Annals. Food Science and Technology
2019

FT-IR SPECTROSCOPY AS METHOD FOR TRANS FATTY ACIDS QUANTIFICATION-A SHORT REVIEW

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Abstract
The trans fatty acids (TFA) quantification is required due to its harm of human health, regular consumption can increase the risk of diseases. To reduce the intake, consumers must be informed about the negative impact on their health and the TFA content labelled. Countries as the US, Canada, and Denmark impose a maximum usage of TFA in products for a consumer higher safety. The GC and IR methods are commonly used for TFA quantification, and through this review, it was highlighted the importance and the advantages of IR procedure. FT-IR is a non-invasive, rapid and powerful technique superior to other techniques in noise-signal ratio, spectral resolution, samples treatment, and detection sensitivity. The present work addresses a comprehensive review of different IR techniques, a comparison, and the election of the most efficient one. ATR-FT-IR methods turned out to be easier and faster for the trans fatty acids quantification at its specific region (991-945 cm^{-1}). One of the main approaches of this review is the rapidity of IR methods, the analysis can be executed directly on the fat without the need for specific preparation of samples.

Key words: ATR-FT-IR, trans fatty acids, quantification, edible oils, food

Received: 30.07.2019 Reviewed: 23.09.2019 Accepted: 30.09.2019

INTRODUCTION

Trans fatty acids (TFA) are unsaturated fatty acids with at least one isolated double bond in a *trans* or *E* configuration. The most common natural unsaturated fatty acids have their double bonds in a c*is* configuration, the *trans* ones being less common (Fig.1) (Mathias D. 2016). There are three types of TFA based on its sources: the first ones, naturals, (Lucarini M. et al 2017) derives from the bacterial transformation in the rumen of ruminants of unsaturated fatty acids ingested by animals. (Mensink P.R. 1990). TFA represent 3-5% of the total fat content of milk and beef fat (Lucarini M. et al 2017). The other ones, result during the partial hydrogenation process, when liquid vegetable oil is converted into solid or semi-solid fats, such as shortening or margarine (Mozaffarian D. et al 2006). The third TFA category is produced due to the high frying temperature.

Usage of *trans* fats in the food industry has an economic argument due to their lower purchase price and their capacity to increase the shelf-life of the products (doughnuts, chips, margarine, candy, fries, etc.) (Albuquerque T.G. et al 2011). The consumption of those can harm human health, increase the risk of heart disease (Lopez-Garcia E. et al 2005), diabetes (Mozaffarian D. et al 2009) and cancer (Albuquerque T.G. et al 2011). Compared to unsaturated fatty acids, which elevate LDL (Low-Density Lipoprotein) cholesterol, TFA also lower HDL (High-Density Lipoprotein) (Willett W.C. et al 1993).
Dietary TFA should thus be limited to a maximum percentage of 1% of the total energy consumption of a person (2-3 g/ day). (Mathias D. 2016) To reduce risks related to TFA consumption, numerous researches have been carried out regarding the decrease or elimination of TFA from all products (Albuquerque T.G. et al 2011).

In Japan, mentioning TFA on food labels is not mandatory and there are also no limit when it comes to consumer safety. On the other hand, the US (US FDA 2003) and Denmark have using restrictions and label inscription is mandatory. (Massoba M.M. et al 2007). FDA (Food and Drug Agency) and CFIA (Canada Food Inspection Agency) are the first two organizations who required to include TFA on the label, and the first two countries to introduce the mandatory declaration of TFA (Canadagazette 2003) (US FDA 2003). Denmark was the first country in the world to introduce a limitation for *trans* fatty acids (TFA) in foods. (Restrepo J.B. et al 2016).

The tendency is to reduce *trans* fatty acids (TFA) from the population`s diet. (Aldai N. et al 2013).

Nowadays in many countries, the demand for sensitive and accurate methodologies to determine the quantity of trans fat in food products and dietary supplements has increased. Infrared (IR) spectroscopy and gas chromatography (GC) methods were chosen for the rapidity of quantification of total TFA in fats and oils (Massoba M.M. et al 2007).

This review is trying to exalt the differences between analytical analysis used to quantitate *trans* fats, the advantages of IR spectroscopy versus GC method.

