attenuated to minimum and meet the determination requirements for CO.

Table 1. Effect of τ value on the CPMG echo decay curve

τ / μ s	T2/ms	SP/a.u
150	1000	11.603
200	1500	3.43
250	2000	1.217

Note: τ - Half echo time; T2-Transverse relaxation time; SP-Signal modulus value.

3.1.3. Half-echo Time (τ)

Echo count were the echo number after the signal collection. In general, enough echo count was needed to relax the signal completely. With the increase of echo count the distribution interval of T2 were enlarged and signal amplitude of corresponding echo curve were attenuated gradually. As show in Table 2, Signal amplitude attenuated to 8 when the echo count was 4000, which indicate that echo count was too small to attenuate the signal amplitude completely. Along with the echo count reach to 6000 and 8000, the signal amplitude was attenuated to 2 and 1 respectively. Finally, select the 8000 as the most suitable echo count.

Table 2. Effect of τ value and EchoCount on the CPMG echo decay curve

EchoCount	T2/ms	SP/a.u
4000	1600	9.011
6000	2400	2.034
8000	3200	1.008

Note: T2-Transverse relaxation time; SP-Signal modulus value.

3.2. The Correlation between Total Polar Compounds (TPC) and LF-NMR Data

The determination of TPC indicate that the content of TPC in fresh CO were 4.58%, with the prolonged duration the level of polar compounds in fried CO were keeping elevated and reach to reject point up to 24 h. At the end of frying process, the content climbed to 42.49%. The accumulation of TPC and frying time were significantly correlated during whole frying process, this finding was accordance with the research of Liang et al. [33].

Figure 2 were the typical T2 spectrum of fresh and fried CO, three peaks were successively marked as T21, T22 and T23, the area of each peak were represented as S21, S22 and S23 respectively. As can be seen from Figure. 2, the T2 spectrum of fresh refined CO were consisted by T22 and T23, while a new peak was generated in fried oil, Wang et al. [32] reported similar results. As show in Table 3, the S21 were increased with the extend of frying time, while the S22 and S23 present a decreased tendency. The decrease rate of S23 were 2 times of S22, this could be ascribed to the quicker degradation of unsaturated fatty acid, because generally S22 and S23 in typical T2 spectrum were represent the level of saturated and unsaturated fatty acid respectively. The S21, area of new oxidized compounds differentiate from Triglyceride composition, were increased during frying process and present significant correlation with frying time ($y=2.7516x+10.605$, $R^2=0.9717$). The content increase of compounds characterized by similar valve of T21 strengthen the signal response of LF-NMR

and the result the heighten of the corresponding area (S21) consequently [32,34,35].

Besides, the starting time of T21, T22 and T23 were all shrink with the increase of frying time and showed good correlation with it ($R^2 > 0.96$). Otherwise, research focused on soybean oils attested that there was no obvious regular changes of starting time of T23 [30], this difference could be attributed to the oxidation and per-oxidation product generated from complex chemical reaction, such as oxidation, splitting and hydrolysis. Xiong Guohuan [36] and Wang Yongwei [32] maintains that the shorten of transverse relaxation time were closely associated with compounds that generated in frying process and characterized by large size, complex construction, long chain and huge molecular, which will generate bucking effect resulted from nuclear spin of local magnetic field by increase the overall intermolecular interaction force and binding effect. Finally, results in the shorten of transverse relaxation time. In addition, the polymerization degree of oxidation products that

produced during frying were enlarged with the prolonged process and the relaxation of hydrogen proton in magnetic field were affected by intermolecular interaction, ultimately resulting in a overall migration of T2 spectrum [26].

The content of total polar compounds (TPC) and S21 were correlated closely with frying time and Fan Zhixiong [37] insist that S21 in T2 spectrum could reflect the quality of fried oils without the effect of antioxidant [31]. Hence, the LF-NMR technology could be applied in rapid detection of edible oils effectively [26,30]. As depicted in Figure 3, the greater correlation was existed between total polar compounds (TPC) and S21, which in accordance with the finding of Shen et al. [38]. LF-NMR analysis technology that depended on this theory were applied in predict the total polar compounds in fried oils, which means that LF-NMR analysis technology based on spectroscopy could replaced the classic column chromatography based on chemical method as the rapid detection way to determine the total polar compounds in fried oils.

