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A Methodology Study on the Optimal Detection of Oil and Moisture Content in Soybeans Using LF-NMR and Its 2D T_1-T_2 Nuclear Magnetic Technology

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Abstract: In this study, we aimed to provide an accurate method for the detection of oil and moisture content in soybeans. Introducing two-dimensional low-field nuclear magnetic resonance (LF-2D-NMR) qualitatively solved the problem of overlapping component signals that one-dimensional (1D) LF-NMR techniques cannot distinguish in soybean detection research. Soxhlet extraction, oven drying, LF-NMR spectrum, and LF-NMR oil and moisture content software were used to detect soybean oil and moisture content. The comparison showed that the LF-NMR oil and moisture content software was faster and more accurate than the other methods. The specific identification of the oil and moisture signals of soybean seeds using longitudinal relaxation time (T_1) and transverse relaxation time (T_2) successfully solved the problems of less mobile water, overlapping free water, and oil signals. Therefore, LF-2D-NMR can complement conventional LF-NMR assays, and this study provides a new method for the analysis and detection of moisture and oil in soybeans.

Keywords: T_1-T_2; LF-NMR; LF-2D-NMR; soybean; oil and moisture content

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1. Introduction

China is a large consumer of edible vegetable oil, and soybean oil is necessary in the lives of its residents. China mainly relies on soybean imports from countries such as Brazil and the U.S. With trade frictions between China and the U.S. and the impact of coronavirus disease 2019, governments have taken closing epidemic prevention measures. Hence, the flow channels of agricultural trade have been blocked, resulting in a soybean supply crisis for the foreign trade industry [1]. Owing to the constraints of production resources and technology, many research institutions have devoted themselves to the innovative breeding of soybean varieties to meet the growing domestic demand for soybeans. The Heilongjiang Academy of Agricultural Sciences crossbred a new high-oil and high-yielding variety of soybean called “Heinong 531” [2]. In addition, the world’s first cholesterol-lowering soybean variety with a Chinese genetic background is “Dongnong 358”. It was developed at Northeast Agricultural University and it has a variety of health uses [3]. The national soybean standard stipulates that soybeans with a moisture content of less than 13% and a crude fat content of more than 20% are considered high-oil soybeans. After experimental harvest, it has become increasingly important to quickly and cost-effectively select high-quality soybeans with a high oil content.

The entry points for mainstream screening of crops primarily focuses on crop appearance characteristics such as fullness of seeds and absence of pests and diseases, combined with the application of crop seed selection techniques such as optical characterization methods, X-ray techniques, and machine vision techniques. However, few studies use high and low oil content and moisture content as entry points for soybean screening [4–6] The oven-drying method (105 °C) is the standard arbitration method for moisture detection in the Chinese grain and oilseed industry (acquisition, storage, marketing, transfer, processing, and import/export), and the Soxhlet extraction method is the international standard method for crude fat detection. Zhu et al. developed a near-infrared (NIR) spectral model of soybean moisture and crude fat content to rapidly determine soybean quality [7]. However, this method must be combined with chemometrics, and the modeling process is complex and time-consuming [8]. In contrast, Sørland et al. demonstrated that low-field nuclear magnetic resonance (LF-NMR) is a fast and accurate alternative to oven and solvent extraction for determining crude or total fat and moisture content in biological systems. Moreover, it does not require complex post-processing of the test data, reducing the tedium of the test [9]. LF-NMR has been proven to simplify the test and provide a fast and accurate moisture content measurement of single maize seeds [10].

As a non-destructive and non-invasive measurement technique, LF-NMR has been promoted in food agriculture, energy geotechnical, and life science fields [11–13]. It aims to reflect the changes in the water–oil content of a sample by obtaining the relaxation time of hydrogen protons in a radio frequency (RF) field using an appropriate pulse sequence and quantifying the intensity of the proton mobility in this state [14–17]. The relaxation times are classified into two types: T_1 for spin–lattice or longitudinal relaxation time and T_2 for spin–spin or transverse relaxation time. The corresponding commonly used sequences are the inversion recovery (IR) and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. The oil and moisture content software developed with LF-NMR is a professional software for processing data in the food, research, education, and other related industries. It is based on segmented (SEG)-CPMG, which adds several 180° pulses with different echo intervals to the original CPMG sequence. This implies that two 180° pulses are added after the 90° pulse; the first 180° interval is the echo time (TE) and the second 180° interval is TE2. When TE and TE2 are the same, this is a common CPMG sequence. This establishes the signal amplitude of the oil (or water) and the signal value of the oil (or water) mass to derive the oil (or water) content standard curve of the test sample. During logging, early NMR based on a one-dimensional (1D) NMR technique measured only the T_2 transverse relaxation time of the formation pore fluid and could not distinguish whether these signals were from oil or water. Moreover, and their T_2 signals overlapped when oil, gas, and water were present at the same time. The ability of two-dimensional (2D) NMR technology to identify components has been proven to be stronger than that of 1D-NMR [18,19].

