# Characterization of Sacha Inchi (*Plukenetia volubilis* L.) Oil by FTIR Spectroscopy and $^1$H NMR. Comparison with Linseed Oil

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**ABSTRACT:** Three oil samples obtained from sacha inchi (*Plukenetia volubilis* L.) seeds were studied by means of FTIR and $^1$H NMR. Frequency data of the most significant bands of the IR spectrum of this oil are given. These data show that sacha inchi oil has a high degree of unsaturation. The same fact is deduced from the ratio between the absorbance of the bands due to the stretching vibrations of the *cis* olefinic CH double bonds at 3010.5 cm$^{-1}$ and to the methylene symmetrical stretching vibrations at 2855.1 cm$^{-1}$. The proportions of monounsaturated, polyunsaturated, and saturated acyl groups were predicted from the frequency of some IR bands, and these were in satisfactory agreement with the values obtained through FAME generation and their quantification by GC. Likewise, simple observation of the $^1$H NMR spectra provided a great deal of information about sacha inchi oil, with regard not only to the relative proportions of the different acyl groups but also to their nature. Thus, the presence of γ-linolenic acyl groups was discounted. Furthermore, the area of some $^1$H NMR signals was used to determine the proportion of saturated and mono-, di-, and triunsaturated acyl groups, which also were in satisfactory agreement with the values obtained by classical methods. IR and $^1$H NMR determinations take very little time in comparison with classical methods and do not require chemical manipulation or transformation of the sample. A comparison was also made between the compositions of sacha inchi and linseed oil. Both oils are important sources of the healthful n-3 linolenic acyl groups, and sacha inchi also contains high proportions of the n-6 linoleic acyl groups.

Paper no. J10400 in *JAOCS 80,* 755–762 (August 2003).

**KEY WORDS:** Characterization, composition, Fourier transform infrared spectroscopy, $^1$H nuclear magnetic resonance, sacha inchi oil.

Sacha inchi (*Plukenetia volubilis* L.), also named Inca inchi, is a wild, climbing, semiwoody, perennial, oleaginous plant of the Euphorbiaceae family that grows in the tropical jungles of America at altitudes of between 200 and 1500 m. It is also known as "Inca peanut," "wild peanut," or "mountain peanut." This legume has seeds of a lenticular shape, which are rich in oil and proteins and contain heat-labile substances with a bitter taste. It has traditionally been consumed by the Indians of Peru. It was probably cultivated by the pre-Incas and the Incas because representations of this plant and of its fruits have been found on vessels in Inca tombs; although it has been in danger of extinction, projects are now underway—developed by several universities, industries, local institutions, and farmers—to recover its cultivation.

The Amazon natives obtain flour and oil from sacha inchi seeds. These products are used in the preparation of different meals and beverages; roasted seeds and cooked tender leaves are also consumed. However, this plant has rarely been studied, and its importance from the nutritional and functional point of view is still a subject of research. To our knowledge, only one paper has been published on the composition of its seeds (1); these authors have indicated that this plant can be considered as a potential new crop for some forest regions of South America, because it contains 35–60% oil and around 27% proteins rich in cysteine, tyrosine, threonine, and tryptophan.

Recently, the health and nutritional importance of the n-3 polyunsaturated acyl groups, such as the docosahexaenoic present in fish lipids or the α-linolenic acyl groups present in some vegetable oils (2), has been commented on. In fact, these kinds of acyl groups are known to provide protection against cardiovascular disease, rheumatoid arthritis, cancer, and possibly the severity of viral infections (3). Owing to these benefits, the manufacture of foods supplemented with n-3 acyl groups, such as infant formulas and clinical nutrition products, has increased considerably and, at the same time, so has the demand for oils rich in the n-3 polyunsaturated acyl groups. In this context, the study of sacha inchi oil as raw material for the food and nutraceutical industry could be considered.

The proportions of the various acyl groups in sacha inchi oil, determined by a classical method, were reported in the only paper published about the composition of sacha inchi seeds (1); however, the presence of γ-linolenic acyl groups was not discounted.

FTIR and $^1$H NMR are nondestructive techniques that are very useful in the study of edible oils and fats (4,5). Thus, FTIR has been used for the characterization of edible oils and fats as well as for determining chemical parameters such as PV and iodine value, among others (6–14). In the same way, $^1$H NMR has been used in studies of the characterization, authentication, and quality assessment of some edible oils, as well as for determining parameters such as iodine value, among others (15–22). In this paper, sacha inchi oil was

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studied by means of the previously mentioned techniques; the composition of this oil was determined using methods based on these techniques, and the results obtained were compared with those obtained by classical methods. The presence or absence of γ-linolenic acyl groups in sacha inchi oil was elucidated, and similarities and differences with linseed oil were commented on.

