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FTIR Spectroscopy for Evaluation and Monitoring of Lipid Extraction Efficiency for Murine Liver Tissues Analysis †

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Abstract: Over the past several decades, growing research on lipids and lipidomic technologies have shown the important role played by lipids in many different situations. A powerful technique used for lipids detection and characterization in biological tissues is Fourier Transform Infrared (FTIR) spectroscopy. The main goal of the present work is to exploit FTIR spectroscopy as a tool for monitoring lipid extraction efficiency by evaluating three different lipid extraction methods in murine liver tissues. In particular, infrared spectra have been obtained in the 4000–600 cm^{-1} wavenumber region and the contributions of different functional groups have been evidenced. The ratio values estimated using the absorbance of selected bands related to different liver constituents have been used for a quantitative comparison of the efficiency of the different extraction methods

Keywords: lipids; murine liver tissue; extraction methods; FTIR spectroscopy

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1. Introduction

Over the past several decades, growing research on lipids and lipidomic technologies have shown how the perception of lipids has changed. Lipids are functionally versatile molecules in plants, animals, and humans. They are certainly key components of the cell membranes and a source of energy, but they also play an essential role in physiology and pathophysiology, in signal transduction between cells and body metabolism and act as diagnostic and/or prognostic biomarkers of different diseases [1]. Many studies have shown the relationship between altered lipid metabolism and type 2 diabetes mellitus (T2DM) or metabolic disease as Nonalcoholic fatty liver disease (NAFLD) or neurodegenerative disease as Parkinson's Disease or Atherosclerosis (a risk factor for ischemic stroke) [2–4]. A powerful technique used for lipids detection and characterization in biological tissues is Fourier Transform Infrared (FTIR) spectroscopy [5]. The main goal of the present work is to exploit FTIR spectroscopy as a tool for monitoring lipid extraction efficiency by evaluating three different lipid extraction methods [6]. FTIR spectroscopy is used to monitor the extraction efficiency of the Folch [7], Bume [8] and modified Bume [9] methods in murine liver tissues. In particular, infrared spectra will be obtained in the 4000–600 cm^{-1} wavenumber region and the contributions of different functional groups will be evidenced. The ratio values estimated using the absorbance of selected bands related to different liver constituents will be used for a quantitative comparison of the efficiency of the different extraction methods.

2. Materials & Methods

Murine liver tissues were used in the experiments. Animals were kept in standard temperature and humidity conditions. At sacrifice, the animals were deeply anesthetized

by overdose of Tanax (0.1 mL intrapulmonary). The tissues investigated (50–100 mg) were collected and immediately frozen at −20 °C. All animal studies were carried out in accordance with the principles and procedures outlined in the National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the Italian Ministry of Research and the Italian Ministry of Health.

Extraction of lipid using Folch method [7] was performed using frozen tissue collected into the test tube. 4 mL of methanol was added to the test tube and the sample was homogenized for 3 min at 15000 rpm. After the addition of 1,5 mL of chloroform, the solution was stirred for 10 min at room temperature. 3 mL of water was added to methanol/chloroform mixture and the sample was centrifuged at 2500 rpm for 5 min at 24 °C. Then, the upper layer was removed, and the lower chloroform phase (containing lipids) was collected in a new test tube, flushed with nitrogen, capped, and stored at −20 °C.

