



Analytical Methods

Characterisation of the lipidic components of margarines by ^1H Nuclear Magnetic Resonance



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ABSTRACT

In this work, ^1H Nuclear Magnetic Resonance (^1H NMR) has been used to study the lipidic fraction of margarines of different compositions, determining simultaneously both major components, which is to say triglycerides, and other minor ones, such as 1- and 2-monoglycerides, 1,2-diglycerides, vegetable stanols and sterols, and sorbic acid. The results show a wide variety of acyl group compositions, with polyunsaturated groups ranging from 22% to 50%, monounsaturated from 23% to 50%, and saturated from 21% to 55%. 1,2-Diglycerides and vegetable stanols and/or sterols have been detected in all the studied margarines, in concentrations varying between 2.73 and 26.06 mmol/kg, and between 3.40 and 240.01 mmol/kg, respectively. A good agreement has been found between the results obtained by ^1H NMR and some composition data of these margarines, showing the usefulness of this technique to analyse the lipidic composition of margarine in a quick and easy way.

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1. Introduction

^1H Nuclear Magnetic Resonance (^1H NMR) has demonstrated to be very useful in determining the composition in acyl groups of vegetable oils (Guillén & Ruiz, 2003; Guillén & Uriarte, 2012a, 2012b). Moreover, this technique has also been employed to study other oil components that are usually present in much lower concentrations than triglycerides, such as mono- and di-glycerides (Alonso-Salces, Holland, & Guillou, 2011; Alonso-Salces et al., 2010; Sacchi, Paolillo, Giudicianni, & Addeo, 1991; Sacchi et al., 1996), free fatty acids (Satyarthi, Srinivas, & Ratnasamy, 2009) or antioxidant compounds such as phytosterols (Alonso-Salces et al., 2010, 2011; Dais, 2010; Kurata, Yamaguchi, Ohtsuka, & Nagai, 2005) and tocopherols (Alonso-Salces et al., 2010). The usefulness of ^1H NMR is reinforced by the simplicity of the sample preparation, the speed of the analysis and the great deal of information that is obtained in a very few minutes. All these advantages make ^1H NMR a very valuable tool, not only for investigation purposes, but also for quality control.

Among fatty foods, margarine has not received as much attention as edible oils. This is a spreadable fatty foodstuff which can be used for direct consumption, but also as a food ingredient in food such as cakes or biscuits, both at home and in industry. In

fact, margarine is being increasingly used due to the advantages that it offers over butter in relation to its fat composition; it is obtained mainly from vegetable oils and fats, so its content in mono- and poly-unsaturated acyl groups is higher than that of butter and, in addition, margarine does not contain cholesterol. However, the fat content of margarine can be as high as that of butter (minimum 80%) and, for this reason, it is more and more usual to find margarines with lower fat contents, commonly called light margarines. Furthermore, as a consequence of the current interest in the development of functional foods that could contribute to the health of consumers, there is a growing trend towards enriching this product with ingredients that seem to be beneficial at the cardiovascular level, such as polyunsaturated acyl groups (Nair, Leitch, Falconder, & Garg, 1997) or phytosterols (Marangoni & Poli, 2010).

Most of the studies on margarine focus on physicochemical parameters such as moisture and free fatty acid content (Pop, 2010), fatty acids, sterols and tocopherols (Kanematsu, Maruyama, Kinoshita, Niiya, & Imamura, 1976a, 1976b; Kohiyama, Maruyama, Kanematsu, & Niiya, 1990). Regarding the use of NMR for the study of margarines, it must be noted that there are only a few scientific works where this technique has been used for this purpose. Most of them report the use of ^{13}C NMR to study the triacylglycerol composition of margarines, the distribution of fatty acids in the glycerol structure and/or the content of *trans* fatty acids (Cengarle, Pranzetti, & Carta, 1992; Lie Ken Jie, Lam, Pasha, Stefenov, &

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Marekov, 1996; Sacchi, Addeo, Musso, Paolillo, & Giudicianni, 1995). Concerning ^1H NMR, it is worth pointing out the work of Schripsema (2008), who studied the polar and the apolar fraction of margarine by means of this technique. This author gave quantitative data of linoleic groups and also of 1,2-diglycerides and sorbic acid, but neither the study of the whole acyl group composition nor that of vegetable sterols and/or stanols was accomplished.

Considering all the above, the usefulness of ^1H NMR to study the lipidic fraction of different margarines is tested in this work. The study is focussed on the determination of the different types of acyl groups and of some minor components such as mono- and di-glycerides, and certain vegetable stanols and sterols, whose determination can be of interest.

2. Materials and methods

2.1. Samples

The samples subject of study were three commercial margarines, selected because of their differences in both lipid proportion and composition: a "light margarine" with a fat content of 40%, enriched with omega-3 acyl groups and vegetable stanols (LMS), a margarine with 98% of fat defined as "margarine for frying" (MF), and a third margarine with an intermediate fat content (60%) (IM).

The amount of margarine used for the study was approximately 0.16 g in all cases. Margarine MF was analysed directly, without further pre-treatment. The presence of detectable levels of water in margarines LMS and IM could distort the signals of the ^1H NMR spectra to a certain extent; for this reason, the aliquots taken from these latter were subjected to a liquid-liquid partition between deuterated chloroform (1 ml) and water (1 ml), and the study was carried out with the chloroform phase, after dessication with anhydrous sodium sulfate.

