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Composition of Glyceride Esters of Lauric Acid by FTIR Band Shape Analysis

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Synthesis of glyceride esters of a fatty acid produces a mixture of isomers that are difficult to separate and analyze, requiring high temperature GC in most cases particularly for long-chain esters. In this paper, we present a fast estimation of the composition of the glyceride esters of lauric acid and glycerol (monolaurin, dilaurin, and trilaurin) by FTIR band shape analysis. The method uses the fact that the carbonyl stretching regions of the pure glycerides have different band shapes, which implies any composite band of a mixture of glycerides may be resolved into the component peaks due to each glyceride. The carbonyl band region was fitted with five component peaks using a commercial peak-fitting program. The peak at 1745 cm^{-1} is characteristic of trilaurin whereas the peaks at 1740 cm^{-1} and 1731 cm^{-1} provide a unique height ratio for mono- and dilaurin. Calibration curves were prepared and a system of two equations may be solved to obtain the composition of mono-, di-, and trilaurin. This method was tested with known mixtures of the glycerides yielding estimates within ± 10 % composition units.

Keywords: glycerides, lauric acid, infrared, band shape, monolaurin

INTRODUCTION

The Philippines supplies about 65% of the world's coconut oil, which take up about 90% of the coconut process industry [1]. Despite being one of the top producers of such commodity, the utilization and further applications for coconut oil are still lacking. One promising development is the discovery of coconut oil's anti-bacterial and anti-viral activity and this is believed to be due to its C-12 (dodecanoate or laureate) content [2]. Recent studies also revealed that it was not only the fatty acid that is responsible for its anti-microbial properties, but also due to the lauryl monoglyceride which showed the largest activity against bacteria and viruses compared with other fatty acid esters [3]. This monoglyceride has been synthesized by esterification of lauric acid and glycerol [4-7]. The reactions usually yield a mixture of the mono-, di- and triglycerides. To analyze such mixture, chromatographic methods such as high performance liquid chromatograph [8] and high temperature gas chromatography [6,9] were used.

In this paper, we present a quick estimation of the composition of the triglycerides (monolaurin, dilaurin, and trilaurin) by FTIR band shape analysis.

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The procedure may find use in monitoring reactions, such as in production or kinetics study. For example, industrial scale production would benefit from such a quick assay to assess how the production process is going.

EXPERIMENTAL

Materials. Standard samples of glycerol 1-monolaurate (1-monolaurin), glycerol dilaurate (isomers of 1,2 dilaurin and 1,3 dilaurin), and glycerol trilaurate (trilaurin) were purchased from Sigma Chemicals Co. Reagent grade diethyl ether was obtained from J.T. Baker.

Preparation of calibration samples. Standard stock solutions of 10 mg/mL of pure monolaurin, dilaurin, and trilaurin were prepared. Solutions for the calibration curve were prepared by mixing corresponding volumes of the stock solutions as shown in Table 1.

Table 1. Standard (calibration) solutions of glycerides of lauric acid

Mixtures	Mass % proportion
ML:DL	50:50
	75:25
	25:75
ML:TL	50:50
	75:25
	25:75
ML:DL:TL	50:25:25
	25:50:25
	25:25:50

Legend: ML: Monolaurin, DL: Dilaurin, TL: Trilaurin

Esterification of glycerol and lauric acid. Reactants glycerol and lauric acid were placed in the reaction chamber in a ratio of 4:1 together with molecular sieve, a lipase (Novozyme 435), and solvent (acetone). The mixture was heated and maintained at 50.0 0C (± 0.1) using a thermostated water bath and magnetically stirred. After 8 hours, the mixture was suction filtered using a fritted funnel. The solvent was then evaporated under vacuum. This product mixture was redissolved in diethyl ether and the unreacted lauric acid extracted twice using 0.5 M KOH solution. The ether solution was evaporated under vacuum.