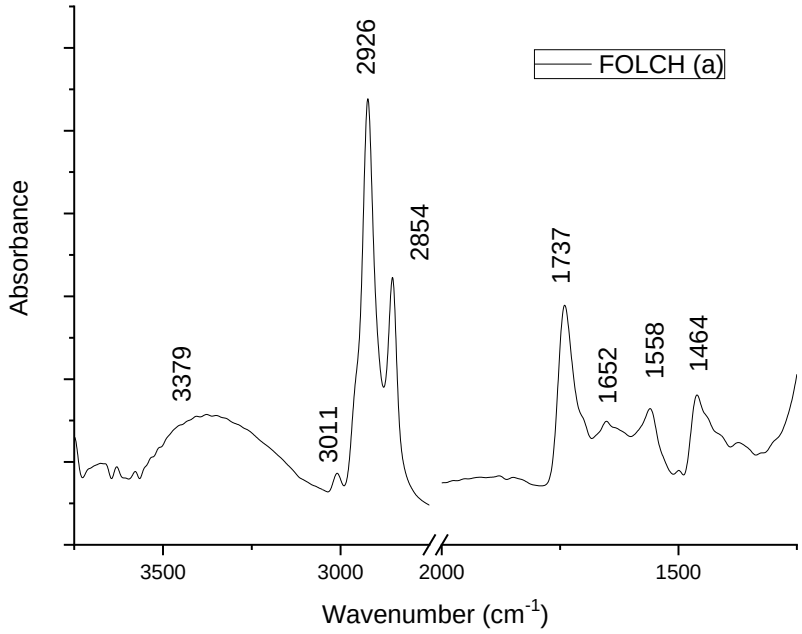
For Bume method [8], frozen tissue was collected into the test tube. 500 μL BUME mixture (butanol: methanol [3:1]) was added to the test tube and the solution was vortexed for one minute. After the addition of 500 μL heptane: ethyl acetate [3:1] and 500 μL 1% acetic acid (to induce phase separation), the sample was centrifuged for 10 min at 4000 g and then lipids were recovered from the organic (upper) phase. The upper organic layer was collected and placed into a new test tube. A second phase extraction was performed adding 500 μL heptane: ethyl acetate [3:1] to water (lower) phase. After the addition of heptane: ethyl acetate, the solution mixture was vortexed and centrifuged at 4000 *g* for 10 min and the resulting upper organic layer was collected and combined with the previous organic layer. The lipid extract was flushed with nitrogen, capped and stored at −20 °C.

For modified Bume method [9], frozen tissue was collected into the test tube. Butanol/methanol (300 μL, [3:1], *v*/*v*) was added to the test tube and the solution was vortexed for one minute. Heptane/Ethyl Acetate (150 μL, [3:1], *v*/*v*) was added to the tube and the solution was vortexed again for another minute. Another 150 μ heptane/Ethyl Acetate was added, and after vortexing for 1 min, 300 μL of 50 mM LiCl was added to the test tube to induce phase separation. After this step, the resulting solution was vigorously vortexed for another minute. Then the solution was centrifuged at 2700 *g* for 10 min. The upper organic layer was collected and placed into a new test tube. The remaining aqueous layer was re-extracted twice, first with 320 μL of heptane/Ethyl Acetate [3:1], then with 250 μL of the same solvent. After each addition of heptane/Ethyl Acetate, the solution mixture was vortexed and centrifuged at 2700 *g* for 10 min, and the resulting upper organic layers were collected and combined with the previous organic layer. The lipid extract was flushed with nitrogen, capped, and stored at −20 °C.

For FT-IR spectral analysis, the lipid extracts were resuspended in methanol and drops of a few microliters were placed on metallic IR-reflective surface and left to dry and used for spectra acquisition. FTIR spectra were obtained using a Perkin Elmer Spectrum One spectrometer. All spectra were collected using 64 scans in the range from 4000 to 1000 cm^{-1} with a 4 cm^{-1} spectral resolution.

3. Results and Discussion

In Figure 1 the FTIR spectra of lipid obtained from three different techniques are reported. They show similar profiles with some differences in shape and in the relative intensites of different peaks.


FOLCH (a)
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1558
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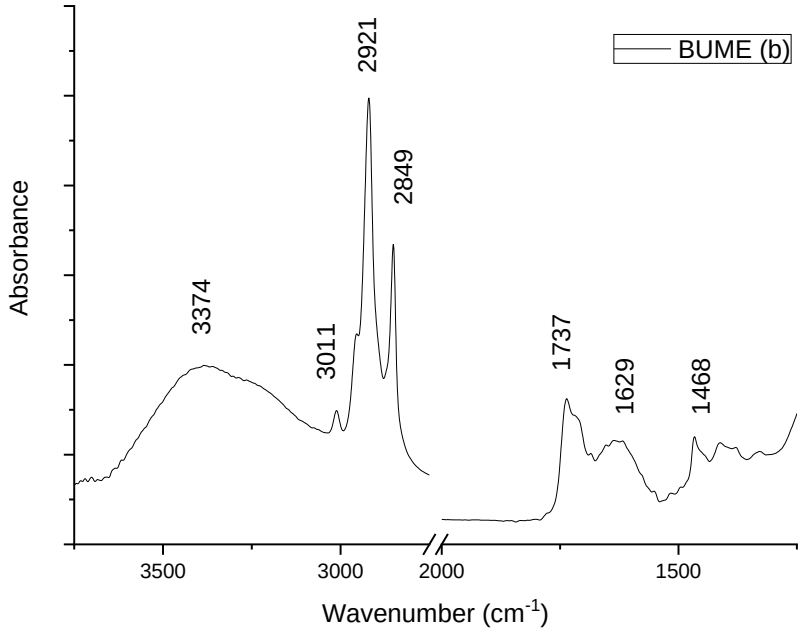
3500
3000
2000
1500
Wavenumber (cm-1)


