Oxidation in Fish Lipids During Thermal Stress as Studied by ^{13}C Nuclear Magnetic Resonance Spectroscopy

Isabel Medina*, Raffaele Sacchi[1], Italo Giudicianni[2], and Santiago Aubourg

Instituto de Investigaciones Marinas del CSIC, E-36208 Vigo, Spain

ABSTRACT: ^{13}C Nuclear magnetic resonance spectroscopy has been applied to elucidate the mechanism of lipid oxidation occurring during thermal treatment of fish. Effects of temperature and time of processing have been studied by means of a model system of lipids, extracted from salmon (*Salmo salar*) muscle, to simulate industrial conditions of canning. Unsaturated fatty acids located at the *sn*-2 position of the glycerol moiety were the most prone to oxidative damage. Regarding the mechanism of the reaction, results inferred from olefinic and methylenic resonances indicated a higher susceptibility of the allylic sites closest to the carbonyl group, followed by those placed near the methyl terminal group. Unsaturations located in the middle of the carbon chain did not show much damage. The glyceryl region provided an unusual resonance at 53.4 ppm, which could be assigned to a hydroxylic compound formed during process. *JAOCS 75*, 147–154 (1998).

KEY WORDS: ^{13}C NMR spectral regions, fish lipids, oxidation, thermal processing.

Many studies have described the important role that alterations of lipids during processing of foods have on the quality of the final product (1,2). Lipid damage is often focused on the reactivity of polyunsaturated fatty acids (PUFA) because it can produce a significant number of polar compounds, including volatile derivatives by action of heat *via* the process of lipid oxidation (3–5). The highly unsaturated fatty acid composition renders fish flesh extremely susceptible to oxidation and rapid degradation during processes that involve thermal treatment.

Although some information is available on the effects of strong thermal processing, such as canning, on lipid composition of fish muscle, it is essentially related to lipolysis and the amounts of free fatty acids formed (6,7). Regarding oxidative damage, some studies have described the volatile profiles produced during heat treatments of fish to establish a relationship with the quality of canned muscle (8,9). Elevated temperatures during the canning process can stimulate and accelerate the interaction and decomposition of many intermediate products. In addition, the qualitative and quantitative extension of this reaction depends on the fish species processed (9). These points render fish lipid oxidation during heating extremely difficult to analyze.

In the evaluation of a mechanism of fish lipid oxidation and in the assessment of its overall flavor quality, different methodologies have been employed (10). Most of these methods are based on the formation of specific compounds or on the modification of others. However, their evolution is so rapid that the use of these approaches in fish thermal processing has not been very useful. The main advantage of ^{13}C nuclear magnetic resonance (NMR) spectroscopy in lipid studies over the classical procedures is the simultaneous determination of compounds formed and of the acyl compounds degraded and the study of the mechanisms involved in such reactions (11,12). ^{13}C NMR spectroscopy has been successfully employed in the monitoring of free fatty acid release after fish thermal processing (12,13).

The oxidative reaction involves both a degradation of acyl compounds and a modification in the olefinic nuclei due to the formation of conjugated dienes and hydroperoxides. The aim of this work was to employ ^{13}C NMR spectroscopy to study the lipid oxidation that occurred during the heating of fish muscle. To simulate industrial conditions of canning, lipids extracted from salmon white muscle were heated in a model system at different times and temperatures. After thermal stress, ^{13}C NMR spectra were recorded both on raw and heated lipids. To elucidate the mechanism and extension of the oxidation, different spectral regions were carefully studied.

EXPERIMENTAL PROCEDURES

Raw material. Samples of salmon (*Salmo salar*) fresh muscle were purchased at a commercial market (kept on ice for 1 d).

Lipid extraction. Lipids were extracted from raw muscle by the Bligh and Dyer (14) method. Lipid extracts were stored at –20°C in chloroform until analysis; propyl gallate was used

*To whom correspondence should be addressed at Instituto de Investigaciones Marinas del CSIC, Eduardo Cabello 6, E-36208 Vigo, Spain. E-mail: medina@iim.csic.es.

[1]Current address: Dipartimento di Scienza degli Alimenti, Università di Napoli Federico II, I-80055 Portici, Italy.

[2]Current address: Centro Interdipartimentale di Metodologie Chimico-Fisiche, Università di Napoli Federico II, Via Mezzocannone 16, 80134 Napoli, Italy.

as antioxidant. Lipid content was determined by a gravimetric method (15).

Model systems of thermal processing. To study the effect of processing temperatures, 100 mg of salmon lipids was incubated for 60 min at 115 (usual conditions employed in canneries, critical lethality = 7), 130, and 150°C. To study the effect of processing times, 100 mg of salmon lipids was incubated at 115°C for 60, 80, 100, and 120 min.

Peroxide value determination. Peroxide values were measured in duplicate by AOAC Method 28:026 (16).

^{13}C NMR spectroscopy. Full ^{13}C NMR spectra and high-resolution spectra of the olefinic region of raw and heated lipids were recorded on an AC-270 Bruker (Karlsruhe, Germany) spectrometer, operating at a ^{13}C frequency of 67.88 MHz. Spectra were recorded at concentrations of 10–20% wt/vol by dissolving 50–100 mg lipid in 0.5 mL chloroform-*d* (Aldrich Chemical, Milwaukee, WI) and using controlled temperatures of 30 ± 0.1˚C to obtain the best chemical shift and relaxation rate reproducibility. Two different sets of acquisition parameters were used. The full ^{13}C NMR spectrum was recorded with 256–3000 scans, at a 200-ppm spectral width, 16 K data points (with resulting digital resolution of 2.7 Hz/pt and 0.37 s acquisition time), relaxation delay of 2 s, and 45˚ pulse width. All free induction decays (FID), prior to Fourier transformation (FT), were filtered using exponential multiplication (line broadening of 2 Hz) for sensitivity enhancement. Chemical shifts were indirectly referred to tetramethylsilane (TMS, $\delta = 0$ ppm) by using the central resonance of chloroform-*d* ($\delta = 77.00$ ppm).

