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ANALYSIS

Quantification of *trans* fatty acids in food products by GC, ATR-FTIR and FT-NIR methods

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Summary

The obligation to label food products with the content of trans fatty acids has prompted analysts to take a critical look at the official methods of analysis and to develop improved methods. This article examines the advantages and disadvantages of the official methods and compares them with a method using Fourier transform near-infrared spectroscopy.

Introduction

Labelling of the *trans* fatty acid content of food products has now been mandated both in Canada and the USA (1,2). The effective date to declare the *trans* fatty acid content in the nutrition label of foods and dietary supplements is December 2005 and January 2006 in Canada and the USA, respectively. Currently, there are two official methods for the quantification of *trans* fatty acid accepted by the American Oil Chemists' Society (AOCS) and the Association of Official Analytical Chemists (AOAC): namely, capillary gas chromatography (GC) (3) and Fourier transform infrared (FTIR) spectroscopy in conjunction with the attenuated total reflectance (ATR) cell (4,5).

FTIR spectroscopy instruments have advantages over dispersive spectrometers (4,5). They offer high signal-to-noise ratio, better wavelength precision through the use of an internal reference laser, and higher energy throughput allowing the efficient use of different sample handling techniques, such as ATR and computing capabilities. Recently, Fourier transform near-infrared (FT-NIR) spectroscopy has also been applied for the analysis of total *trans* fatty acids (6), and the potential has been demonstrated to extend it to determination of individual fatty acids, including *trans* fatty acids (7,8).

Comparison of official methods

Of the two official methods, that based on GC is far more time consuming since all samples need to be converted to volatile fatty acid derivatives, generally fatty acid methyl esters (FAME). In contrast, FTIR measurements are taken directly on fat samples using the ATR cell; prior extraction of fat from food samples is required, but no derivatization, or use of solvents. The GC method provides both identification and quantification of individual *trans* fatty acids, but the accuracy and reliability of the method depends on the resolution and identification of all *trans* fatty acids. It is for this reason that very long, highly polar capillary GC columns are recommended to maximize the resolution of as many fatty acid isomers as possible (9,10).

The GC method is really put to the test when analysing fats and oils that are heated, oxidized, or extensively processed by partial hydrogenation, particularly when these fats and oils have complex fatty acid compositions, as is the case with fish oils. The many *trans* fatty acid isomers formed under these conditions provide a challenge for the GC analyst since standards are often not available. On the other hand, the ATR-FTIR method provides the total *trans* fatty acid content irrespective of the location of the *trans* double bonds in the molecule. Complementary techniques are required to analyse non-volatile fatty acid polymers or oxidized fatty acids containing *trans* double bonds, that would be measured by IR but are missed if we measure only FAME that elute from the GC column.

An additional problem arises when two (or more) non-conjugated *trans* bonds are present in the same fatty acid molecule. By GC, the concentration of such a fatty acid is multiplied in accordance with the number of these bonds; in contrast, the IR absorption intensity would be greater than one *trans* bond, but the number of double bonds is not necessarily additive (11).

Cis/*trans* conjugated fatty acids are a unique case since they are excluded from the *trans* labelling requirement in the USA (2). While easily resolved by GC, they provide a challenge for ATR-FTIR because

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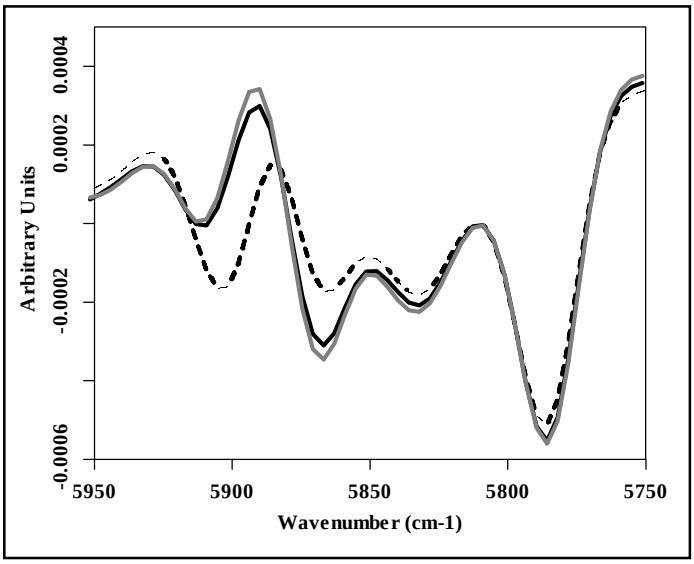

Figure 1. Second-derivative FT-NIR spectrum of soybean oil and extracted oil from salad dressing (see text for details).

they exhibit two absorption bands (near 986 and 950 cm^{-1} respectively) situated one on each side of a single isolated *trans* fatty acid band (near 966 cm^{-1}) (11). Measurement of the second derivative of ATR-FTIR spectra has been proposed to provide a better resolution of these closely-associated absorption bands, and we will describe this in the November 2004 issue of *Lipid Technology*.

