APPLICATION NOTE

No. AN50723

Rapid Analysis of *Trans* Fat Content Using a Fourier Transform Infrared Spectrometer

Key words

ATR, FTIR, gas chromatography, infrared, oil analysis, *trans* fat

Introduction

Evidence continues to mount on the adverse affects of human consumption of *trans* fatty acids. A report by the US Food and Drug Administration (FDA) concluded that consumption of *trans* fat contributes to increased LDL ("bad") cholesterol levels, increasing the risk of coronary heart disease. The FDA estimates that publicizing the *trans* fat content of processed food products would prevent up to 1,200 cases of heart disease and up to 500 deaths each year.[1]

Trans fats are found in many common food products in the form of hydrogenated oils. The use of hydrogenated oils is widespread because they improve the consistency and shelf-life of many processed food products, especially baked goods.

Nutrition Facts

Serving Size 1 cup (228g)
Servings Per Container 2

Amount Per Serving	
Calories 260	Calories from Fat 120
	%**Daily Value***
Total Fat 13g	20%
Saturated Fat 5g	25%
Trans Fat 2g	
Cholesterol 30mg	10%
Sodium 660mg	28%
Total Carbohydrate 31	10%
Dietary Fiber 0g	0%
Sugars 5g	
Protein 5g	

Vitamin A 4%	•	Vitamin C 2%	
Calcium 15%	•	Iron 4%	

* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values me be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300g	300g
Sodium	Less than	2,400mg	2,400 mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 1: Revised label, from the FDA, which includes information on trans fat

Countries around the world, led by Denmark, the United States, and Canada, have begun to enact strict guidelines requiring food manufacturers to list *trans* fatty acid content on the packaging, as shown in Figure 1. The importance of this labeling task places strict requirements on the analysis – speed, reliability, and robustness are essential. Certified methods by the American Oil Chemist Society (AOCS) or the Association of Official Analytical Chemists (AOAC)) entail infrared (IR) spectroscopic or gas chromatographic (GC) analysis.

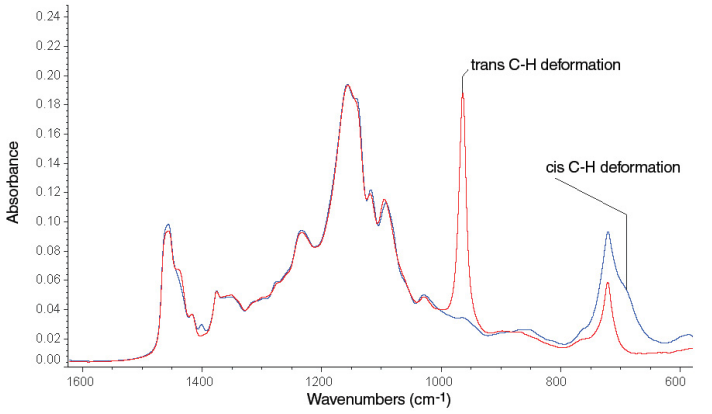

Figure 2: FTIR spectra of *cis* and *trans* fats shows spectral differences distinguishable using FTIR

Most present analyses require extraction to isolate the fats. GC methods are commonly used, due to the availability of GC equipment and its sensitivity. GC methods (AOCS Ce 1f-96 and AOAC 996.06) have good sensitivity down to 0.5%, but the triglycerides must be broken down to release the fatty acids, which must then be converted to fatty acid methyl esters (FAME) for injection. In addition, overlap of *cis* and *trans* peaks causes fat values to be underestimated, so fractionation of the *cis* and *trans* isomers using TLC or HPLC must be done, adding significant time and effort.

The infrared features of the *cis* and *trans* molecular configurations occur in different spectral regions, so no interference occurs. This can be seen in Figure 2, which shows overlaid infrared spectra of trielaidin (100% *trans*) and triolein (100% *cis*). However, the traditional infrared analysis method, Cd 14-95, also uses FAME derivatives, and requires carbon disulfide (CS_2) as a dilution solvent. This is cumbersome, limited in sensitivity, and the use of CS_2 is objectionable due to its odor. Although the method specifies applicability down to 0.5%, the *trans* peak is a shoulder on a large peak in the fat spectrum, making accurate measurement difficult, so the method may only work to 5%.

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A newer infrared method was developed based on a heated horizontal attenuated total reflectance (ATR) accessory and is specified in methods AOCS Cd 14d-99 and AOAC 2000.10. The ATR method is easy, rapid, and reproducible. Direct analysis of the *trans* isomer in the fat sample can be completed without weighing or preparation of FAME derivatives, and no smelly solvents are needed. The small sampling area of modern single bounce ATR accessories only require sample volumes of 50 µL or less, allowing for a reasonable sample size when extracting fat from food.