To record the high-resolution ^{13}C NMR spectra of the olefinic region, the following acquisition parameters were used: 5000 scans, 10 ppm spectral width, 32 K data points (with resulting digital resolution of 0.04 Hz/pt and 23.2 s acquisition time), relaxation delay of 2 s, and 45–90˚ pulse width.

Distortion Enhancement Polarity Transference (DEPT) spectra were run with a pulse angle of 135° to obtain simultaneous identification of all protonated carbons.

Integrations of NMR intensity were repeated three times for each spectrum, and a relative standard deviation of less than 5% was found.

Trichloroacetyl isocyanate (TAI) reactions. A few drops (a small excess) of TAI (Fluka, Buchs, Switzerland) were added to the NMR tubes according to Bose and Srinivasan (17), and the ^{13}C NMR spectra were repeated.

Statistical analysis. Data obtained were subjected to the analysis of variance (ANOVA) one-way method according to Sokal and Rohlf (18).

RESULTS AND DISCUSSION

Different spectral regions provided useful information about the mechanism of the oxidative reaction and the degradation compounds formed.

Carbonyl region. The carbonyl spectral region has become a practical tool for the study of acyl stereospecific changes occurring in fish lipids during processing (11,12). Stereoselectivity of fatty acid oxidation can be easily studied from the carbonyl resonances of triacylglycerols (TG), in which docosahexaenoic acid (DHA) (22:6n-3) peaks are shifted upfield from the two complex envelopes that contain saturated (ST) and monounsaturated (MU) fatty acids in the *sn*-1,3 and *sn*-2 positions, respectively (Fig. 1). Table 1 shows the ratio between the NMR intensities for the ST–MU fatty acids and PUFA in the two positions of the glycerol backbone. Data obtained showed a diminution in the intensities of all *sn*-2 fatty acids, resulting in an increase of the *sn*-1,3/*sn*-2 ratio after heating lipids at 115°C for 60 min (usual conditions of fish canning). These results agree with previous findings that were obtained after thermal hydrolysis of fish lipids (12,13). As for the mechanism of hydrolysis, physical damage of acyl chains esterified to the secondary hydroxy group due to a heat effect may be considered. All values calculated for heated samples were significantly greater than those of raw lipids. In particular, ST–MU fatty acids located at positions *sn*-1,3 were the most resistant to thermal oxidation as can be inferred from the *sn*-1,3/*sn*-2 ratios during the process.

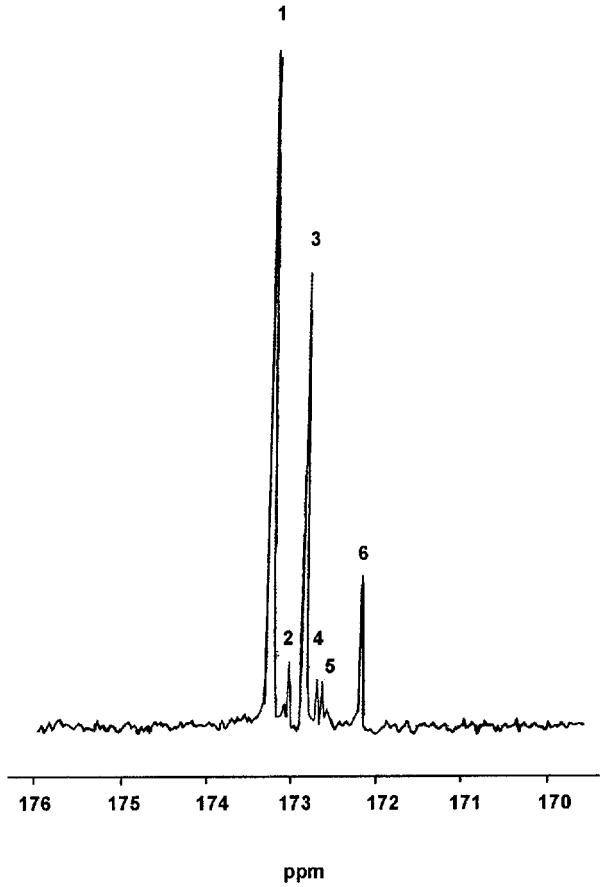


FIG. 1. Expansion of the carbonyl spectral region of salmon lipids. Labeled peaks are assigned as: 1. *sn*-1,3 saturated (ST) and monounsaturated (MU), 2. *sn*-1,3 eicosapentaenoic acid (EPA), 3. *sn*-2 ST and MU, 4. *sn*-2 EPA, 5. *sn*-1 docosahexaenoic acid (DHA), and 6. *sn*-2 DHA.

When times exceeded 60 min or temperatures were higher than 115°C, the effect on the *sn*-2 position remained more ambiguous because some coefficients decreased slightly (Table 1). Although all values calculated during processing confirmed the susceptibility of the *sn*-2 position, strong industrial conditions can probably promote damage on the *sn*-1,3 positions to affect the ratios between resonance intensities. In addition, overlapping signals corresponding to oxidation products must be considered because they influence the calculated ratios as well. Fish lipid incubation at high temperatures during longer times produces an ample variety of oxidation products (9,19). Structural changes due to the formation of these hydroperoxides and conjugated dienes have to result in new carbonyl signals that are presumably located close to those of nondamaged TG. Carbonyl resonances of unsaturated fatty acids did not show significant shift differences related to their corresponding hydroxy derivatives (20). Taking into account the variety of different oxidation products likely to be formed, sensitivity and resolution achieved by the use of ^{13}C NMR were not sufficient to distinguish these minor signals that likely overlap those corresponding to nondegraded compounds. The formation of diglycerides or monoglycerides [resonances between 174.0 and 172.5 ppm (11)] may also modify *sn*-1,3/*sn*-2 ratios. However, there was no lipid hydrolysis because no resonances corresponding to free fatty acids were detected [carbonyl signals at 177.3-176.5 ppm (11)].