Conventional magnetic resonance imaging (MRI) is the superposition and accumulation of cross-sectional signals. The selected cross-sections are processed by phase encoding, and frequency encoding, of Fourier-transformed data. LF-2D-NMR shows Laplace changes, and the molecular motion properties (longitudinal relaxation time T_1, transverse relaxation time T_2, diffusion coefficient D, etc.) are related to the relaxation spectrum. However, the original signal obtained by sampling a two-dimensional sequence is generally not directly usable. The 2D spectrum obtained after inversion has a clear and intuitive meaning [20]. The 1D experiments use a constant interval between pulses, allowing the evaluation of longitudinal or transverse relaxation. However, in multi-dimensional experiments, the signal is measured as a function of two or more independent variables. Spin systems can evolve via different relaxation mechanisms [21]. Researchers have found that the 1D LF-NMR spectrum technique is limited in the information it can obtain for complex samples, and its high overlap effect also affects the detection performance. Greer et al. demonstrated that 2D technology can be used to detect the oxidation of unsaturated fatty acids in edible oils and is convenient for assessing the authenticity of soy sauce and its adulteration, which can provide richer sample information [22,23]. In food agriculture, researchers have made use of the emergence of LF-NMR techniques, primarily using 1D spectrum information in combination with MRI techniques to analyze changes in sample moisture content flow. There are fewer studies and applications that reflect the oil content of samples [24–27]. Traditional 1D spectral analysis is fast and non-destructive. However, when 1D analysis is performed for different substances in the same sample, the peaks of various components tend to overlap, leading to misclassification. The LF-2D-NMR technique, which combines T_1 and T_2, can be used to visualize differences in the sample components.

In summary, this study investigated the conventional and novel methods for detecting soybean seed oil and moisture content. It involved the specific identification of the oil and moisture signals of soybean seeds using T_1-T_2.

2. Materials and Methods

2.1. Test Materials

Six soybean varieties were selected for the trial: Zhonghuang 13, Zhonghuang 35, Zhonghuang 39, Xudou 20, Zhonghuang 57, and Huadou 14. They were purchased from a local agricultural market (Shenyang, China). A random sampling method was used to select 120 seeds of a uniform size with smooth skin. The samples were grouped, numbered, and weighed using an electronic balance.

2.2. Testing Instruments

The NMR spectrometer (NMI20-015V-I) was purchased from Niumai Electronic Technology Co. Ltd. (Shanghai, China) with a magnetic field strength of (0.5 ± 0.08) T, a RF pulse frequency of 21 MHz, a magnet temperature of 32 °C, and a probe coil diameter of 15 mm.

The Sartorius BSA124S-CW ten-thousandth electronic balance was purchased from Sartorius Instrument System Co., Ltd. (Beijing, China) and calibrated with a maximum weighing value of 120 g, a minimum weighing value of 0.01 g, and an accuracy of 0.0001.

The DK-S26 electric-heated thermostatic water bath was purchased from Jinghong Test Equipment Co. Ltd. (Shanghai, China) with a power consumption of 1500 watts and a temperature control range of RT+5~100 °C.

The DHG-9053A electrothermal constant-temperature air-blowing drier was purchased from Yiheng Scientific Instruments Co. Ltd. (Shanghai, China) with a power consumption of 800 watts and temperature control range of RT+10~200 °C.

The steel laboratory hood was purchased from Meikali Laboratory Equipment Co. Ltd. (Liaoning, China) with a ventilation air volume of 0.3~0.5 M/S and ventilation air velocity of 1500 M^3/H.

The Dragon TopPette single-channel adjustable pipette gun was purchased from Dragon TopPette Xingchuang Experimental Instrument Co. Ltd. (Beijing, China) with a minimum volume of 2 μL and maximum volume of 20 μL.

The YDS-2 portable liquid nitrogen tank was purchased from Jinfeng Liquid Nitrogen Container Co. Ltd. (Chengdu, China).

2.3. Test Methodology

The test methods and comparison angles are plotted as a control chart in Figure 1. The test samples were selected from commercially available soybeans with a moisture content below 13%. However, the LF-NMR oil and moisture software required samples to have a moisture content of less than 10% when tested; therefore, a drying step was added as a pre-treatment (Section 2.3.3). Non-destructive testing based on the LF-NMR technique was compared with destructive testing based on the national standard method. With conventional T_1 and T_2, there is often a problem of signal overlap; therefore, LF-2D-NMR was used.

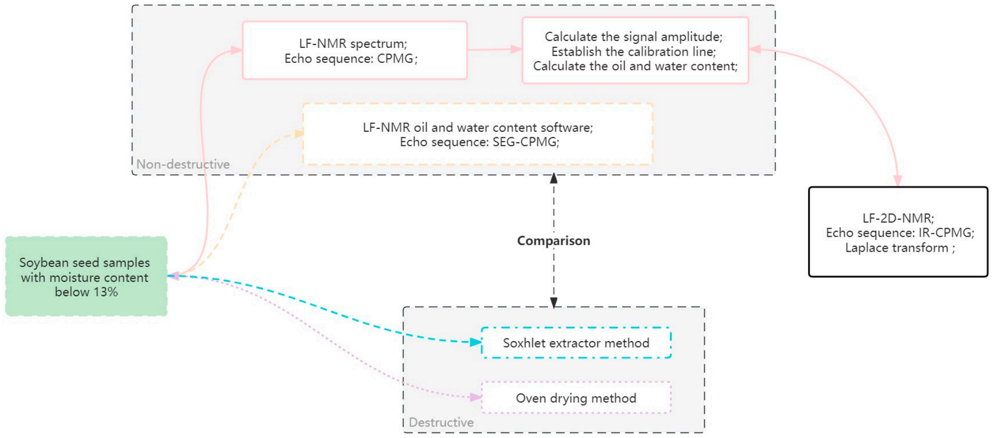

Figure 1. All test methods are plotted into non-destructive and destructive sections for comparison, with LF-2D-NMR presented separately as a new point. The soybean seeds comply with international trade regulations (moisture content below 10%), and those above the value were pre-treated by drying.