## MATERIALS AND METHODS

*Samples.* Sacha inchi oil samples, obtained from roasted seeds, were provided by Agroindustrias Amazónicas (Lima, Perú). Two were crude oils (crude 1, SIC1; crude 2, SIC2), and the third was a semirefined oil (refined, SIR). In addition, three different samples of edible linseed oil (L1, L2, and L3) were acquired in local supermarkets; in addition, γ-linolenic acid, from Aldrich (Milwaukee, WI), was used as standard to detect the presence of γ-linolenic acyl groups in the sacha inchi oil.

*FTIR spectroscopy.* FTIR spectra of the oil samples were acquired on a Bruker Vector 33 spectrometer interfaced with a personal computer operating under Windows NT. All spectra were recorded from 4000 to 500 $cm^{-1}$ with a resolution better than 4 $cm^{-1}$, co-adding 32 interferograms, with an accuracy in the frequency data of better than 0.01 $cm^{-1}$. A film of a small amount of sample (approximately 2 μL) was deposited between two discs of KBr, avoiding the presence of air, as in previous studies (11–14). Duplicate spectra were collected from each sample. The frequency value of each band was obtained automatically by the software; this procedure avoids the experimental errors associated with the subjectivity of external operators. The assigment of bands to a specific functional group vibration mode was made by comparison with previous studies of edible fats and oils (12,14). The absorbance of some bands was measured automatically by taking the baseline from 3750 to 2472 $cm^{-1}$, as in previous papers (12), to avoid the experimental errors associated with the subjectivity of having external operators.

*$^1$H NMR.* The $^1$H NMR spectra were recorded on a Varian 300 Plus spectrometer operating at 299,862 MHz. Each oil sample, weighing 0.2 g, was mixed with 400 μL of deuterated chloroform and a small proportion of tetramethylsilane as internal reference; this mixture was introduced into a 5-mm-diameter tube. The acquisition parameters were: spectral width, 5000 Hz; relaxation delay, 3 s; number of scans, 32; acquisition time, 3.744 s; and pulse width, 90°, with a total acquisition time of 3.37 min. The experiment was carried out at 25°C. Signals assignments were done as in previous studies (22). The area of the signals was determined by using the equipment software, and integrations were done three times to obtain average values.

*Generation of FAME and their quantification by GC.* FAME were prepared by esterification of an aliquot of the sample in the presence of 1% sulfuric acid in methanol, following a modified procedure based on method C included in the Regulation EEC/2568/91 (EEC 1991), and were extracted with hexane (23). FAME were separated and quantified by injecting 1 μL of the hexane solution into a PerkinElmer Autosystem gas chromatograph equipped with an FID and using helium as the carrier gas. The capillary column used was a SUPELCOWAX 10 (30 m length, 0.53 mm i.d., and 1.0 μm thickness) coated with polyethylene glycol. The chromatographic conditions were as follows: injector temperature, 250°C; detector temperature, 250°C; oven temperature, set initially at 60°C (1 min hold), increased to 200°C at 20°C/min (1 min hold), increased to 240°C (14 min hold). Identification of the different peaks was done by comparison with the retention times of standards. Quantification was carried out by area normalization.

## RESULTS AND DISCUSSION

Figure 1 shows the IR spectrum of SIC1 oil. This spectrum is similar to that of the other two samples, SIC2 and SIR, and the three differ from the spectra of other vegetable oils either in the exact frequency and absorbance of the bands or in the presence or absence of some bands. These variations are due to the different length and degree of unsaturation of the acyl groups in the TG constituents of each vegetable oil.

As Figure 1 shows, the spectrum of SIC1 has a weak band **a** associated with the overtone of the glyceride ester carbonyl absorption at 3471.21 $cm^{-1}$. Band **c**, resulting from the stretching vibration of the *cis* olefinic CH double bonds, is found at 3010.57 $cm^{-1}$ (3010.54 $cm^{-1}$ in the refined sample); the high value of the frequency of this band indicates its richness in polyunsaturated acyl groups (11). Among the several oils studied in our laboratory, only linseed oil shows a band frequency as high as this; for example, olive oil shows values near 3005.4, rapeseed oil near 3007.5, and corn oil near 3008.8 $cm^{-1}$. The two bands, **e** and **f**, resulting from the methylene asymmetrical and symmetrical stretching vibrations appear at 2927.14 and 2855.09 $cm^{-1}$, respectively. The frequency values of these lat-

