# D$_2$O assisted FTIR spectroscopic analysis of moisture in edible oil

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A R T I C L E   I N F O

A B S T R A C T

D$_2$O-assisted moisture analysis of edible oils was investigated. The acetonitrile extract of the oil samples was split into two parts. The spectrum of one part was taken as is, another was recorded after addition of excess D$_2$O. Changes in spectral absorption of the H—O—H bending band (1600–1660 cm$^{-1}$) was used to calculate moisture in oil samples. To effectively depleting absorption of water in the acetonitrile extract, a 30-fold excess of D$_2$O is required. The typical OH-containing constituents in oil did not show significant interference on the H/D exchange. Validation experiments by using five oils with five levels of moisture spiked (50–1000 μg/g) suggested that the prediction tracked the spiked amounts well. The results of variance analysis indicate that there is no difference in terms of analytical methods and oil types used ($p < 0.001$). The D$_2$O method developed is generally applicable to the accurate analysis of moisture at trace levels (<100 μg/g) in edible oils.

## 1. Introduction

Water is one of the most important factors that determine the quality of edible oils. A water content of more than 0.2% (2000 μg/mL) in refined oil substantially enhances hydrolytic reactions and subsequently accelerates auto-oxidation and rancidity reaction, resulting in off-flavor and the reduction of smoke point of the oil. Karl Fischer (KF) titration is still considered to be the gold standard for water analytical methods (Panda et al., 2022). Recently, Fourier transform infrared (FTIR) spectroscopy has been found to have enormous potential as an alternative to the predominantly wet analytical techniques used to determine key quality parameters associated with edible fats and oils (Li et al., 2018).

Che Man and Mighani (Che Man and Mighani, 2000) applied FTIR spectroscopy to the determination of moisture in crude palm oil using the 3600–3200 cm$^{-1}$ O–H stretching band. Later, this research group also built a FTIR-attenuated total reflectance (ATR) method for determination of moisture in palm-oil-derived soaps (Rohman and Che Man, 2009). And more recent works carried a similar analysis on olive oil (Cerretani et al., 2010), transformer oil and lubricating oil (Sim and Jeffrey Kimura, 2019). These partial-least-squares-based methods, which are product-specific, require extensive calibration effort and inherently lack sensitivity because of the short path length associated with ATR accessories. By using transmission spectroscopy, one gains

sensitivity because of the longer path lengths available, in combination with differential spectroscopy, matrix effects can be minimized. Hence, an alternative FTIR-based transmission method using a solvent extraction procedure and the H—O—H bending band at 1680–1600 cm$^{-1}$ was developed (Al-Alawi et al., 2005; Al-Alawi et al., 2006) and automated (van de Voort et al., 2007). This method is based on the extraction of solvent-immiscible samples with dry acetonitrile (AN) and the measurement of the extracted moisture. It uses the differential spectrum obtained by subtracting the spectrum of dry AN from that of the sample's AN extract. It was found to work reasonably well for high-moisture samples (>500 μg/mL). Its reproducibility is limited to ±50 to 80 μg/mL. Similarly, van de Voort, Tavassoli-Kafrani, and Curtis (van de Voort et al., 2016) developed a simple moisture analysis method based on the measuring spectral absorption of CO$_2$ at 2335 cm$^{-1}$, which was produced from stoichiometric reaction of toluenesulfonyl isocyanate and water in oil. This method omitted moisture extraction, which facilitated its practical implication. However, solubility of carbon dioxide in oil mixture and disturbance of carbon dioxide from the air should be cautious. Hence, this method was only applied to oil samples with moisture of 100–1000 μg/mL. To precisely measure the trace moisture (<100 μg/mL) in oil samples by using FTIR coupled with solvent extraction procedure, co-extraction of substances other than water from samples must be concerned. Ng and Mintova (Ng and Mintova, 2011)
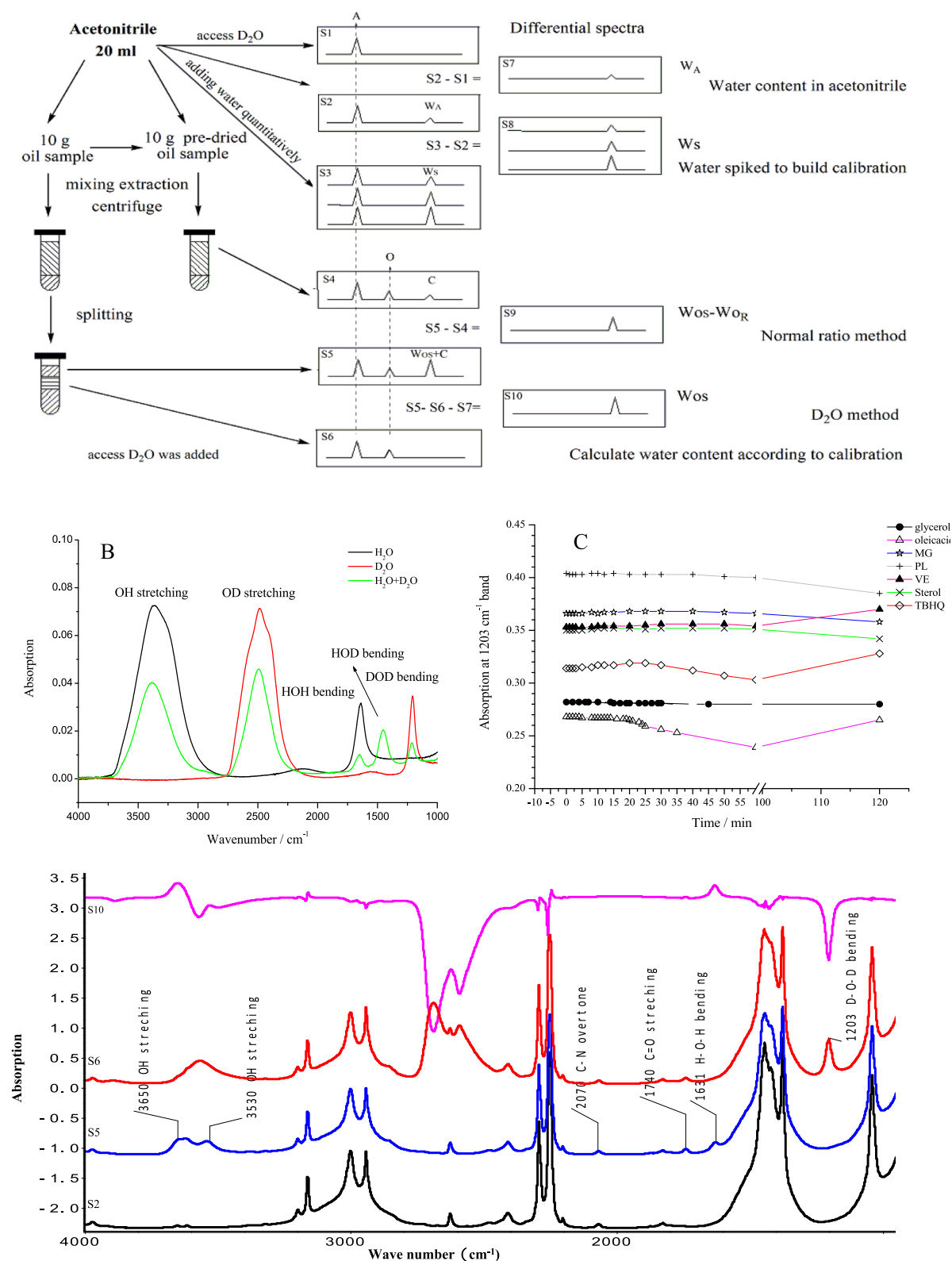
**Fig. 1.** General analytical protocol for the analysis of water content in edible oils **(A)**, FTIR spectroscopy of $H_2O$, $D_2O$ and their mixture at ratio of 1:1**(B)**, H/D exchange capacity of polar OH-containing components in edible oil **(C)**, and Spectra diagram on analysis of moisture in oil samples by $D_2O$ assisted FTIR spectroscopy combined with acetonitrile extraction using 1050 μm cell **(D)**. (S1) dry acetonitrile, (S5) acetonitrile oil extract, (S6) acetonitrile oil extract with addition of $D_2O$, and (10) differential spectrum obtained by S6-S5. *Note: A*, spectral contribution of dry acetonitrile; *O*, minor spectral contribution of the oil extracted by dry acetonitrile; *C,* spectral contribution of co-extracted substances by dry acetonitrile from oil; $W_A$ is residual water in dry acetonitrile, *Ws is* spectral contribution of spiked water in acetonitrile; $W$OR is the residual water in the dry oil, and $W$OS is water in the oil sample.

