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Characterization of Rapeseed Oil Using FTIR-ATR Spectroscopy

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Abstract: Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy was employed to characterize rapeseed oils. The spectral features of rapeseed oils were first investigated. Spectral data was processed using principal component analysis (PCA) and linear discriminant analysis (LDA) to discriminate the oils from three cultivars of rapeseeds. As a result, 100% discrimination accuracy was obtained by LDA. Furthermore, the applicability of FTIR-ATR spectroscopy to characterize the changes of rapeseed oils caused by thermal treatment was studied. The rapeseed oil at 60 °C was regularly subjected to spectral measurement, and the spectral changes induced by thermal treatment were analyzed and discussed. This study had demonstrated the good performance of FTIR-ATR spectroscopy in characterizing rapeseed oils.

Key words: Rapeseed oil, FTIR-ATR, characterization.

1. Introduction

Rapeseed oil is the third most important source of vegetable oil after soybean oil and palm oil [1]. Vegetable oils are predominantly composed of tri-fatty acid esters of glycerol, commonly referred to as triacylglycerols (TAG) [2]. Analysis of rapeseed oils shows the TAG level reaches 91.8%-99.1% of the total lipid, and the remaining is made of complex mixtures of minor compounds including phospholipids, free fatty acids, unsaponifiables, tocopherols, chlorophylls and sulfur [3]. The composition of rapeseed oils varies depending on rape cultivars, the agronomic and climatic conditions. Characterization of rapeseed oil is of great importance in analytical assessment of oil quality and nutritional values, detecting some possible alteration or adulteration [2] and understanding the chemical behaviors of oils during storage and processing. The most common methods for analysis of rapeseed oils are gas chromatography (GC) and high performance liquid chromatography (HPLC) [4]. However, both the methods are expensive, time-consuming and require a pre-separation step prior to analysis. Fourier transform infrared spectroscopy (FTIR) has been verified as a rapid and powerful tool for quality evaluation of vegetable oils [5-7].

In the framework of FTIR, transmission and attenuated total reflectance (ATR) are two types of accessories commonly used for oil and fat analysis [8]. For transmission measurements, oil samples are placed between two well-polished disks, typically made of KBr, creating a very thin film (0.01-1 mm). Sometimes, the samples have to be dissolved in solvents such as CCl_4 or CS_2 before sample placement [9], to avoid complete absorption. However, it is difficult to clean halide disks after testing, and the resultant spectra are easily contaminated since the halide disks fog easily due to abruption of moisture from environment and sample [10]. In comparison to the transmission measurements, ATR provides a more convenient and superior means of sample handling since neat oil samples can simply spread onto the surface of ATR crystal and be wiped off after spectral collection. Besides, ATR technique inherently provides a short

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effective path length, thus making ATR particularly useful in the analysis of strongly absorbing sample such as neat oils [11]. At present, Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy has been successfully used in both qualitative and quantitative analysis of edible fats and oils, involving chemical characterization [12-16], identification and authentication [17-20], and quantification of free fatty acids, *trans* fats, iodine value and saponification number [21-24]. Most of the studies based on FTIR-ATR are conducted on the oils of sunflower, olive, soybean, cottonseed, peanut, sesame, corn, butter, etc., but there are only a few reports on FTIR-ATR in rapeseed oil analysis [25].

In this study, FTIR-ATR spectroscopy was employed to characterize rapeseed oils from different cultivars. The effect of thermal treatment on rapeseed oils was also investigated. The differences in their spectral characteristics caused by rapeseed cultivars and thermal treatment were analyzed.

2. Materials and Methods

2.1 Sample Preparation

Rapeseed oil samples were prepared by Soxhlet extraction with anhydrous ether for 8 h. Three rapeseed cultivars commonly grown in China (No. 5 Deyou, No. 521 Kele and No. 10 Qinyou) were provided by Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. Each cultivar of rapeseed was subjected to the oil extraction and the extraction was conducted in triplicate. After the oil extraction, the three cultivars of rapeseed oil were directly subjected to spectral measurement. Following the spectral measurement, the rapeseed oil of No. 5 Deyou was particularly selected and exposed to thermal treatment. This cultivar of rapeseed oil was stored in thermostable plastic bottles (diameter 15 mm and height 40 mm) and placed uncovered at 60 °C in a Model 88-1magnetic stirrer (Guohua Electric Appliance, China). Duplicate samples were removed at the interval time of 6 h, 12 h, 1 d, 2 d, 4 d and 6 d. The samples were cooled in a desiccator for at least 30 min before proceeding to spectral scanning.