FTIR spectra. A sample is dissolved in diethyl ether and the solution is smeared onto a KBr plate. The smeared sample was dried under an IR lamp for 30 to 60 seconds. (It is important to dry the sample under the lamp, because residual ether was observed to distort the carbonyl region of the band.) The FTIR spectra were obtained using a Shimadzu FTIR-8201 PC model at 4 cm-1 resolution using a DTGS detector using blank KBr as background; 40 scans were averaged for each spectral measurement.

Band Shape Analysis

Processing Raw FTIR spectra. The FTIR raw spectrum was first baseline corrected using the software SpectraCalc by Galactic Inc., particularly the region between 2000 cm-1 to 1550 cm-1, which contains the carbonyl stretching frequency. These spectral regions were then converted to the specific file format applicable to the band shape analysis program.

Analysis using PeakFit v4.12 (trial version). Curve fitting analysis was performed using the spectral processing software PeakFit v4.12 (trial version) by Seasolve Software Inc.[10]. The carbonyl region of the spectrum was analyzed using the following typical steps:

1. Autofit peaks I residual was chosen. Component peaks were automatically recognized based on the local maxima in the smooth data stream. Hidden peaks were then optionally added where peaks in the residuals occur. These additional component peaks were chosen based on the peaks observed in the pure glycerides.
2. Default settings that were kept include the baseline fitting at 3% Linear, D2 and the smoothing option at 1% using the option Savitsky-Golay. The peak type was set to spectroscopy and the Voigt function was then applied at 1.5% amplitude. Prior to fitting, the software was also set to automatically refine the shape and allow residuals to be added.
3. The component peaks add up to a composite band that overlaps the original spectrum. The center position was kept to within ± 2 cm-1 of the assigned peak taken from the spectra of the pure glycerides. These component peaks were then locked at specific wavenumbers and peak widths.

4. A nonlinear least squares curve fit analysis was done with at least 200 iterations to a maximum of 500 iterations. The software uses the Levenberg-Marquardt algorithm (more information on this is described in the software).

RESULTS AND DISCUSSION

Determination of component peaks in pure standards. The FTIR spectra of the pure monolaurin (ML), dilaurin (DL), and trilaurin (TL) are shown in Figure 1. The band around 3200-3400 cm^{-1} is assigned to the O-H stretching vibration. As expected, this band is absent in TL but present in the DL and ML. The ML structure has two free hydroxyls and only one for the DL, and this fact is also observed in a higher normalized band for the ML. The O-H stretching vibration for the ML was also broader and appeared at a lower frequency compared with that of the DL.

The carbonyl stretching frequency region (1650-1730 cm^{-1}) for the three glycerides have different band shapes as shown in the zoom-in on Figure 2. The carbonyl band shape for esters was studied by Blume et al. [11] wherein they reported shifting of the carbonyl vibration to lower frequencies due to hydrogen bonding interactions with a polar solvent. The same trend is observed here where the TL showed the band with the highest C=O stretching frequency at 1745 cm^{-1}. The TL band was also the most symmetric indicating only one type of carbonyl vibration. In comparison, the C=O bands of the ML and DL were shifted to lower frequencies, were broader and clearly indicate overlapping component peaks at 1740 and 1730 cm^{-1}. These observed red shifts in the C=O band are thus also explained by H-bonding interactions with the free hydroxyls in ML and DL. These identified peaks were then used as basis for the curve fitting analysis discussed below.

Band shape analysis and curve fitting are usually done in analyzing unresolved spectral bands to be able to extract quantitative information from the spectrum [12,13]. The absorbance band is nonlinear least squares fitted with the sum of a number of component peaks that have been identified for specific species or molecular groups. The relative concentrations of these groups are then calculated from the relative component peak areas.