determined the moisture in lubricant using FTIR spectroscopy combined with dimethylsulfoxide extraction. The water content of fresh oil itself was not considered, and the potential dilution problem due to the miscibility of oils was neglected. Although the underlying absorption was improved to some degree by using a fresh DMSO extract of the oil as reference, its relative standard deviation (SD) was found to be high as approaching 5% in validation experiments (the level of moisture in samples was not clear).

As we noted in our latest study, the ultimate sensitivity, reproducibility, and accuracy of the method is dependent on spectroscopic dilution correction used to compensate for the miscibility of the oil samples in acetonitrile (van de Voort et al., 2011). They also depend on the use of gap-segment second-derivative spectra to minimize the associated possibility of spectral interference from absorption by the oils (Mossoba et al., 2011), as well as the correct selection of reference (Meng et al., 2012). To eliminate the potential interference at 1631 cm$^{-1}$ from co-extraction compound, i.e., matrix absorption, the solvent extract of the dry oil would be ideal reference. However, the oil sample extract and its dry oil extract are generally obtained separately, thus, these processes inevitably result in deviation. In addition, the residual moisture in dry oil often results in a decrease in analytical accuracy. To prepare this reference dry oil, relatively large volume of samples is required, however, the availability of samples is often insufficient. This limits the application of the dry solvent extract of the oil as reference and decreases the accuracy of FTIR analysis. Moreover, this approach is clearly impractical as a routine analytical procedure.

In this study, we took AN extract of oil samples with addition of D$_2$O as reference, developed a D$_2$O method for accurate determination of trace moisture in edible oil. The addition of excess D$_2$O can eliminate the bending absorption of H—O—H at 1631 cm$^{-1}$ while the matrix absorption caused by co-extraction left there. By subtracting this reference spectrum from spectrum of AN sample extract, the matrix absorption can be easily deducted, leading to more accurate determination of moisture. Moreover, D$_2$O dosage and interference by potential co-extracted components from oil with respect to D/H inter-exchange reaction were investigated. The analytical performances of D$_2$O method were subsequently assessed and compared with the normal ratio (NR) approach.

## 2. Materials and methods

### 2.1. Materials

Refined soybean oil, sunflower oil, rapeseed oil, camellia oil, and extra virgin olive oil, were obtained from a local market in Hangzhou, China. These oils were pre-dried with activated 4 Å molecular sieves (280 °C for 5 h) for a minimum of 2 weeks. Obtained dry oils were further passed through an activated silica gel column to any residual moisture, then kept in a desiccator to prevent moisture absorption. Dry AN and dioxane (certified 20–100 μg/mL H$_2$O, Sigma, St. Louis, MO, USA) were stored over activated sieves and dispensed from a re-pipetter (Hirschmann-Laborgerate, Germany), the inlet port of which was protected with drierite to prevent moisture ingress from the ambient air. Dry AN was used as the extraction solvent for moisture, while dioxane was used as a water-miscible solvent carrier to facilitate the distribution of defined amounts of water to the dry edible oils. Deuterium oxide (99.99% D) was also obtained from Sigma.

