Rapid ATR-FTIR method for monitoring the release of major components of clove essential oil encapsulated into a complex organic matrix

JOSÉ DANIEL WICOCHEA RODRIGUEZ[1], Emanuelle Gastaldi[1], Thierry Ruiz[1], Peggy Rigou[2], Stéphane Peyron[1] and Pascale Chalier[1]

[1] *IATE, UMR 1208 INRA – CIRAD – Montpellier SupAgro – Université de Montpellier, 2 Place Viala, 34060 Cedex, Montpellier, France*
[2] *SPO, UMR 1083 INRA – 2 Place Viala, 34060 Cedex, Montpellier, France*

Abstract

Fourier transform infrared (FTIR) attenuated total reflection (ATR) spectroscopy is a rapid and non-destructive technique that provides an overall infrared fingerprint of a matrix and/or sample. It can prevent time consuming analysis for monitoring changes in an aroma profile and/or aroma compounds transfer (1).

In this study, clove essential oil (EO), previously identified by GC-MS, was encapsulated into an organic solid matrix mainly composed of proteins and polysaccharides. The controlled release of major aroma compounds of the essential oil was followed using a FT-IR method and compared to conventional gas chromatography methodology. The quantification by FTIR was performed after a calibration procedure carried out by depositing pure clove oil and its major components at different concentrations on a constant mass of matrix without essential oil. Several Partial Least Squares (PLS) regression calibration models were optimized on the specific bands of aroma compounds to determine the best correlation ($R^2 > 0.90$) between the predicted and reference values of clove essential oil major compounds. The limit of detection (LOD) and limit of quantification (LOQ) were determined and the release of major compounds of clove EO proved to be similar as the results obtained using GC-FID. Such ATR-FTIR method can be used as an alternative rapid method for the identification and quantification of major aroma compounds in complex organic samples.

Introduction

The common method for essential oils (EOs) quantification is extraction followed by a gas chromatography analysis which is expensive and time consuming. Fourier transform infrared (FTIR) attenuated total reflection (ATR) spectroscopy is a rapid and non-destructive technique which provides an overall fingerprint of a sample and can be considered as an interesting alternative way for the rapid quantification of EOs or monitoring their evolution in a complex medium (1). ATR-FTIR has already been successfully used for EOs characterization but to our knowledge there are no previous investigations that have been conducted on the quantification of EO encapsulated in an organic complex matrix. Indeed, the presence of complex matrices can compromise the analysis by affecting the sensitivity and the specificity of method due to spectra superposition of the targeted compounds with the matrix fingerprint.

The aim of this study was to assess the potential of ATR-FTIR method applying a partial least square (PLS) model and a cross validation with a GC quantitative analysis to monitor the release of clove EO encapsulated in a complex matrix.

Experimental

Matrices elaboration and GC-MS analysis of Clove EO

An organic powder with high content in polysaccharides and proteins was mixed with distilled water and clove EO (Golgemma, Esperza, France). The mixture was processed and dried at a low temperature to obtain a matrix with the ability to retain and release aroma compounds. Clove EO was characterized by a GC-MS (ISQ, ThermoScientific, Austin, Texas, USA) equipped with a DB-WAX polar capillary column (30 m, 0.25 mm i.d. x 0.25 µm of thickness). Helium was used as carrier gas with a flow rate of 1.2 ml/min. The GC-MS oven temperature was kept at 40 °C for 5 min and programed to 260 °C at a rate of 2 °C/min. One µL of diluted sample (dilution 1/100) was injected at constant temperature of 250 °C via split injector (1:20). Spectra were obtained in the electron impact mode (70ev) in full scan mode with a range between 40-500 amu. Identification of components was based on the calculated Kovats indexes estimated by simultaneous injection of alkanes and comparison of spectra with mass spectra libraries (NIST 2.0/Wiley/INRA). Quantification of the identified compounds was expressed as percentage by directly calculating from peak areas.

ATR-FTIR method

A FTIR Nicolet 6700 spectrometer (Thermo Scientific) in Attenuated Resonance mode has been used. Clove EO major aroma compounds used as standards were purchased from Sigma-Aldrich and analyzed directly by deposing around 13 mg of aroma compound over the diamond crystal of a Smart DuraSamplIR accessory (Thermo Scientific). Spectral data were recorded from 64 scans with a resolution of 2 cm^{-1} in the range of 800-4000 cm^{-1} wavenumbers.

For establishment of standard curves, organic matrices free of clove EO were elaborated. Different known amounts of clove essential oil were deposited into the several matrices by using a precision pipette and stored in closed glass jars at room temperature until analysis. The matrices were grinded using a laboratory mortar and analyzed directly by the ATR-FTIR spectrometer. In parallel, the EO was extracted from the matrix (18h at constant stir and room temperature) using hexane in the presence of 100 µL of 2-heptanol at 3 g/L as internal standard. The organic extracts were analyzed and the major components previously identified using standard components were quantified by a GC-FID (Varian CP-3800 GC, Les Ulis, France).

Controlled release of EO from matrices and validation of FTIR method

The organic matrices loaded with clove EO were put into a Memmert oven HPP IPP plus (Buchenbach, Germany) maintained at controlled temperature of 25 °C and relative humidity at 72 % during a period of 34 days. At interval times, samples were removed and analyzed by the ATR-FTIR method and the GC-FID method for comparison purpose.

