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Quality detection of tea oil by ^{19}F NMR and ^{1}H NMR

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SUMMARY: The nuclear magnetic resonance (NMR) technique was applied to monitor the quality of tea oil herein. The adulteration of virgin tea oil was monitored by ^{19}F NMR and ^{1}H NMR. The ^{19}F NMR technique was used as a new method to detect the changes in quality and hydroperoxide value of tea oil. The research demonstrates that ^{19}F NMR and ^{1}H NMR can quickly detect adulteration in tea oil. High temperature caused a decrease in the ratio D and increase in the total diglyceride content. Some new peaks belonging to the derivatives of hydroperoxides appeared at δ-108.21 and δ-109.05 ppm on the ^{19}F NMR spectrum when the oil was autoxidized and became larger when the hydroperoxide value increased. These results have great significance in monitoring the moisture content, freshness and oxidation status of oils and in detecting adulteration in high priced edible oils by mixing with cheap oils.

KEYWORDS: *^{1}H NMR; ^{19}F NMR; Hydroperoxides; Quality detection; Tea oil*

RESUMEN: ***Determinación de la calidad del aceite de té mediante ^{19}F RMN y ^{1}H RMN***. En este trabajo se utiliza la técnica de resonancia magnética nuclear (RMN) para controlar la calidad del aceite de té. La adulteración del aceite de té virgen se controló mediante las técnicas de ^{19}F RMN y ^{1}H RMN. La técnica de ^{19}F RMN se utilizó como un nuevo método para detectar los cambios en la calidad y el índice de hidroperóxido del aceite de té. La investigación demuestra que las técnicas ^{19}F RMN y ^{1}H RMN pueden detectar rápidamente la adulteración del aceite de té. La alta temperatura provoca una disminución en la proporción D y un aumento en el contenido total de diglicéridos. Algunos picos nuevos, pertenecientes a derivados de hidroperóxidos, aparecieron a δ-108,21 y δ-109,05 ppm en el espectro de ^{19}F RMN cuando el aceite se autoxidaba e incrementaban cuando aumentaba el índice de hidroperóxido. Estos resultados tienen gran importancia en el seguimiento del contenido de humedad, de la frescura y del estado de oxidación de los aceites y en la detección de la adulteración de aceites comestibles de alto valor con aceites baratos mediante el uso de ^{19}F RMN y ^{1}H RMN.

PALABRAS CLAVE: *^{1}H RMN; ^{19}F RMN; Aceite de té; Detección de calidad; Hidroperóxidos*

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1. INTRODUCTION

Camellia oleifera, commonly called oil tea tree, belongs to the Theaceae family and has been widely cultivated in China for a long time (Qin *et al*., 2018). Camellia oil is widely referred to as tea oil and is commonly utilized for cooking in China (Tu *et al.*, 2017). It is rich in unsaturated fatty acids such as linolenic, linoleic and oleic acids (Weng *et al*., 2018). Its oleic acid content is similar to that found in olive oil, reaching up to 70%. In addition, there are also some unsaponifiable compounds such as tocopherols, squalene, phytosterols, and flavonoids present in tea oil (Memon, 2011; Xiao *et al*., 2016). These nutrients can easily be digested and absorbed by the human body, and are beneficial to lowering cholesterol, preventing and treating hypertension, and cardiovascular diseases (Wang *et al.,* 2012; Lee and Yen, 2006). Tea oil has been reported to exhibit antioxidant activity (Zhou *et al.,* 2018), and is also known as "Oriental Olive Oil", which has been very much preferred by consumers in China. Among plant oils, the low-temperature and cold-pressed tea oil has a higher price than others because it retains nutrients as much as possible. Thus, some unscrupulous merchants adulterate tea oil with other low-price plant oils in order to make higher profits.

The adulteration of tea oil by other low-cost oils damages consumer interest. There are some certain analytical methods for detecting the adulteration and quality of oils, such as GC-MS, ultraviolet spectroscopy, infrared spectroscopy and nuclear magnetic resonance (NMR) spectroscopy (Li *et al.,* 2016; Gurdeniz and Ozen, 2009; Zhou *et al.,* 2015). However, the NMR technique, especially ^{1}H NMR (Sacchi *et al.,* 1997), has become a favorable choice (Santos *et al*., 2018), owing to its fast and effective approach over the traditional methods like GC-MS. Andrade *et al.* (2012) analyzed the degree of unsaturation of combined and free fatty acids in several plant oils (soybean, corn, sunflower, canola, linseed, cottonseed and jatropha) using ^{1}H NMR, which was found to be satisfactory when compared to other conventional methods. Jiang *et al.* (2018a) used ^{1}H NMR as a fast method to determine soybean oil deterioration during deep frying and discovered that it is similar to the conventional gas chromatography method for analyzing secondary oxidation products. ^{1}H NMR can be also used to determine the chemometric characteristics of extra-virgin olive oil (EVOO) in order to identify the specific compounds responsible for olive oil characteristics (Ingallina *et al*., 2019). Shi *et al.* (2018) used ^{1}H NMR combined with chemometrics for the rapid detection of adulteration in tea oil, and also confirmed the efficacy of this method in terms of speed and accuracy.

Our laboratory has previously established a new technique for monitoring the quality and adulteration of olive oil by ^{19}F NMR (Zhou *et al.,* 2015). Jiang et al. (2018b) used this method combined with ^{1}H NMR to detect EVOO adulteration successfully. As far as we know, no study based on a ^{19}F NMR approach for the detection of the quality of tea oil according to temperature and time changes has been reported. In this work, the quality and adulteration of tea oil using ^{19}F NMR was studied and specifically, the determination of moisture content and the detection of oxidation with temperature were conducted in order to expand the application of NMR techniques for the assessment of plant oil quality.