2.2 FTIR-ATR Spectral Measurements

A FTIR spectrometer (Nicolet 6700, Thermo Scientific, USA) equipped with a 45 °C ZnSe ATR accessory (Bruker, Germany) was utilized. The oil samples directly spread onto the ATR ZnSe crystal with a pipette for spectral measurements. After each measurement, the ATR crystal was carefully and thoroughly cleaned with pure acetone followed by ethanol to eliminate the presence of oil residues. The washed crystal was then rinsed with deionized water, wiped with cotton and dried under nitrogen gas, to ensure a clean crystal surface before measuring the next sample. The FTIR-ATR spectra was recorded in the wavelength range of 650-4,000 cm^{-1} using 32 scans per sample at a resolution of 4 cm^{-1} and a mirror velocity of 0.32 cm/s. The background spectrum of the empty ATR crystal was collected for spectra intensity normalization before proceeding to scan the sample.

3. Results and Discussion

Fig. 1 shows the spectra of three cultivars of rapeseed oils with a total of nine samples.

Well-resolved peaks presented in the spectra are correlated to constituents in rapeseed oils. TAG is a major component in edible oils, and thus is dominant in the spectra. The major bands associated with TAG functional groups included the peak a, at 3,010 cm^{-1}, corresponding to the C-H stretching of *cis* double bonds; the peak b, at 2,924 cm^{-1}, corresponding to the methylene C-H asymmetrical stretching; the peak c, at 2,856 cm^{-1}, corresponding to the methylene C-H symmetrical stretching; the peak d, at 1,746 cm^{-1}, corresponding to the ester C=O stretching; the peak, at 1,465 cm^{-1}, corresponding to the methylene C-H bending; the peak h, at 1,375 cm^{-1}, corresponding to the methyl C-H symmetrical bending; the peaks i, j, k and l, near 1,240, 1,160, 1,120 and 1,110 cm^{-1}, respectively, assigned to the ester C-O stretching; the peak o, at 721 cm^{-1},

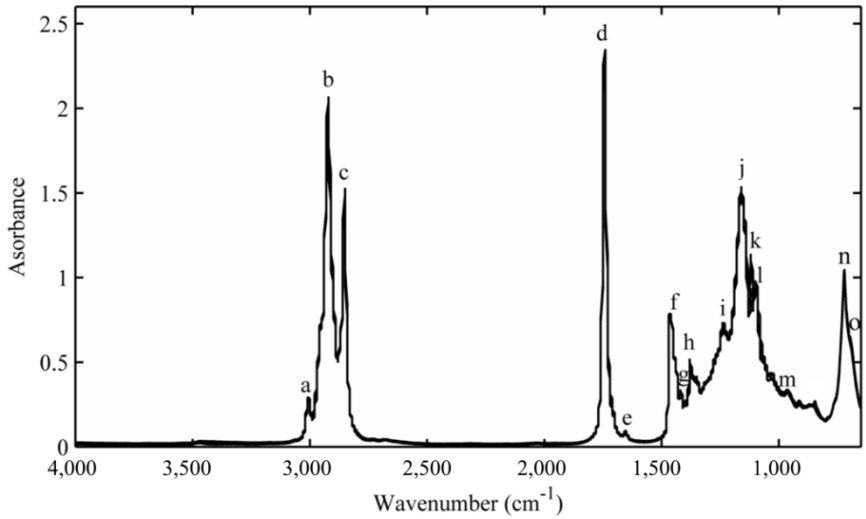

Fig. 1 FTIR-ATR spectra of rapeseed oils.
The peak letters correspond to those indicated in Table 1.

Table 1 The assignment of peaks in the FTIR-ATR spectra of rapeseed oils.

Peak	Wavenumber (cm^{-1})	Functional group	Vibration mode
a	3,010	=C-H (*cis*-)	Stretching
b	2,924	-C-H (CH_2)	Asymmetrical stretching
c	2,856	-C-H (CH_2)	Symmetrical stretching
d	1,746	-C=O (ester)	Stretching
e	1,650	-C=C- (*cis*-)	Stretching
f	1,465	-C-H (CH_2)	Bending
g	1,420	=C-H (*cis*-)	Bending
h	1,375	-C-H (CH_3)	Symmetrical bending
i	1,240	-C-O	Stretching
j	1,160	-C-O	Stretching
k	1,120	-C-O	Stretching
l	1,110	-C-O	Stretching
m	966	-HC=CH- (*trans*-)	Bending out of plane
n	721	-CH_2-	Rocking
o	690	-HC=CH- (*cis*-)	Bending out of plane

resulting from the C-H rocking [6, 12, 15, 26]. Besides, very weak peak e, around 1,650 cm^{-1}, was assigned to the *cis* C=C stretching; the peaks g, at 1,420 cm^{-1} and m, at 966 cm^{-1}, resulting from the CH_2 bending of *cis* C=C bonds and the C-H bending of *trans* C=C bonds, respectively [6, 26]. Finally, a shoulder peak o, at 690 cm^{-1} indicated the CH bending of *cis* C=C bonds [27] as summarized in Table 1.