2.3.1. LF-NMR Oil and Water Content Software

Suzhou Newmark Electronic Technology Co. (Suzhou, China) provided the oil and water content software instructions. As the T_2 relaxation time of the aqueous copper sulfate solution is very similar to the T_2 relaxation time of crop seeds, the calibration sample in the water content test of seeds is generally an aqueous copper sulfate solution. Soybean seeds were taken for spectrum pre-tests, the T_2 relaxation spectrum was obtained by inversion, and the T_2 of the bound water peak and less mobile water peak were observed in the spectrum to be approximately 1 ms. The concentration of the copper sulfate solution was calculated according to Equation (1). The formula depends on the test instrument used.

$$\mathrm{y} = 0.0032\mathrm{x} + 0.0022 \tag{1}$$

where y denotes the $1/T_2$ value of the copper sulfate solution, x is the concentration of copper sulfate solution, and the calculated concentration of copper sulfate solution was 311.8 mmol/L. Several test tubes of matching caliber were chosen. An SEG-CPMG pulse sequence was used as the test apparatus. The oil used for calibration was loaded into the test tubes at the following weights: 0, 0.016, 0.033, 0.045, and 0.06 g. In addition, 311.8 mol/L copper sulfate solution was loaded in other tubes at 0, 0.0136, 0.0242, 0.036, and 0.0517 g, respectively. As the solution height was lower than 10 mm, resulting in an insufficient signal volume, equal pieces of clean tissue paper were plugged into the vial, and the calibration solution was injected into the tissue paper for the test. Currently, the mass of the copper sulfate solution cannot be used as pure water quality to establish a calibration line. The formula for calculating the pristine water quality of the aqueous copper sulfate solution specimen is as follows Equation (2).

$$\mathrm{MW} = \mathrm{MX} - \left(\mathrm{C} \times \mathrm{MX} \times 10^{-6} \times 159.5\right) \tag{2}$$

where MW is the mass of pure water, MX is the mass of the copper sulfate solution, and C is the concentration of the configured copper sulfate solution. Pure water masses of 0, 0.01292, 0.02408, 0.0343, and 0.0491 g were calculated. The oil calibration line was established without additional calculation of the refined oil mass. Mass calibration was selected, the pure water mass and oil mass were entered for calibration, and the soybean oil content and water content calibration lines were obtained for the measurement of soybean seeds. Calibration lines have a service life, and a calibration line can be prepared using the instrument for a maximum of one month.

2.3.2. LF-NMR Spectrum Tests

Each soybean seed was loaded into a test tube at 15–20 mm with the sample height controlled, and the free induction decay (FID) pulse sequence was used to determine the central frequency of the magnetic field and the hard pulse width. The seed pellets were placed in a tube, centered on a permanent field magnet coil, and analyzed for T_2 with three replicates. The parameters of the CPMG pulse sequence were set as follows: main resonance frequency (SF) = 21 MHz, offset frequency (O1) based on each test, 90° pulse RF pulse width (P1) = 17 μs, spectral width (SW) = 200 kHz, TE = 0.25 ms, repetition time (TR) = 0.25 ms, repetition time (TW) = 2000 ms, number of echoes (NECH) = 3000, number of repetition samples (NS) = 16. The spin–echo signals generated from three repeated acquisitions were imported into the NMR inversion fitting software for each inversion, and the average values were used as the sample relaxation times. T_1 was obtained by inversion recovery. The NMR inversion software was set to 100,000 iterations to perform detailed inversion of the obtained T_2 decay curve [26]. The signal amplitude at the water/oil peak was used for the calculation.

To ensure the accuracy of the comparison, the spectrum and software detection methods used the same batch of mass gradients of soybean oil and copper sulfate solution to establish the calibration line. The same masses of copper sulfate solution were used for the spectrum test, and the inversion of the signal amplitude corresponding to the peaks of moisture and oil was obtained. The same pure water and peak signal amplitude copper sulfate solution were used to establish a linear relationship to obtain the water calibration line, pure oil quality, and peak signal amplitude to develop the calibration line steps, as described above. Based on the test data for curve fitting, using R squared (R^2) tests. The calculation formula is as follows (Equation (3)).

$$\mathrm{R}^2 = 1 - \frac{\sum_\mathrm{i}(\hat{\mathrm{y}}_\mathrm{i} - \mathrm{y}_\mathrm{i})^2}{\sum_\mathrm{i}\left(\mathrm{y}_\mathrm{i} - \bar{\mathrm{y}}\right)^2} \tag{3}$$

where y_i is the actual measured value, $\hat{\mathrm{y}}_\mathrm{i}$ is the predicted value obtained after fitting the standard line, and $\bar{y}$ is the actual average value. If R^2 was <0.99, the test was restarted. The result was a water–oil standard curve. The signal amplitudes of soybean seed oil and moisture after the spectrum test were substituted separately into the standard curve, and then the water and oil contents of the spectrum were calculated.