One key requirement for the ATR method is the use of *trans*-free reference fat for background correction, to eliminate the sloping baseline and shoulder peak measurement seen in the Cd 14-95 method. The method states it can be applied down to *trans* levels of 1%. However, the reference must approximate the fatty acid profile of the fat sample or it can adversely affect the methods accuracy, particularly near the limit of quantification. This means the user must select a *trans*-free fat that is similar to the fat sample being analyzed.

Materials and Methods

Data was collected on a previous generation Thermo Scientific Nicolet FTIR spectrometer. Samples were run on a heated diamond ATR accessory (100 scans, 4 cm^{-1} resolution, at 65 ºC). Trielaidin (100% *trans*) and triolein (0% *trans*) standards supplied by Nu-Chek Prep were used to prepare the calibration standards per the official method AOCS Cd 14d-99. The peak at 966 cm^{-1} is due to the out- of-plane C-H deformation about *trans* double bonds. The area of peak 966 cm^{-1} was used to quantify the *trans* content, using a linear regression generated in the Thermo Scientific TQ Analyst™ software. Standards from 1 to 50% *trans* are shown overlaid in Figure 3 along with a 100% trielaidin, and the resultant calibration is shown in Figure 4.

Conclusion

Food manufacturers can use the infrared ATR technique for rapid determination of the *trans* fat content of the fats and oils used in the manufacture of food products. This analysis is instrumental in helping them comply with the food labeling requirements set by various countries throughout the world to help promote healthy eating habits. To help manufacturers meet these requirements, instrumentation companies have developed systems that can be used to quantify the *trans* fat content of edible fats and oils using FTIR spectroscopy.

The high quality of the data, and the resulting calibration curve, show the excellent dependability of the FTIR method. FTIR spectroscopy is an important tool in analytical laboratories due the speed and sensitivity of analysis, in addition, to the ease of use of ATR accessories. For the analysis of lipid material, FTIR provides unique information difficult to obtain using other techniques.

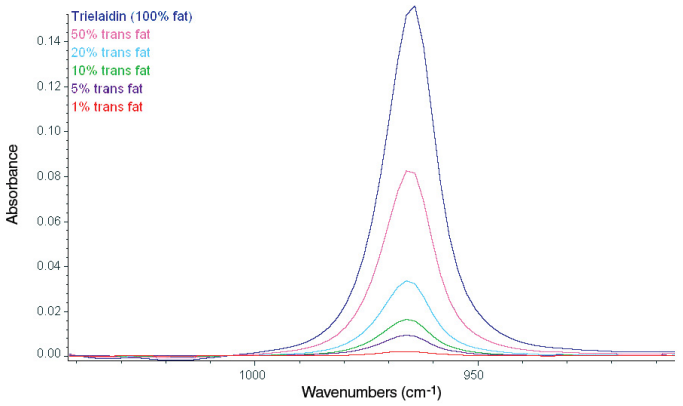

Figure 3: FTIR spectra overlay of trans fat from 1 - 50% with trielaidin (100% *trans*) standard

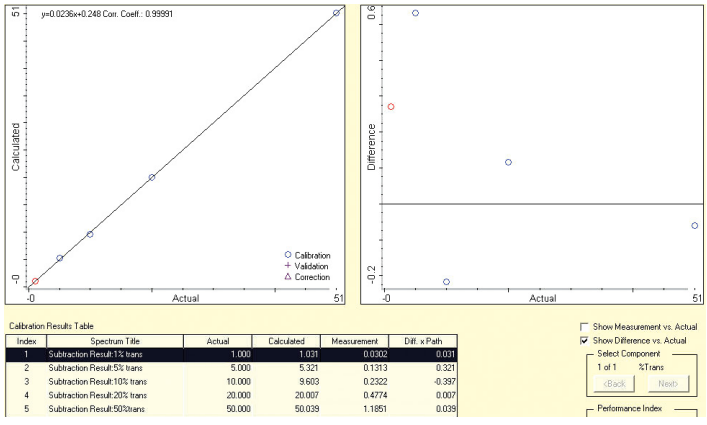

Figure 4: Thermo Scientific TQ Analyst linear regression calibration for *trans* content

References

1. FDA Web site: http://www.fda.gov/oc/initiatives/transfat/

Find out more at **thermofisher.com/FTIR**