The overall spectral appearance of the all rapeseed oil samples was quite similar. It was very difficult to differentiate the three varieties of rapeseed oils based on the spectra alone. Therefore, principal component analysis (PCA) was performed to facilitate an overview of the distribution of three varieties of rapeseed oils. The first and second principal components (PCs) accounted for 99.89% and 0.09% of the total variance, respectively. The score plot of the first two PCs showed the three classes of rapeseed oils, which could be clearly distinguished from one another (Fig. 2). To further evaluate the class assignment, linear discriminant analysis (LDA) was applied on the two PCs by calculating the Mahalanobis distance, which is the distance between each sample from all the centers of the three groups of samples [28, 29]. As a result, the

assignment success rate obtained was 100%, which showed the two PCs could completely discriminate these three different rapeseed oils.

The lipid oxidation is a major cause of quality deterioration of vegetable oils. The oxidation could cause changes in the chemical composition of vegetable oils, which in turn alters the oil quality attributes such as texture, taste, shelf life, appearance and nutritional profile, consequently resulting in the development of rancid flavors and potentially toxic compounds [30]. The process of lipid autoxidation at ambient temperature (25 °C) has been well understood, but it is still complex and variable due to the oil types and oxidation conditions. Thermal stress is considered an important driving factor causing oxidative reactions of lipids. Therefore, the spectral characteristics of rapeseed oils resulting from thermal treatment were investigated to further understand the lipid oxidation behaviors. In this case, the rapeseed oil of No. 5 Deyou was chosen for this study.

Fig. 3a shows the FTIR-ATR spectra of the rapeseed oil of No. 5 Deyou after exposed to thermal treatment at 60 °C at different incubation time. Prominent changes in the spectra of rapeseed oils were observed at 3,500, 3,010, 1,650, 966 and 690 cm^{-1}. Fig. 3b shows the evolution of absorbance intensity at those wave numbers. Around 3,500 cm^{-1}, a weak broad peak occurred and the corresponding absorbance increased when the thermal treatment prolonged. This peak could be associated with O-H stretching vibration of hydroperoxides as the oxidation of polyunsaturated lipids underwent peroxidation reactions with oxygen and formed lipid hydroperoxides [31]. The absorbance at 3,010 cm^{-1} associated with the C-H stretching vibration of *cis* C=C

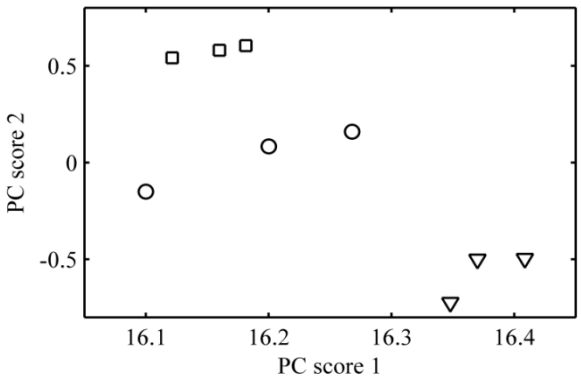

Fig. 2 The score plot of PCs obtained from the FTIR-ATR spectra of three cultivars of rapeseed oils. □: Deyou No. 5; △: No. 521; ○: No. 10 Qinyou.