2. MATERIALS AND METHODS

2.1. Chemicals

All solvents were of reagent or analytical grade. Hexafluorobenzene (99%), 4-tert-butylphenol, pyridine and chloroform-d were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). The deriving fluorine reagent (4-fluorobenzoyl chloride, purity: 98%) was purchased from Sigma-Aldrich (Shanghai, China). The rest of the reagents used in the experiment were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Samples

Two tea oils were prepared in our lab: one was extracted with petroleum ether (bp range 60-90 °C) at room (or low) temperature (TOL) and the other was extracted by the Soxhlet method with petroleum ether (TOS) and then the solvent was removed by a vacuum rotary evaporator in a water bath at 30 °C. Other commercial plant oils were purchased from the local supermarket. The samples were stored at room temperature away from light.

5, 10, 15, 20, 25, 30, 35, 40 and 45% refined tea seed oil were separately added to TOL.

2.3. Sample preparation for NMR analysis

0.1 mL hexafluorobenzene in a stock solution of pyridine and CDCl3 mixed in a ratio of 1:1.5 (v/v) was mixed with 0.5 g 4-tert-butylphenol. Hexafluorobenzene was used as reference material because the chemical shift was observed at δ164.90 ppm n the ^{19}F NMR analysis. And 4-tert-butylphenol was as a quantitative standard in the ^{19}F NMR analysis. 0.1 Gram oil sample was put in a 4 mL centrifuge tube mixed with 0.4 mL stock solution. The resulting solution was transferred to a 5 mm NMR tube and 30 μL of deriving reagent were added. Then the reaction mixture was left to react for 0.5 h in the tube at room temperature away from light. After completion, the ^{19}F NMR spectra of the samples were determined immediately.

20 μL oil sample were dissolved in 0.4 mL $CDCl_3$ with 0.03% trimethylsilane (TMS). The resulting solution was transferred to a NMR tube and then the ^{1}H NMR spectra were recorded.

2.4. NMR spectroscopy experiments

All NMR recordings were conducted on a Bruker AVANCE III HD 600MHz spectrometer, operating at 564 and 600 MHz for the ^{19}F and proton nucleus, respectively. Typical spectral parameters for this ^{19}F NMR experiment were as follows: 90° pulse width = 19.3 μs, sweep width = 100 kHz, relaxation delay = 1 s, memory size = 64 K. 32T transients were accumulated for each spectrum. For all FIDs, line broadening of 0.3 Hz was applied and drift correction was performed prior to Fourier transformation.

Typical spectral parameters for ^{1}H NMR experiment were shown as: 16 scans and 4 dummy scans for each free induction decay, 32K for time domain points with a spectral width of 12.0 ppm, 90° pulse width of 9.0 μs, acquisition time of 2.7 s and relaxation delay of 1.0 s.

2.5. Effect of temperature on tea oil

20 Grams of TOL were divided into two equal parts, one of which was spiked with 0.02% *tert*-butylhydroquinone (TBHQ) and the other without any treatment. The samples were placed on a Rancimat instrument for heating and oil samples were taken every 4 h at 80, 100, 120, 140 °C for a total of 24 h,

2.6. Oven test, reducing autoxidized tea oil, determination of POV

40 Grams of tea oil were placed in a clean beaker, and the beaker was placed in a 63 ± 1 °C oven. The peroxide value was measured every 5 days, and the peak between δ-108 ~ δ-110 ppm on a ^{19}F NMR spectrum was measured and calculated.

Two grams of tea oil were dissolved at 63 °C in the oven after the 35 days in 30 mL solvent (acetic acid: $CHCl_3$ = 3:2) and reduced by KI for 3 min and then washed with 100 mL water 3 times to remove the acetic acid. Anhydrous sodium sulfate was used to dry moisture, while $CHCl_3$ in the solution was dried on a rotary evaporator.

The method of peroxide value measured by titration (Stuffins and Weatherall, 1945) was used according to the following formula:

$$\text{POV}\ \ (\text{g}/100\text{g}) = ((V - V_0) \times \text{c} \times 0.1269)/\text{m} \times 100$$

Where V is the volume of sodium thiosulfate standard solution consumed by the sample, V_0 is the volume of sodium thiosulfate standard solution consumed by blank sample, c is the concentration of sodium thiosulfate standard solution, m is the weight of the oil. The formula for the hydroperoxide value between δ-108 and δ-110 ppm measured by ^{19}F NMR is

$$\text{n}(\text{mmol}/100\text{g}) = ((A/A_i) \times N)/m \times 100$$

Where: A is the area of the peak between δ-108 and δ-110 ppm, Ai is the area of the peak of 4-tert-butylphenol, N is the millimolar amount of 4-tert-butylphenol, m is the weight of oil.

2.7. GC-MS experiments

The methyl esterification method for samples before GC-MS was carried out. 50 Milligrams of oil sample dissolved in 4 mL n-hexane (chromatographic grade) were put in a tube with stopper. Then 200 μL of 2 M KOH-CH_3OH were added and the tube was shaken vigorously for 1 minute for methyl esterification. Then it was left to stand for 5 min to allow solid-liquid separation. One gram of sodium hydrogen sulfate monohydrate was added to the solution to neutralize potassium hydroxide, followed by immediate analysis of the supernatant by GC-MS (ISO 5509:2000, ISO 5508:1990).

The GC-MS analyses were performed on a Shimadzu GC2010A (Kyoto, Japan) gas chromatography. A Rtx®-Wax capillary column (30 m length, 0.25 mm i.d., and 0.25 μm film consisting of cross-bond polyethylene glycol (Restek)) was used. The conditions of the GC-MS analysis were as follow: column temperature = 140 to 250 °C, rate = 4 °C/min, injection temperature = 220 °C, carrier gas = nitrogen, column flow (nitrogen flow rate) = 1.36 mL/min, injection volume = 0.5 μL, split ratio = 30:1.

Mass spectroscopy conditions: ion source temperature = 230 °C, interface temperature = 280 °C, ionization voltage = 0.2 kv.

The peak area normalization method was used to calculate the relative content.

