NMR analysis of the triglyceride composition of cold pressed oil from *Camellia reticulata* and *Camellia japonica*

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Abstract: Oil from Camellia species, mainly *C. oleifera*, is used in cooking and cosmetics in Asia. The relative composition of the fatty acid components of the triglycerides of oil from *Camellia reticulata* and *Camellia japonica* is studied by NMR.

INTRODUCTION

The genus *Camellia* is indigenous to eastern and south eastern Asia and comprises over 250 woody evergreen species. In Western countries, camellias are grown mainly as ornamental plants. Most are cultivars of *C. japonica*, and the rest cultivars of *C. reticulata* and *C. sasanqua*. In Galicia (NW Spain, figure 1), one of the more important camellia producing-regions in Europe, about 2,5 million camellia plants are produced each year in nurseries for houseplant and gardening being mainly exported to Belgium, The Netherlands, France, United Kingdom and Portugal markets.

Figure 1. Geographic location of Galicia

In Asia, some species, mainly *C. oleifera*, are used to obtain oil from their seeds. Oil from *C. oleifera* (tea seed oil) is used extensively in China as cooking oil (Yu et al., 1999). Similarly to olive oil, tea seed oil is rich in polyunsaturated fatty acids, mainly oleic acid (Shyu et al., 1990),

and is generally known to aid cholesterol loss and resistance to stress (Fu and Zhou, 2003). *Camellia* species cultivated in Western countries as ornamentals (*C. japonica*, *C. reticulata* and *C. sasanqua*) have oil-rich seeds (Chen et al., 2008), but the study of these oils have received little attention.

Nuclear magnetic resonance (NMR) has become a useful tool to study edible oils and fats, as it requires a very small sample, whose preparation is very simple, and it takes little time (Guillén and Ruiz, 2001). ^{1}H and ^{13}C NMR techniques have been successfully used to characterize olive oil, providing information of minor olive oil components, acyl distribution and positional allocation of glycerol tri-esters (Mannina and Segre, 2002). The aim of this work was to study the triglyceride composition of oil from *C. reticulata* and *C. japonica* cultivated in NW Spain by NMR analysis.

RESULTS

The structure of the mayor triglycerides present in oil is shown in figure 2, outlining in different colors the most singular kinds of hydrogens from the NMR shifts point of view. The tertiary hydrogen in the glycerin moiety (brown) could be used to quantify the ratio of saturated to unsaturated esters, since there is only one hydrogen for each triglyceride molecule. Vinylic hydrogens (magenta) have a characteristic chemical shift, and could be used to determine the ratio of saturated to unsaturated esters, whereas bisallylic hydrogens (green, blue) could be used to differentiate the nature of the unsaturated components.

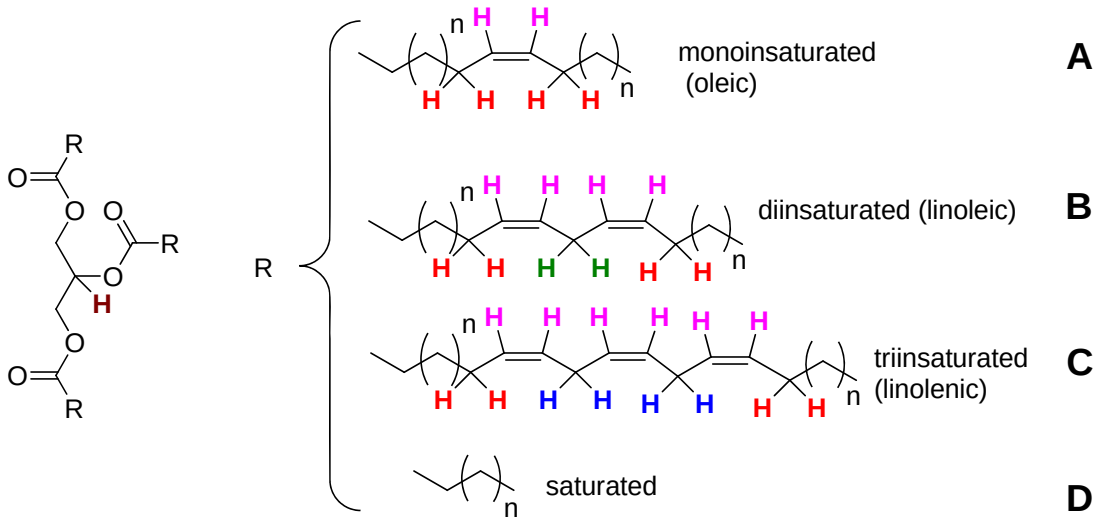

Figure 2

Thus, the 300 MHz (7.0 T) spectra for both samples of oil: *Camellia japonica* (Figure 3) and *Camellia reticulata* (Figure 4) was studied. Unfortunately the spectra obtained did not allow integrating accurately the tertiary hydrogen (**H**) of the glycerine moiety (δ ~5.25 ppm), because

its shift is very near to vinyl hydrogens (δ ~5.35 ppm), and no difference was obtained between green and blue bisallylic hydrogens (δ ~2.80 ppm).