In spite of these effects, values calculated for the different thermal treatments were always greater than those of raw lipids, and the highest ratios obtained for ST–MU *sn*-1,3/DHA *sn*-2 (12.74) and DHA *sn*-1,3/DHA *sn*-2 (0.80) at 115°C for 120 min showed clearly that PUFA esterified in the *sn*-2 position were more prone to suffer thermal oxidative damage than fatty acids esterified in the *sn*-1,3 position.

Olefinic region. The study of the olefinic signals in a ^{13}C NMR spectrum of a fish oil is especially complicated because of the high number of signals and their quite similar chemical shift values (21,22). In particular, olefinic resonances that correspond to PUFA of salmon lipids could not be totally resolved by recording the spectrum under the conditions of low-resolution NMR described above (Fig. 2A).

Recent papers have suggested the use of high-resolution spectral conditions, related to a restricted spectral width, longer acquisition times and higher digital resolution (see the Experimental Procedures section), to assign all olefinic resonances in the ^{13}C NMR spectra of fish lipids (23,24). On this basis, the olefinic regions of the spectra of salmon lipids, both raw and heated, were carefully investigated by recording high-resolution spectra under the appropriate experimental conditions. Figure 2B shows the typical pattern obtained for salmon lipids. Assignments were made according to data previously reported (23,24).

To elucidate which allylic sites are more labile to oxidative reaction during thermal stress, the intensities of olefinic resonances corresponding to MU fatty acids, DHA and eicosapentaenoic acid (EPA) (20:5n-3) were carefully measured. The attack of oxygen at the allylic site will also be accompanied by variations of the chemical shifts of the olefinic resonances, and consequently, changes the intensities of the nearest olefinic nuclei to this position. Figure 3 shows the relative decrease of the intensities of DHA resonances obtained for lipids that were subjected to the strongest thermal treatment, compared to those found in raw lipids. Unsaturations nearest to the carbonyl group seem to be the most susceptible to oxidation. Temperature showed higher effects than time and resulted in a reduction in the intensities of C_{20} and C_{19} olefinic carbons as well. This finding seems to indicate that oxidative reaction occurs first at the double bonds nearest to the carbonyl group, and then at the unsaturations closest to the methyl terminal group. The intensities of C_{17} and C_{16} were less. The 10% reduction detected for C_6 and C_5 nuclei of EPA may support this hypothesis.

No single compound is formed from oxidative deterioration of fish muscle (19). Different hydroperoxides, hydroxy, and conjugated compounds are likely produced, given an ample variety of signals in the olefinic spectral region. The decreases detected in olefinic resonances were not very big and ranged between 0–15%. Taking into account the variety of compounds formed and the complexity of this spectral region, the sensitivity of the ^{13}C NMR technique was not enough to detect and quantify these signals in such minor concentrations. Only for salmon lipids subjected to stronger thermal conditions (150°C for 60 min) could some minor resonances at 131.1, 131.4, and 133.2 ppm be singled out. These signals may be assigned to olefinic resonances of hydroxy fatty acids in accordance with results reported by Knothe and Bagby (25). As for the carbonyl spectral region, prior isolation of hydroperoxides or conjugated dienes would be necessary to determine these new resonances exactly. Higher field spectrometers may also be required (26).

Glyceryl region. As reported previously, glyceryl regions provide easy characterization of the nuclei related to different

TABLE 1
Ratios Between the Fatty Acid Content (% mole fraction) in the Two Positions of the Glycerol Moiety[a]

		Heated: time effect (115°C)				Heated: temperature effect (60 min)		
	Raw	60 min	80 min	100 min	120 min	115°C	130°C	150°C
ST-MU *sn*-1,3/ST-MU*sn*-2	1.52[a]	3.16[b]	2.54[c]	2.38[c]	1.76[d]	3.16[b]	2.59[c]	1.63[a]
ST-MU *sn*-1,3/DHA *sn*-2	4.44[a]	7.65[b]	4.97[c]	5.97[d]	12.74[e]	7.65[b]	6.03[d]	5.41[d]
DHA *sn*-1,3/DHA *sn*-2	0.39[a]	0.53[b]	0.40[a]	0.42[a]	0.80[c]	0.53[b]	0.42[a]	0.50[b]

[a]Mean of three determinations. Values with different letters in the same row are significantly different, $P < 0.01$; ST, saturated; MU, monounsaturated; DHA, docosahexaenoic acid.