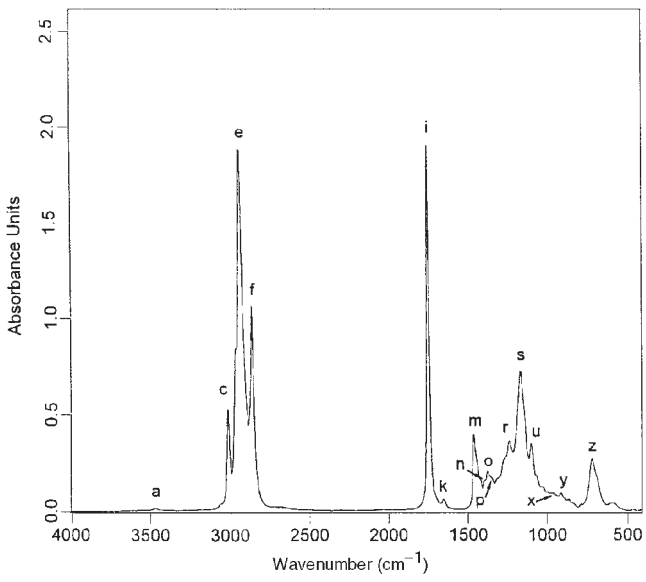


**FIG. 1.** FTIR spectrum of sacha inchi crude 1 (SIC1) oil.

ter bands in sacha inchi oil are also similar to those of linseed oil and are the highest observed among all vegetable oils previously studied in our laboratory (11); this is probably due to the high proportion of polyunsaturated groups in the sample.

The ratio of absorbances of bands **c** and **f** was taken as a measure of the degree of unsaturation of the oil (4,24,25). This ratio is near 0.50 for SIC1, SIC2, and SIR, showing the high degree of unsaturation of this oil in relation to other edible oils such as olive (A**c**/A**f** = 0.17, where A is absorbance), rapeseed (A**c**/A**f** = 0.23), and corn (A**c**/A**f** = 0.27) oils.

Band **i**, resulting from the stretching vibration of the C=O group of TG, appears at 1746.08 $cm^{-1}$; this value is one of the lowest observed in vegetable oils and is characteristic of oils with a high degree of unsaturation. The small band **k**, assignable to disubstituted *cis* C=C of the unsaturated acyl groups, appears at 1653.9 $cm^{-1}$. Band **m**, resulting from the bending vibrations of the $CH_2$ and $CH_3$ aliphatic groups, appears at 1461.81 $cm^{-1}$; and band **n**, tentatively assigned to rocking vibrations of the CH bonds of *cis*-disubstituted olefins, is found at 1419.57 $cm^{-1}$. The frequency value of the two latter bands is also related to the degree of unsaturation of the sample; thus, small frequency values for band **m** and high values for band **n**, in relation to the values of other vegetable oils, indicate a significant degree of unsaturation in the oil sample.

Band **o**, which appears at 1395.28 $cm^{-1}$, is difficult to assign. In spite of this, it was reported in previous papers (11,13) that the frequency of this band is very closely related to the proportion of mono- and polyunsaturated acyl groups in the oil sample. The equations obtained in that study, relating the frequency (F) of band **o** (F**o**) and the percentage of the monounsaturated acyl groups (%M) or of the polyunsaturated acyl groups (%P), were as follows:

$$F\mathbf{o} = 1394.90 + 9.90\cdot10^{-2}\%M, \quad n = 8, \quad R = 0.9910 \quad [1]$$

$$F\mathbf{o} = 1402.61 - 8.43\cdot10^{-2}\%P, \quad n = 8, \quad R = 0.9223 \quad [2]$$

From the frequency data of band **o** of the SIC1 spectrum, and using these equations, the predicted proportion of monounsaturated and polyunsaturated acyl groups in this oil is around to 3.8 and 86.8%, respectively (see Table 1).

Band **p**, at 1376.73 $cm^{-1}$, resulting from symmetrical bending vibrations of the $CH_3$ groups, as well as bands **r**, at 1238.26 $cm^{-1}$, and **s**, at 1163.45 $cm^{-1}$, both associated with the stretching vibration of the C–O ester groups and with the bending vibration of the $CH_2$ group, do not show great variations among the different vegetable oils. The IR spectra of the majority of vegetable oils studied in our laboratory show a band **t**, near 1118–1120 $cm^{-1}$, whose frequency increases and intensity decreases as the degree of unsaturation of the sample increases in such a way that this band does not appear either in the linseed or in the sacha inchi oil spectra. The absence of band **t** in an oil spectrum indicates that the oil is highly unsaturated.