Gas chromatography is commonly utilized to determine the FA composition of all fat-containing products. (Khan M.U. et al 2017) Compared with ATR-FT-IR that has minimum or no sample preparation, GC is a laborious technique, takes a lot of time and during the preparation and analysis of the samples it involves hazardous utilization of chemicals (Lucarini M. et al 2017). Some of the most important advantages of the GC method are: it has better sensitivity, giving detailed info of the composition and it`s suitable for low *trans* fats levels. Due to the availability of equipment and sensitivity, GC is a handy method. There are plenty of official methods, most of them different for each country (Background discussion) as AOCS Ce 1f-96 (AOCS 2002), AOAC 996.06, (Miller J. et al 2007) AOCS Ce 1h-05 or 1j-07 (Khan M.U. et al 2017). GC-FID and MS are two of the most used techniques for TFA determination, often helping on the labelling.

Replacing the glass columns with the capillary ones, GC evolved as a method, by giving greater peak resolution for *cis*/*trans* isomers (Albuquerque T.G. et al 2011).

As a modern solution, IR method was developed. This method has less negative impact on the environment, without needing special preparation of the sample or massive quantities of solvent (Baker J.M. et al 2014). The time was significantly reduced compared to the laborious chromatography method. According to the wavelength range, IR is divided in three parts: far-infrared (50-700 cm^{-1}) (Lindenmaier R. et al 2017), mid-infrared (400-4000 cm^{-1}) (Smith, B.C. 1999) and near infrared (NIR) (3500- 14000 cm^{-1}) (Cascant M.M. et al 2019).

When we speak about rapidity and minimum extraction, FT-IR methods constitute a significant advance for the TFA quantification. American Oil Chemists' Society (AOCS) and AOAC International adopted a few official methods (Priego-Capote F. et al 2004): AOCS Cd 14d-99, AOAC 200.10 and the updated one Cd 14e-09. The last one, adopted in 2009, is trying to diminish the analysis time at less than 5 min, giving more accurate results and is suitable for TFA quantification in food with lower TFA concentration (< 2% of total FA).

Thanks to IR methods, the costs were reduced by consuming less extractant (only 15-20 mL) or using directly the neat oil or fat (Mossoba M.M. et al 2014). It is worth emphasizing that the FT-IR method can be used as a screening method in the USA and Canada and it is good for quality control. ATR-IR technique is widely used in TFA quantification and decreases the sample handling problems (Priego-Capote F. et al 2004) by putting the sample directly on the diamond ATR crystal. (Sherazi S.T.H. et al 2009).

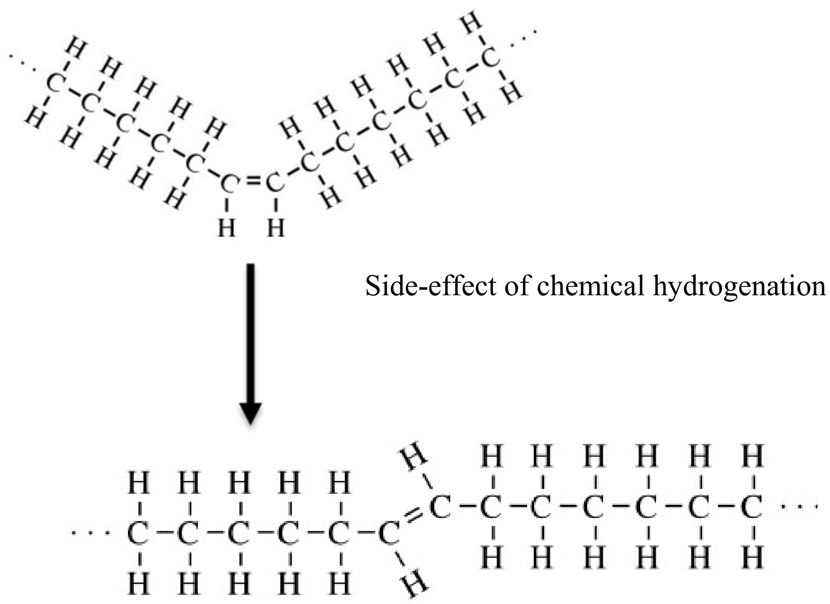

Fig.1 *Cis* (above) and *trans* (below) configuration (http://www.indiana.edu/~oso/Fat/trans.html)

Infrared spectroscopy – Transmission

One of the common techniques used to determine the amount of the TFA, in natural or artificial fats, is Fourier – transform infrared spectroscopy (FT-IR). It provides information via infrared radiations that pass through the sample and monitoring the characteristics vibration of the chemical bonds of the functional groups of molecules.

We can consider that two different FT-IR spectra are not the same for two different chemical structures (Lucarini M. et al 2017), this, because it can believe to be a „fingerprint analytical technique" (Jaiswal P. et al 2014) for the structural identification of compounds.

