Qualitative Analysis of Edible Oils using Low Field ^{1}H NMR Spectroscopy and Multivariate Statistical methods

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Abstract. Low field ^{1}H Nuclear Magnetic Resonance(LF NMR) Spectroscopy is an efficient tool to capture a research sample's content and purity.LF NMR is employed for the qualitative analysis of Edible oils. Edible oils used for the study include Coconut, Groundnut, Olive, Mustard, Rice bran and Soyabean oil. Principal Component Analysis(PCA) is used to build a model initially that could classify the oils based on their chemical composition. This model built using PCA could capture 96% of total variance in data. Linear Discriminant Analysis(LDA) was used to build a model that could classify with 100% accuracy. The results provide successful proof for detecting adulteration and further classification of edible oils using Low field ^{1}H NMR Spectroscopy in conjunction with Multivariate Statistical methods such as PCA and LDA.

Keywords: Low field ^{1}H NMR Spectroscopy · Multivariate Statistical methods · Principal Component Analysis(PCA) · Linear Discriminant Analysis(LDA) · Adulteration detection · Classification · Edible Oils

1 Introduction

Edible vegetable oils, due to their high nutritional value and substantial health benefits, are an integral part of cuisine, both in domestic and food manufacturing sector. Due to lesser profit margins of selling the edible oils in pure form, manufacturers of the oil resort to adulterating the pure oil with less expensive oils. Some of the less expensive oils include refined oils, that are hazardous to health. Due to increased awareness from consumers,producers and policy makers, food authentication has gained importance in the recent years [8].

Different analytical techniques are used to detect the presence of adulterants in edible oils. Some of the methods used include Near Infrared(NIR) spectroscopy[5], Mass spectroscopy, High Performance Liquid Chromatography (HPLC)[15], Synchronous fluorescence[14] , Fourier Transform Infrared(FTIR)

spectroscopy[23],Nuclear Magnetic Resonance(NMR) spectroscopy[22], Gas chromatography[15][2] and Vibrational spectroscopy[6]. These analytical techniques have their own pros and cons based on their performance parameters such as sensitivity and their expenditure. Low field Nuclear Magnetic Resonance(LF NMR) spectroscopy forms a preferred option that is used in industry for food quality assessment, because of its low cost and rapid detection technique.

Research was carried out to assess the oil content, moisture levels as well as microstructure changes in french fries , using LF NMR spectroscopy. The study comments on the influence of fatty acid composition on the behavior of oil absorption and surface morphology.[24]. LF NMR as a powerful tool was used in the detection of adulteration of peanut oil.Quality changes that occur in peanut oil upon adulterating it with various oils were studied using LF NMR spectroscopy[26]. A comparative study between low field and high field NMR was done to detect the adulteration of cold pressed rape seed oil with refined oils, and to quantitatively detect adulteration percentage. Though high field NMR spectra produced better classification results, LF NMR still produced competitive classification results in detecting the presence of adulterants[17].

The main aim of this study is to classify edible oils that are available in Indian Market and to detect the presence of adulterants in them using Low Field -Nuclear Magnetic Resonance spectroscopy(LF NMR). The oil samples used for study include Coconut, Groundnut, Olive, Mustard, Ricebran and Soyabean oil. Multivariate Statistical data analysis techniques are applied on the acquired spectra for qualitative analysis of expensive oils adulterated with less expensive oils. For e.g., Mustard oil being adulterated with Rice bran oil, Virgin Olive oil being adulterated with Soyabean oil etc.,

Introduction section covers the issue of adulteration and the need for detecting adulteration using various techniques. NMR analysis section defines the molecular process involved and the advantages of LF NMR spectroscopy along with literature review. Materials and methods section provides insight into the sample preparation, processing and acquisition of LF NMR spectra. Results of the experiment by applying Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) and their optimization for better results is discussed in the results and discussion section.