Band **u**, associated with the stretching vibration of the C–O group in esters, appears in SIC1 at 1100.16 $cm^{-1}$. The frequency of this band also is closely related to the proportion of monounsaturated and polyunsaturated acyl groups in the sample (11). Equations obtained in our previous paper relating these variables were as follows:

$$F\mathbf{u} = 1100.46 - 4.87\cdot10^{-2}\%M, \quad n = 8, \quad R = 0.9908 \quad [3]$$

$$F\mathbf{u} = 1096.68 + 4.13\cdot10^{-2}\%P, \quad n = 8, \quad R = 0.9176 \quad [4]$$

By taking the frequency of band **u** of the SIC1 oil spectrum, and using these equations, a 6.2% proportion of the monounsaturated acyl groups and an 84.2% proportion of the polyunsaturated acyl groups were predicted (see Table 1). These proportions are very close to those predicted using the frequency data of band **o**. The average values of both approaches give, for SCI1 oil, 5.0 and 85.6% proportions of mono- and polyunsaturated acyl groups, respectively, and a 9.4% proportion of saturated acyl groups, obtained by difference. As in Table 1, these proportions are close to those obtained through FAME formation and subsequent separation and quantification by GC, and also to those found by other authors (1).

As in most of vegetable oils, the SIC1 spectrum has two weak bands, **x** and **y**, at 968.5 and 914.9 $cm^{-1}$, respectively. The first is assigned to the out-of-plane bending vibration of isolated *trans* olefins. The second, although difficult to assign, is closely related to the degree of unsaturation of the

**TABLE 1**
**Calculated Proportions of Monounsaturated (M), Polyunsaturated (P), and Saturated (S) Acyl Groups in Sacha Inchi Oils[a]**

| | F**o** (%) | | | F**u** (%) | | | Average (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | P | S | M | P | S | M | P | S |
| $SIC1_{FTIR}$ | 3.8 | 86.9 | 9.3 | 6.2 | 84.3 | 9.5 | 5.0 | 85.6 | 9.4 |
| $SIC1_{FAME\text{-}GC}$ | — | — | — | — | — | — | 8.9 | 86.0 | 5.0 |
| $SIC2_{FTIR}$ | 4.1 | 86.6 | 9.3 | 6.0 | 84.5 | 9.5 | 5.0 | 85.5 | 9.4 |
| $SIR_{FTIR}$ | 2.6 | 88.4 | 9.0 | 6.2 | 84.3 | 9.5 | 4.4 | 86.3 | 9.3 |
| $SIR_{FAME\text{-}GC}$ | — | — | — | — | — | — | 7.9 | 87.0 | 5.0 |
| SI[b] | — | — | — | — | — | — | 9.6 | 82.0 | 7.7 |

[a]From FTIR frequency values (F) of bands **o** and **u** and the average values together with proportions obtained from FAME quantified by GC and others taken from the literature. SIC1, sachi inchi crude oil 1; SIC2, SI crude oil 2; SIR, SI semirefined oil.
[b]Data from Reference 1.

sample; bearing in mind that pure tristearin, tripalmitin, trilinolenin, and trilinolein show this band frequency at 920.55, 916.53, 915.70, and 914.14 $cm^{-1}$, respectively, and that triolein shows only a shoulder at 903.73 $cm^{-1}$ (13), the frequency value of this band (914.87 $cm^{-1}$) in the sacha inchi oil spectra can be considered typical of an oil sample rich in the polyunsaturated acyl groups.

As already noted, the IR spectra of SIC2 and SIR oil samples have a great similarity to that of SIC1, showing almost unappreciable variations either in the frequency or in the absorbance of the bands. Table 1 gives the proportions of mono- and polyunsaturated acyl groups calculated from the **o** and **u** band frequencies, together with the average values thus obtained for these samples, as well as those determined through FAME formation and subsequent separation and quantification by GC. The results obtained by both methods are in satisfactory agreement and also agree with those reported by other authors (1). Obviously, the similarity between the spectra of these samples is a consequence of the similarity in their compositions.

In short, the FTIR spectra of sacha inchi oil show that it is highly unsaturated. The frequency values of the different bands are close to those of linseed oil. The proportions of the different acyl groups calculated from the frequency data are close to those determined through FAME formation and subsequent separation and quantification by GC, and also to those previously found by other authors (1). It is noteworthy that this information was obtained from the FTIR data directly from the oil sample, in the few minutes that the acquisition of the IR spectrum took. However, with the approach used above, it was not possible to calculate the proportions of the linoleic and linolenic acyl groups separately, which would afford more accurate information in relation to the similarity or difference between sacha inchi and linseed oils.