GC combined with FT-NIR

A more recent development of the FT-NIR method for *trans* fatty acid analysis offers the potential to combine the speed of the ATR-FTIR method, and the specificity of the GC method but without the time-consuming sample preparation of the latter (7,8). In fact, FT-NIR measurements have been taken of samples with or without prior extraction of the fat (7). Unlike the ATR-FTIR method that shows a highly characteristic *trans* absorption at 966 cm–1, the FT-NIR absorption consists of overlapping and broad overtone and combination bands arising from the same fundamental absorptions occurring in the FTIR region.

Advances in the instrumentation and computing power for chemometrics analysis over the last ten years have favoured FT-NIR, making possible the characterization of, for example, foods, pharmaceuticals and plastics for quality control and quality assurance purposes. However, it should be noted that the sample size required for FT-NIR analysis is considerably larger (1–2 g) compared with ATR-FTIR (1–50 mg) or GC (5–20 mg). A custom-made fibre optic probe for FT-NIR would permit the analysis of smaller sample sizes.

There is an additional fundamental difference between FT-NIR and GC analysis of fats and oils. In the GC method, fats and oils are first converted to their respective FAME, then resolved on a GC capillary column, and finally each separated fatty acid is analysed individually. In the FT-NIR analysis, fats and oils can be analysed directly without the need to resolve individual fatty acids, which makes the FT-NIR technique dependent on the matrix. In other words, with FT-NIR, a mathematical model representing a particular class of products is first developed and validated. This model can then be used to analyse any unknown sample within the same family. Essentially, a model developed for oils could not be used for margarines, and one for margarines would not work for oils.

Potential for FT-NIR

The development of each specific model may be time-consuming and thus considered a drawback for the FT-NIR method, but this model needs to be developed only once, whereas in GC each sample must be converted to its respective FAME and analysed independently. The FT-NIR method also shows great potential as a rapid tool to detect impurities and adulteration. The FT-NIR absorption is representative of combinations and overtones of fundamental FTIR bands, and changes in the absorption spectrum reflect differences in composition that can easily be distinguished from that of the native sample. **Figure 1** shows the effect of residual solvent present in the extracted fat from a sample of salad dressing.

The peak profile in the second-derivative FT-NIR spectrum changed significantly because of small amounts of chloroform and methanol in the sample (see the dotted line). A typical profile for vegetable oils was observed (see the black line) after the chloroform and methanol were evaporated by warming the flask containing the sample. In this case, the soybean oil spectrum has been included in Figure 1 for comparison (see the grey line). The fatty acid composition determined for the extracted salad dressing oil was remarkably similar to that of soybean oil.

Typical samples with low or high *trans* fatty acid contents were quantified using all three methods (GC, ATR-FTIR and FT-NIR), and the results are shown in **Table 1**. In general, the GC and FT-NIR results were similar which may not be surprising since the FT-NIR matrices were developed based on the GC findings. On the other hand, the ATR-FTIR findings were generally lower for low-*trans* products and rather similar when the *trans* levels were high (Table 1).

One explanation of this discrepancy may be the lower limit of *trans* determination by ATR-FTIR (4). This problem is overcome in the GC method by increasing the sample load when minor fatty acid isomers need to be measured with greater confidence.

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Concentrating the IR signal of a neat sample is not possible unless prior fractionation of the compounds in question is undertaken, but this would defeat the very simplicity and rapidity of the IR method compared to GC.

Conclusions

In conclusion, all three methods for *trans* determination (GC, ATR-FTIR and FT-NIR) have inherent advantages and disadvantages. It is premature to indicate which of the methods is most accurate, since several differences in response remain unexplained and need to be clarified.

The choice of method will depend on each specific set of requirements and circumstances. If few samples require analysis with a maximum of compositional information, the GC method is clearly the best choice. On the other hand, if only the total *trans* content is required for routine analysis, then one may consider ATR-FTIR or FT-NIR. The FT-NIR method has the advantage that it provides not only the total *trans* content, but also extensive information about the fatty acid profile of the product. However, it should be noted that the FT-NIR method first requires the preparation of a specific model for each product type, and that this requires comparisons with accurate GC determinations.

Efforts are in progress to conduct a collaborative study using FT-NIR to determine the fatty acid amount and composition of products, including their *trans* fatty acid content, in order to establish it as an official FT-NIR method. We will discuss ATR-FTIR further in the November issue of *Lipid Technology*.

Table 1. *Trans* fatty acid content analysis of oils by GC, ATR-FTIR and FT-NIR methods (PH = partially hydrogenated).

Sample	% *Trans* fatty acids		
	GC	ATR-FTIR	FT-NIR
Walnut	1.99	1.10	1.96
Canola	1.65	0.76	1.47
Soybean	2.08	1.40	2.03
PH soybean 1	27.27	27.14	26.41
PH soybean 2	51.57	50.28	49.85

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