2.8. Statistical analyses

All statistical analyses were determined using IBM SPSS 22.0. The experiments were performed in duplicate, and values were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. ^{19}F NMR analysis

Our lab previously found a novel method to detect the quality and adulteration of olive oil using the ^{19}F NMR technique (Zhou *et al*., 2015). The main principle of the method is based on the derivatization of the active hydroxy groups like diglycerides (DGs) and water with 4-fluorobenzoyl chloride, and the integration of the appropriate peaks in the ^{19}F NMR spectrum in the MestReNova. The deriving reagent and the intermediate products between 4-fluorobenzoyl chloride and the stock solution peaks were observed at δ-105.05 and δ-103.60 ppm, respectively. The peak at δ-107.45 ppm was attributed to 4-tert-butylphenol, the internal standards in this experiment. The peaks at δ-107.95 and δ-107.86 ppm belong to the α- and β-hydroxyl groups of 1,2-DG and 1,3-DG, respectively. The water peak was attributed to δ-110.42 ppm. The quality of tea oil was analyzed by calculating the characteristic peak appearing on the ^{19}F NMR spectrum.

3.2. Diglyceride content

The DG content is an important indicator of the quality of oil, and it can be detected by ^{19}F NMR. The contents of 1,2-DGs, 1,3-DGs, total diglycerides (TDGs) and the ratio D (1,2-DGs to TDGs) in tea oil and other plant oils are summarized in Table 1. After comparing five different kinds of tea oils, it was found that the fresh TOL had a high D ratio (0.77) and lower TDGs (1.45%). The content of TDGs (2.14%) and D ratio (0.27) of refined tea oil showed the opposite trend. Clearly, the D ratio is a significant indicator for judging the quality of oils because it usually occurs from the isomerization of 1,2-DGs to 1,3-DGs during oil storage and refining. A study by Vigli (Vigli *et al.,* 2003) showed that the D ratio of extra virgin olive

Table 1. Compositional parameters of tea oil and some other plant oils determined by ^{19}F NMR Spectroscopy.

Sample	1,3-DGs	1,2-DGs	Total DGs	D
TOL	0.33 ± 0.01	1.12 ± 0.10	1.45 ± 0.11	0.77 ± 0.01
TOS	0.63 ± 0.06	0.85 ± 0.15	1.49 ± 0.21	0.57 ± 0.02
tea oil from the supermarket 1	1.31 ± 0.07	0.54 ± 0.01	1.86 ± 0.06	0.29 ± 0.02
tea oil from the supermarket 2	1.30 ± 0.09	0.62 ± 0.02	1.92 ± 0.11	0.32 ± 0.01
Refined tea oil	1.57 ± 0.06	0.57 ± 0.01	2.14 ± 0.07	0.27 ± 0.01
Extra virgin olive oil	0.58 ± 0.10	0.68 ± 0.00	1.26 ± 0.10	0.54 ± 0.04
Soybean oil	0.24 ± 0.06	0.10 ± 0.02	0.34 ± 0.08	0.31 ± 0.01
Refined soybean oil	0.67 ± 0.03	0.34 ± 0.02	1.01 ± 0.01	0.34 ± 0.02
Rapeseed oil	2.02 ± 0.22	0.80 ± 0.13	2.82 ± 0.34	0.28 ± 0.01
Palm oil	3.01 ± 0.14	1.09 ± 0.07	4.10 ± 0.10	0.27 ± 0.03
Corn oil	2.16 ± 0.05	1.22 ± 0.11	3.38 ± 0.14	0.36 ± 0.02

[a] Data are expressed as mean ± standard deviation (n=2).
[b] TOL, tea oils extracted at low (room) temperature; TOS tea oils extracted by Soxhlet method.

oil (EVOO) freshly extracted from normal mature olives should be close to 1. Although the D ratio in all oil samples decreases with storage time, the closer the D ratio is to 1, the fresher the oil. When two kinds of tea oil based on extraction temperature were compared, TOL had a higher D ratio, indicating it has better quality and freshness. Usually, commercial tea oil is inevitably affected by temperature during the production process, especially the refining process. Table 1 shows the D ratio of TOL (0.77) > TOS (0.57) > tea oil from the supermarket (0.29, 0.32) > refined tea oil (0.27). This phenomenon is supported by the present results, that is, the quality of tea oil is affected by temperature and freshness. EVOO is a high quality cold-pressed plant oil because of its nutritional value and health benefits. The D ratio of TOL in our lab is much higher than all EVOO samples detected in our previous papers (Zhou *et al*., 2015; Jiang *et al*., 2018b) because all EVOO samples were imported from Spain and Italy to Shanghai at least 1.5 years after being prepared. In addition, the 1,3-DG content of all the other plant oils was higher than 1,2-DGs, and the D ratio D was about 0.3 which is in agreement with the study by Zhou *et al*. (2015). This indicates that the extraction temperature has a great influence on the quality and freshness of plant oil. The lower the extraction temperature is, the better the quality of the oil.

3.3. Moisture content

Moisture content is also a significant indicator for judging the quality of oils (Hu *et al*., 2008). In order to study the moisture content of tea oil by ^{19}F NMR, tea oil was placed in the oven at 63 °C until its mass was constant, then cooled to room temperature. 0, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1% distilled water was added to the tea oil, and shaken vigorously. ^{19}F NMR was subsequently used to detect the moisture content. The moisture contents in the tea oil were detected at 0, 0.008 ± 0.002, 0.015 ± 0.003, 0.042 ± 0.005, 0.055 ± 0.002, 0.060 ± 0.004, 0.059 ± 0.005%, respectively by ^{19}F NMR. The water contents detected by ^{19}F NMR agree well with those added. The detection of moisture content is usually done together with volatile matters in oils according to the official methods of AOAC (1997, method Cd 8b-90). But in some cases, volatile matters are desired flavors for some edible oils, such as EVOO, or Chinese traditional ground sesames oil. So ^{19}F NMR can directly detect the specific moisture contents in oils. As expected, the solubility of moisture in oil is very low. When the content of added moisture reaches the saturated state of oil dissolution, the excess water will layer from the oil quickly and easily. According to the results of the moisture content detected by ^{19}F NMR, when the content of added water reaches 0.06%, the moisture content becomes saturated in the oil. When over 0.06% water is added to oil, high deviation happens.