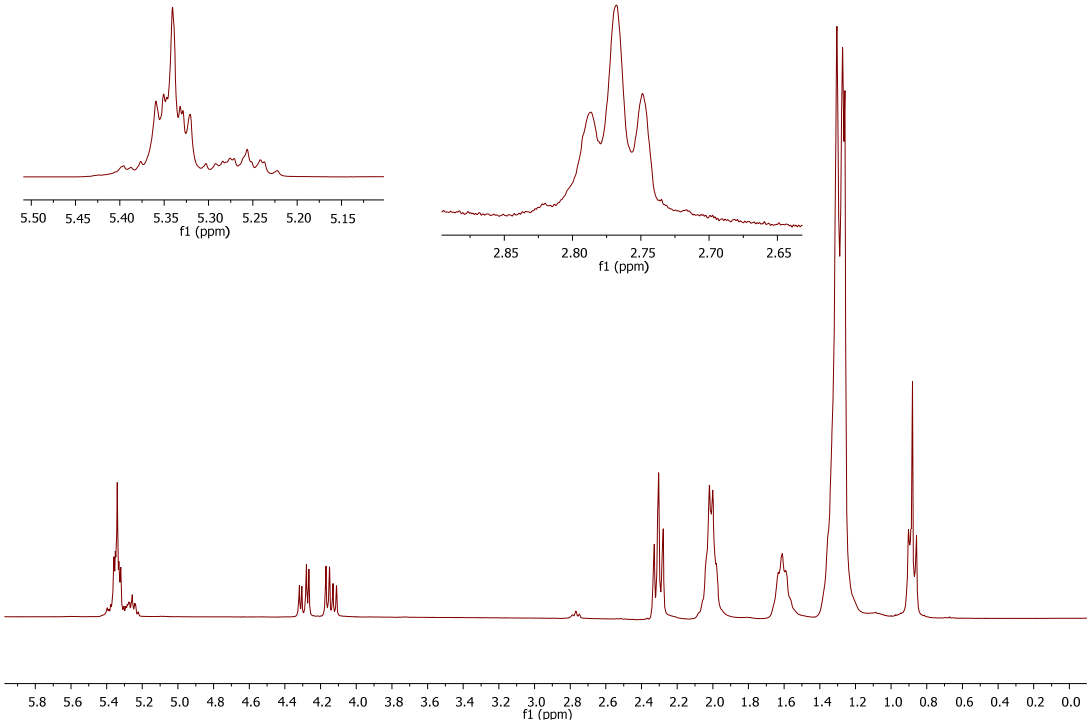

Figure 3. *Camellia japonica* ^{1}H-NMR (300MHz)