### 2.2. Instrumentation

The FTIR spectrometer used in this study was a Bomem Work IR (Bomem, Quebec City, Canada) equipped with a deuterated triglycine sulfate (DTGS) detector. It was purged with dry air using a Balston dryer (Balston, Lexington, MA, USA). The spectrometer was controlled by an IBM-compatible Pentium 150 MHz PC running proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, Canada). A 1000 mm CaF$_2$ transmission flow cell (International Crystal Laboratories, Garfield, NJ, USA) equipped with luer fittings was employed for sample handling. The cell inlet was connected to a 10 cm 18-gauge stainless-steel aspiration needle via a flexible silicone tubing, and the outlet line was connected to vacuum via a trap fitted with a valve to allow loading and emptying of the cell. All spectra were collected by co-adding 32 scans at a resolution of 4 cm$^{-1}$ and a gain of 1.0.

### 2.3. Calibration

Calibration was performed according to our method in a previous work (Meng et al., 2012). Moisture standards were prepared by the gravimetric addition of water to dry AN, and their spectra were taken and recorded as **Spectra 3(S3)**. The spectrum of dry acetonitrile was recorded as **Spectrum 2(S2)**. Do spectral subtraction by S3-S2, differential spectrum was obtained and recorded as **Spectra 8(S8)**. This differential spectrum was taken the second derivative of by the 5–5 gap-segment method, obtaining second derivative spectra. The absorption intensity of the band at 1631 cm$^{-1}$ (Absorption$_{1631}$, H—O—H bending) in this second-derivative spectra was measured, and its relation to the added moisture was simulated via linear regression, and a calibration equation H$_2$O content (μg/g) = 15.04 × Abs$_{1631}$ + 4.10 ($R^2$ = 0.9999, SD = 3.3 μg/g) was obtained. This calibration curve was used to calculate the moisture in the AN extract of samples, and this moisture content was converted into the moisture content of the original oil (μg/g).

### 2.4. Analytical protocol

Moisture analysis was carried out in a standardized manner using AN and oil at 2:1 (v/v) ratio (Fig. 1**A**). Ten milliliters of the oil sample or its "dried" form (Dry oil) was added to a tared 30 mL centrifuge tube, which was then weighed. Subsequently, 20 mL of dry AN was added from a pre-calibrated re-pipette, and the weights were recorded to ±0.0001 g. The tubes were capped, shaken on a vortex mixer for 60 s, and then centrifuged for 10 min at ~3300*g* to separate the oil and AN phase. The spectrum of AN dry oil extract was taken directly and marked as **Spectrum 4(S4)**. The AN extract of oil sample was divided into two parts, one part was aspirated into the transmission flow cell directly, and its spectrum was recorded as **Spectra 5(S5)**. For the other part, excess D$_2$O was added, and the spectra of the mixture were taken as reference spectrum and recorded as **Spectra 6(S6)**. Performing spectral subtraction of S6 (reference spectrum 2 produced from addition of D$_2$O) from S5 (spectrum of AN sample extract), obtained differential spectra were taken the second derivative of by the 5–5 gap-segment method, and the absorption of the band at 1631 cm$^{-1}$ (Absorption$_{1631}$, H—O—H bending) in the second-derivative spectra was measured, calculate moisture according to calibration equation. This D$_2$O assisted FTIR spectroscopic method for determination of moisture in oil was referred to as **D$_2$O method**. For **normal ratio (NR) method**, what is different compared with **D$_2$O method lies in the selection of reference spectrum, i.e.,** the spectrum of AN dry oil extract (**S4**) was taken as reference spectrum 1, hence differential spectrum was obtained by subtracting **S4** from **S5** (spectrum of AN sample extract)**.** The other operations were the same as D$_2$O method did. Worth noting is that all of the differential spectra used were not obtained by using a 1:1 spectral subtraction, but by using a ratio between the reference and the extract overtone bands measured at 2080–2060 cm$^{-1}$. This ratio was used to account for any displacement due to miscibility of the oil in AN or for any density changes (van de Voort et al., 2007).

### 2.5. Validation

The accuracy of the D$_2$O method developed was assessed by using five types of dry oils, each spiked with five levels of moisture, ranging from 0 to 1000 μg/mL. All the 25 oil samples were determined by using
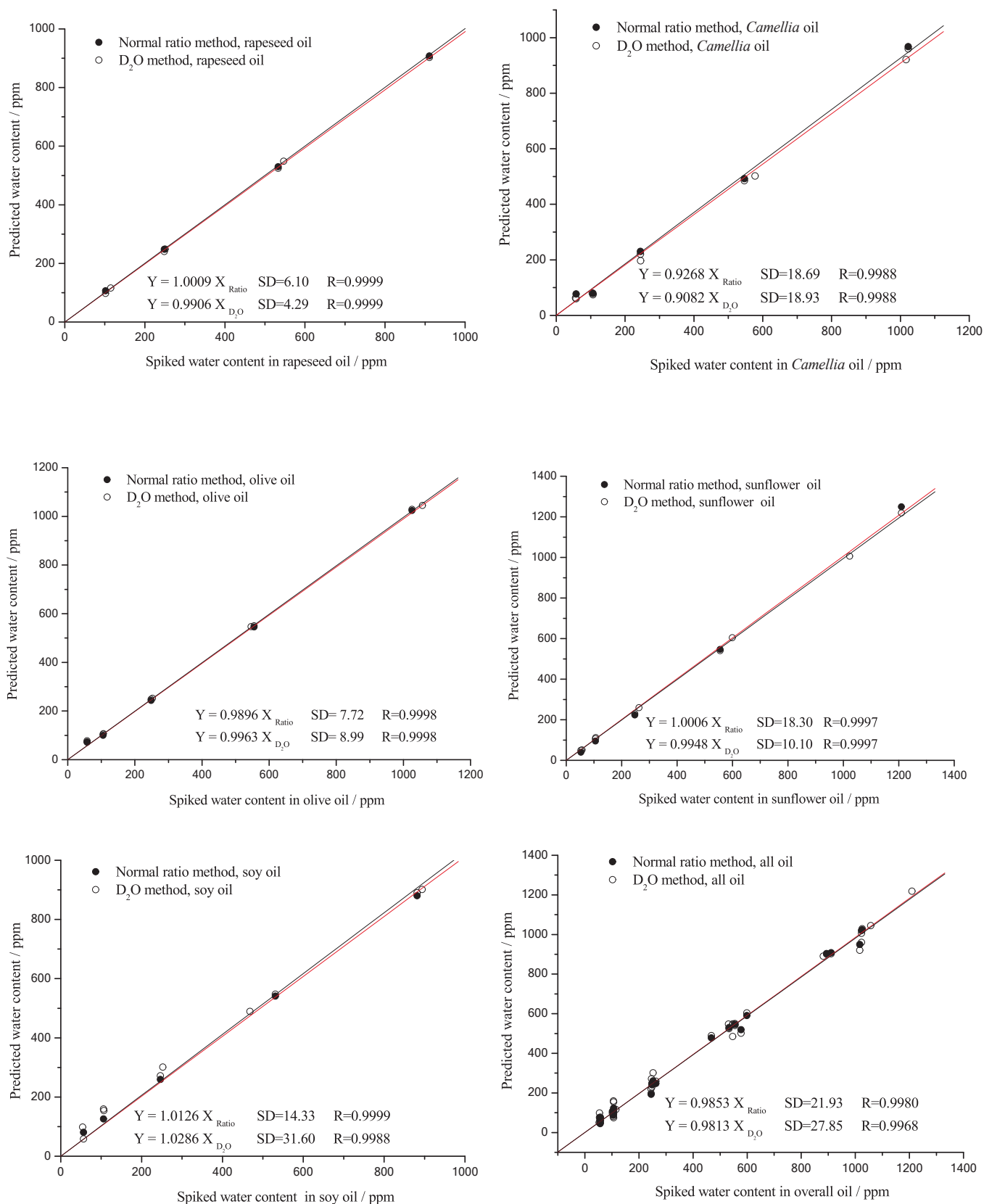
**Fig. 2.** Comparison between the added value -of water and predicted values based on D$_2$O method and normal ratio method.