3.4. Adulteration of tea oil with refined tea oil

Refined tea oil has the same fatty acid composition of tea oil prepared at a low temperature, but it is much cheaper, so some unscrupulous merchants add refined tea oil to tea oil to make more profit. In addition, there are some minor nutritional components in tea oil that are lost during refining. Some researchers have suggested that the D ratio can be utilized as an index to distinguish different grades of the same kind of oil such as olive oil (Jiang *et al.,* 2018b). Hence, using ^{19}F NMR to detect adulteration in cold-extracted tea oil with refined tea oil is feasible.

In Figure 1, the contents of 1,3-DGs, TDGs increased and D ratio decreased with the adulteration level. The D ratio and the adulteration level showed good correlation (r = 0.9653). It can be seen that it is feasible to use ^{19}F NMR to detect the incorporation of refined tea oil into TOL. Therefore, the D ratio is a key parameter for determining whether TOL is adulterated with refined tea seed oil or not. The higher the D ratio is, the fresher the tea oil.

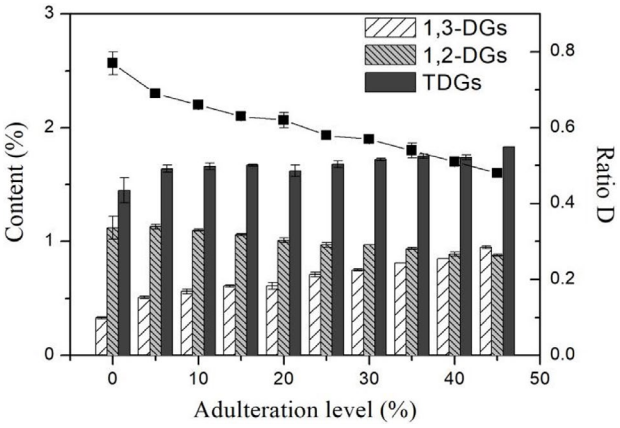

Figure 1. 1,2-DGs (%), 1,3-DGs (%), TDGs content (%), D ratio of the adulteration of tea oil with refined tea oil determined by ^{19}F NMR. Values are mean ± standard deviation (n=2).

3.5. Adulteration of tea oil with other plant oils

3.5.1. Determination of fatty acid composition

^{1}H NMR has been demonstrated as a method for determining the fatty acid composition of oil (Shi *et al.,* 2018). The contents in unsaturated fatty acids (oleic, linoleic and linolenic acids), saturated fatty acids (SFAs) and squalene in plant oils can be obtained by calculating the integral peak area on the ^{1}H NMR spectrum (Figure 2). The assignment of fatty acid signals has been established by Castejón *et al.,* (2014). According to the various signal intensities appearing in the ^{1}H NMR spectra, the peak at δ1.68 ppm in the ^{1}H NMR spectrum of tea oils belongs to squalene. In comparison with another study on the ^{1}H NMR spectrum of squalene, the peak was identified as methyl protons belonging to the CH_3-17 and CH_3-29 of squalene (Mannina *et al.,* 2009; Shi *et al.,* 2019).

According to Jiang *et al.* (2018a), all spectra of different plant oils have similar shape but different peak intensities. So according to the diversity of the fatty acid composition of plant oils, the detection of adulteration in tea oil can be carried out. Then the ^{1}H NMR technique was used to detect the adulteration of tea oil with other plant oils, and GC-MS served as a standard method.

3.5.2. Adulteration of tea oil with soybean oil

Soybean oil, which is much cheaper than TOL and may be used to adulterate TOL, has a different fatty acid composition compared to tea oil. As shown in Figure 3a, in addition to SFAs, the other three parameters, linolenic, linoleic, and oleic acids are all in good agreement with the adulteration level. With an increase in the adulteration level, the contents of linolenic and linoleic acids also increased, whereas the content of oleic acid decreased. GC-MS is a traditional method for detecting oleic acid content in adulterated TOL. The sensitivity and veracity of ^{1}H NMR in determining fatty acid composition can be compared to GC-MS. When the adulteration level reached 45%, the linolenic, linoleic and oleic acid contents according to ^{1}H NMR were 2.63, 28.12 and 52.17%. The content of linolenic, linoleic and oleic acid by GC-MS were 2.53, 29.10 and 54.85%, respectively. The differences in linolenic, linoleic and oleic acid values were 0.1, 0.98 and 2.68%, which showed about 3.95, 3.37 and 4.89% deviation from the data measured by GC-MS. It can be seen that the fatty acid contents measured by ^{1}H NMR were consistent with GC-MS. So ^{1}H NMR can accurately detect the adulteration in tea oil with soybean oil more rapidly than GC-MS.

Comparing the linear equation of linolenic, linoleic and oleic acids by ^{1}H NMR and GC-MS,

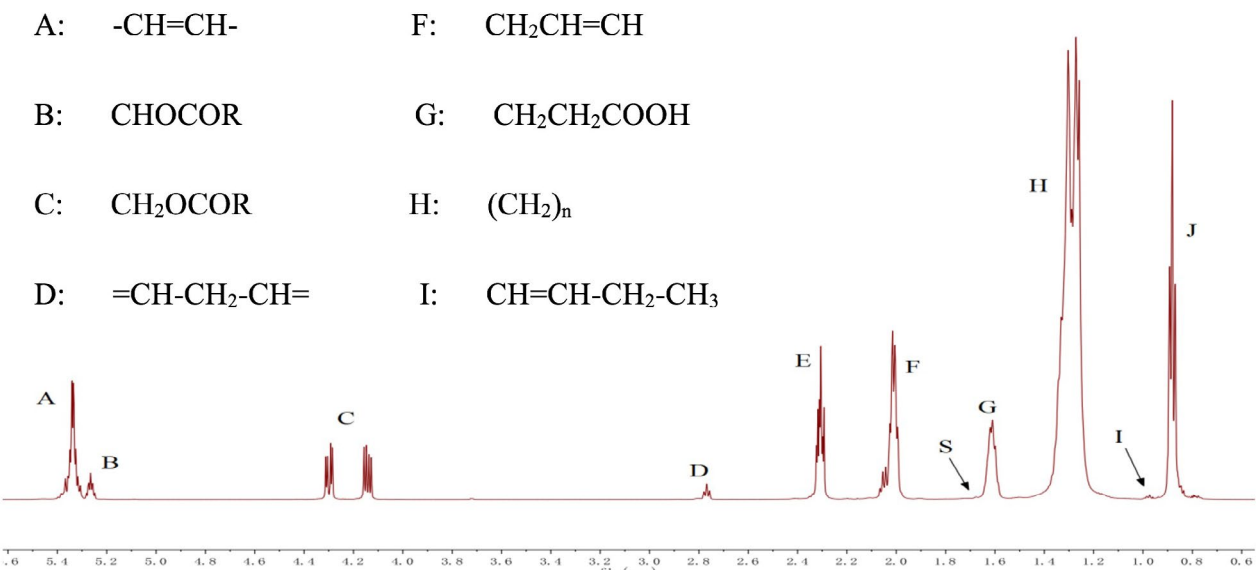

FIGURE 2. 600 MHz ^{1}H NMR spectrum of Camellia Oil.