2.3.3. Oven-Drying Method

Following the pre-test, the soybeans were dried for 8 h to achieve a constant weight for the official test. Soybean kernels were numbered, weighed, dried at 105 °C for 8 h in the oven, and taken out and weighed, and the difference was calculated to obtain the moisture content. The mass of kernel moisture (MKM) and kernel moisture content (KMC) were calculated using Equations (4) and (5).

$$\mathrm{MKM} = \mathrm{FW} - \mathrm{DW} \tag{4}$$

$$\mathrm{KMC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{FW}} \tag{5}$$

where FW is the fresh weight of the seeds and DW is the dry weight of the seeds [10].

2.3.4. Soxhlet Extraction Method

Soybeans, to which liquid nitrogen was added, were manually crushed into powder. The filter paper cartridge was removed from its package and loaded into the extraction cylinder of a Soxhlet extractor. The fat flask was removed at the end of extraction, heated in the oven (100–150 °C) to a constant weight, and the percentage of fat content was calculated.

2.3.5. LF-2D-NMR Technology

The soybeans were placed in tubes. Standard oil samples were used for calibration, and the FID pulse sequence was used to determine the central frequency of the magnetic field and hard pulse width. The IR-CPMG sequence was selected. The number of reversal times (NTI) was set to 20, the number of reversal recovery times (VDLI) was set to 20 according to a logarithmic distribution, TE was set to 3 × P2, the number of echoes (NECH) was set to 3000, and the test time was approximately equal to the waiting time multiplied by the number of accumulations multiplied by the number of reversal times. Each sample was measured once, and the generated echo signal was inverted in the NMR spectrum analysis software. Furthermore, it was pseudo-colored and normalized to obtain a 2D-NMR map, with the color shades indicating the signal magnitude and the data and images recorded.

2.4. Statistical Analysis

A paired sample *t*-test and Pearson correlation analysis were carried out for a set of observations using IBM Statistical Package for the Social Sciences (SPSS) Statistics v. 23.0 (SPSS Inc., Chicago, IL, USA). All figures were plotted using OriginPro 2018 (Microcal, Piscataway, NJ, USA), Paint 3D (Microsoft Corporation, Redmond, WA, USA), and Excel 2018 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussion

3.1. Analysis of Soybean Oil and Moisture Content Testing Methods

Table 1 lists the standard curves for oil and moisture content. This illustrates that the R^2 values of both methods based on the LF-NMR technique were greater than 0.99, which is very effective. When the water mass is 0 (empty extraction), the H atoms of the air moisture produce a signal amplitude, as shown in the local enlargement of Figure 2A, which affects the establishment of the standard curve. However, this situation was ignored when the ratio of the sample water mass to the air moisture mass was sufficiently large.

Table 1. Comparison of the results of different oil and water content testing methods.

Content Type	Method	Standard Curve	R^2
Moisture content testing	Spectrum method	y = 1930.2x + 1.7203	0.999
	Oil and water content software testing method	y = 3907.7x + 3.5069	0.997
	Oven-drying method		
Oil content testing	Spectrum method	y = 1241.9x + 1.7577	0.994
	Oil and water content software testing method	y = 4262x − 0.9541	0.999
	Soxhlet extraction method		

Note: The signal amplitude at the peak of the moisture/oil content was the dependent variable, and the moisture/oil mass was the independent variable. R^2 indicates the degree of standard curve fitting.

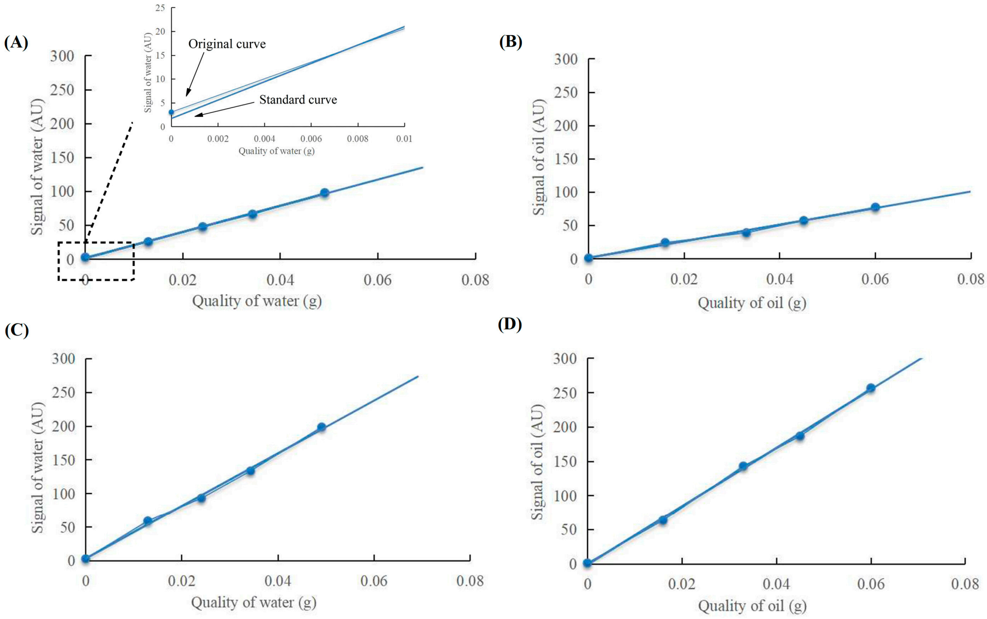

Figure 2. Standard curves for moisture and oil content in the spectrum method and the LF-NMR software test method. (**A**,**B**) Scale graphs of the spectrum moisture and oil content method; (**C**,**D**) scale graphs of the software moisture and oil content method.