Figure 2 shows the $^1H$ NMR spectrum of SIC1 oil. This spectrum contains the 10 signals typical of the vegetable oil spectra. The area of these signals is proportional to the number of each kind of hydrogen atoms present in the sample. Thus, methylic protons of saturated, oleic (n-9), and linoleic (n-6) acyl groups, together with other potential n-6 acyl groups, give signal **1** between 0.83 and 0.93 ppm; this signal results from the overlapping of the triplets of the methylic proton signals of the three acyl groups just mentioned. As indicated in a previous paper (22), saturated and oleic (n-9) acyl groups give a triplet signal, with chemical shifts at 0.856, 0.879, and 0.900 ppm, and linoleic (n-6) together with other n-6 acyl groups gives a triplet signal, with chemical shifts at 0.866, 0.889, and 0.911 ppm, clearly differentiated from the others. Amplification of signal **1** allows one to clearly distinguish the six peaks corresponding to the two triplets (see Fig. 3). As shown in a previous paper (22), the observation of this signal allows one to deduce whether the proportion of saturated and n-9 acyl groups is higher or lower than the proportion of n-6 acyl groups. In Figure 3, which shows this signal for SIC1 and for linseed 1 (L1) oils, both oils clearly contain very different proportions of these acyl groups. Thus, it is evident that the dominant peak in L1 corresponds to the saturated and n-9 acyl groups, at 0.879 ppm, whereas in SIC1 the dominant peak is that corresponding to the n-6 acyl groups, at 0.889 ppm.

Signal **2**, shown in Figure 2, is a triplet, attributed to the methylic protons of the n-3 acyl groups, that appears between 0.94 and 1.00 ppm; the great intensity of this signal, compared to signal **1**, indicates that one of the main groups in SIC1 oil is the n-3 acyl group; this is also true in L1 oil, as shown in Figure 3.

From the areas of signals **1** and **2**, and taking into account that each acyl group, independent of its degree of unsaturation, contains the same number of methylic hydrogen atoms, the proportion of n-3 acyl groups can be determined on the one hand, and, on the other, the proportion of saturated, n-9, and n-6 acyl groups together can be determined. The results