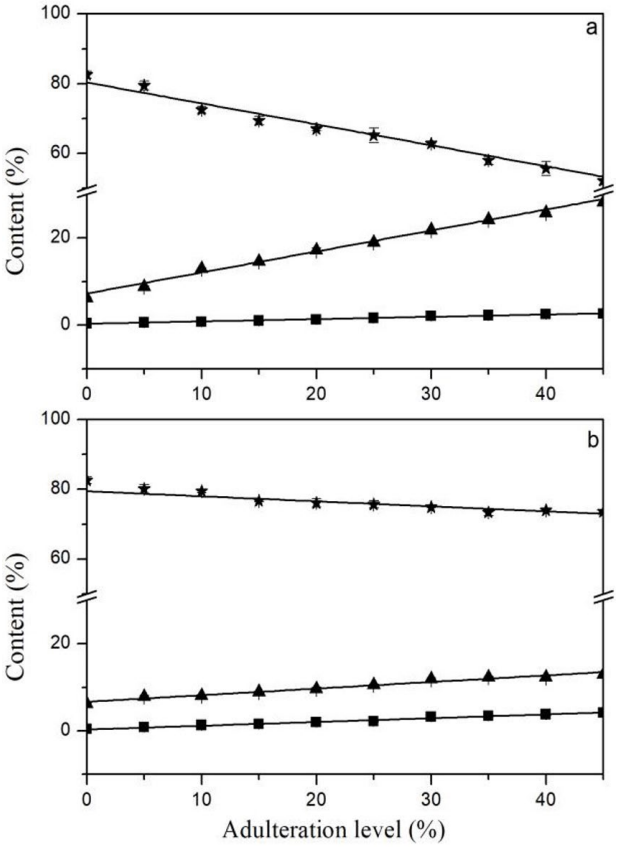

FIGURE 3. Four parameters (oleic, linoleic, linolenic acids and SFAs) in the adulteration of tea oil with other plant oils determined by ^{1}H NMR. (a) adulteration of tea oil with soybean oil, b) adulteration of tea oil with rapeseed oil) (■ linolenic acid, ▲ linoleic acid, ★ oleic acid). Values are mean ± standard deviation (n=2).

it can be found that the contents of linolenic and linoleic acid show the best relationship (R=0.9889) with the adulteration level. According to the linear equation (y = 0.0537x + 0.3111) for linoleic acid, the adulteration level can be accurately calculated.

3.5.3. Adulteration of tea oil with rapeseed oil

Rapeseed oil is an edible oil containing erucic, oleic, linoleic and linolenic acids, tocopherols and sterols (Lambelet *et al.,* 2003). Generally, there are about 14-19% oleic acid and 31-55% erucic acid in traditional rapeseed oil, and erucic acid is bad for the growth and development of the human body (Clement and Renner, 1977). The average content in high-oleic rapeseed oil is about 61%, which is similar to that in tea oil. Therefore, this kind of rapeseed oil was chosen for adulteration to detect the sensitivity of the ^{1}H NMR method. As shown in Figure 3b, the fatty acid composition (oleic, linoleic and linolenic acids), especially the content of oleic acid, are all in good relationship with the adulteration level. The various fatty acid contents were consistent with those detected by GC-MS. When the adulteration level reached 10%, the linolenic acid contents were 1.05 and 1.31% as detected by ^{1}H NMR and GC-MS, respectively. The biggest difference in the linolenic acid value was 0.26%, which was about a 24.62% deviation from the data measured by GC-MS. When the adulteration level reached 45%, the linolenic acid contents were 4.13 and 4.13% as detected by ^{1}H NMR and GC-MS, respectively. This shows no deviation from the data measured by GC-MS. Comparing linoleic oleic acids detected by ^{1}H NMR and GC-MS, the biggest differences in value were 1.65 and 2.29%, which were about 17.40 and 3.21% deviation from the data measured by GC-MS. So ^{1}H NMR can also detect the adulteration of tea oil with rapeseed oil more quickly than GC-MS.

Therefore, there is no significant difference between the two methods used for detecting the adulteration of tea oil. This phenomenon may be applied to determining the content of 18-chain fatty acids in soybean oil and rapeseed oil. Rapeseed oil has more 18 carbon chain fatty acids than soybean oil. The more 18 carbon chain fatty acid content present in oils, the more sensitive the ^{1}H NMR detection method is (Knothe *et al.*, 1996).

3.6. Effect of temperature on the quality of tea oil

The temperature during extraction, transportation and storage of tea oil has a certain impact on its quality. High temperatures can lead to a decline in the quality of tea oil, which often leads to the loss of some nutrients and speedy autoxidation, isomerization. High temperature can also cause an increase in peroxides in oils. All these influences can be detected by ^{19}F NMR.