3.2. Numerical Analysis of Different Methods for Detecting the Oil and Water Content of Soybeans

The Chinese National Standard for Soybeans requires the moisture content of traded soybeans to be no higher than 10%. Therefore, this test statistically eliminated soybean data with moisture content higher than 10% from the results. In addition, meeting the assumption of error normality results in a normal error. The Shapiro–Wilk test combined with average histograms and Q-Q plots was used to verify that the three methods satisfied the normality assumption [27].

Table 2 shows the results of the oven drying and Soxhlet extraction methods, which are used as national standards for detecting soybean moisture and oil content. The results of the other two methods were tested using matching sample *t*-tests to develop an analysis of variance. The difference between the oven-drying method and the software moisture content method was slight for moisture content, and the Cohen's d value was more significant than 0.5; the difference was average. For oil content, the difference between the Soxhlet extraction method and the software oil content method was also slight, and the Cohen's d value was greater than 1. Therefore, the software moisture and oil content method differed less than the national standard method. Simultaneously, the correlation analysis of soybean moisture and oil content test results of other methods was conducted, and a heat map of the correlation coefficients is shown in Figure 3. As shown in Figure 3A, the Pearson correlation coefficient value between the oven-drying method and software moisture content method was the largest, whereas the correlation coefficient value between the oven-drying method and spectrum moisture content method was the smallest. The correlation coefficient between the Soxhlet extraction method and the software oil content method was the highest in Figure 3B. The combination of variance and correlation analyses indicated that the results of the national standard method were comparable to those of the software oil and moisture content method.

Table 2. Results of matched samples *t*-test for different assays.

Matching Variables	Matching Difference	Cohen's d
Oven-drying method for matching spectrum moisture content	1 ± 0.262 ***	1.094
Oven-drying method for matching software moisture content	−0.349 ± −0.051 ***	0.65
Soxhlet extraction method for matching spectrum oil content	−1.593 ± 0 ***	1.854
Soxhlet extraction method for matching software oil content	0.67 ± −0.086 ***	1.151

Note: Matched differences are presented as mean ± standard deviation. *** represents 1%, significance levels. Cohen's d value represents the level of variance.

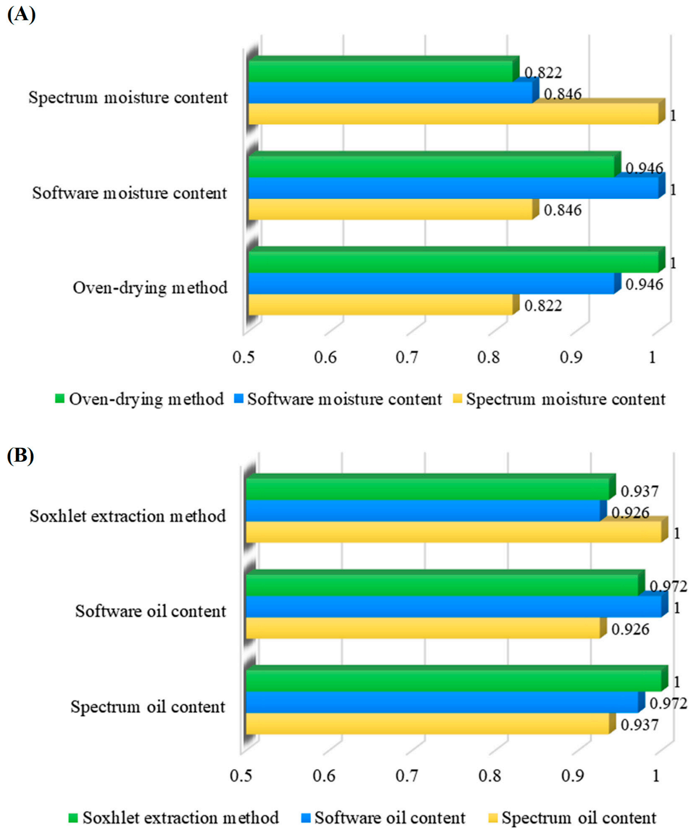

Figure 3. (**A**) Pearson correlation coefficient for the three soybean moisture content test results and (**B**) Pearson correlation coefficient for the three soybean oil content test results. All analyses showed a significance level of 1%.