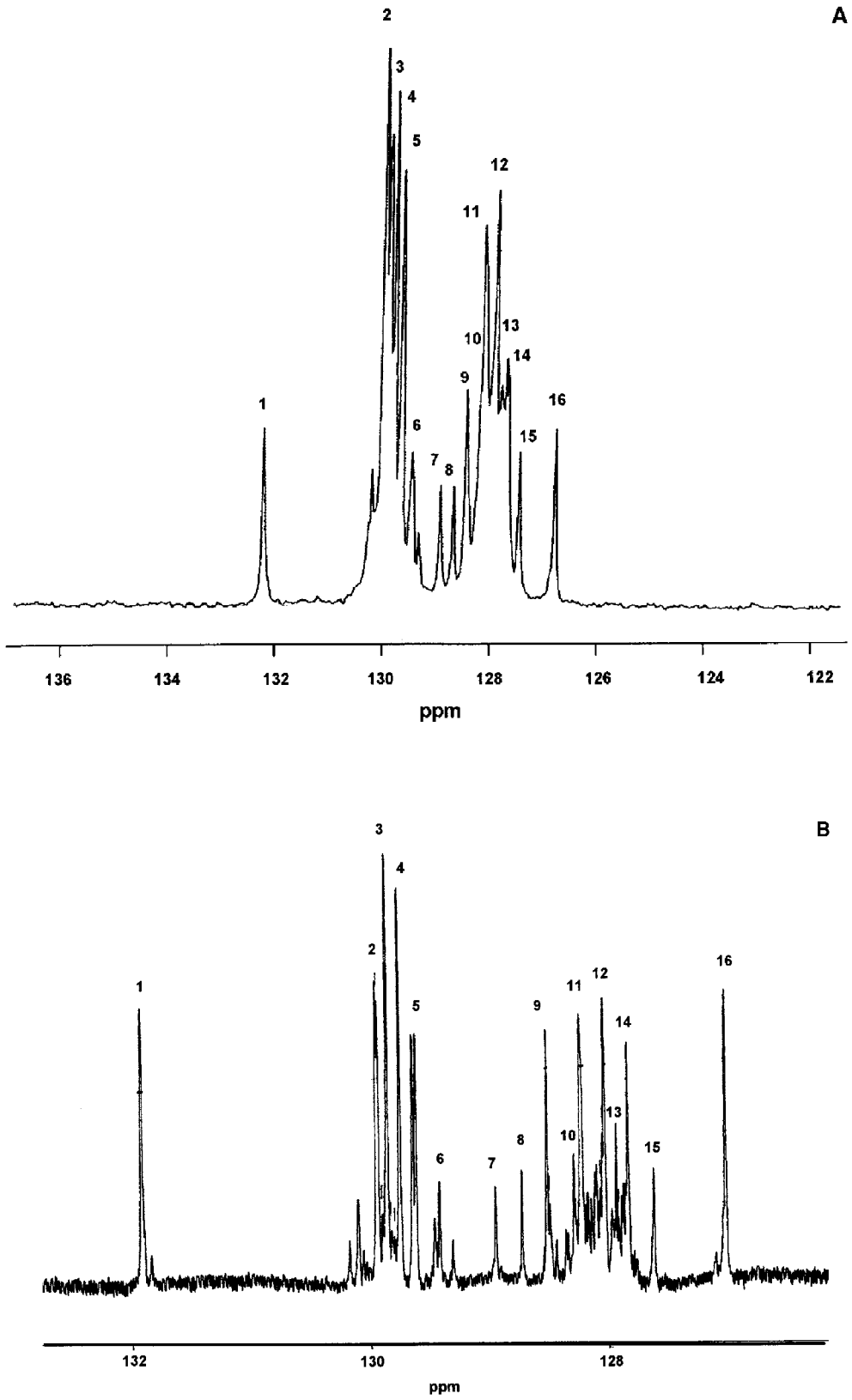


FIG. 2. Expansion of the olefinic spectral region. A. Low-resolution spectrum. B. High-resolution spectrum. Labeled peaks are assigned as: 1. C_{20} of DHA, 2. C_{10} of 18:1n-9 and 16:1n-7, 3. C_{12-14} of 20:1n-9 and 22:1n-11, 4. C_{11-13} of 20:1 n-9 and 22:1n-11, 5. C_9 of 18:1n-9 and 16:1n-7, 6. C_4 of DHA, 7. C_5 of EPA, 8. C_6 of EPA, 9. C_{17} of DHA, 10. C_7 of DHA, 11. C_{14-10} of DHA, 12. C_{11-13} of DHA, 13. C_8 of DHA, 14. C_{16} of DHA, 15. C_5 of DHA, and 16. C_{19} of DHA. See Figure 1 for abbreviations.

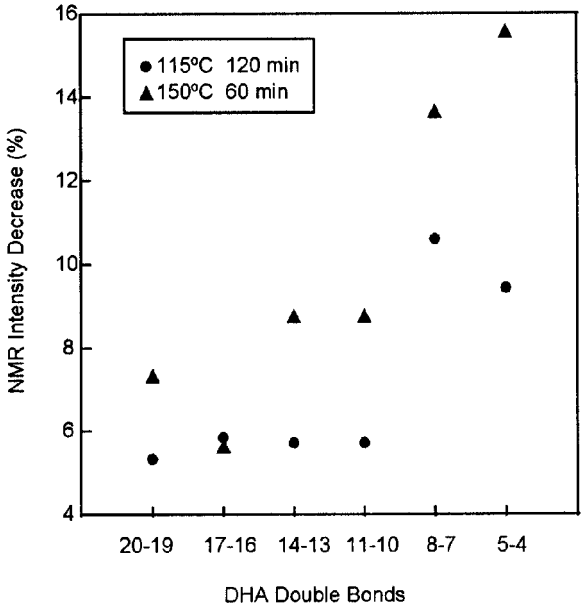


FIG. 3. Relative diminution of DHA olefinic resonances of heated lipids related to raw lipids. Intensities of each resonance were expressed as ratios relative to the olefinic signal of C_9 of 20:1n-9, taking this fatty acid into account as reference. Values for each unsaturation were calculated as means of the two olefinic nuclei. Abbreviation: NMR, nuclear magnetic resonance. See Figure 1 for other abbreviations.

glyceride species (11,27). In addition to resonances corresponding to TG, phospholipids and cholesterol, a resonance at 53.4 ppm was detected in heated samples at times and temperatures higher than usual (Fig. 4A). Signals of diglycerides or monoglycerides were not found according to results of the carbonyl region, in which there were no free fatty acid resonances.

To test the potential hydroxylic character of this carbon, heated lipids were treated with TAI to yield carbamates. This compound, usually employed in NMR studies, is an effective acylating agent, which reacts quantitatively with hydroxylic compounds and allows determination of the number and type of hydroxylic groups in a molecule (17). Although TAI can react with amine or phenol groups as well, evolution of the 53.4 ppm signal during the thermal process indicated a hydroxylic character. It is not likely that signals of new phospholipids or phenols increase in such concentrations during thermal stress of fish lipids (Fig. 4A).