Table 2 shows the data obtained from the curve fitting of the carbonyl peaks of the pure glycerides

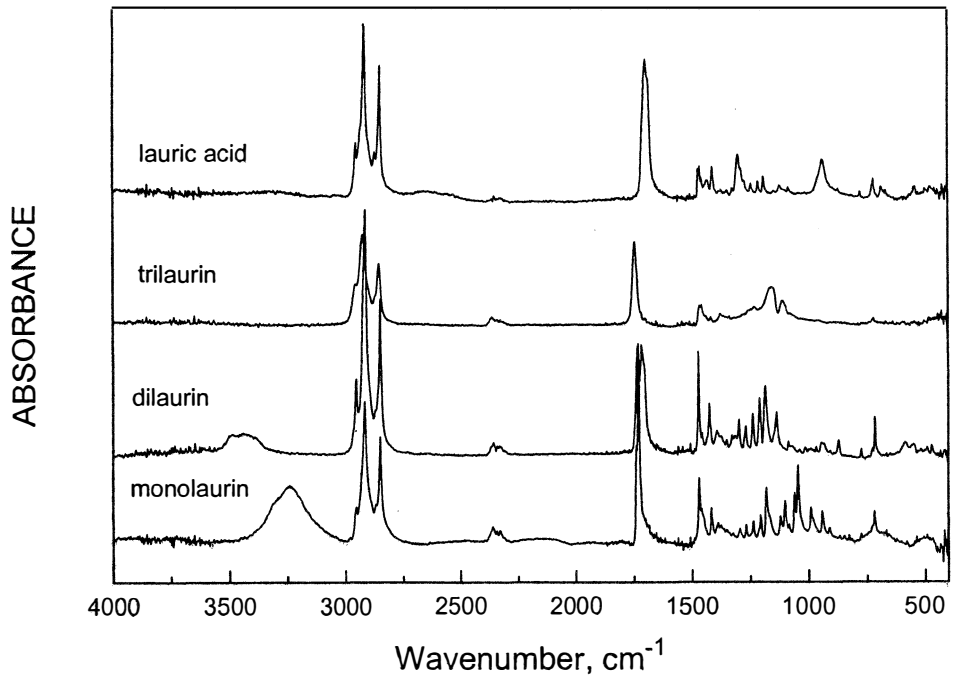

Fig. 1. *FTIR normalized spectra of (bottom to top) pure monolaurin, dilaurin, trilaurin and lauric acid. The IR spectra were vertically offset from each other for comparison purposes.*

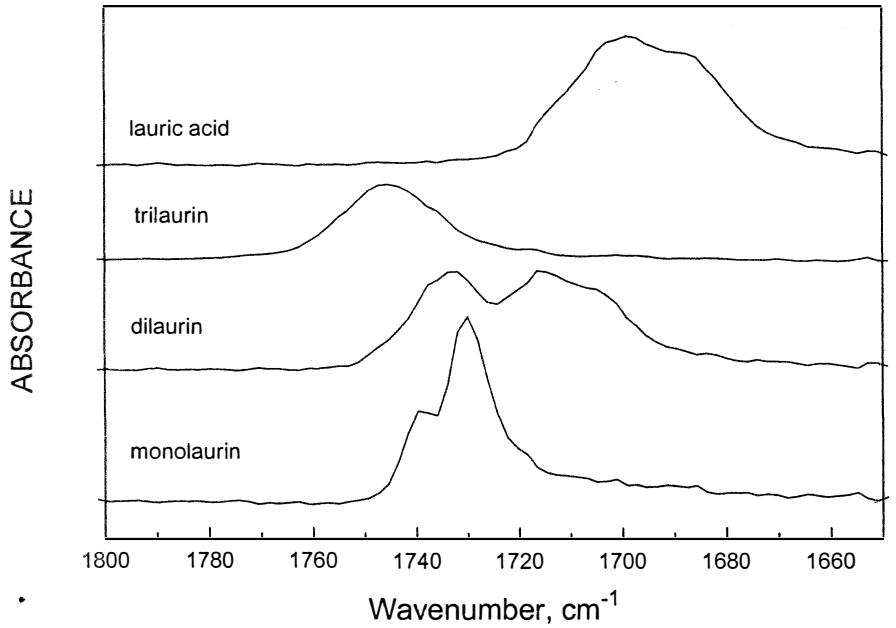

Fig. 2. FTIR spectra at regions 2000 cm-1 to 1550 cm-1 showing the major carbonyl peaks of pure (bottom to top) monolaurin, dilaurin, trilaurin and lauric acid. The IR spectra were vertically offset from each other for comparison purposes.