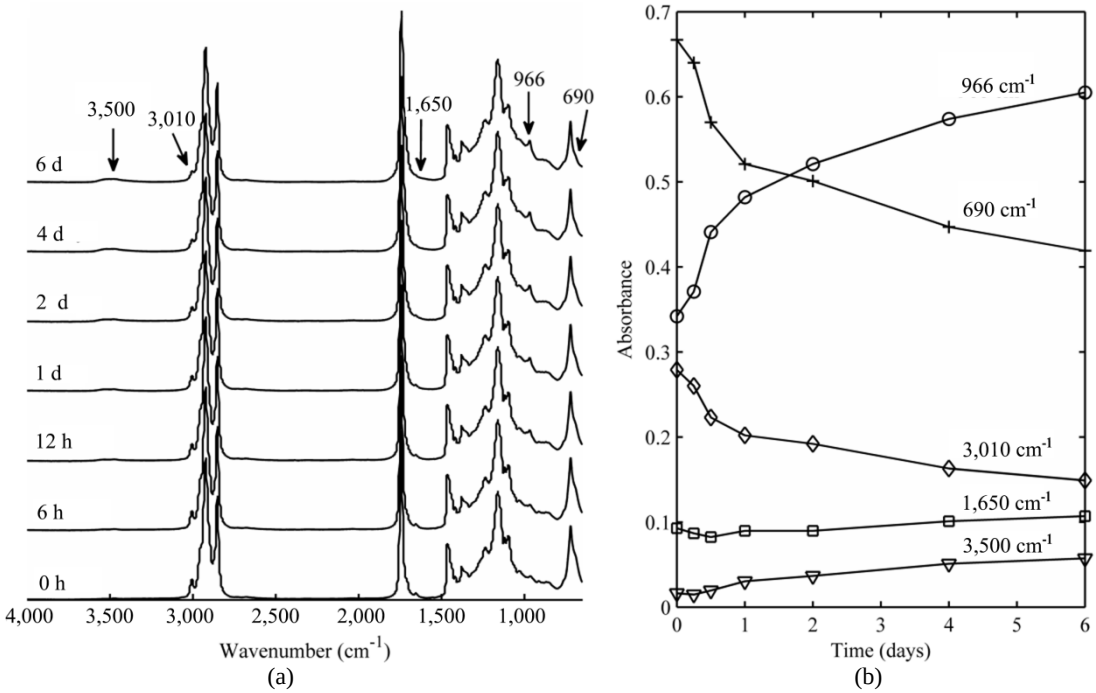

Fig. 3 (a) FTIR-ATR spectra of rapeseed oils with different periods of thermal treatment and characteristic wavebands indicated by the arrows. (b) The absorbance changes of characteristic wavebands over the whole period of thermal treatment.

was found diminished, particularly at the preliminary stage of lipid oxidation. The depletion of the *cis* C=C was the characteristic of lipid oxidation under moderate conditions [31]. A weak peak at 1,650 cm^{-1}, associated with stretching vibration of *cis* C=C underwent some interesting changes. There was a slight decrease at first, then followed by a slight increase. According to the spectra, however, the weak peak at 1,650 cm^{-1} became invisible as the oxidation process advanced. The explanation for this change was relatively complex. The initial decrease could be attributed to the net loss of C=C bonds [31]. As for the later decrease, thermal oxidation of unsaturated oils was followed by considerable isomerisation of C=C. The isomerisation generated the bonds of *trans* C=C and conjugated C=C systems [32], and thus explained the absorption increase of the band around 1,675 cm^{-1}, which probably overlapped with the band of aldehydes and ketones at 1,730-1,680 cm^{-1} resulting from hydroperoxides.

Major changes of FTIR-ATR spectral took place at 966 cm^{-1} and 690 cm^{-1} as the oxidation rate increased. The rapid increase in absorbance at 966 cm^{-1} indicated the formation of *trans* C=C. The peak at 966 cm^{-1} was the characteristic absorption of the C-H bending vibration of *trans* fat and had been used for quantitative calibration of *trans* fat [9, 23, 25]. It is worth noting that the increase at 966 cm^{-1} was, nearly to the same degree, accompanied by the decrease at 690 cm^{-1}, which could possibly provide a new tool to quantify the *trans* fat. The changes in the shoulder peak at 690 cm^{-1} was seldom investigated in the previous literature. The changes in the oxidized rapeseed oil spectral was due to the C-H vibration of *cis* C=C [27]. This phenomenon could indicate a conversion of *cis* C=C to *trans* C=C when rapeseed oil that contained high amounts of unsaturated fatty acids, especially oleic acid, exposed to high temperature, tending to be vulnerable to thermal isomerization and degradation.

4. Conclusions

Rapeseed oils showed a rich absorption in FTIR-ATR spectra and this spectral information could be used to differentiate rapeseed oils from different cultivars. The chemical changes of rapeseed oil induced by thermal treatment could be monitored by FTIR-ATR spectroscopy. This finding had shown the potential of FTIR-ATR spectroscopy as a versatile tool for characterization of rapeseed oils.

Acknowledgments

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