A direct method for *TFA* quantification is the FT-NIR method, dependent on the matrix, which means that it isn't similar for different types of fats (oils, margarine). In the table 1 it can be see the results, comparing all three methods, used by Azizian H. and co. in 2004. Between GC and FT-NIR there's a similitude due to the FT- NIR matrices that are developed based on the GC findings. On the other hand, the ATR-FT-IR lower results explanation is the limit of the determination of the *trans* fat. (Azizian H. et al 2004).

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

Table 1. *Trans* fatty acid content analysis of oils by GC, ATR-FT-IR and FT-NIR methods (PH = partially hydrogenated) (Azizian H. et al 2004)

Mossoba M.M. and co. compared the transmission-FT-IR (Mid-Infrared) method with the ATR (seven-bounce crystal) one, and it came out that both of them are equally satisfactory, as GC, for TFA quantification (Mossoba M.M. et al 2014).

Infrared spectroscopy- ATR

For the quantitative determination of isolated TFA, FT-IR is used and it also measures the *trans* peak area at its specific region (991-945 cm^{-1}) (Lucarini M. et al 2017), (Mossoba, M.M. et al 1991) except FT-NIR (1000-4000 cm^{-1}) (Azizian H et al 2004) exemplified in Fig.2 (A) (Schimadzu App. News) and (B) (Azizian H et al 2005). Even if transmission method is a good one, researchers developed ATR-FT-IR which extends and amplifies the range of IR spectroscopy (Lucarini M. et al 2017).

Via ATR-FT-IR spectroscopy you can make rapid and reproducible measurements for quantitative and qualitative analysis of food products containing *trans* fats. For the total determination of TFA, in fats, were adopted a few officials' methods like AOCS Cd 14-99, AOAC 2000.10 and AOCS Cd 14e-09. (Mossoba M.M. et al 2008) M.U. Khan and co. did analysis, using the last official method listed above, on variety of products and hydrogenated fats from the Indian market related to GC-FID (Fig. 3 (B)): the correlation coefficient using regression plot was R = 0,9950 (Fig.3 (A)).

The results show that the hydrogenated fats have a high content in *trans* fats, the highest level is at one of the hydrogenated fats, PHO3 = 4,73%, comparatively with the highest level found on the biscuit 2 = 3,83% (Khan M.U. et al 2017).

Mahesar S.A. and co. used Single-bounce, horizontal attenuated total reflection (SB-HATR FT-IR) as a spectroscopy method with the help of PLS regression on cereal-based foods (breakfast cereals, crackers, and cakes) on the Pakistan market. The results show that in all products, hydrogenated oils and fats may have been used.

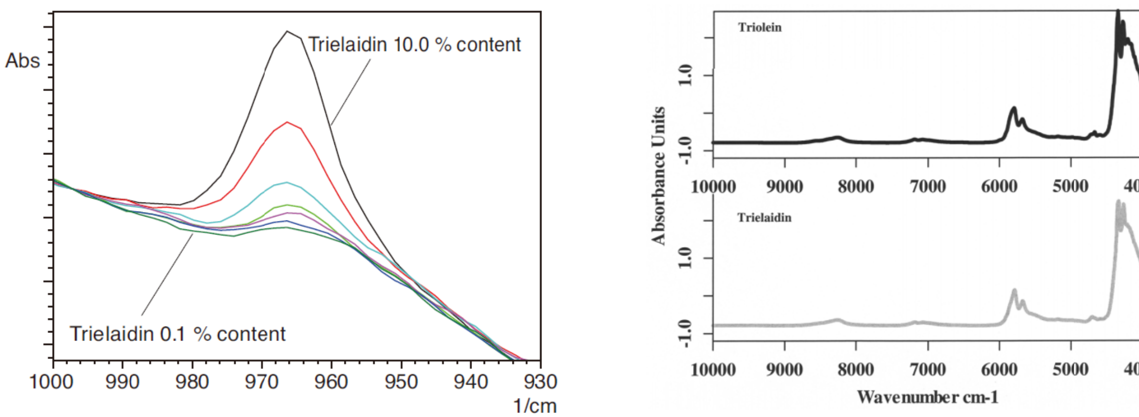

Fig.2 (A) *Trans* fatty acid peak region selected for calibration curve (Schimadzu App. News); **(B)** Fourier transform NIR (FT-NIR) absorption spectra of triolein (black line) and trielaidin (grey line) in the spectral region from 4,000 to 10,000 cm^{-1}. (Azizian H. et al 2005)