2 NMR Analysis

NMR spectroscopy helps in determining the chemical composition of a sample under study by obtaining the overall structure of the molecule. It does so by examining the nucleus of the molecule that we wish to study by placing it in a magnetic field. The nucleus which is dense and positively charged when subjected to a strong magnetic field, the protons in them start spinning.

Different nuclei have different spins and those nuclei with spin equals $\frac{1}{2}$ can exist in one of the two possible states. This nature is possessed by the following isotopes : ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P. The hydrogen nuclei is the most commonly

found nuclei in all organic compounds and thus the most frequently analyzed nuclei. Hence the name ^{1}H NMR Spectroscopy.

Let us consider the magnetic field B_0 is applied along the *z axis* at equilibrium. A short Radio Frequency(RF) pulse is applied at right angle to the magnetic field. This RF pulse flips down the protons to *xy plane* and continue to precess. This process continues until RF pulse is present and the protons comeback to original position upon stopping the RF pulse. This process is known as Free Induction Decay(FID). The precession happens at a particular frequency known as Larmor frequency or precessional frequency described by the equation,

$$\nu = \frac{\gamma B_0}{2\pi} \tag{1}$$

where

- ν refers to Larmor or precessional frequency
- γ refers to gyromagnetic ratio , constant for a given isotope
- B_0 refers to magnetic field strength

Based on the electron density distribution, the electron creates its own magnetic field which opposes the applied magnetic field B_0. A proton with higher electron density around it, experiences a lower magnetic field and hence lower Larmor frequency and vice versa. This flipping down of magnetization vector from *z axis* to *xy plane* and continued precession induces a current in the receiver coil. This signal when detected and amplified results in a sine wave of decreasing magnitude, explaining the re-alignment of protons in the nucleus to*z axis*. This process is known as Free Induction Decay(FID). The FID is representation in time domain. On application of Fourier Transform, the time domain is converted to frequency domain, and results in NMR spectrum.

To describe the returning back of H proton from excited magnetic state to equilibrium,the terms longitudinal relaxation time (T_1) and transverse relaxation time(T_2) are used.T_1 relaxation, also known as relaxation of the spin-lattice, involves the transfer of energy in between excited nuclear spins and lattice. It results in decrease of overall energy. Spin-spin relaxation, otherwise known as (T_2) relaxation, refers to the transactions amid nuclear magnetic moments (spins)[7]

Nuclear Magnetic Resonance (NMR) Spectroscopy is useful technique to analyse food matrices,which are quite complex in nature. It is a non- destructive and rapid method that can be used to detect adulteration in food products[13]. It can provide information over a wide range of components in a single analysis and has fast data acquisition rate[10]. Nuclear Magnetic Resonance has emerged as an effective tool for qualitatively detecting the adulterants present in the oil samples. Based on the chemical environment, NMR helps to decide the molecular

structure through ppm scale resonance frequency shifts[21]. NMR in combination with chemometrics is a useful tool in controlling the food quality of products such as edible oils, milk, honey, paprika powder etc.,[10] [1] [19] [3].

2.1 Low Field NMR Spectroscopy

However, NMR spectroscopy instrumentation requires high-field homogeneous magnetic fields[11] with superconducting magnets that require cryogenic cooling for probes, which makes them quite expensive [18]. Low field Nuclear Magnetic Resonance (LF NMR) makes use of magnetic fields of lower strength to capture the spectra.But they do have an intrinsic drawback of limited sensitivity due to minor variations in thermodynamic equilibrium concentrations of the spin states [4]. The low sensitivity can cause measurement of small shifts in resonance frequency difficult.Shim pulses are used in LF NMR spectrometers to overcome the inhomogeneity in permanent magnet [21]. Thus it makes LF-NMR Spectroscopy suitable for routine analysis,that are low cost and suitable in industrial settings.They provide smaller footprint when compared with High field NMR spectroscopy. The benchtop spectrometer that is used for the study is cryogen free, having a permanent magnet of strength 1.4T, from NMReady-60 Pro(Nanalysis, Canada). The standard NMR tubes used has a dimension of 5mm.