2.2. Study by ^1H NMR

The ^1H NMR spectra of the samples were registered in a Bruker Avance 400 spectrometer operating at 400 MHz. Margarine MF was mixed in a 5 mm diameter tube with 400 μl of deuterated chloroform which contained 0.2% of non deuterated chloroform and a small amount (0.03%) of tetramethylsilane (TMS) as internal references. In the case of margarines LMS and IM, 600 μl of the corresponding chloroform extracts were added to the RMN tube.

Acquisition parameters were: spectral width 5000 Hz, relaxation delay 3 s, number of scans 64, acquisition time 3.744 s, and pulse width 90°, with a total acquisition time of 12 min 54 s. The experiments were carried out at 25 °C. It must be noted that, as well as the relaxation delay of 3 s, relaxation delays of 30 and 60 s were also assayed but differences among the spectra acquired were not observed; therefore, given that the increase in this parameter considerably extended the analysis time, the lowest value (3 s) was selected.

Compounds such as 1,2-dioleoylglycerol, 1,3-dioleoyl-rac-glycerol, 1,3-dilinoleoyl-rac-glycerol, dioleoylglycerol, dipalmitin, 1-oleoyl-rac-glycerol, 1-linoleoyl-rac-glycerol, 2-oleoylglycerol, 2-monopalmitin, sorbic acid, β -sitosterol, Δ 5-stigmasterol and stigmastanol, acquired from Sigma Aldrich (St. Louis, MO, USA), together with Δ 5-campesterol (Larodan, Sweeden), were used as standard compounds for identification purposes. Likewise, trilinolein and trilinolenin were acquired from Sigma Aldrich (St. Louis, MO, USA) for quantitative purposes.

The quantification of mono- and di-glycerides, stanols and sterols, and sorbic acid was carried out, as in previous studies (Guillén & Goicoechea, 2007), by considering the area of the non deuterated

chloroform signal (7.29 ppm); this is always present in a small proportion (0.2%) in the used deuterated chloroform and can be taken as internal standard. All these determinations are possible because the area of the ^1H NMR signal is proportional to the number of protons that generates the corresponding signal.

All the determinations were done in duplicate in order to obtain a mean value with the corresponding standard deviation for each of the components studied.

3. Results and discussion

3.1. Information obtained from the visual observation of the ^1H NMR spectra of the studied margarines

3.1.1. About triglycerides

Triglycerides are the major components of margarine lipids. Fig. 1 shows the ^1H NMR spectra of the lipids of margarines LMS, IM and MF. All of them have been plotted at a fixed value of absolute intensity for comparative purposes. Each one of the signals is produced by the different types of hydrogen atoms present in the lipids of these margarines. The profile of these spectra is very similar to that of vegetable oils; this is not strange, since the margarines studied come mainly from vegetable oils and fats. Concretely, signals C, D, E, F, G, I, J, K, M and N are present in the spectra of vegetable oils (Guillén & Ruiz, 2003). The assignments of these signals are given in Table 1.

By observing the ^1H NMR spectra of the margarines subject of study with the naked eye, it is possible to know some features of their composition. Fig. 2a shows some of the main spectral signals in the three margarines studied. From the methylic protons of acyl groups (signals C and D), certain information can be extracted. Thus, as signal C shows, in margarines LMS and MF, the most abundant acyl groups are oleic plus saturated, which give a triplet centred at 0.880 ppm, linoleic groups being in lower proportions (triplet centred at 0.890 ppm). In addition, the three margarines contain linolenic groups, which give the triplet centred at 0.974 ppm (signal D), sample LMS being the richest and MF the poorest in this type of acyl chains.

Likewise, from signal E (due to methylenic protons in position β or further, from olefinic groups, or in position γ , or further, from carbonyl groups inside the triglyceride molecule), some information can also be obtained. As can be observed in Fig. 2a, margarine LMS has the highest proportion of oleic groups; this is deduced by the higher intensity of the shoulder at 1.267 ppm. In addition, an increasing proportion of saturated groups (signal at 1.258 ppm) from margarines LMS to MF can also be anticipated from the observation of signal E.

The enlargement of signal G of mono-allylic protons, representative of all the unsaturated acyl groups present in the samples, makes it possible to distinguish the signals corresponding mainly to oleic, at 2.002 and 2.018 ppm, linoleic, at 2.039 and 2.057 ppm, and linolenic acyl groups, at 2.097 ppm, in the three samples studied, although in different proportions. The three kinds of acyl groups have four mono-allylic protons; for this reason, it is possible from the visual observation of signal G to know that the main unsaturated groups in margarines LMS and IM are oleic and linoleic, respectively, the proportion of linoleic being of a similar order to that of oleic in margarine MF.

Finally, signal J of bis-allylic protons provides information about the presence and proportions of polyunsaturated acyl groups. Linolenic groups, which contribute four bis-allylic protons, give a triplet centred at 2.803 ppm; this overlaps with the triplet of linoleic groups centred at 2.768 ppm, which contribute two protons. The comparison of this signal in the three margarines shows that the highest proportion of linolenic relative to linoleic acyl groups

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