After reaction with TAI, resonances corresponding to hydroxylic carbons show significant shifts. In our experiment, after TAI addition, the resonance at 53.4 ppm was shifted (Fig. 4B). Because TAI reacts exothermically also with primary, secondary and tertiary amines, such as the polar heads of phospholipids, a shift of the resonance corresponding to the trimethyl group of phosphatidylcholine (54.5 ppm) was also observed (Fig. 4B). Regarding the new signals detected after reaction, the three new carbons introduced resonate at a lower magnetic field than chloroform, as do the C atoms of the unreacted TAI (17). Therefore, resonances at 92.0 and 92.3 ppm were singled out as corresponding to TAI carbons of the unreacted reagent and that affected by the environment of the hydroxylic nucleus, respectively. So were signals at 163.23, 160.26, 158.62, and 156.80 ppm. In the olefinic spectral region, two resonances at 124.7 and 124.02 ppm were also detected. These signals could be olefinic nuclei shifted high field by about 4.5–7 ppm. This result may agree with the TAI-induced shifts of olefinic carbons in allylic alcohols reported by Bose and Srinivasan (17). Although these authors have also reported a downfield shift of about 2.5–4.5 ppm for the carbinol carbon in unsaturated alcohols, hydroxyl carbons in fatty acids will likely be affected by the positions of the carbonyl group and other unsaturations, resulting in a different shifting.

This previous assignment as a hydroxyl carbon was supported by a DEPT spectrum (Fig. 4C). The partially relaxed spectrum from the inversion-recovery experiment showed as positive signals those corresponding to CH_2 nuclei, and as negative signals those corresponding to CH and CH_3, including the resonance at 53.4 ppm (CH-OH).

Knothe and Bagby (25) have assigned the ^{13}C NMR spectra of different allylic hydroxy groups in several fatty compounds, ST and unsaturated as well. Accordingly, spectra of salmon lipids heated at stronger conditions than usual (115°C during 60 min) showed resonances at 72.7–73.5 ppm that could be attributed to specific hydroxy-bearing carbons. As previously mentioned, the corresponding signals in the carbonyl and olefinic spectral regions likely overlapped, and they could not be detected in our experiments.

The regression between the peroxide value and the NMR intensity of the resonance at 53.4 ppm was calculated for each sample. NMR intensities were measured by taking into account the trimethyl resonance of phosphatidylcholine at 54.5 ppm as reference. Results indicate a satisfactory agreement between NMR and the iodometric assay ($R^2 = 0.87$). The Y intercept (–0.195) was not significant (P value >0.05). The slope (0.251) showed significance and indicated an error of 0.3% between both techniques, probably due to differences in the sensitivity of the methodology employed.

The signal at 53.4 ppm was also found in tuna lipids extracted from overprocessed canned muscle, and it was equivocally attributed to nuclei corresponding to the solvent (11). Figure 5 shows the evolution of the intensity of resonance at 53.4 ppm during the heating process. Because raw and heated lipids treated under the usual conditions of time and temperature did not show this signal, the possibility of employing it to assess the quality of overprocessed thermal foods must be considered.

Methylenic region. Study of the methylene intensities pertains to nuclei near the carbonyl and methyl terminal groups, allylic and bisallylic signals, and the rest of the -CH_2 nuclei. Figure 6 shows the identification of the methylenic region of raw salmon lipids according to Gunstone (22). Table 2 summarizes the ratios of the different resonances taking into ac-