In LF-NMR, water can be classified into three categories based on the different states of water contained in soybeans. The first is stable water that is bound to proteins and other macromolecules through hydrogen bonds. It decays quickly, and it is difficult to separate. The second is bound water, which exists in the internal tissue interstices by capillary action, has strong mobility, and acts as a solvent to support the grain for a series of biochemical reactions (decomposition, transport, and synthesis of substances) The third is called free water [28]. Moreover, there is water with motility between stable and bound water, either present inside the cells and tissues or outside the cells, or in the form of an interconversion between bound and free water, or less mobile water. The results of the different detection methods are plotted in the radar diagram in Figure 4. Figure 4A shows that the spectrum moisture content method results were the smallest, and those of the software moisture content method and oven-drying method were similar. This is because the drying time is too long to ensure the complete removal of free water, and most of the combined water is converted into free water by evaporation, which leads to soybean skin rupture, protein denaturation, and the inability to remove soybean unsaturated fatty acids by thermal oxidation to generate macromolecules. Simply, excessive drying makes the soybean mass relatively larger than that of complete drying and the difference in the calculation of the water content of the results is small. The oven-drying method detects values including the total evaporation of free water and free water contained in the seeds themselves, which is mostly converted from bound water. The software water content detection object includes all hydrogen atoms of water contained in soybean seeds, i.e., the superposition of bound water, less mobile water, and free water; while the water content of the spectrum method calculates the water content by detecting the signal amplitude of bound water, and the signals of free water and oil peaks easily overlap, making the spectrum water content measurement value low [29,30]. The reason for the large difference between the measured values of the Soxhlet extraction method and the software oil content method in Figure 4B is that there is a problem of excessive reflux and drying times during the extraction of crude fat. The repeated high-temperature heating of the fat flask (60–105 °C) makes the activity of soybean fat oxidase high. The more hydroperoxides that are generated, the larger the crude fat mass. The most noticeable result for the oil content of the spectrum method is detection of oil peaks, difficulty in flowing water peaks, and other problems that generate signal overlap; however, the difference is not significant. It was concluded that the detection values of the oven-drying and Soxhlet extraction methods were consistent with the oil and water content measurements of the software method.

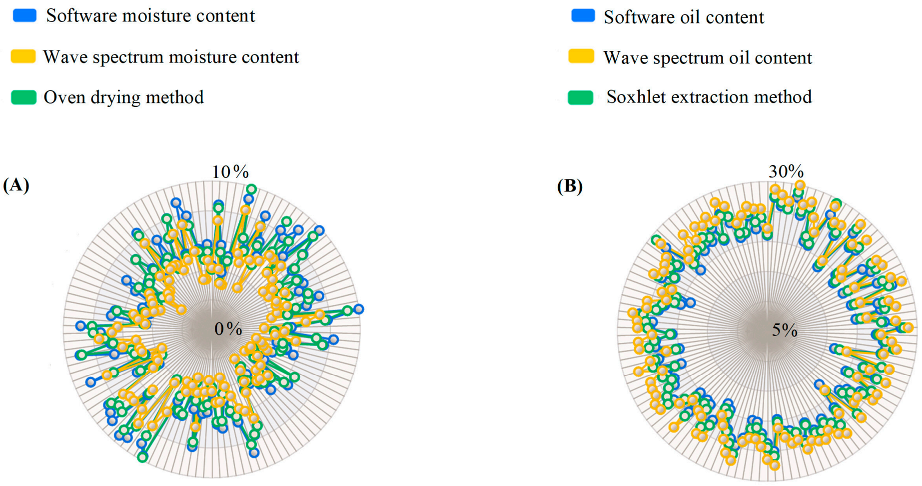

Figure 4. (**A**) Radar plot of the results of the three methods for the moisture content of soybeans and (**B**) radar plot of the results of the three methods for the oil content of soybeans.

The incomplete detection of free and bound water should be minimized when performing relevant water content measurements. There are existing examples of combining oven-dried fried foods using a spectrum method to determine the oil–water content and applying it to frying systems [31]. However, LF-NMR oil and moisture content software is a new technology in the market and is based on the SEG-CPMG sequence to measure the oil and moisture content of the test material. This has the advantage of being less time-consuming and more accurate compared with the traditional oven-drying method, which is time-consuming and labor-intensive, and the Soxhlet extraction method, which destroys the seed.

3.3. One-Dimensional LF-NMR Mapping Analysis

The T_1 spectrum is shown in Figure 5A; the range of the T_{13} peak before and after drying was almost unchanged, which can be considered as the oil peak without a water signal. However, the total signal amplitude was slightly reduced after drying because the total number of hydrogen protons in the system was reduced as the fatty acid unsaturation of the oil increased with heat. The overall signal amount of the oil was relatively low [32]. After drying, the soybean bound water transformed into new, less mobile water, corresponding to the disappearance of T_{11} (Figure 5A) and the appearance of a small new peak, i.e., T_{11} was the original soybean bound water peak. Simultaneously, the relaxation time of T_{13} was more than 1000 ms, and its signal amplitude was small, analyzed as a trace of free water or signal noise, and therefore ignored. In Figure 5B, the T_2 relaxation spectrum was observed for the soybean without drying treatment (10% moisture content), containing four peaks: the bound water peak T_{21} (0.1–1 ms), less mobile water peak T_{22} (1–10 ms), and the overlapping part of the oil and free water peaks T_{23} and T_{24} (10–1000 ms) [29,30]. On one side, after drying, T_{21} gradually decreased and shifted to the right. This combined with the similarly reduced T_{22} to form a new bound water peak, indicated that some bound water was converted to less mobile water after the high-temperature drying of the soybean seeds to maintain their metabolic activities. On the other side of the same drying process, oil peaks and free water peaks overlapped and T_{23} and T_{24} left shifted to form a new peak. Oil is known to be unaffected by chemical exchange, the conversion of non-free water to free water under high temperature conditions, and oil signal overlap, making the new peak relatively increased compared to the original peak signal amplitude, that is, proving that in the T_2 spectrum there is peak signal overlap problem. This indicates that 1D does not distinguish between the components of the problem of overlap.