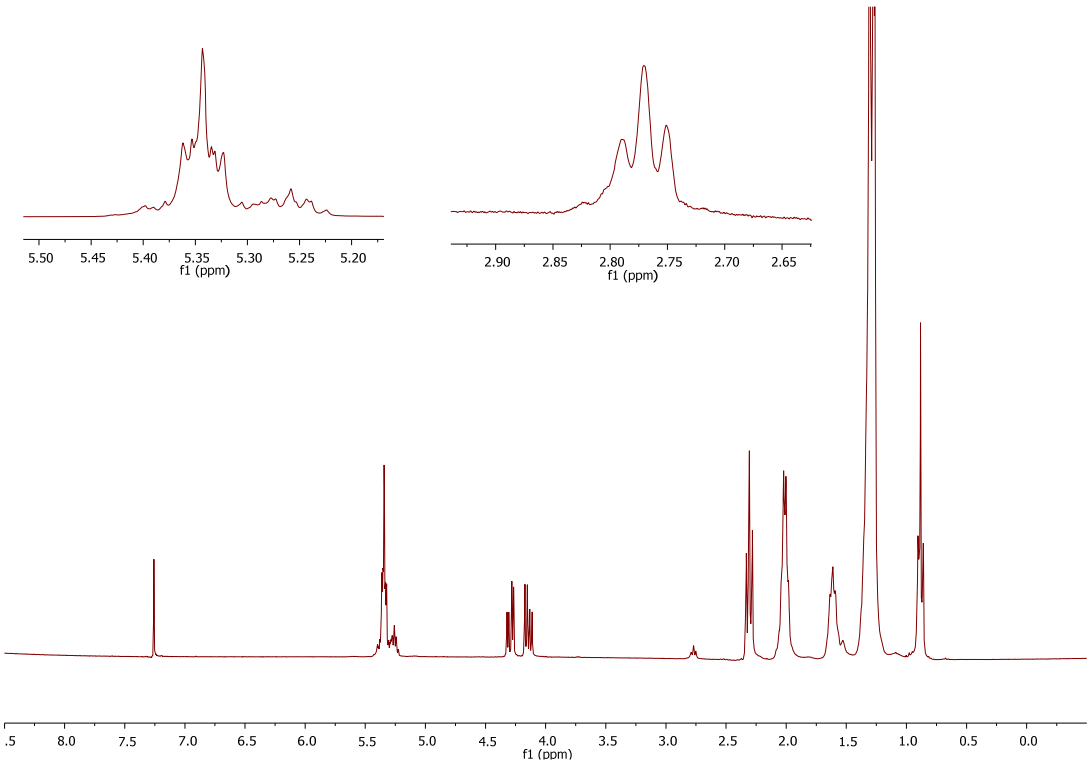

Figure 4. *Camellia reticulata* ^{1}H-NMR (300MHz)

However, the use of a 750 MHz (17.6 T) spectrometer to analyze the samples allowed the separated integration of the signal of tertiary **H** in glycerine moiety and vinyl hydrogens of the fatty acid chains. The enhancement of resolution allowed setting a relationship among the integral values of these hydrogens. Therefore the following equations were defined, being **A**, **B**, **C** and **D** the proportion of each kind of fatty acid involved in the triglyceride structure of oil.

vinylic hydrogens integral= 2A+4B+6C
linoleic bisallylic hydrogens integral=2B
linolenic bisallylic hydrogens integral=4C
A+B+C+D=3

For C*amellia japonica* (Figure 5), once the peak of **H** was normalized to 1, the signal of vinyl hydrogens integrated 5.26 (δ 5.37-5.50 ppm), and the bisallylic hydrogens 0.36 and 0.02, corresponding to linoleic and linolenic acids, respectively.

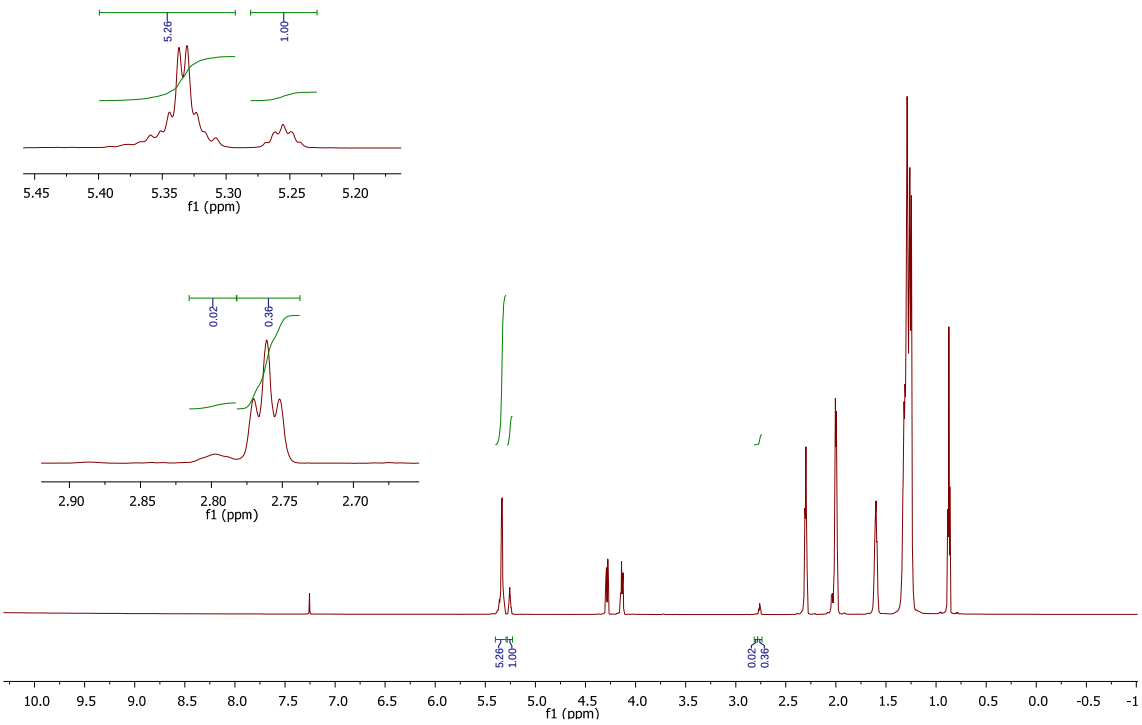

Figure 5. 750 MHz ^{1}H-NMR of oil from *Camellia japonica*

The measured integrals can be summarized as: **H=5.26, H=0.36, H=0.02, H=1.** Therefore the values of each kind of acid in oil from *Camellia japonica* are: **A** 75.75%, **B** 6.0%, **C** 0.17%, and **D** 18.67%, for oleic, linoleic, linolenic and saturated fatty acids, respectively.