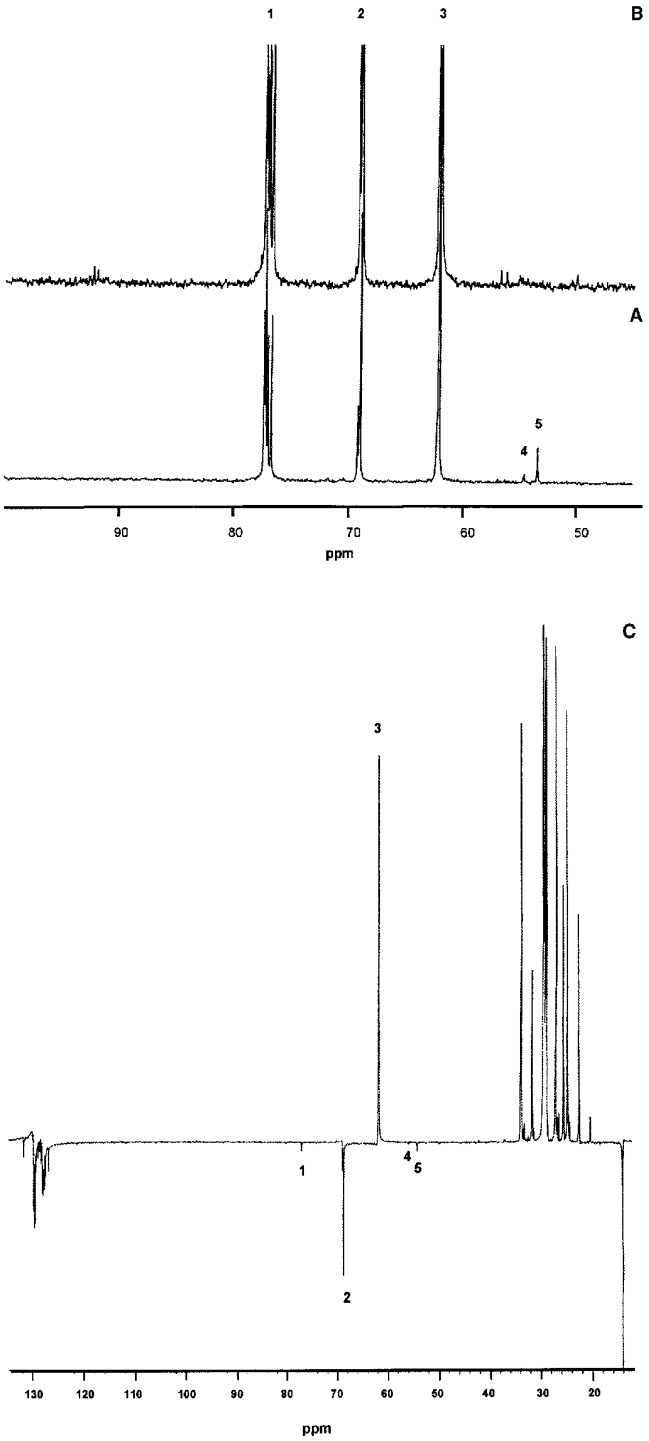


FIG. 4. Expansion of the glyceryl spectral region. A. Heated lipids. B. Heated lipids after reaction with trichloroacetyl isocyanate. C. DEPT spectrum of heated lipids. Labeled peaks are assigned as: 1. solvent, 2. *sn*-2 triglyceride (TG), 3. *sn*-1,3 TG, 4. trimethyl resonance of phosphatidylcholine, and 5. -CH-OH.

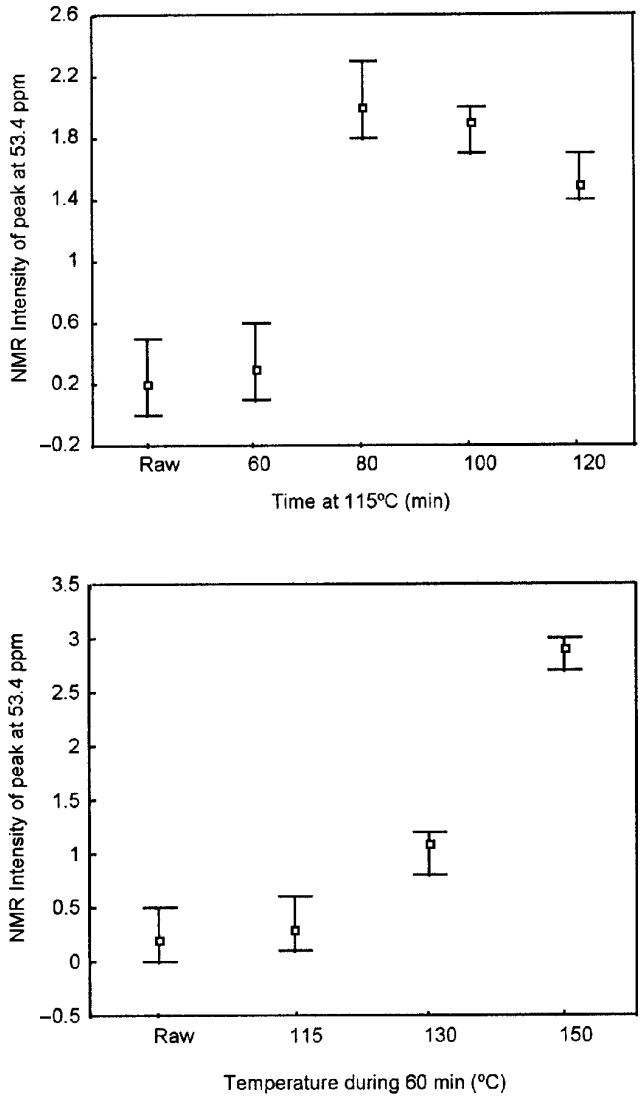


FIG. 5. Evolution of the resonance corresponding to hydroxylic nuclei at different heating conditions, expressed as ratios of intensities related to the trimethyl resonance of phosphatidylcholine (signal at 54.5 ppm).

count the signal at 31.9 ppm, corresponding to the n-3 carbon of saturated and *n*-9,11 fatty acids, as reference. As can be inferred, both high temperatures and long times of processing produced important decreases in intensities of the resonances of the carbons nearest to the carbonyl group: C_2 and C_3 of all fatty acids, and resonances of C_4, C_5 and C_6 of 22:5n-3, 18:4n-3 and 20:5n-3, respectively. However, there were no important changes in the n-2 signal of n-3 PUFA. On the other hand, high temperatures showed a higher effect on allylic and bisallylic nuclei than long times. Results obtained confirmed the previous affirmation, formulated on the basis of the olefinic resonances, concerning faster degradation at the position nearest to the carbonyl group.

Methyl region. The n-3 PUFA content can be obtained by using distinctive signals because those corresponding to n-3 PUFA are shifted low-field (14.16 ppm) with respect to those corresponding to n-6 PUFA and all other fatty acids (14.00 ppm) (22). The total n-3 PUFA content is then simply calcu-

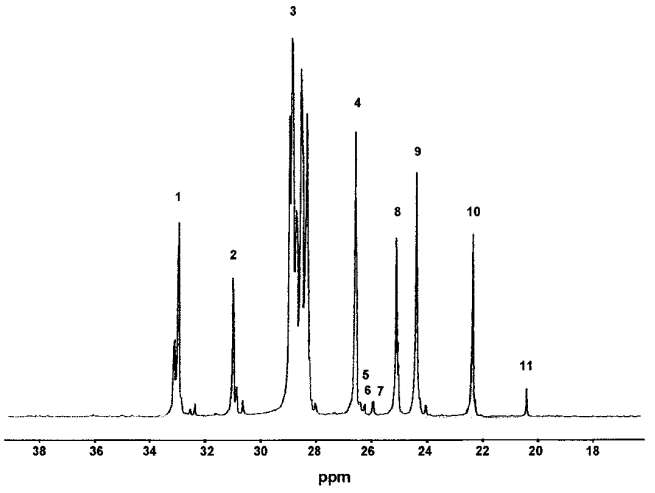
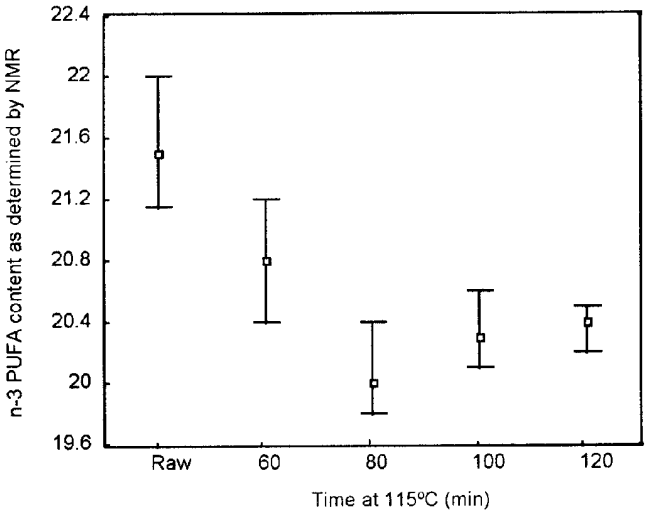