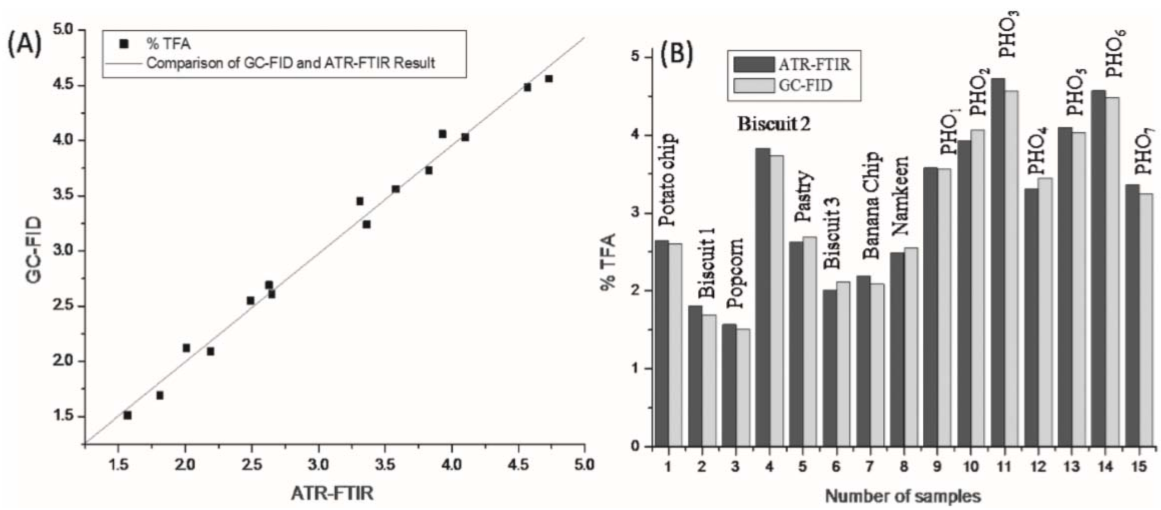

Fig.3 (A) Linear regression plot of ATR-FT-IR and GC results; **(B)** Comparison of GC-FID and ATR-FT-IR results (Khan M.U. et al 2017)

Trans fats levels ranging from 2,5 % to 16,3 %, the highest registered on F4 (cake) and the opposite on F8 (corn chips). As in the previous case, the table below (Table 1) highlights a good comparison of TFA from FT-IR and GC determination. (Mahesar S.A. et al 2010).The single-bounce attenuated total reflectance (SB-ATR) was also adopted by Sherazi S.T.H. and co. in 2009 and Yu X. and co. in 2011, in both cases they took edible oils as analysis samples. The first ones showed that industrially edible oils (canola, coconut, soybean, sunflower cooking oils) are containing a massive quantitates of *trans* fats, the maximum value registered at one of the hydrogenated fats, with 19,19%. SB-ATR, as method, impose a limitation of use, because of the oil high viscosity, the samples are difficult to handle and the techniques unsuitable in high sensitivity analysis. (Sherazi S.T.H. et al 2009). Based on this fact, *n*-hexane is considered an ideal solvent for edible oils, and the test presented by Chen L. and his team in 2018 attest its force of using.

In Fig.4 (A) it can be clearly seen the absorbance peak at 1743 cm^{-1} for soybean oil, and the absence of it in the *n*-hexane.

To see if *n*-hexane is also suitable for different edible oils, they compared colza oil, corn oil, and sunflower oil with soybean oil. Fig.4 (B) Using the ATR-FT-IR method, it wasn't found any different peak positions between them (Chen L. et al 2018).

In 2011 Yu X. and co. developed two new techniques, to improve the ATR-FT-IR method, one using spectral reconstitution (SR), and the other, based on generalized 2D correlation spectroscopy (Yu X. et al 2011).

After a few years Lirong X. and co., in 2015, gave a better solution for *TFA* quantification, introducing polyethylene (PE) film-based FT-IR, a much more convenient procedure due to its lower time spent per analysis. Even if it`s not as precise or sensitive as the SR FT-IR technique, the newest alternative proposes to decrease the price and a better solution comparative to ATR-FT-IR. In this case, the difficulties of sample handling are not a problem anymore, all of them were mixed with hexane in order to reduce the viscosity and therefore facilitate the procedure. For the validation, the results obtained were compared to the GS-MS ones, and it was found that they have almost the same accuracy (Lirong X. et al 2015).

Taking into consideration that some of the *trans* fats are produced during heating or frying, Chen L. measured, through the ATR-FT-IR technique, the quantity of oil absorbed externally by starchy food. Their study highlights the feasibility of this IR accessory, it can be potentially applied for quantification or identification of chemical composition, in this case, *trans* fats, directly on the material surface (Chen L. et al 2018). (Mahesar S.A. et al 2010)

Table 2. Lipid content and trans fat of cereal-based food determined by FT-IR and GC.