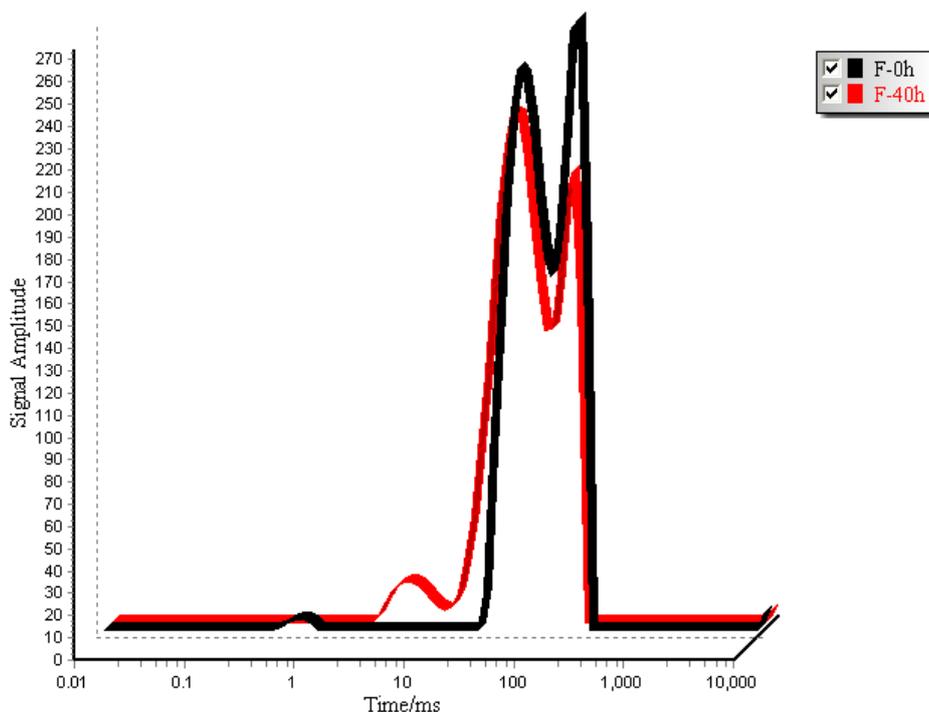


Figure 2. Typical transverse relaxation time(T2) spectrum of Camellia seed oil

Table 3. Change of Starting Time of peak and character peaks area of fried Camellia seed oil (n=6)

Time/h	Starting Time of peak/ms			Character Peaks Area		
	T21	T22	T23	S21	S22	S23
0	5.21±0.12	26.63±0.98	157.68±0.65	18.27±0.34	2165.59±3.54	915.13±2.01
4	5.01±0.28	23.16±0.45	153.73±0.76	25.68±0.38	2137.33±2.78	897.74±2.45
8	4.71±0.03	22.04±0.36	150.87±1.05	33.70±0.76	2124.92±4.02	866.37±3.01
12	4.49±0.45	21.16±0.45	147.78±0.87	37.00±0.56	2079.28±3.64	837.7±2.93
16	4.44±0.11	20.61±0.76	144.39±2.03	54.17±0.50	2054.78±3.97	825.06±1.99
20	4.29±0.43	19.64±0.65	142.79±1.05	59.87±0.08	2056.28±2.23	798.81±3.45
24	4.10±0.32	18.74±0.34	140.31±1.05	66.01±0.36	2046.07±3.06	765.57±1.67
28	4.04±0.18	17.74±0.45	139.99±0.97	85.31±0.88	2045.31±2.09	744.03±2.57
32	3.77±0.36	16.30±0.56	136.69±0.67	96.99±0.94	2034.85±3.78	718.63±2.09
36	3.36±0.22	14.17±0.56	133.98±1.65	118.6±0.99	2020.94±3.67	699.16±1.67
40	3.01±0.06	12.17±0.76	129.19±2.06	126.41±1.05	2001.51±4.02	657.1±1.01

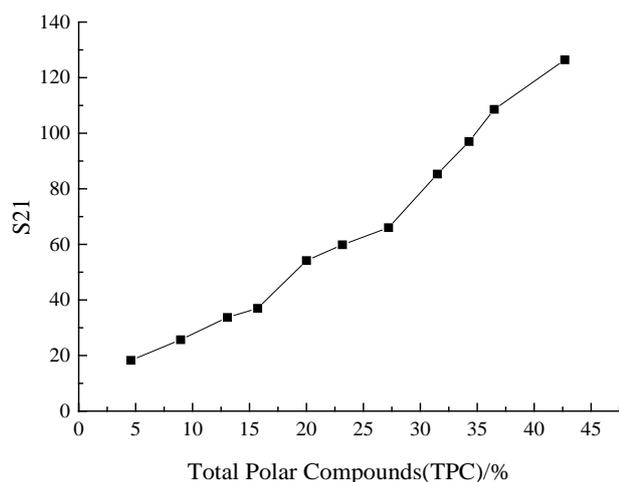


Figure 3. The correlation between total polar compounds (TPC) and S21

4. Conclusion

1). The optimistic parameters of LF-NMR determine the T2 of CO were: TR=1500 ms, τ =250 μ s, EchoCount=8000, NS=4 times, TD = 754038, SW=250 kHz.

2). The content of polar compounds in fried CO were keeping elevated during deep frying and reach to reject point up to 24 h. S21 in LF-NMR T2 spectrum were increased with the prolonged duration of frying, otherwise, S22 and S23 were decreased. The start time of T21, T22 and T23 were all shorted. The greater correlation was existed between TPC and S21, LF-NMR could replace the classic column chromatography as the rapid detection way to determine the TPC in fried oils.

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Statement of Competing Interests

The authors have no competing interests

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