following the procedure outlined in the Methodology. The data in Table 2 can be better understood using Figure 3 wherein the calculated component peaks and the resulting superposed band are plotted together with the original FTIR spectrum.

As discussed earlier, the TL appears to be largely a single peak which is distinct from the other two glycerides (Figure 3c), although a small additional component band had to be added to fix the broad tail end towards the low frequency region of the band. The fact that trilaurin lacked H-bonding sites in its molecular structure implies that the C=O band is "free" and appears at a higher frequency with little overlap with the DL and ML C=O band region (Figures 3a and 3b). Because of the absence of significant interference with the other bands, a calibration curve could be directly created for trilaurin (Figure 4a) allowing for a direct quantification of trilaurin the mixture.

In comparison, ML and DL have the same component peaks at 1740 and 1731 cm^{-1}. The monolaurin and dilaurin component bands overlapped, although it was found that the ratio of the absorbance at 1740 cm^{-1} to that of 1731 cm^{-1} is different for mono- and dilaurin. This ratio was used to construct a calibration curve for the relative compositions of monolaurin and dilaurin (Figure 4b). Aside from these component peaks, the broad tail end of the C=O bond necessitates additional component peaks to improve the agreement of the calculated superposed band with the original spectrum.

Therefore, a complete quantification of a mixture of the mono-, di-, and trilaurin is possible using the % trilaurin calculated from the 1745 cm^{-1} peak and the relative ratio of mono- to dilaurin using the 1740 and 1731 cm^{-1} peak, where a system of two equations (Equations 1 and 2) was set up as discussed further below.

In summary, the peaks at 1745, 1740, and 1731 cm^{-1} were identified in the pure glyceride spectra and these were used in the analysis of mixtures. However, to improve the curvefit, two additional peaks were added in the low frequency tail end of the spectrum. To correct for varying amounts of sample smeared on the KBr plate, the spectra were normalized relative to the C-H stretching band at 2850 cm^{-1}, which is common to all spectra.

Table 2. Absorbance of different component peaks and correlation coefficient (R^2) of the fitted curve after at least 200 iterations of standard glyceride solutions.

Glyceride	Peak centers, cm^{-1} (Absorbance value)					R^2
Monolaurin	-	1739.2 (0.0513)	1730.2 (0.1911)	-	1707.0	0.9871
Dilaurin	-	1740.2 (0.0443)	1731.9 (0.0825)	1715.3 (0.0874)	1703.3	0.9929
Trilaurin	1745.4 (0.0775)		-	1716.8	-	0.9939