**Table 1**
Predicted water content by $D_2O$ method under various amount of $D_2O$ added.

| Acetonitrile volume (mL) | Spiked content of water in acetonitrile (µg/mL) | Mass of water in 10 mL acetonitrile (mg) | $D_2O$ volume Added (µL) | $D_2O/H_2O$ mass ratio | DF | $D_2O$ | Predicted water content (µg/mL) | Relative standard deviation (%) |
|---|---|---|---|---|---|---|---|---|
| 10 | 1050 | 10.5 | 0 | 0 | | 8.535 | 141.0 | 86.57 |
| 10 | 1050 | 10.5 | 10 | 1 | 0.9987 | 38.121 | 475.4 | 54.72 |
| 10 | 1050 | 10.5 | 20 | 2 | 0.9982 | 48.772 | 646.8 | 38.40 |
| 10 | 1050 | 10.5 | 40 | 4 | 0.9979 | 58.472 | 803.0 | 23.52 |
| 10 | 1050 | 10.5 | 100 | 10 | 0.9931 | 67.3 | 950.1 | 9.51 |
| 10 | 1050 | 10.5 | 200 | 20 | 0.9855 | 70.787 | 1015.2 | 3.31 |
| 10 | 1050 | 10.5 | 300 | 30 | 0.9775 | 72.092 | 1046.1 | **0.37** |
| 10 | 1050 | 10.5 | 400 | 40 | 0.9716 | 72.55 | 1060.8 | 1.03 |
| 10 | 1050 | 10.5 | 600 | 60 | 0.9518 | 73.056 | 1094.3 | 4.22 |
| 10 | 1050 | 10.5 | 1000 | 100 | 0.9336 | 72.89 | 1115.4 | 6.23 |
| 10 | 1050 | 10.5 | 20,000 | 200 | 0.8820 | 70.432 | 1144.0 | 8.95 |

Note: Each sample was analyzed in duplicate. Results are expressed as means. Initial water content of the acetonitrile was 135.48 µg/mL.
DF, dilution factor for the addition of $D_2O$.

both NR method and $D_2O$ method. Each sample was determined in duplicate. The water concentrations predicted by two methods were compared, and compared with the amounts spiked. The resulting regression equations and statistics of the predicted moisture and added moisture were compared with respect to oil type and determination method.

### 2.6. Statistical analysis

Statistical analysis was applied by using SPSS 11.0 (Cohort Software, Minneapolis, MN, USA). The general model procedure, three-way analysis of variance (ANOVA), was applied to the water content of the edible oils to test statistical differences. Oil sample, analytical method, and water level of the extract were considered as sources of variation. Mean values of the water content from duplicates for each extract were considered in the latter statistical analysis. Significance was set at $p <$ 0.05.

## 3. Results and discussion

### General statement

A method that combines AN extraction with FTIR spectroscopy was developed to determine the water content of fats and oils by using the 1700–1600 cm$^{-1}$ H—O—H bending band in our previous studies (Meng et al., 2012). By using differential spectra, the ratios of any moisture present in the dry AN used to extract water from the sample, as well as other spectral contributions of the oil and its co-extracted substances that may be miscible with the AN, should be obtained to determine the moisture extracted from the sample. Therefore, the sensitivity, reproducibility, and accuracy of the method are ultimately dependent on the selection of the reference spectrum to a great degree. The use of dry acetonitrile as reference can simplify the analytical procedure and afford an acceptable analytical accuracy for oil samples with moisture levels of more than 500 µg/mg. However, this simplified ratio method cannot eliminate the spectral contribution of oils and unknown polar constituents co-extracted by AN. These contributions were called matrix absorption. To cope with this matrix absorption, using the AN extract from the corresponding dry oils as reference is fitting (NR method). This NR method is applicable to samples with very low moisture levels (~100 µg/mg) and showed higher accuracy and lower limit of detection as evidenced by our previous study (Meng et al., 2012). Nevertheless, the analytical results are slightly lower than the actual water content of the samples due to overcompensation for residual water in the dry oil. In addition, for the NR method, each sample to be tested require extracting twice to prepare the differential spectra for moisture determination, i.e both raw oil sample and its dried sample were required to be subject to extraction by AN. As a routine quantitative analytical tool, it is tedious

and clearly impractical. Hence, we here introduced a new reference spectrum based on $D_2O$-treated AN extract of the oils. Addition of excess $D_2O$ eliminated the H—O—H bending absorption at 1631 cm$^{-1}$ in the spectra of the AN sample extract. While the potential spectral contributions from co-extract compound other than water, including acetonitrile, minor oil, and co-extracted polar substances, also called matrix absorption, still remained there. By using this reference spectrum, matrix absorption could be deducted completely, which would effectively enhance the analytical performances. The absolute water content of the dry acetonitrile can also be determined.