BUME (b)
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Wavenumber (cm-1)

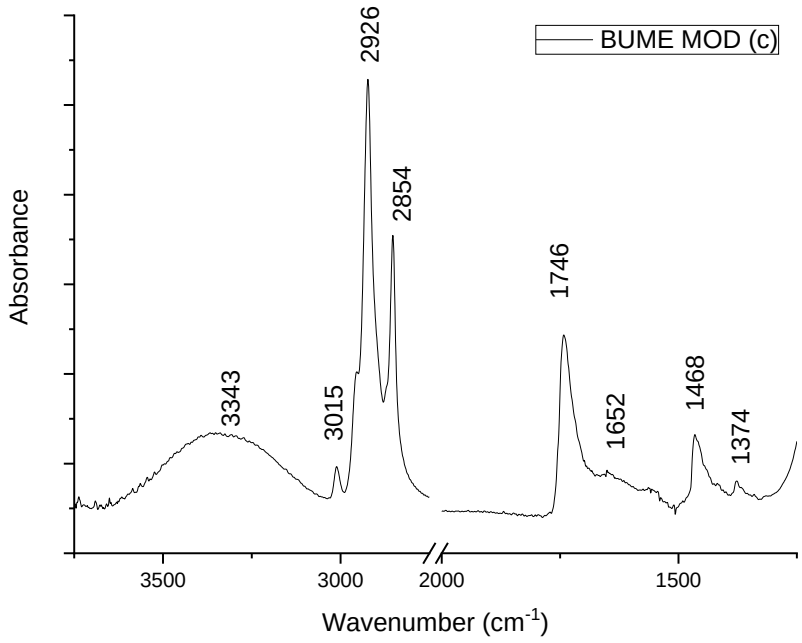

Figure 1. FT-IR spectra of samples obtained with the three different extraction methods.

In Figure 1 the FT-IR spectra of the samples obtained using the different extraction methods are reported. The contributions due to functional groups related to lipids are clearly evident. In addition, some peaks ascribed to proteins are also present. In Table 1, the assignments of all the main peaks present in the spectra are shown.

As is evident all the three used methods are quite efficient in lipid extraction. In order to exploit the information given by FT-IR spectra in a more quantitative way, the ratio between the CH_2 asymmetric stretching peak (located at ≈2926 cm^{-1} and related to lipid content) and the Amide A peak (placed ≈ 3300 cm^{-1} and due to protein content) can be evaluated. This ratio assumes the values 2.75 ±0.14, 2.5 ±0.13 and 3.94 ±0.20 for samples extracted using Folch, Bume and Bume modified methods, respectively. These values allow us to consider Bume modified method as the most efficient in lipid extraction.

Table 1. Main peaks present in the spectra of Figure 1 and assignments according to Ref. [10].

Peak Position (cm^{-1})			Assignments
Folch Method	**Bume Method**	**Bume Mod Method**	
3379	3374	3343	N-H stretching (Amide A)
3011	3011	3015	C-C unsaturated fatty acids
2926	2921	2926	CH_2 asymmetric stretching
2854	2849	2854	CH_2 symmetric stretching saturated fatty acid
1737	1737	1746	ester C-O stretching
1652	1629	1652	C O stretching (Amide I)
1558			N H bending (Amide II)
1464	1468	1468	CH_2 bending

4. Conclusions

In the present paper, we investigated the efficiency in lipid extraction of three different methods by using FT-IR spectroscopy. All the three procedures show good performances. The evaluation of the ration between the intensity of two peaks related to lipid and protein contributions indicate that Bume modified method is characterized by the higher efficiency. Further investigations are in progress for confirming these results. Among the three different extraction systems used in this study, the Bume and Bume modified methods are superior to the Folch method in terms of simplicity, through-put, automation, solvent consumption, economy, health and environment yet delivering lipid recoveries fully comparable to or better than the Folch method.

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Informed Consent Statement:

Data Availability Statement: Data are available on request.

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Conflicts of Interest: The authors declare no conflict of interest

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