FIG. 6. Expansion of the methylenic spectral region. Resonances are labeled as: 1. C_2 of all fatty acids, 2. ω3 of saturated and n-9,11 fatty acids, 3. other CH_2, 4. allylic, 5. C_6 of 22:5n-3, 6. C_5 of 18:4n-3, 7. C_4 of EPA, 8. bisallylic, 9. C_3 of all fatty acids except DHA, 10. C_3 of DHA, and 11. ω2 of all n-3 polyunsaturated fatty acids (PUFA). See Figure 1 for other abbreviations.

lated by referring the intensity of the signal at 14.16 ppm to the sum of the intensities of both signals according with Medina *et al.* (13). A slightly lower content of n-3 fatty acids was observed at usual processing conditions (Fig. 7). These results are in agreement with those previously reported for canned tuna muscle, where a diminution in the n-3 content was detected after thermal sterilization (28). In these compounds, high temperature showed more influence than the processing time (Fig. 7). In fact, processing time did not produce significant changes in the n-3 content. Damage in other unsaturated acids that do not belong to the n-3 series must also be considered.

Taking into account the relative decrease of the olefinic resonances, the behavior of n-1 and n-3 carbons confirmed the fact that unsaturations nearest to the carbonyl group are more susceptible to oxidation than others. The correlation between n-3 PUFA content and the intensity of signals corresponding to C_{20} and C_{19} olefinic carbons during heating confirmed the accuracy of data obtained ($R^2 = 0.83$).



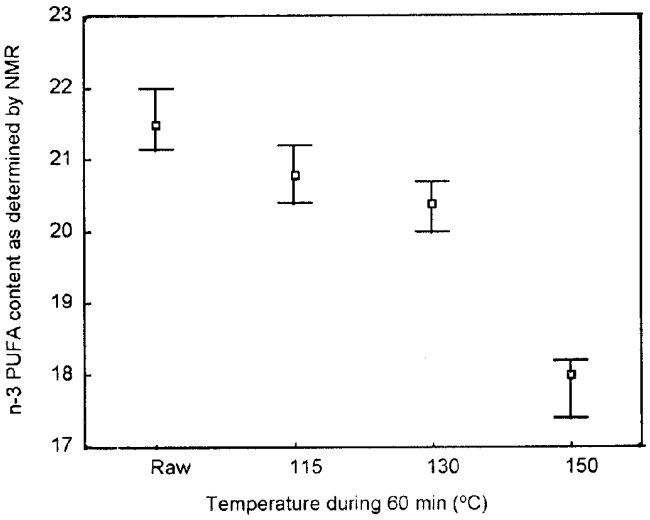


FIG. 7. Evolution of the n-3 PUFA content at different heating conditions, expressed as mol% fraction, determined from the intensity of n-1 carbons by ^{13}C NMR. See Figures 3 and 6 for abbreviations.

TABLE 2
Intensities of Methylenic Nuclei Referring to the Intensity of n-3 Methylenic Resonance of Saturated and n-9,11 Fatty Acids[a]

	Raw	Heated: (115°C, 120 min)	Heated: (150°C, 60 min)
C_2	2.04	1.56	1.41
Allylic	1.73	1.88	1.46
Bisallylic	1.46	1.25	1.31
C_3—ST	2.48	1.80	1.77
C_3—DHA	1.65	1.28	1.33
n-2—n-3 PUFA	0.21	0.20	0.21
C_6—22:5n-3	0.17	0.08	0.11
C_5—18:4n-3	0.23	0.09	0.10
C_4—EPA	0.21	0.11	0.11

[a]EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids. See Table 1 for other abbreviations.

^{13}C NMR spectroscopy has proven its usefulness as a rapid, nondestructive and complete technique for the study of lipid damage during food processing. Data regarding the type of reaction involved, its mechanism, and the nature of degradation products formed can be direct and simultaneously inferred from the lipid sample without prior separations. The combination of high-resolution ^{13}C NMR experiments, to improve sensitivity and resolution, and low-resolution experiments, to obtain direct and rapid analyses of the acyl and lipid class composition, with ^{1}H NMR (29) asserts

the use of NMR spectroscopy for control and monitoring of lipid oxidation during a wide variety of food processes.

ACKNOWLEDGMENTS

We acknowledge financial support for Research Project ALI 90-0773 provided by the Comisión Interministerial de Ciencia y Tecnología (CICYT), Research Project CEE UP. 3-783 (of DG XIV), and a research grant from the Departamento de Postgrado (CSIC) (to IM). Mr. V. Piscopo is gratefully acknowledged for technical assistance with the spectrometry.