Fig. 1**A** illustrates the basic protocols used in the study. Firstly, the spectrum of acetonitrile (AN) was directly recorded as Spectrum 2(S2), after addition of excess $D_2O$ to AN, the spectrum of obtained mixture was recorded as Spectrum 1(S1), the differential spectrum obtained by S2-S1, Spectrum7(S7), was used for calculation of residual moisture in dry AN. The dry AN was gravimetrically spiked with various level of water, then their spectra were taken and recorded as Spectra 3(S3). S3 minus S2 produced Spectra 8(S8), which was used to build the calibration curve. The spectrum of AN extract of the dried sample oil was recorded as Spectrum 4 (S4, reference 1). The AN oil sample extract was split into two parts. FTIR spectra of one part were directly recorded as Spectra 5 (S5). Another part was treated with excess $D_2O$, then the spectra of mixture were taken as Spectra 6 (S6, reference 2). The differential spectra **S9** and **S10**, obtained by making spectral subtraction from **S5** against **S4** (reference 1) or **S6** (reference 2), were used for water content calculation. The detailed sources for spectral absorption at 1631cm$^{-1}$ reflecting water content in each spectrum were parsed as following.

*For solvent acetonitrile:*
**Spectrum 1(S1):** Acetonitrile with added with excess $D_2O$
Absorption at 1631 cm$^{-1}$ = A (1)
**Spectrum 2(S2):** Dry acetonitrile
Absorption at 1631 cm$^{-1}$ = A + $W_A$ (2)
**Spectrum 7 (S7):** S2 − S1
Absorption at 1631 cm$^{-1}$ = $W_A$ Calculation of residual water in dry acetonitrile (3)
*For calibration:*
**Spectra 3(S3):** Acetonitrile gravimetrically spiked with various level of moisture
Absorption at 1631 cm$^{-1}$ = A + $W_A$ + $W_s$ (4)
**Spectra 8 (S8):** S3- S2
Absorption at 1631 cm$^{-1}$ = $W_s$ Built calibration curve (5)

**For determination of water in oil samples:**

**Spectra 4(S4):** Acetonitrile extract of the dried oil samples
Absorption at 1631 cm$^{-1}$ = A + $W_A$ + O + $W_{OR}$ + C (6)
**Spectra 5(S5):** Acetonitrile extract of oil samples

**Table 2**
Water content of the different oils as obtained by various analytical methods.

| Oil sample | Water concentration level | Analytical method (μg/mL) | | |
|---|---|---|---|---|
| | | Spiked moisture | Normal ratio Method | $D_2O$ method |
| 1- Olive oil | 1 | 57.49 | 73.04 | 77.39 |
| | | 57.68 | 66.83 | 71.47 |
| | 2 | 105.27 | 99.66 | 104.54 |
| | | 106.33 | 101.51 | 106.58 |
| | 3 | 248.41 | 243.29 | 248.12 |
| | | 252.77 | 247.64 | 252.25 |
| | 4 | 555.24 | 545.07 | 549.98 |
| | | 546.43 | 541.54 | 546.59 |
| | 5 | 1025.68 | 1023.83 | 1028.59 |
| | | 1056.95 | 1039.96 | 1044.96 |
| | Average | 401.23 | 398.24 | 403.05 |
| 2. Rapeseed oil | 1 | – | – | – |
| | 2 | 102.1 | 106.7 | 97.35 |
| | | 115.1 | 125.84 | 115.82 |
| | 3 | 248.68 | 248.09 | 240.21 |
| | | 251.04 | 256.17 | 248.45 |
| | 4 | 533.08 | 529.44 | 524.5 |
| | | 547.1 | 553.26 | 548.46 |
| | 5 | 910.8 | 907.63 | 902.33 |
| | Average | 386.84 | 389.59 | 382.45 |
| 3-Camellia oil | 1 | 58.04 | 77.05 | 60.11 |
| | | 56.38 | 47.72 | 62.72 |
| | 2 | 106.65 | 78.89 | 74.3 |
| | | 104.94 | 86.73 | 80.49 |
| | 3 | 244.74 | 230.54 | 220.49 |
| | | 245.37 | 193.2 | 196.86 |
| | 4 | 546.74 | 492.3 | 484.95 |
| | | 577.94 | 519.41 | 501.79 |
| | 5 | 1022.89 | 967.25 | 959.94 |
| | | 1016.45 | 949.07 | 920.69 |
| | Average | 398.01 | 364.22 | 356.23 |
| 4-Sunflower oil | 1 | 53.04 | 39.85 | 49.71 |
| | | 57.03 | 44.3 | 51.23 |
| | 2 | 105.71 | 94.9 | 105.95 |
| | | 106.76 | 94.92 | 111.59 |
| | 3 | 247.57 | 222.98 | 230.04 |
| | | 262.67 | 247.24 | 259.25 |
| | 4 | 555.24 | 546.59 | 540.25 |
| | | 599.22 | 590.57 | 603.93 |
| | 5 | 1209.74 | 1249.08 | 1218.71 |
| | | 1022.95 | 1020.01 | 1005.76 |
| | Average | 421.99 | 415.04 | 417.64 |
| 5-Soy oil | 1 | 56.18 | 80.77 | 58.24 |
| | | 54.39 | 75.44 | 98.32 |
| | 2 | 105.9 | 126 | 159.16 |
| | | 106.83 | 122.74 | 153.92 |
| | 3 | 246.4 | 259.34 | 271.67 |
| | | 252.62 | 260.84 | 301.27 |
| | 4 | 531.1 | 540.6 | 547.58 |
| | | 468.17 | 477.25 | 489.01 |
| | 5 | 881.74 | 880.36 | 890.12 |
| | | 894.1 | 903.55 | 901.12 |
| | Average | 359.74 | 372.69 | 387.04 |
| Overall oil | Average | 394.00 | 387.85 | 389.72 |