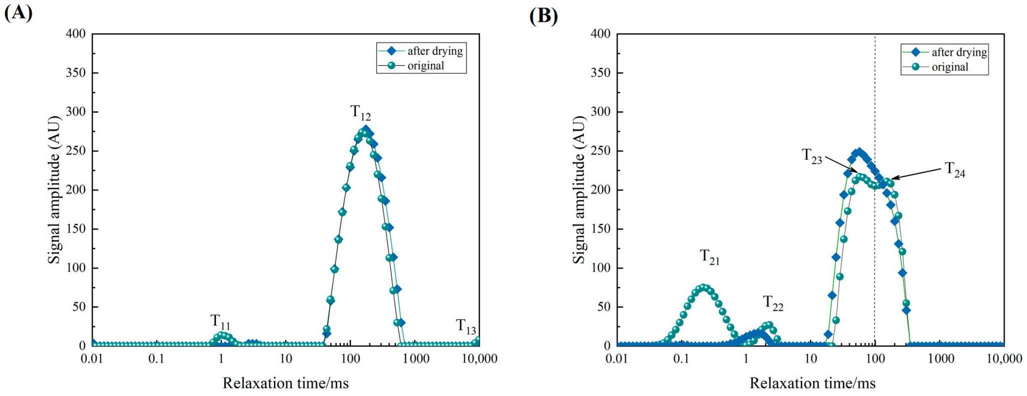

Figure 5. T_1 and T_2 relaxation spectra of the soybeans (**A**) before and (**B**) after drying.

In brief, three types of moisture with different transverse relaxation times, bound water, less mobile water, and free water, exist inside the soybean kernels that meet the trade standards. Changes in their moisture components can be observed in the transverse relaxation spectrum when soybeans are treated with immersion and the effect of neglecting the oil signal in the relaxation spectra under comparison. However, the overall hydrogen atomic weight of dried soybeans is much lower than that of soaked soybeans, and it is necessary to specifically distinguish the oil signal from the water signal, hence, the information obtained from 1D relaxation can no longer meet the experimental needs, thus this study introduces LF-NMR 2D mapping for analysis.

3.4. Results of Qualitative Analysis of LF-2D-NMR Fractions

Figure 6 shows the qualitative analysis of the internal moisture and oil before and after drying of soybeans, where Figure 6A,C show LF-2D-NMR profiles of soybeans before and after drying, and Figure 6B,D show the corresponding X:10°–Y:20°–E:10° views. As the molecular weight/viscosity increases, the T_1/T_2 ratio increased, and the larger the area of regional integration (stronger signal), the higher its content (redder color). The T_{22} and T_{23} spectra, which exhibited signal overlap on a 1D scale, were positioned along the same horizontal axis of T_2 relaxation as the green- and red-coiled sections in Figure 6A. Combining the double superposition of T_{12} and T_{22} signal amplitudes shows that the red-coiled portion is the soybean oil peak, which is adjacent to the oil peak on the left. The lower portion of the green-coiled portion, which has a weaker signal, is the free water peak and the upper portion is the less mobile water peak, both of which have weaker signal amplitudes than the oil peak [33]. During storage, the metabolism of grain crops is influenced by the temperature and humidity of the external environment. Although the quality of soybeans with a moisture content of 10% or less was stable during storage, some deterioration occurred, producing products of oil oxidation and fat hydrolysis, as shown in the area circled in purple in the lower right of Figure 6A [34]. In addition, when performing 2D-NMR, it is essential to note that it is a positively correlated spectrum and that the respective signals of transverse and longitudinal relaxation should be concentrated upon and observed as a whole, rather than interpreting the 1D information of one side as the other in the absence of all signals. The signal growth of the peaks in the yellow circled portion of Figure 6B,D, combined with the new peak (1–10 ms) relaxation time of bound water after drying the soybeans in Figure 5A, proves that it is bound to the water peak. Overheating the instrument during the test also had a negligible effect on the measured values and was ignored here.