REFERENCES

1. Pearson, A., J. Love, and F. Shorland, Warmed-Over Flavour in Meat Poultry and Fish, *Adv. Food Res. 23*:2–74 (1977).
2. Cheftel, J., and H. Cheftel, *Introducción a la Biología y Tecnología de Alimentos,* edited by J. Cheftel, and H. Cheftel, Acribia, Zaragoza, 1976.
3. Chan, H., *Autooxidation of Unsaturated Lipids,* Academic Press, New York and London, 1987, pp. 1–16.
4. Hsieh, R., and J. Kinsella, Oxidation of Polyunsaturated Fatty Acids: Mechanism Products and Inhibition with Emphasis on Fish, *Adv. Food Res. Nutr. 33*:233–241 (1989).
5. Frankel, E.N., Volatile Lipid Oxidation Products, *Prog. Lipid Res. 22*:1–13 (1982).
6. Hale, M.B., and T. Brown, Fatty Acids and Lipid Classes of Three Underutilized Species and Changes Due to Canning, *Marine Fish Rev. 45*:4–6 (1983).
7. Aubourg, S.P., C. Sotelo, and J.M. Gallardo, Changes in Flesh Lipids and Fill Oils of Albacore (*Thunnus alalunga*) During Canning and Storage, *J. Agric. Food Chem. 38*:809–812 (1990).
8. Przybylski, R., N.A.M. Eskin, and L.J. Malcolmson, Development of a Gas Chromatographic System Trapping and Analyzing Volatiles from Canned Tuna*, Can. Inst. Sci. Technol. J. 24*:129–135 (1991).
9. Yasuhara, A., and T. Shibamoto, Quantitative Analysis of Volatile Aldehydes Formed from Various Kinds of Fish Flesh During Heat Treatment, *J. Agric. Food. Chem. 43*:94–97 (1995).
10. Warner, K., and N.A.M. Eskin, *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, AOCS Press, Champaign, 1994.
11. Sacchi, R., I. Medina, S. Aubourg, I. Giudicianni, L. Paolillo, and F. Addeo, Quantitative High-Resolution ^{13}C NMR Analysis of Lipids Extracted from the White Muscle of Atlantic Tuna *(Thunnus alalunga), J. Agric. Food Chem. 41*:1247–1253 (1993).
12. Medina, I., R. Sacchi, and S. Aubourg, ^{13}C NMR Monitoring of Free Fatty Acid Release After Fish Thermal Processing, *J. Am. Oil Chem. Soc. 71*:479–482 (1994).
13. Medina, I., R. Sacchi, and S. Aubourg, A ^{13}C NMR Study of Lipid Alterations During Fish Canning. Effect of Filling Medium, *J. Sci. Food Agric. 69*:445–450 (1995).
14. Bligh, E., and W. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
15. Herbes, S., and C. Allen, Lipid Quantification of Freshwater Invertebrates: Method Modification for Microquantification, *Can. J. Fish. Aquat. Sci. 40*:1315–1317 (1983).
16. AOAC, *Official Methods of Analysis*, 14th edn., Association of Official Analytical Chemists, Washington, D.C., 1984, Method 28:026.
17. Bose, A.K., and P.R. Srinivasan, Trichloroacetyl Isocyanate as an *in situ* Derivatizing Reagent for ^{13}C NMR Spectroscopy of Alcohols, Phenols and Amines, *Tetrahedron 31*:3025–3029 (1975).
18. Sokal, R., and F. Rohlf, *Biometry,* W. Freeman and Co., San Francisco, California, 1981, pp. 208–270.
19. Frankel, E.N., Formation of Headspace Volatiles by Thermal Decomposition of Oxidized Fish Oils vs. Vegetable Oils, *J. Am. Oil Chem. Soc. 70*:767–772 (1993).
20. Knothe, G., M.O. Bagby, R.E. Peterson, and C.T. Hou, 7,10-Dihydroxy-8(*E*)-octadecenoic Acid: Stereochemistry and a Novel Derivative, 7,10-Dihydroxyoctadecanoic Acid, *Ibid. 69*:367–371 (1992).
21. Gunstone, F.D., The ^{13}C NMR Spectra of Oils Containing ν-Linolenic Acid, *Chem. Phys. Lipids 56*:201–207 (1990).
22. Gunstone, F.D., High-Resolution NMR Studies of Fish Oils, *Ibid. 59*:83–89 (1991).
23. Aursand, M., and H. Grasladen, Interpretation of the ^{13}C NMR Spectra of Omega-3 Fatty Acids and Lipids Extracted from the White Muscle of Atlantic Salmon (*Salmo salar*), *Ibid. 62*:239–251 (1992).
24. Sacchi, R., I. Medina, L. Paolillo, and F. Addeo, High-Resolution ^{13}C NMR Olefinic Spectra of DHA and EPA Acids, Methyl Esters and Triacylglycerols, *Ibid. 69*:65–73 (1994).
25. Knothe, G., and M.O. Bagby, Assignment of ^{13}C NMR Signals in Fatty Compounds with Allylic Hydroxy Groups, *J. Am. Oil Chem. Soc. 73*:661–663 (1996).
26. Sacchi, R., M. Patumi, G. Fontanazza, P. Barone, P. Fiordiponti, L. Mannina, E. Rossi, and A.L. Segre, A High-Field ^{1}H Nuclear Magnetic Resonance Study of the Minor Components in Virgin Olive Oils, *Ibid. 73*:747–758 (1996).
27. Sacchi, R., F. Addeo, I. Giudicianni, and L. Paolillo, Nuclear Magnetic Resonance in the Analysis of Olive Oils, *Riv. Ital. Sostanze Grasse 56*:171–178 (1989).
28. Sacchi, R., I. Medina**,** S. Aubourg, and L. Paolillo, ^{1}H NMR Rapid and Structure Specific Determination of n-3 Polyunsaturated Fatty Acids in Fish Lipids, *J. Am. Oil Chem. Soc. 70*:225–228 (1993).
29. Claxon, A.W.D., G.E. Hawkes, D.P. Richarson, D.P. Naughton, R.M. Haywood, C.L. Chander, M. Atherton, E.J. Lynch, and M.C. Grootveld, Generation of Lipid Oxidation Products in Culinary Oils and Fats During Episodes of Thermal Stressing: A High-Field ^{1}H NMR Study, *FEBS Lett. 355*:81–90 (1994).

[Received November 14, 1996; accepted October 30, 1997]