Absorption at 1631 cm$^{-1}$ = A + $W_A$ + O + Wos + C (7)

**Spectra 6**(S6): Acetonitrile extract of oil samples with added excess $D_2O$

Absorption at 1631 cm$^{-1}$ = A + O + C (8)

**Spectra 9**(S9): S5 − S4 Calculation for the content of water in the oil sample, **NR method**

Absorption at 1631 cm$^{-1}$ = Wos − $W_{OR}$ (9)

**Spectra 10**(S10): S5 − S6 − S7 Calculation for the content of water in the oil sample, **D$_2$O method**

Absorption at 1631 cm$^{-1}$ = WoS (10)

Where:

A = Spectral contribution of dry acetonitrile.

O = Minor spectral contribution of the oil extracted with dry acetonitrile.

**Table 3**
Results of three-way analysis of variance of the water content of edible oils as determined by various methods.

| Source | Type III sum of squares | df | Mean Square | *F* value | Significant level |
|---|---|---|---|---|---|
| Corrected Model | 16152057.3 (a) | 71 | 227493.8 | 181.7 | 0.000 |
| Intercept | 20459919.7 | 1 | 20459919.7 | 16342.3 | 0.000 |
| Water conc (WC) | 15229598.4 | 4 | 3807399.6 | 3041.2 | 0.000 |
| Oil type (OT) | 52070.9 | 4 | 13017.7 | 10.4 | 0.000 |
| Analytical method (AM) | 971.2 | 2 | 485.6 | 0.39 | 0.680 |
| WC × OT | 177575.2 | 15 | 11838.3 | 9.4 | 0.000 |
| WC × AM | 8319.0 | 8 | 1039.9 | 0.83 | 0.579 |
| OT × AM | 12881.8 | 8 | 1610.2 | 1.29 | 0.265 |
| WC × OT × AM | 16923.9 | 30 | 564.1 | 0.45 | 0.991 |
| Error | 86385.4 | 69 | 1251.96 | | |
| Total | 37665125.1 | 141 | | | |
| Corrected Total | 16238442.7 | 140 | | | |

$^a$ $R^2$ = 0.995 (adjusted $R^2$ = 0.989); *df*, degrees of freedom; *F*, Snedecor's statistic function; *p*, probability.

C = Spectral contribution of substances co-extracted with water by dry acetonitrile.

$W_A$ = Spectral contribution of residual water in dry acetonitrile.

$W_{OR}$ = Spectral contribution of residual water in the dry oil.

Ws = Spectral contribution of spiked water in acetonitrile.

$W_{OS}$ = Spectral contribution of water in the oil sample.

Clearly, the results from equation (8) more precisely reflect the real content of moisture in oil sample, indicating that the $D_2O$ method based on differential spectrum **S10** should have the highest analytical accuracy. NR method based on differential spectrum **S9,** reflected by Equation (7), has slight bias due to overcompensation of underlying absorption by residual water in the dried oil. In addition, for oil samples determination by NR method, preparation and extraction of dried oil samples are additionally required. While $D_2O$ method omitting this tedious requirement, and should exhibit more accurate analytical performances compared with the NR method.

### 3.2. The $D_2O$/$H_2O$ exchange reaction in solution and the infrared spectroscopy of mixture

The $D_2O$/$H_2O$ exchange reaction eliminated the bending absorption of water in AN oil extract, which make accurate compensation of matrix absorption possible. This is the premise of the $D_2O$ method developed to accurately determine the trace moisture in edible oil. Hence, the D/H exchange reaction in AN and the related FTIR absorption of reaction products are to be investigated. To simplify the analysis, $D_2O$/$H_2O$ exchange reaction in water solution was first examined. The FTIR spectra of $D_2O$, $H_2O$, and the $D_2O$/$H_2O$ mixture at ratio of 1:1 are taken and shown in Fig. 1B. The spectrum of $H_2O$ contained three bands, a narrow band at 1631 cm$^{-1}$ identified as H—O—H bending absorption, a prominent, unresolved multiple bands centered at approximately 3350 cm$^{-1}$ assigned to O—H stretching and a weak, broad band centered at approximately 2134 cm$^{-1}$. While for the $D_2O$ spectrum, the unresolved double band centered at 3350 cm$^{-1}$ of O—H stretching became a slightly better resolved pair of peaks centered at 2490 cm$^{-1}$ (O–D stretching in $D_2O$). The H—O—H band centered at 1631 cm$^{-1}$ shifted to 1205 cm$^{-1}$ replaced by D–O–D bending. Each of these bands for the $D_2O$/$H_2O$ mixtures clearly presents a single band; in each case, the wavenumber shifted significantly higher as the mole fraction of $H_2O$ increased (Lappi et al., 2004). In other words, the isolated O—H and O–D oscillator frequencies appear resolved at wavenumbers higher than those of the

**Table 4**

Results of multiple comparisons among various groups for each factor (µg/mL).

| Water concentration level | N | Water content (µg/mL) | Oil type | N | Water content (µg/mL) | Analytical method | N | Water content (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| 1 (50 µg/mL) | 24 | 60.64 [a] | 3 (Camelia) | 30 | 372.82[a] | $D_2O$ method | 47 | 389.72[a] |
| 2 (100 µg/mL) | 30 | 109.69[b] | 5 (Soy) | 30 | 373.16a | Normal ratio method | 47 | 387.85[a] |
| 3 (250 µg/mL) | 30 | 248.67[c] | 2 (Rapeseed) | 21 | 386.29[a] | Spiking value | 47 | 394.00[a] |
| 4 (500 µg/mL) | 30 | 534.94[d] | 1-(Olive) | 30 | 400.84[ab] | | | |
| 5 (1000 µg/mL) | 27 | 989.28[e] | 4-(Sunflower) | 30 | 418.23[b] | Significant | | 0.799 |

Note, Student–Newman–Keuls α = 0.05, For any pairwise comparisons in the same row, the same letter indicates no difference between the two groups, different letters marked suggest statistical differences among various groups.

symmetric and asymmetric normal modes. It is quite interesting that a new band centered at ~1450 cm$^{-1}$, which can be attributed to HOD, appeared in the bending region of the spectrum, but not in the stretching region.