3 Materials and Methods

3.1 Sample preparation and processing

The oil samples used for study include Coconut, Groundnut, Olive, Mustard, Ricebran and Soyabean oil. The oils were purchased from IICT Hyderabad and stored at room temperature. The samples were diluted using deuterated standard chloroform ($CDCl_3$) and a standard solution of deuterated Dimethyl Sulfoxide (DMSO d6). The purpose of using solvents is to produce a more defined spectrum (narrow line width) when compared with using pure oil samples as such for testing.A fraction of 480 μl of adulterated oil, succeeded by 120 μl of $CDCl_3$, was titrated directly into Nanalysis standard 5mm tubes.

3.2 Acquisition of LF ^{1}H NMR spectra

Proton 1D spectra were recorded at 20° C using NMReady-60 Pro(Calgary,Canada) Benchtop Spectrometer operating at 60 MHz. The spectral acquisition parameters include: Time domain 65536 points, 16 scans,spectral width 12 ppm, acquisition time 190 seconds. All the files were stored in a separate folder in "comma separated values" (.csv). The analysis of data was performed using The Unscrambler Software(Version 10.5, CAMO Software Inc.,).

4 Results and Discussion

4.1 Preprocessing Methods

The pre-processing techniques are used to remove scattering and other distrubances in the spectra captured. Other disturbances include presence of outliers, difference due to measurement geometry etc[9]. The resultant spectrum after applying pre-processing technique can be used for building models with better classification ability. Since carrying out the analysis using The Unscarmbler software was difficult with time domain 65536 data points, the initial step was to extrapolate to timedomain 4096 data points. Figure 1 shows the spectra obtained before pre-processing on extrapolated data points.

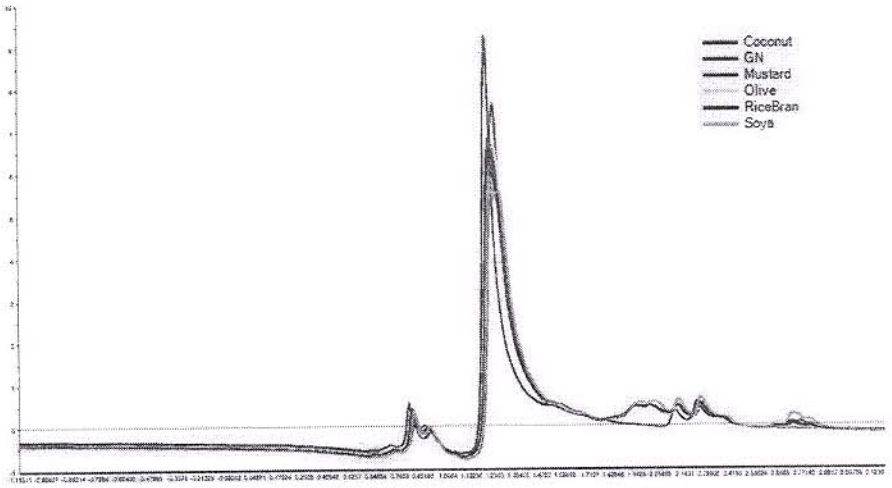

Fig. 1. Raw spectra before pre-processing.

The Standard Normal Variate(SNV) is applied as pre-processing technique to the extrapolated data points. In SNV, each spectrum is transformed by subracting the mean of the spectrum from corresponding individual spectrum and dividing by the standard deviation of the spectrum. It helps in removing the multiplicative interferences of light scatter and particle size[16] and brings all spectra to the same scale[9]. The SNV is calculated as follows:

$$\bar{x_i} = \frac{\sum_{j=1}^{m} x_{ij}}{m} \tag{2}$$

$$\sigma = \sqrt{\frac{\sum_{j=1}^{m} (x_{ij} - \bar{x_i})^2}{m-1}} \tag{3}$$