Chemometric analysis

Spectra treatments were performed using Omnic v7.3 and TQ Analyst v7.3 softwares (Thermo electron). The most prominent spectral band specific of clove EO was selected for the PLS analysis. Calibration models were validated by a cross validation (leaving 40% out) using a regression PLS calibration algorithm. Limit of detection (LOD) and limit of quantification (LOD) was calculated with the guidelines of the International Conference on Harmonisation (ICH) (2).

Results and discussion

Essential oil characterization

The major components of clove essential oil and their relative proportion were identified by GC-MS. Eugenol represents 62.52 % ± 0.35 of total oil, acetyleugenol 23.62 % ± 0.42, β-caryophyllene 9.77 % ± 0.18, α-caryophyllene 1.39% ± 0.02, caryophyllene oxide 0.98% ± 0.01, methyl salicylate 0.91% ± 0.014 and allylphenol 0.47% ± 0.01. These components are the most outstanding major compounds in the EO composition as generally described in literature (3).

ATR-FTIR method.

First, the FTIR spectral bands of clove EO were compared with the spectra of an uncharged matrix. As no overlapping with the pure matrix spectra are observed for the ranges of the spectra from the 1250 to 1600 cm^{-1} wavenumbers, a band was selected in this region allowing a good sensitivity of the analysis. For quantification, the 1514 cm^{-1} stretching aromatic C=C vibration (4) was preferentially selected. This band was found for three of the clove EO major compounds (eugenol, acetyleugenol and 4-allyphenol) according to the aroma compounds characterization by ATR-FTIR. These 3 phenolic compounds represent 86% of the essential oil, which explains the proportional increase of the 1514 cm^{-1} peak to 5 concentrations included in the standard curve (Figure 1). This stretching band was chosen to elaborate the PLS regression, a normalization was carried out according to the peak height of CH$_2$ stretching band at 2853 cm^{-1} that corresponds to the organic pure matrix.
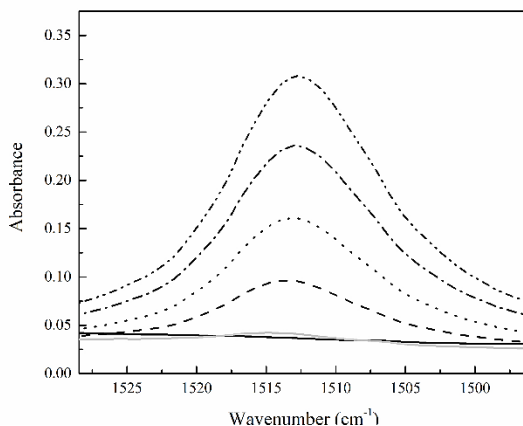

Figure 1: Increase of the spectroscopic selected band (1514 cm^{-1}) proportionally to concentration.

Quantitative analysis of trapped EO by ATR-FTIR.

For the development of the quantification model, averaged spectra issued from 35 samples were correlated with a GC-FID standard curve for which average values were obtained from 5 samples (Figure 2). Although the different amounts of analyzed samples between the two methods (13 mg of matrix for ATR against 300 mg for GC) the correlation between the ATR-FTIR and GC-FID analysis was satisfactory since PLS regression model produced a high coefficient of determination (R^2) and RMSECV and RMSEP values were 0.0120 and 0.0162 g/g respectively, indicating that the residuals of the calibration data are low and the model gives a good estimation on how it built the data for unknown samples. LOD (0.003 g of clove EO/g matrix) and LOQ (0.010 g of clove

EO/g matrix) were determined by multiplying the standard deviation measured on 15 blank samples by 3 and 10 times respectively, and dividing the result value by the slope of the ATR-FTIR standard curve.

Controlled release of EO from matrices and validation of the ATR-FTIR method

The release of clove EO at controlled relative humidity and temperature was followed both GC-FID and ATR-FTIR methods. It was found that the chemometric method could be applied to predict the release of major compounds of clove essential oil, including eugenol, acetyl eugenol and 4-allylphenol. The fact that the quantification does not cover the totality of the compounds and that small changes in aromatic profile occurred as observed by GC analysis could result from variability within samples.
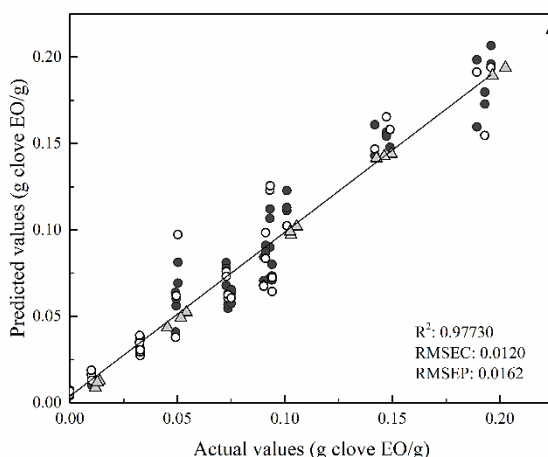

Figure 2: Results of PLS cross validation essay: ●PLS calculated value, ○ PLS validated value, ▲ GC-FID validation.

The proposed ATR-FTIR protocol demonstrated to be a reliable and robust quantitative method compared to the GC despite the complexity of the organic matrix studied. The ATR-FTIR method can be used to survey the release of the selected aroma compounds of essential oils trapped in an organic matrix or study the influence of specific external factors over the matrix.

References

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