The high-wavenumber band that peaked at 3350 cm$^{-1}$ has been assigned to the symmetric and asymmetric stretches in water (dos Santos et al., 2022). In most matrixes or at high concentrations of water, the two O—H stretching absorptions merge into a single broad band because of hydrogen bonding (Buckingham et al., 2008). Acetonitrile, a non-hydrogen-bonding aprotic polar solvent, impedes water association via hydrogen bonding up to concentrations of ~2000 µg/mL. Beyond this concentration, the separation deteriorates as hydrogen bonding forces overtake acetonitrile solvation. In acetonitrile solution (Fig. 1C, 1D and Fig. 2), this unresolved O—H stretching band shifts to 3536 cm$^{-1}$,if the water content is not high enough (generally up to 2000 µg/mL) to form hydrogen bonds, then the peak splits into two peaks centered at 3654 and 3618 cm$^{-1}$. Our previous water analysis method using FTIR spectroscopy was based on these OH stretching bands because of its high sensitivity with differential spectra. However, the OH stretching bands are prone to interference by compounds containing active hydroxyl. Later, the H—O—H bending band (centered at 1631 cm$^{-1}$) was found to be more fitting because it is unique to water molecules. Quantitation based on band at 1631 cm$^{-1}$ showed higher sensitivity due to minimization in the underlying absorption caused by the second-derivation of differential spectra. In AN solution, the related D–O–D, H—O—D bending absorption were located at 1205 and 1490 cm$^{-1}$. Among them, H—O—D bending overlapped with acetonitrile.

### 3.3. Reactivity of the potential hydroxyl group-containing constituents from oil against $D_2O$

The key consideration related to the suitability of acetonitrile is its aprotic nature, which ensures that it does not undergo deuterium exchange and provides a vehicle for moisture extraction and for facilitating the focus on deuterium exchange with water, the target molecule. In AN oil extract, besides $H_2O$, oil and its minor constituents could be coextracted. Hence, these coextracted constituents maybe react with $D_2O$. If this undesired hydrogen–deuterium exchange reaction of coextracted constituents happened, albeit more slowly, it would directly produce $H_2O$ one hand. The other hand, this reaction would also consume part of the $D_2O$, thus altering $D_2O/H_2O$ reaction equilibrium, leading to uncomplete omitting of H—O—H bending absorption. Both aspects all possibly resulted in significant analytical deviation. Of all potentially interfering substances coextracted from oil, free fatty acids (FFAs), alkyl alcohols, glycerol, partial glycerides, phospholipid, tocopherol, and the antioxidant *tert*-butylhydroquinone (TBHQ), etc. OH-containing compounds, are particularly deserved concern. To evaluate the H/D exchange activity of these potential interfering constituents, acetonitrile was separately spiked with glycerol, oleyl alcohol, oleic acid, glycerol monolaurate (monoglyceride), tocopherol, soy lecithin, and TBHQ to mimic their normal levels in the acetonitrile oil extract. A defined excess of $D_2O$ was then added and mixed. The FTIR spectra of the mixture were recorded at fixed time intervals. H—O—D (1450 cm$^{-1}$) and D–O–D (1205 cm$^{-1}$) bending absorptions were measured, and the absorbance at 1205 cm$^{-1}$ was plotted against holding time (Fig. 1C). Because the H—O—D bending band overlaps with the C—H vibration of acetonitrile, only the $D_2O$ bending band was monitored. Apparently, glycerol was the most stable. It hardly reacted with $D_2O$ for as long as 2 h. The next most stable components were lecithin, monoglyceride, tocopherol, and phytosterol. After 60 min kept, their absorption at 1205 cm$^{-1}$ band showed slight fluctuation, but it recovered in 120 min. Oleic acid and TBHQ showed relative high H/D exchange activity, but they still remained inert to $D_2O$ within previous 30 min. This stable period was long enough to guaranteed completing the acquisition of the deuterated reference spectrum. In addition, the content of FFA and TBHQ in edible oil was minor and their extraction ratios by acetonitrile were not high, so their potential interferences were negligible. Our current data and later validation results all suggested that most of the OH-containing constituents in edible oil are stable in H/D exchange. Hence, specific concerns on their potential interference with water analysis are not necessary.

### 3.4. The amount of $D_2O$ required by H/D interchange reaction to completely eliminate the H—O—H bending absorption

Apparently, previous $D_2O/H_2O$ interchange reaction laid the foundation for determination of water in oil by $D_2O$ method. Whether the $H_2O$ in AN sample extract could be exchanged completely or not determines the analytical accuracy of $D_2O$ method developed. To achieve higher accuracy, the amount of $D_2O$ must be sufficiently excessive. How much $D_2O$ added is necessary? To answer this question, AN (with water content of 1050 µg/mL determined by KF method) was analyzed with $D_2O$ method developed. The amount of $D_2O$ added was varied from 1- to 200-fold of that of $H_2O$ in the AN to be analyzed. The results and relative standard deviation are listed in Table 1. Results suggested that the optimal $D_2O$ dosage is around 20- to 60-fold that of water in AN. Under these cases, the relative standard deviation (RSD) falls within ~5%. Especially for 30-fold excess $D_2O$ case, the RSD produced was <0.5%. This agrees well with the calculated value. It is reported that the equilibrium constant for the $H_2O/D_2O$ interexchange reaction at 25 °C is 3.76 (Duplan et al., 2005). Theoretical calculation according to this equilibrium constant of 3.76, to achieve 99.0–99.9% conversion of H—O—H to H—O—D, the amount of $D_2O$ required should be 27.01- to 27.5-fold that of $H_2O$ in reactant. Hence, a 30-fold excess of $D_2O$ is suitable with respect to accurate determination of trace moisture in oil by $D_2O$ method.