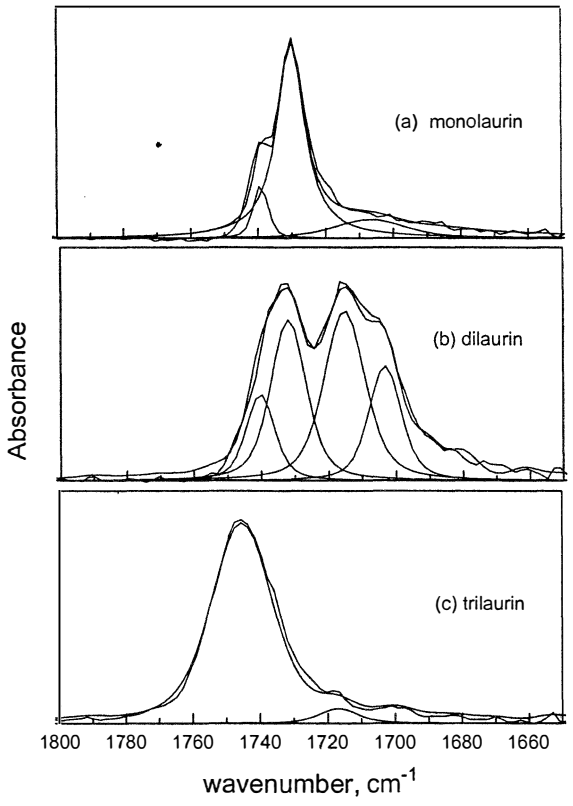

Fig. 3. C=O stretching of pure monolaurin (a), dilaurin (b), and trilaurin (c) spectra resolved into the components peaks.

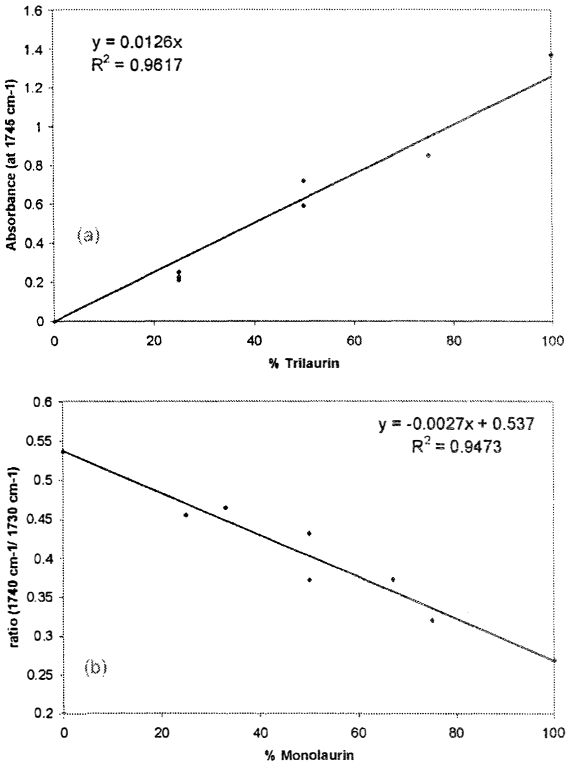

Fig. 4. (a) Plot of normalized absorbance or peak height vs. % Trilaurin and (b) plot of ratios of absorbance or peak height at 1740 cm^{-1} to peak at 1730 cm^{-1} vs. % Monolaurin. The derived regression line, equation, and correlation coefficients are also shown. The calibration for Trilaurin was forced to zero because of absence of peak at 1745 cm^{-1}.

Quantification of relative concentrations of the glycerides. Known mixtures of monolaurin, dilaurin and trilaurin (Table 1) were prepared. The FTIR spectra of the mixtures were then obtained. Using the curve fitting analysis, each carbonyl peak was resolved into five component peaks among which the absorbance (or peak height) at peaks around 1745 cm^{-1}, 1740 cm^{-1} and 1730 cm^{-1} were used to set-up the calibration curve.

In order to verify the validity of the calibration curve, spectra containing the mixtures of known percentage of the three glycerides were taken. Using the same method of analysis, the peak heights of the component peaks were then obtained. In order to determine the predicted percentages of glycerides in the sample, the peak height around 1745 cm^{-1} was substituted to the equation from the trilaurin calibration curve. Likewise, the peak height ratio 1740 cm^{-1} to 1730 cm^{-1} peak was substituted to the equation from the ML/DL calibration curve. To get the final mono- and dilaurin composition in the mixture, the following equations were used:

$$\% ML_{tr} = (100 - \%TL) * \%ML_{rel} \quad (1)$$

$$\%DL_{tr} = (100 - \%TL - \%ML_{tr}) \quad (2)$$

where:

%TL = the percent trilaurin determined using the equation from trilaurin calibration curve