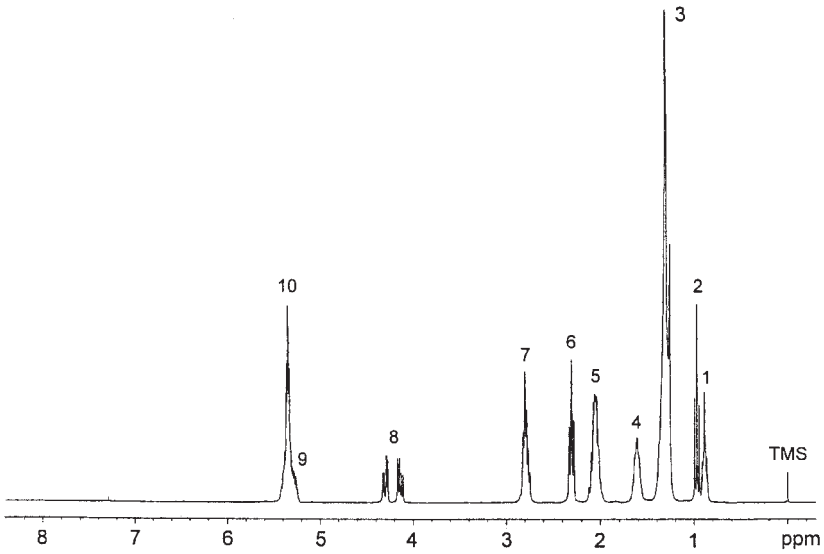


**FIG. 2.** $^1H$ NMR spectrum of SIC1 oil. TMS, trimethylsilane; for other abbreviation see Figure 1.

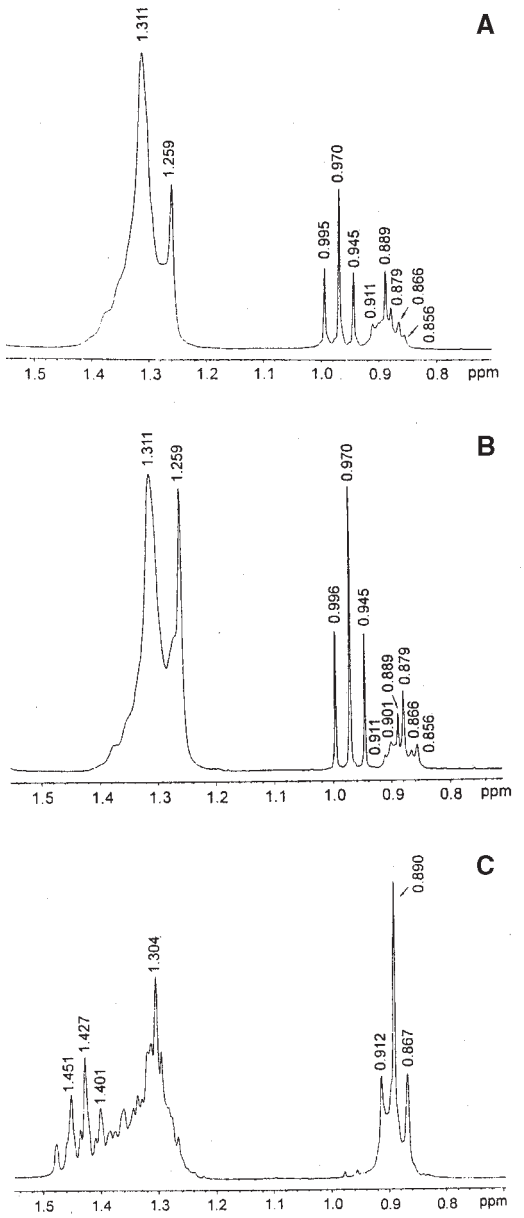


**FIG. 3.** Expansions of the region between 0.70 and 1.55 ppm of the $^1$H NMR spectra of (A) SIC1 oil; (B) linseed 1 (L1) oil, and (C) γ-linolenic acid. For abbreviation see Figure 1.

obtained indicate that in SIC1 the proportion of n-3 acyl groups is close to 47.4%, and the proportion of saturated, n-9, and n-6 acyl groups together is close to 52.6%; similar results were obtained for SIC2 and SIR oil samples. In the L1, L2, and L3 oil samples, the proportions of the different acyl groups, determined from the $^1$H NMR methylic proton signal area, are, as Table 2 shows, nearly 54–55% for the n-3 acyl groups and nearly 45–46% for the saturated, n-9, and n-6 acyl groups. All these results are in satisfactory agreement with those obtained through FAME quantification and also with those obtained by other authors (1,26); however, the method used here to obtain and quantify FAME apparently gives slightly higher proportions of the n-3 acyl groups than the method based on $^1$H NMR and that used by other authors (1).

**TABLE 2**
**Proportions of the Different Acyl Groups in Sacha Inchi and Linseed Oils[a]**

| | S + O + L (%) | Ln (%) | L (%) | O (%) | S (%) |
|---|---|---|---|---|---|
| $SIC1_{^1H\ NMR}$ | 52.6 | 47.4 | 39.3 | 6.1 | 7.2 |
| $SIC1_{FAME\text{-}GC}$ | 48.5 | 51.5 | 34.5 | 8.9 | 5.0 |
| $SIC2_{^1H\ NMR}$ | 52.4 | 47.6 | 38.5 | 6.3 | 7.5 |
| $SIR_{^1H\ NMR}$ | 53.1 | 46.8 | 38.5 | 7.1 | 7.5 |
| $SIR_{FAME\text{-}GC}$ | 46.7 | 53.3 | 33.7 | 7.9 | 5.0 |
| SI[b] | 54.8 | 45.2 | 36.8 | 9.6 | 7.75 |
| $L1_{^1H\ NMR}$ | 44.9 | 55.1 | 18.5 | 21.1 | 8.9 |
| $L1_{FAME\text{-}GC}$ | 42.2 | 57.8 | 14.2 | 21.7 | 6.2 |
| $L2_{^1H\ NMR}$ | 45.5 | 54.5 | 18.9 | 17.1 | 5.6 |
| $L3_{^1H\ NMR}$ | 45.9 | 54.1 | 14.7 | 19.7 | 11.5 |
| $L3_{FAME\text{-}GC}$ | 41.7 | 58.3 | 14.8 | 19.2 | 7.7 |
| L[c] | 49.0 | 51.0 | 16.0 | 23.0 | 10.0 |

[a]Determinated from $^1$H NMR data, together with others through FAME quantification by GC and sources from the literature. Ln, linolenic; L, linoleic; O, oleic; S, saturated; L1, linseed oil sample 1; L2, linseed oil sample 2; L3, linseed oil sample 3; for other abbreviations see Table 1.
[b]Data from Reference 1.
[c]Data from Reference 26.

These results indicate that both kinds of vegetable oils are important sources of n-3 acyl groups, although the linseed oils studied here are even richer in these than the sacha inchi oil. The proportion of n-3 acyl groups from the $^1$H NMR signal area of the methyl protons also was determined previously by other authors (27–29).