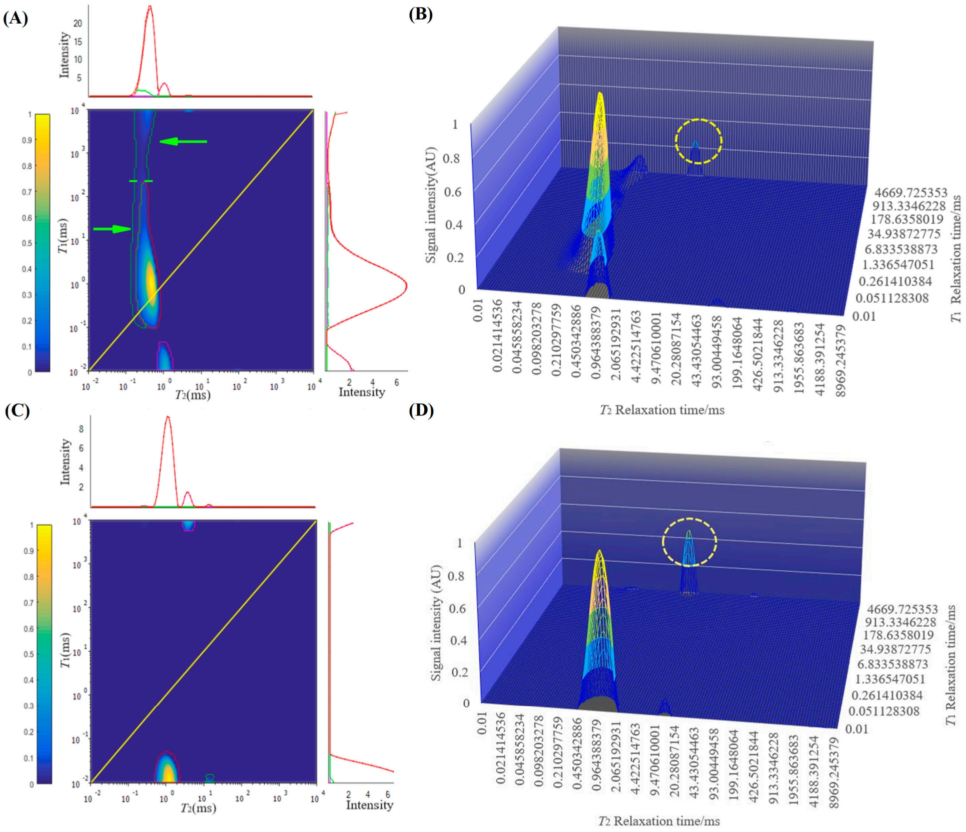

Figure 6. (**A**,**C**) The 2D T_1-T_2 spectra of soybeans before and after drying. (**B**,**D**) Three-dimensional perspectives of (**A**,**C**).

Triglycerides are esters obtained from fatty acids and glycerol, and the most common fatty acids are found in soybean oil, in the form of triglycerides, including linoleic acid C18:2 (53%) and oleic acid C16:1 (23%) [35]. Different heating times and temperatures affect the composition of fatty acids, mainly linoleic and oleic acids, and their indices in edible oils. The T_1-T_2 distribution of edible oils is related to their fatty acid composition [32]. The 2D spectra of soybeans after drying at high oven temperatures (105 °C) are shown in

Figure 6C. The oil peaks decreased and shifted downward. During the high-temperature drying oxidation process, the overlapping effect of the water evaporation signal amplitude was reduced, and the area of the oil peak region decreased. Rearrangement of linoleic acid to form conjugated double bonds occurs at high temperatures. The unsaturated fatty acid content decreases and deteriorates to produce secondary products, which increases the saturated fatty acid content and carbonyl value. An increase in the double bonds increases the inhomogeneity of the hydrogen proton NMR response [32]. The polarity of glycerides is also closely related to the number of hydroxyl groups, and an increase in polarity leads to a relative decrease in the T_2 relaxation time of the system. The higher the molecular weight of the oil in the T_1 direction, the lower the T_1 relaxation time, resulting in a downward shift of the oil peak [32,36].

Overall, LF-2D-NMR is more intuitive for distinguishing between oil and moisture signals in soybean seeds. It is an excellent tool to complement the 1D-NMR description. Moreover, it may be used as a novel analytical method on its own, offering a new perspective on soybean trade and breeding with excellent prospects.

4. Conclusions

Although the Soxhlet extractor method is internationally accepted for calculating the crude fat content of oil seeds, it is time-consuming and labor-intensive, requires a large amount of solvent, and is destructive to seeds. The oven-drying method is a relatively simple process, but it is time-consuming; soybean seeds tend to dry out and the skin breaks during the drying process, and there are doubts about whether the drying process is complete. The advantages of LF-NMR were used to perform the detection, and it was found that the fit of the detection was better, whereas the software detection method had the best results, indicating that this method is feasible. The LF-NMR oil and water content software does not require heavy calculations, a large number of chemical solvents to assist the test, or the destruction of the soybean seed skin and other properties. The LF-NMR spectrum method was based on the peak area and water (oil) quality. Although fast and non-destructive, it has problems with overlapping components affecting signal amplitude and errors in calculating peak area. The 1D detection method suffers from the problem of signal overlap, which renders the detection results inaccurate. This problem was solved by using LF-2D-NMR processing. This study introduces the concept of LF-2D-NMR to address the problem of component overlap in soybean moisture and oil content detections. It also provides a new way of identifying components in soybean seeds by combining T_1-T_2 relaxation information rather than measuring them separately. It deserves to be promoted as an optimal detection method for visualization of soybean oil and moisture. The LF-NMR machine used throughout this experiment has permanent magnets on the main body of the test apparatus that are flexible and easy to move and do not require any special maintenance. This machine is easy to integrate with other equipment and accessories to meet online high-throughput testing requirements. Furthermore, the cost of the instrument is low, the instrument is internally shielded, and no special installation sites are required.

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