Samples	Lipid content (g/100 g)	*trans* (g/100 g of lipid) FTIR	*trans* (g/100 g of lipid) GC	*trans* (g/100 g of food)
F-1	12.7 ± 0.4	13.5 ± 0.3	13.1 ± 0.5	1.7
F-2	23.9 ± 0.3	8.9 ± 0.2	8.3 ± 0.3	2.1
F-3	17.5 ± 0.1	11.6 ± 0.5	11.2 ± 0.6	2.0
F-4	15.8 ± 0.3	16.3 ± 0.5	15.8 ± 0.7	2.6
F-5	21.3 ± 0.4	3.3 ± 0.1	3.1 ± 0.2	0.7
F-6	27.2 ± 0.3	13.4 ± 0.2	12.8 ± 0.3	3.6
F-7	14.8 ± 0.2	10.4 ± 0.3	10.1 ± 0.5	1.5
F-8	8.2 ± 0.2	2.5 ± 0.1	2.3 ± 0.1	0.2
F-9	20.1 ± 0.3	3.9 ± 0.1	3.7 ± 0.2	0.8
F-10	23.6 ± 0.1	15.0 ± 0.3	14.4 ± 0.4	3.5
F-11	16.2 ± 0.2	9.1 ± 0.2	8.8 ± 0.3	1.5
F-13	19.2 ± 0.2	13.0 ± 0.3	12.5 ± 0.4	2.5
F-12	10.3 ± 0.2	6.7 ± 0.3	6.5 ± 0.4	0.7
F-14	26.1 ± 0.3	16.1 ± 0.4	15.7 ± 0.6	4.2
F-15	13.4 ± 0.4	10.0 ± 0.3	9.7 ± 0.4	1.3

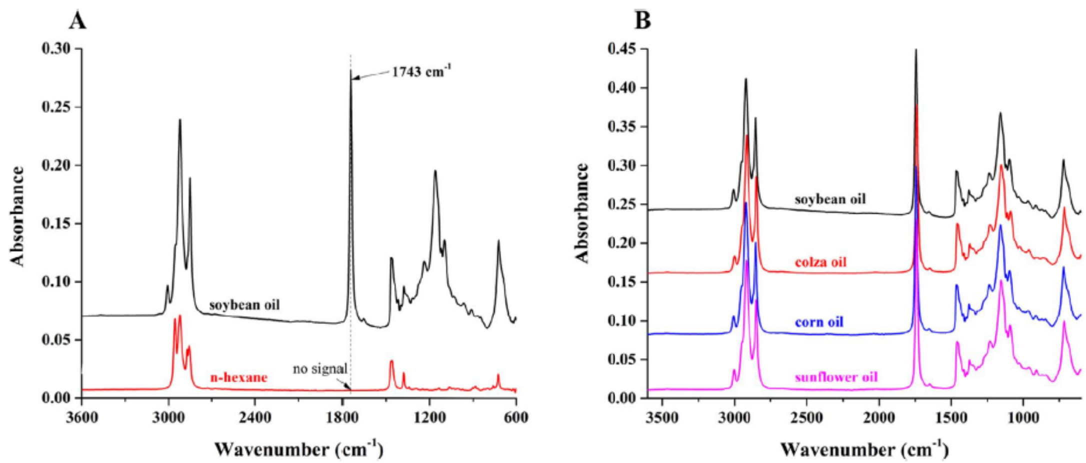

Fig.4 The ATR-FT-IR spectra for soybean oil and *n*-hexane **(A).** The ATR-FT-IR spectra for several edible oils, including soybean, colza oil, corn oil, and sunflower oil **(B)** (Chan L. et al. 2018)

CONCLUSION

The negative impact on human health and the mandatory of TFA labelling triggered the need for new, rapid and accurate methods for the TFA quantification. The IR techniques showed to be a better choice, especially the one using the ATR accessory, all compared with the GC method. In all cases, the correlation coefficient using the regression plot was close to 1, this meaning that IR results are comparable with the GC ones. IR method is a rapid, accurate, with less negative impact, alternative for total determination of *trans* fatty acids. During the research it was found a high TFA quantity in the products/hydrogenated fats from countries where there's no usage limitation. It can be seen the need a regulation who can reduce the TFA intake compared with labelling impositions and it allows to manage all type of products not only the ones with the possibility of packaging.

Acknowledgments

This work was supported by the project 26 PFE/2018 funded by Ministry of Research and Innovation trough Program 1- Development of the National R&D System, Subprogram 1.2- Institutional Performance- Projects for Excellence Financing in RDI

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