Another $^1$H NMR signal present in all edible vegetable oils is signal **3**, shown in Figure 2. This is due to the overlapping of the signals of the methylenic protons, which are in the β position or further in relation to double bonds, or are in the γ position or further in relation to the carbonyl group. The chemical shifts of these methylenic proton signals are between 1.22 and 1.42 ppm. As described in a previous paper (22), this signal shows different shapes, depending on the oil, and can also provide information about the main acyl groups in the sample. In Figure 3 this signal, either in sacha inchi or in linseed oil, has basically two peaks, one sharp, at 1.259 ppm, attributable to the methylenic protons of the saturated acyl groups, and another broad, at 1.311 ppm, attributable to the overlapping of peaks of the methylenic protons of the oleic (n-9), linoleic (n-6), and linolenic (n-3) acyl groups; in addition, in the L1 oil spectrum a shoulder is observable on the peak at 1.272 ppm of methylenic protons of the oleic n-9 acyl groups. The absence of this shoulder in the SIC1 spectrum shows that this oil contains a lower proportion of the oleic acyl groups than L1. In addition, from a comparison of the peak areas at 1.259 and at 1.311 ppm in both oil spectra, it is evident that L1 is richer in the saturated acyl groups than SIC1.

On the other hand, from the shape of signal **3** the presence of γ-linolenic acyl groups in sacha inchi oil can be discounted. This kind of methylenic proton in the γ-linolenic acyl groups gives a characteristic broad multiplet between 1.35 and 1.50 ppm, as shown in the spectrum of γ-linolenic acid in Figure 3. This typical signal also has been observed in the spectrum of

methyl γ-linolenate ester (30). However, it is absent from the $^1$H NMR spectra of sacha inchi oil samples.

Signals **4** and **6** in Figure 2, attributable to methylenic protons in the β or α position in relation to the carbonyl group, appear between 1.52 and 1.70 ppm and between 2.23 and 2.38 ppm, respectively. These signals are not significantly useful to discriminate between oil samples.

Signal **5**, in Figure 2, between 1.94 and 2.14 ppm, is attributable to α methylenic protons in relation to only one double bond, also named allylic protons. Although each one of the oleic, linoleic, and linolenic acyl groups contains four protons of this type, their signals have different shapes (22). The SIC1 oil spectrum (see Fig. 4) has a multiple signal showing the typical peaks of linolenic acyl groups (the signal of trilinolenin is a multiplet with peaks at 2.037, 2.048, 2.072, 2.097, and 2.122 ppm) in which the typical peaks of linoleic acyl groups are also visible (the signal of trilinolein has four peaks at 2.016, 2038, 2.060, and 2.081 ppm), indicating that both are the main acyl groups in this oil. Peaks of the oleic acyl groups hardly contribute to the total signal of SIC1 oil (the signal of triolein is a doublet at 2.001 and 2.020 ppm), showing the low proportion of this group in sacha inchi oil. By contrast, peaks of the oleic acyl groups are clearly observable in the L1 oil signal as this acyl group is in higher proportion in the latter than in SIC1 oil, in agreement with that observed in the methylenic signal between 1.22 and 1.42 ppm. From these results it is evident that a simple observation of this amplified signal **5**, shown in Figure 4, can provide information, in an approximate way, on the proportions of the different unsaturated acyl groups in the sample.

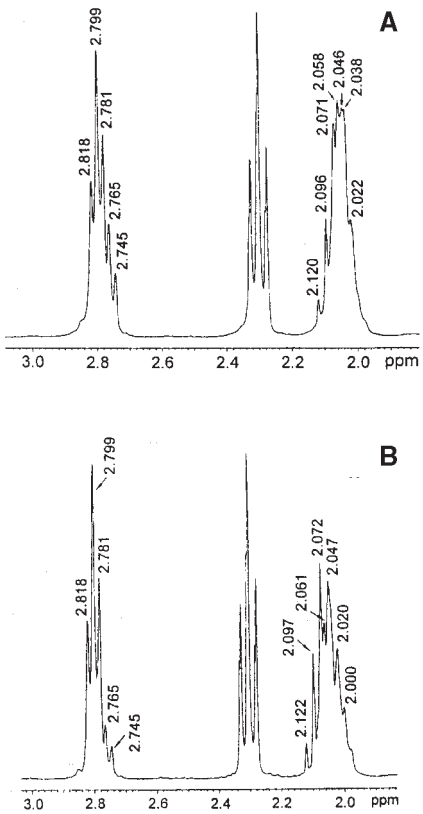


**FIG. 4.** Expansions of the region between 1.90 and 3.10 ppm of the $^1$H NMR spectra of (A) SIC1 oil and (B) L1 oil. For abbreviations see Figures 1 and 3.