Research and results above laid the theoretical foundation for $D_2O$ method to determination of trace moisture in oil. The typical spectra and spectral process during moisture analysis with $D_2O$ methos are illustrated as Fig. 1D. S1 present the spectrum of solvent AN, with no obvious absorption peak at 1631 cm$^{-1}$ was found, indicated that the AN was dry and anhydrous. After extracted the oil sample, the spectra of the AN sample extract are taken as S5. The band at 2060–2080 cm$^{-1}$ was assigned to C—N stretching of AN (Dineshkumar et al., 2022). The finding on little and obvious peaks at 1740 cm$^{-1}$ and 1631 cm$^{-1}$, respectively assigned to C=O and H—O—H bending absorption, suggests that not only water in oil sample were extracted, but also oil and other unknow polar components were co-extracted into AN extract.

After addition of $D_2O$ **(S6)**, $H_2O$ in ACN extract was converted to HOD, this was supported by disappear of H—O—H bending absorption peak at 1631 cm$^{-1}$ (non H—O—H bending absorption from coextracted component at 1631 cm$^{-1}$, i.e matrix absorption should be left there), and appear of HOD bending peak at 1450 cm$^{-1}$(this HOD bending absorption overlapped by C—H overtone band of acetonitrile and oil) and OD stretching peaks at 2690 cm$^{-1}$ and 2450 cm$^{-1}$. In addition, D–O–D bending vibrations at 1205 cm$^{-1}$ is still obvious, indicating that $D_2O$ is a sufficient excess. Making spectra subtraction of **S5-S6** based on the band at 2060–2080 cm$^{-1}$, the differential spectrum **S10** was obtained. Apparently, peaks related with AN, oil were all deducted. Hence, we have reason to believe that the spectral contribution from acetonitrile and co-extracted substances at 1631 cm$^{-1}$ was removed completely. After matrix absorption removal, remained absorption at 1631 cm$^{-1}$ in S10 was ascribed to the real water extracted from the oil sample and the water in dry acetonitrile itself. The latter could be deducted easily by subtracting **S7**. The sample extract and reference for $D_2O$ method were two parts split from the same extract, while those for the NR method were extracted separately, hence the $D_2O$ method should show higher precision as compared with the NR method. All this entire spectral process supported the analytical accuracy of $D_2O$ method, what is left is to subject to validation with actual oil samples.

### 3.5. Validation of $D_2O$ method in comparison with NR method for water content analysis of edible oils

To validate the effectiveness of the $D_2O$ method we developed, five type of oils (rapeseed, sunflower, camelia, olive and soy) were pre-dried over molecular sieves, and each was spiked with moisture at five levels (approximately 50, 100, 250, 500, and 1000 μg/mL) and analyzed in duplicate according to the protocol depicted in Fig. 1A. All these oil samples were also subjected to moisture analysis with NR method. The standard spiking concentrations and predictions are listed in Table 2. The predictions from the NR and $D_2O$ methods were correlated with the spiked amounts for each oil and results were shown in Fig. 2. Clearly, all of the predictions showed good linear correlation with the spiked amounts regardless of oil type and analytical method, with coefficients of 0.96–1.006 and SDs ranging from 4.43 to 18.23. Rapeseed, olive, sunflower, and soy oil showed ideal results; while camelia oil exhibited a slightly higher bias, with coefficients of 0.94 and 0.92 for the NR and $D_2O$ methods, respectively. Global analyses of all the oil samples also showed acceptable predicted accuracy, with coefficients of 0.9865 and 0.9967, as well as overall SDs of 22.16 and 27.75, respectively. Generally, predictions based on the $D_2O$ method are slightly higher than those of the NR method. This agrees with the theoretical analysis based on equations (9) and (10) because of overcompensation of residual water in dry oil by NR method. The mean predictions from both methods are slightly lower than the spiked amounts. However, there are no statistical differences among analytical methods, as shown by the test results for between-subject effects in Tables 3 and 4.

Three-way ANOVA results for the water concentration show a highly significant difference ($p < 0.001$) between water concentrations. Each water concentration level significantly differed from the others, as shown in Table 4. This finding shows that the two methods can differentiate between the five water concentrations regardless of oil type. Furthermore, it suggests that water concentrations as low as ~50 μg/mL can be determined accurately. As expected, the statistical analysis also revealed that the differences in water concentrations in the various oils are highly significant ($p < 0.001$). For the water concentrations from the various analytical methods, no significant differences were found ($p < 0.001$). On the other hand, the interaction between water concentration and oil type was found to be significant ($p < 0.001$). This difference may be attributed to various spiking results. It has nothing to do with analytical method, as verified by the fact that no significant differences were found for interactions between water concentration and analytical method, between oil type and analytical method, as well as between

water concentration, oil type, and analytical method ($p < 0.2$). These experimental and statistical analysis results for the five water concentrations and five edible oils suggest that both NR and $D_2O$ methods are accurate methods for the analysis of trace water in edible oil. There is no difference in analytical results between the two methods. There are also no differences found between the predictions from the two methods and the standard spiking amounts of moisture. The $D_2O$-assisted method we developed in this study is therefore generally applicable to the accurate analysis of trace moisture in edible oils.

## 4. Conclusion

$D_2O$-assisted FTIR spectroscopy combined with acetonitrile extraction is used for the determination of trace moisture in edible oils. The mean error for the water determination is <3%. The technique is simple and more accurate, with respect to omitting the requirement of preparation of dry oil and its separately extraction compared with NR method. The $D_2O$ method is especially suitable for the case that oil sample is unknown and its corresponding dry reference is unavailable. In addition, the $D_2O$-associated FTIR method for moisture analysis is amenable to automation. Although this study has focused on using edible oils as the analytical matrix, the method is also applicable to a wide range of hydrophobic materials, including biodiesel, fuels, and mineral-based oils.

### CRediT authorship contribution statement

**Qin Ye:** Resources, Data curation, Formal analysis, Writing – original draft. **Xianghe Meng:** Writing – review & editing, Project administration. **Linjiang Pang:** Methodology, Visualization.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The authors do not have permission to share data.

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