$\%ML_{rel}$ = the percent monolaurin with respect to the ML and DL mixture

100 = total percent of themixture of the three glycerides

$\% ML_{tr}$ = percent monolaurin in the mixture

$\% DL_{tr}$ = percent dilaurin in the mixture

Table 3 shows a summary of predicted percent glyceride content. The corresponding curve fits are shown in Figure 5a. The predicted composition was off by a mean of ± 6 percentage composition units. For example, for the three trial mixtures tested, the predicted monolaurin compositions were 17 or 22 for the supposedly 25 % ML mixture or 49 % for the known 50 % ML mixture. The mean % error in the predicted value of a glyceride is 18 %. Although this value is unacceptable for strict quantitative measurements, it is a fair estimation of the composition of a mixture of glycerides which is reasonable from infrared spectroscopy data. The method will find use in rapid assays for monitoring of kinetics of esterification reactions or following the progress of reaction in production.

The method is also applied in the analysis of a mixture of glycerides produced from direct esterification of glycerol and lauric acid as shown in Figure 5b. The profile of the carbonyl band for this mixture shows a large peak at 1731 cm^{-1} indicating high monolaurin content. Contrast the band, for example with that for pure monolaurin (Figure 3a). Upon quantification by curvefitting, the estimated composition is 61 % monolaurin, 23 % dilaurin, and 16 % trilaurin.

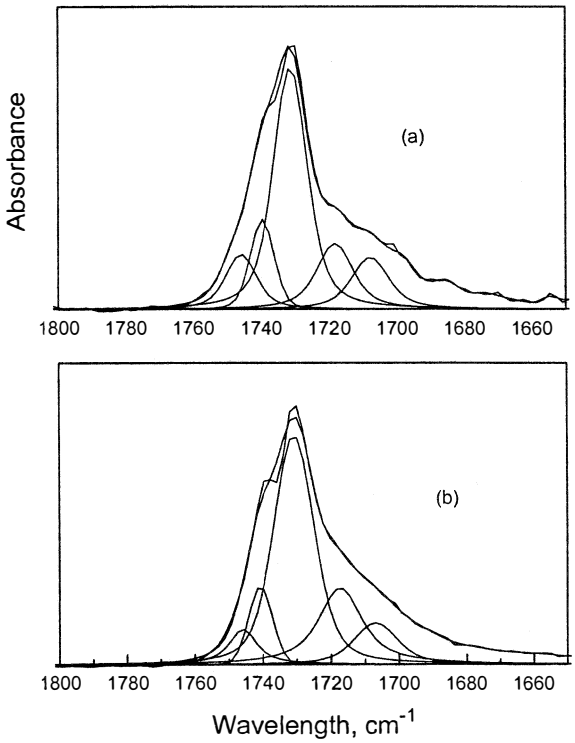

Fig. 5. C=O stretching of glyceride mixtures resolved into several components for (a) a known mixture of mono-, di-, and trilaurin and (b) from esterification of glycerol and lauric acid.

Table 3. Summary of predicted glyceride content using the calibration curve.

Actual % glycerides			Normalized peak height around 1745 cm^{-1}	Relative % ML	Predicted % glycerides		
%ML	%DL	%TL			%ML	%DL	%TL
25	25	50	0.72076	39.1	17	26	57
25	50	25	0.21154	26.7	22	61	17
50	25	25	0.25017	61.1	49	31	20

CONCLUSION

The infrared spectra of the mono-, di-, and triglyceride of lauric acid show different band shapes at the carbonyl region (~1730 cm^{-1}) allowing for a quantitative analysis of a mixture of these glycerides. The carbonyl band was fitted with component peaks chosen from the spectra of the pure mono-, di, and triglyceride. Calibration curves were set-up from standard mixtures of the glycerides. Thus, a quick and relatively cheap method of analysis of glyceride content was achieved, which can estimate to within 6-10 % of the true glyceride composition. This rapid characterization takes away the problems of preparing solutions and using a lot of solvents for chromatographic techniques.

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