3.6.1. The content of TDGs

Figure 4 shows the changes in DG contents and D ratio at 80, 100, 120 and 140 °C within 24 hours in the ^{19}F NMR spectrum when the S/N ratio of the peaks is 8. The D ratio steadily decreased for 16 h and then became stable as heating time increased under 80 °C. Whereas, the D ratio decreased sharply in the first 4 h and then remained stable as temperature increased. The results show that the isomerization of 1,2-

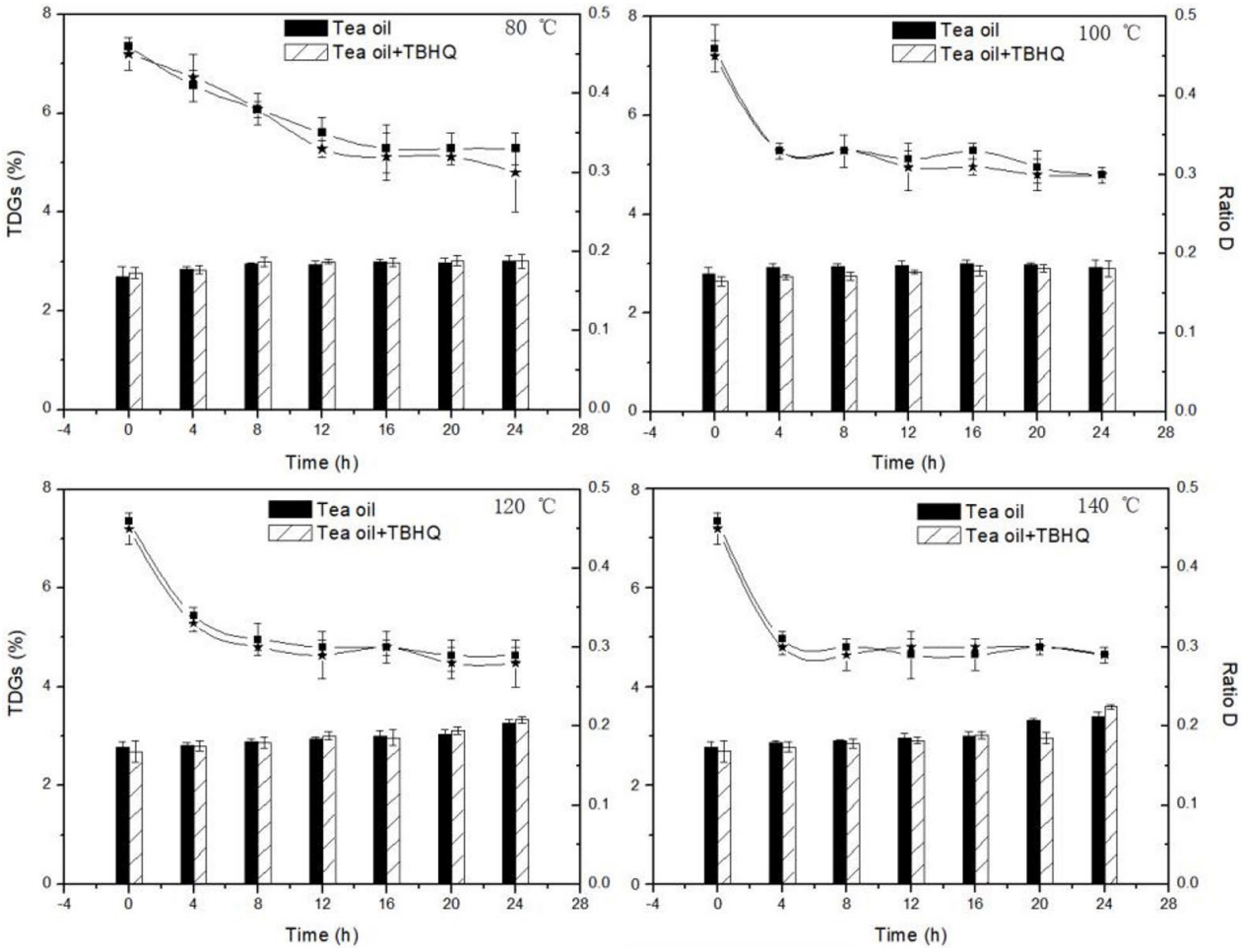

FIGURE 4. The tea oil content of total diglycerides (TDGs) (Columns) and D ratio (Curves) in tea oil and tea oil+tert-butylhydroquinone (TBHQ) heated at 80, 100, 120 and 140 ºC within 24 hours. (■: tea oil; ★: tea oil+TBHQ)

DGs to 1,3-DGs will accelerate when temperature rises. But antioxidants do not affect the speed of the isomerization.

3.6.2. The hydroperoxides

Characteristic peaks at δ-108.21 and δ-109.05 ppm were observed on the ^{19}F NMR spectrum. These peaks are reasoned to belong to the hydroperoxides due to four factors. Firstly, the peak areas of compounds at δ-108.21 and δ-109.05 ppm on the ^{19}F NMR spectrum increased markedly when the heating time increased to 140 °C, as shown in Figure 5. Secondly, the addition of TBHQ to the oil as a positive control greatly inhibited the increase in the peak areas at δ-108.21 and δ-109.05 ppm during 16 to 24 h because TBHQ is a very strong antioxidant and can effectively retard autoxidation of oils, and inhibit the increase in hydroperoxides. But oils commonly contain some natural antioxidants themselves, so oils with and without the addition of antioxidants can retard autoxidation during the first 12 h. But after 12 h, oil without antioxidant addition consumed its own natural antioxidants, and the amount of hydroperoxides increased more rapidly than the oil with the addition of antioxidants. All natural antioxidants, including the ones added, were consumed completely and the hydroperoxides reached maximum levels, as shown in Figure 6. Thirdly, POV (including hydroperoxides and other peroxides), as determined by classic titration is very closely correlated (R2 = 0.943), as shown in Figure 7. Fourthly, it seems that two peaks appear at -109.05 ppm (Figure 8a) after tea oil oxidized for 35 days. But after it was reduced by KI in the acetic acid solution,