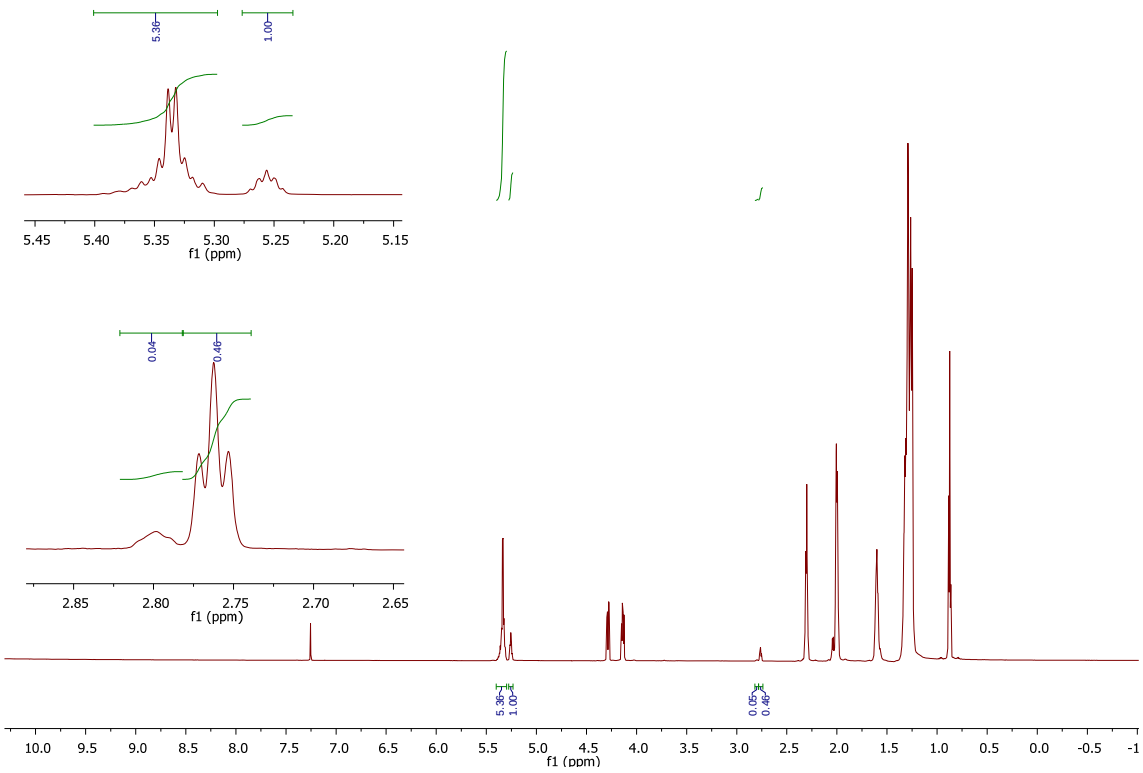

Figura 6. 750MHz ^{1}H-NMR of oil from camellia reticulata

Analogously the results for *Camellia reticulata* (Figure 6) were: **H=5.36, H=0.46, H=0.04, H=1,** resulting the following percentages: **A** 72.75%, **B** 7.67%, **C** 0.33%, and **D** 19.25%, for oleic, linoleic, linonelic and saturated moieties, respectively.

In conclusion, a direct and simple method to evaluate the fatty acid composition oils using 750 MHz ^{1}H-NMR spectroscopy was developed. The protocol has been applied to the analysis of *C. japonica* and *C.reticulata* oils.

MATERIAL AND METHODS

Seeds of *C. japonica* and *C. reticulata* were obtained from mature fruits of healthy plants grown in the live germplasm camellia bank at the Estación Fitopatolóxica do Areeiro (Pontevedra, NW Spain).

After crushing, seeds were cold-pressed for oil, which was stored in amber glass bottles.

NMR sample preparation was done by dissolving 0.05 mL of each oil in 0.5 mL of deuterated chloroform; the solvent was used as internal reference.

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