Signal **7** is attributable to methylenic protons in the α position in relation to two double bonds, also named bis-allylic protons, and appears between 2.70 and 2.84 ppm (see Fig. 2); this signal is attributable to the overlapping of peaks of linoleic (triplet at 2.749, 2.769, and 2.789 ppm) and linolenic (triplet at 2.781, 2.799, and 2.819 ppm) acyl groups (22). In spite of the overlap between the linoleic and linolenic signals, it can be observed in the SIC1 spectrum (Fig. 4) that the area of the signal corresponding to the linolenic acyl groups is somewhat higher than that corresponding to the linoleic acyl groups, and this trait is much more accentuated in the L1 spectrum; however, it must be taken into account that each linolenic acyl group contains four bis-allylic protons, whereas each linoleic acyl group contains only two.

Signal **8** in Figure 2, at 4.10–4.32 ppm, is attributable to the protons on the 1 and 3 carbon atoms of the glyceryl group, and signal **9**, at 5.20–5.26 ppm, is attributable to the proton on the 2 carbon atom of the same glyceryl group. This latter signal overlaps slightly with signal **10**, at 5.26–5.40 ppm, which is attributable to olefinic protons of the different acyl groups (see Fig. 2).

As commented on above, the area of these $^1$H NMR spectra signals is proportional to the number of protons of each type in the sample. Taking into account that the proportion of MG and DG molecules in these oil samples is not significant, the area of signal **8**, corresponding to the four protons on the 1 and 3 carbon atoms of the glyceryl group, was fitted to four units. Thus, the values of the other signal areas refer to a TG molecule, and from the area of the signal of the allylic and bis-allylic protons, the proportions of the linoleic, saturated, and oleic acyl groups can be calculated. If, in the TG molecules of the oil samples, all acyl groups were unsaturated groups, the area of the signal of allylic protons (area of signal **5**) would be very close to 12. For this reason, the proportion of saturated acyl groups in the oil sample can be calculated from the area of the signal of the allylic protons. The proportions determined in this way are given in Table 2. It can be observed that all the sacha inchi oil samples studied contain a very similar proportion of saturated acyl groups, whereas the linseed oil samples contain higher or lower proportions than the sacha inchi oils, although in all cases these proportions can be considered low. From the bis-allylic proton signal area, the percentage of linoleic acyl groups can be calculated, taking into account that (i) the percentage of the linolenic acyl group is known, (ii) that each linolenic acyl group contributes four protons, and (iii) that each linoleic acyl group contributes two. The proportions of linoleic acyl groups obtained (shown in Table 2) indicate that sacha inchi oil is much richer in the linoleic acyl groups than is linseed oil, as deduced from a simple spectral observation. Because the percentage of saturated plus oleic and linoleic acyl groups is known, the proportion of oleic groups can be calculated by the difference; the results

obtained (given in Table 2) indicate that the linseed oils studied here are much richer in the oleic acyl groups than the sacha inchi oils. As shown in Table 2, the degree of agreement between the proportions of the different acyl groups determined directly from the $^1$H NMR spectra and those determined by FAME quantification is satisfactory and is consistent with the results obtained by other authors (1,26). However, the method used here to obtain and quantify FAME seems to enhance the proportions of linolenic acyl groups slightly and to diminish the proportions of saturated and of linoleic acyl groups slightly, in relation to those obtained not only by $^1$H NMR but also by other authors (1,26). The determination of the proportions of the different acyl groups from the $^1$H NMR signal areas, probably using approaches similar to those used here, has been reported before in only two other papers (31,32).

Simple observation of the $^1$H NMR spectra of sacha inchi oil can thus afford a great deal of information in referring not only to the relative proportions of the different acyl groups but also to their nature, permitting the presence of γ-linolenic acyl groups in this oil to be discounted. Furthermore, by using the area of some $^1$H NMR signals, the proportions of the different acyl groups can be calculated, providing results in satisfactory agreement with those obtained by FAME quantification. The determination by $^1$H NMR takes very little time in comparison to classical methods and does not require chemical manipulation or transformation of the sample. Both the sacha inchi and linseed oils studied here have a high degree of unsaturation, although the proportion of n-3 linolenic acyl groups is higher in the second, and the opposite is true for n-6 linoleic acyl groups. Furthermore, the linseed oils studied here are richer in n-9 oleic acyl groups than are the sacha inchi oils. As a consequence, these differences in composition between the two kinds of oil will result in differences in behavior as well as in nutritional properties.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Ministerio de Ciencia y Tecnología MCYT (AGL2000-1696) and of the Universidad del País Vasco (UPV 101.123-EB087/99 and 9/UPV 00101.125.13667/2001). A.R. thanks the Universidad del País Vasco for a predoctoral fellowship. The authors also thank Agroindustrias Amazónicas for providing the samples.

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[Received July 25, 2002; accepted February 28, 2003]