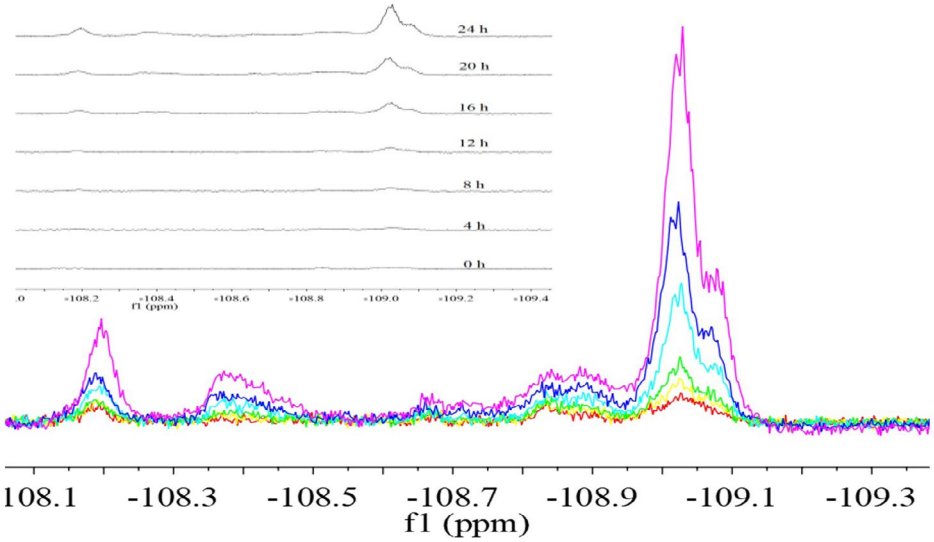

FIGURE 5. 600 MHz ^{19}F NMR spectrum of tea oil heated at 140 °C for 24 h.

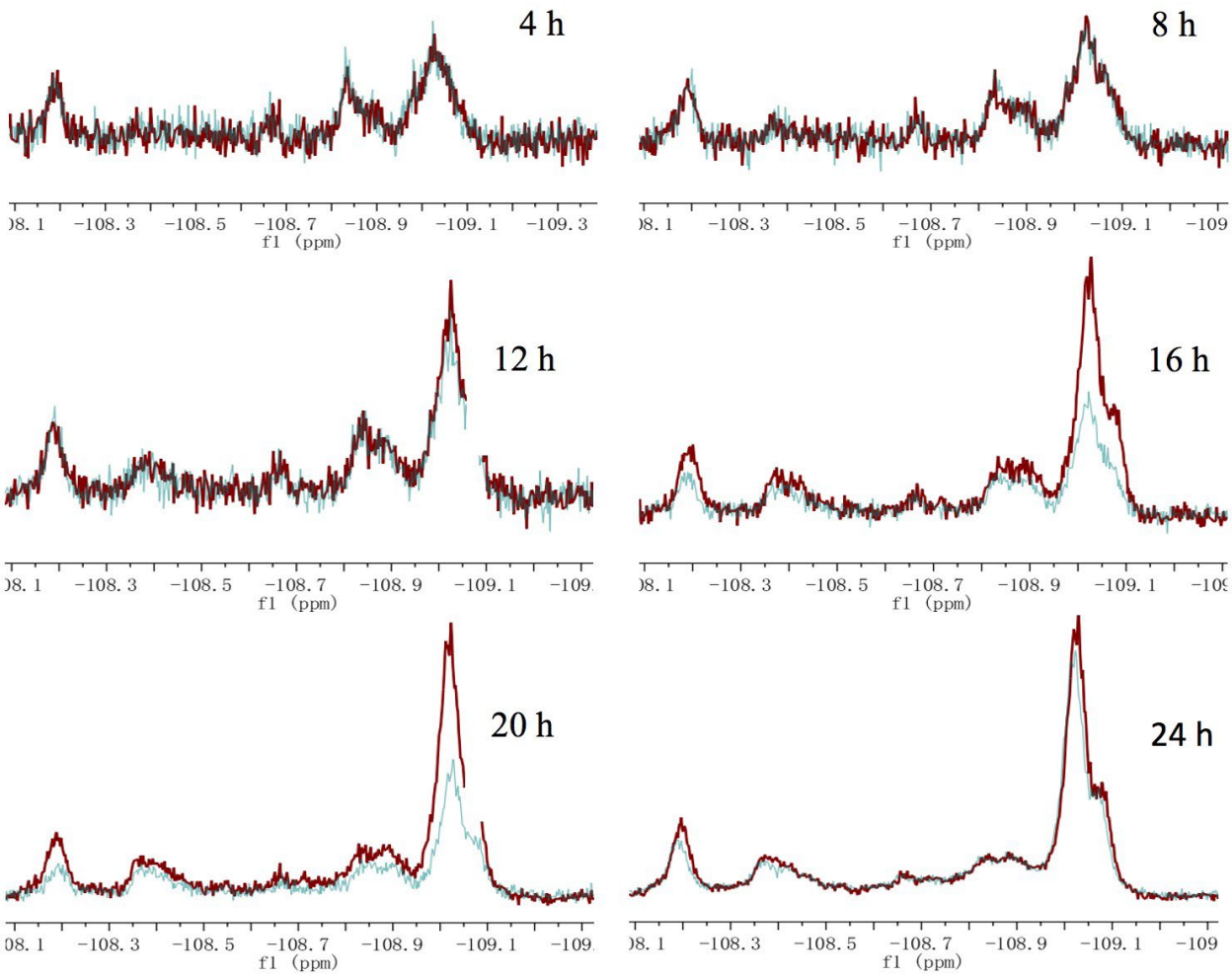

FIGURE 6. The difference between tea oil and tea oil+tert-butylhydroquinone (TBHQ) as detected by ^{19}F NMR during heating at 140 °C for 24 h. (brown: the spectrum of tea oil; blue: the spectrum of tea oil+TBHQ)

two peaks at -109.05 ppm disappeared completely and two new peaks appeared at -109.78 ppm (Figure 8b) and were much more distinguishable than those at -109.05 ppm. This means the hydroperoxides were reduced to related alcohols. The peaks at δ-108.21 ppm showed the same change, disappeared completely and a new peak appeared at δ-109.00 ppm. Figure 9 may explain why the ^{19}F NMR spectrum of autoxidized tea oil appeared in two peaks because the conjugated di-double bonds had a stronger de-shielding effect than the mono-double bond. Also, it is easily explained that two ^{19}F NMR peaks of alcohols are more distinguishable than hydroperoxide ones because the carbon chains of alcohols affect fluorine more than those of hydroperoxides because of one more oxygen atom between fluorine and carbon chains in hydroperoxides.

According to the GB/T11765-2018, the peroxide value for tea oil must be lower than 0.25 g/100g. On the basis of the linear equation y = 7.2077x + 0.1244 for peroxide value measured by titration and ^{19}F NMR, the limit of detection for the hydroperoxide

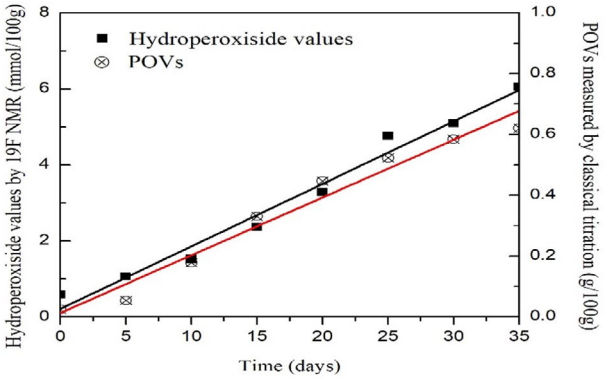

FIGURE 7. The relationship between the peroxide values (POVs) measured by titration and hydroperoxide values by the ^{19}F NMR technique. Values are mean ± standard deviation (n=2).

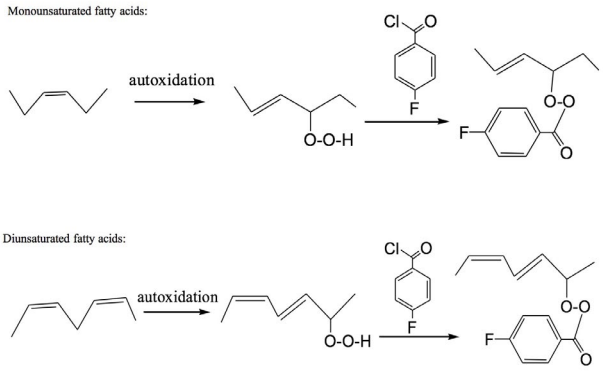

FIGURE 9. Autoxidation of unsaturated fatty acids and the reaction of hydroperoxides of unsaturated fatty acids with 4-fluorobenzoyl chloride.

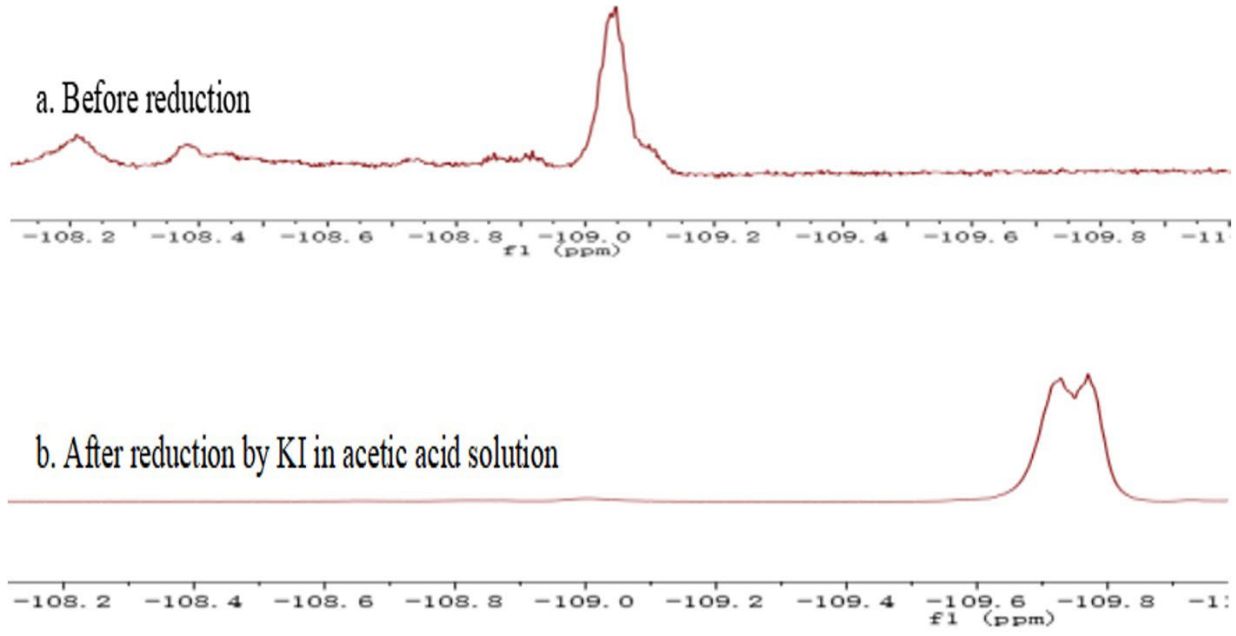

FIGURE 8. The ^{19}F NMR spectra of tea oil stored in the oven at 63 °C for 35 days. (a. Before reduction; b. After reduction by KI in acetic acid solution.)

value level was 1.93 mmol/100g, according to the ^{19}F NMR method. Over this hydroperoxide value (1.93 mmol/100g), the peroxide value for tea oil exceeds regulations and the quality of tea oil is not up to standard.

4. CONCLUSIONS

This is the first time that ^{19}F NMR is used to determine the quality of tea oil, especially the changes in the quality and autoxidation of tea oil. The results from this study demonstrated that ^{19}F NMR and ^{1}H NMR can effectively detect the adulteration of low-temperature extracted tea oil with refined tea oil and other low-price edible oils. The characteristic peaks appearing at δ-108.21 and δ-109.05 ppm on the ^{19}F NMR spectrum belonged to hydroperoxides, and they can be used as an indicator for the determination of the quality and oxidation of tea oil. This phenomenon has great significance for the quality determination of tea oil. Meanwhile, it was found that high temperatures affected the TGDs content and D ratio in tea oil in a short time, and then remained at a certain level within 24 h. In addition, ^{19}F NMR technology can detect long-term dynamic changes of the quality of tea oil. This method is a new, faster and more comprehensive method to determine the quality of tea oil.

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