High-Resolution NMR of Irradiated Almonds

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ABSTRACT: Electron beam-irradiated peeled almonds (dose rate <5kGy) were subjected to a sensory test, ESR, and solid state ^{1}H and ^{13}C NMR spectroscopy using an improved magic-angle probe. In the sensory test, changes were detected only for high radiation doses, and these were similar to those produced by aging. ESR detected significant differences between irradiated and nonirradiated samples, although these differences in free radical content tended to disappear with aging. Slightly higher DG content was detected in the irradiated sample by high-resolution solid-state NMR spectra.

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High-resolution ^{1}H and ^{13}C NMR are currently being used in structural studies and quantitative determinations of edible oils (e.g., Ref. 1). In nuts and seeds the vesicle accumulation of oils determines a certain mobility of glyceride mixtures, and this reduces dipolar interactions and enables the use of NMR spectroscopy techniques (2). Low-field NMR and imaging techniques are thus being used to determine the maturity of seeds through oil and moisture analyses (3,4). It has been shown that cross-polarization magic-angle spinning can narrow the ^{1}H and ^{13}C line widths in some heterogeneous mixtures by removing magnetic susceptibility line broadening. In some cases this enables direct ^{13}C NMR analysis of food samples using solid-state magic-angle techniques (5), although the resolution obtained is insufficient for ^{1}H NMR spectroscopy (6).

Food irradiation is increasingly used as a method of food processing, and in the case of fruit can be used to prevent fungal spoilage (7). In this respect, new analytical methods are needed to assess the quality and safety of irradiated food samples (8–10). The effects of radiation on food depend, among other factors, on the kind of food irradiated, the dose applied, and the conditions of treatment application (11). One of the main effects of ionization is the formation of free radicals, which are responsible for lipid degradation and oxidation, resulting in volatile compounds with objectionable odors. These limit the shelf life of products, even those with fat levels below 1%.

Here we present results on the use of NMR methodology for detecting differences between electron beam-irradiated and -nonirradiated glyceride mixtures, and compare these results with those from sensory and EPR tests. High-resolution magic-angle spinning (HRMAS) NMR spectroscopy is increasingly being used in the field of solid-phase organic synthesis as a tool for the characterization of resin-bound compounds. Furthermore, HRMAS technology enables the study and control of other heterogeneous samples and offers significant improvements in resolution in the ^{1}H and ^{13}C spectra of solid or semisolid food samples (12). Here we describe the use of this method for the analysis of triturated almonds and almond paste to detect changes in composition due to natural aging and to electron beam irradiation processes.

EXPERIMENTAL PROCEDURES

Samples. Almonds (*Prunus amygdalus*: variety Guara) were obtained from the Cooperativa de Frutos Secos del Mañan (Murcia, Spain). Irradiation of peeled almonds was performed with accelerated electrons (Ionmed, Tarancón, Spain) at different doses (1, 3, and 5 kGy). Irradiation treatments were carried out using a Rhodotron (I.B.A., Louvain-la-Neuve, Belgium) circular electron accelerator (Ionmed) with an energy level of 10 MeV. The variability of the real dose of irradiation absorbed by the samples was less than 1% of the programmed dose applied. The dosimeters also verified the homogeneity of the dose and validated the irradiation process. The samples were held at 20°C for 140 d prior to the sensory test evaluation. They were then stored under a nitrogen atmosphere at 2°C before being subjected to NMR and ESR analyses, which, unless otherwise noted, were performed in the next 60 d. Aliquots of the samples were ground to about 0.8 mm grain size in a small agate mortar. PV values (meq O_2/kg of seed) were obtained in triplicate according to an AOAC method (13).

Statistics. Tests for significant differences were carried out using the General Linear Model of the SPSS (version 11.0) statistical package (SPSS, Chicago, IL). ANOVA was conducted for irradiation doses as the factor. When differences were significant, multiple comparisons were made using Tukey's test.

Sensory test methodology. Sensory rating was conducted by a selected and trained panel composed of five judges with some expertise in food tasting. The ability and sensitivity of the tasters were assessed using as standards sucrose for sweetness, caffeine for bitterness, and rancid almonds for the detection of rancidity. Training and selection of the tasters and preparation

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and conditions of the taste booths were done according to the Spanish-European norms UNE 87 (14). The global quality attribute was evaluated with respect to the acceptability of the product by the consumer using 5-point structured scales ranging from 1, "very disagreeable," to 5, "very agreeable."

ESR analysis. X-band ESR spectra were recorded at room temperature and at a microwave power of 0.25 mW. A modulation amplitude of 5 G at 100 KHz was used to enhance the weak ESR signal. The sample tubes were filled with 2 g of ground almonds, and the intensity of the signal was calculated from the integral of the absorption spectra. All measurements were performed by carefully adjusting the sample tubes in the same place as the resonance cavity in order to allow comparison between the obtained signals: At least three coincident measurements were taken for each sample.

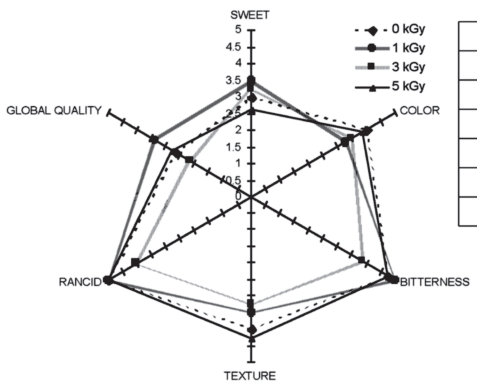
NMR analysis. All NMR spectroscopic studies were performed with a Bruker DMX 500 NMR spectrometer equipped with an HRMAS accessory and a $^{1}H/^{13}C/^{2}H$ gradient probe. HRMAS NMR spectra were recorded with 4-mm ZrO_2 rotors (detection volume 60 μL), and with a hemispherical Teflon insert in the bottom. In all experiments samples were spun between 5 and 8 kHz. Approximately 30 mg of the almond samples was packed into an HRMAS rotor, and a small amount of D_2O (lock signal) was added directly inside the rotor. The spectra were acquired at a temperature of 300 K and referenced to the solvent peak; the sodium salt of 4,4-dimethyl-4-silapentane sulfonic acid was used as an external reference. The ^{1}H NMR spectra were obtained with the one-pulse standard sequence with 256 transients (acquisition time 0.188 s; time between pulses 1.5 s). The ^{13}C NMR spectra were obtained with the standard pulse sequence with 0.9–1 s acquisition time, 0.2 s recovery delay, and 3000–6000 transients; 3 Hz line broadening was applied. A total of 256 increments with two transients and 1000 data points with a spectral width of 3600 Hz were acquired in both dimensions for the 2-D COSY spectra (gsCOSY pulse sequence). A zero filling up to $1 \cdot 10^3$ data points in the F1 dimension and multiplication with a sine bell function in both dimensions were performed. Direct ^{1}H–^{13}C correlations were obtained from the standard gradient heteronuclear single quantum correlation (HSQC) spectra pulse sequence. Heteronuclear spectra were recorded with 2048 data points in t2 and 512 increments in t1, 16 scans per increment being added. Spectral windows for ^{1}H and ^{13}C dimensions were 5430 Hz and 17,500 Hz, respectively.

RESULTS AND DISCUSSION

Sensory tests. Figure 1 shows the numerical assessment given by the panel of tasters. There was a decrease in the parameter "global quality" for both control and irradiated samples, and long storage (4 mon at 20°C) also had a negative effect on the samples, both control and irradiated. This was verified by analysis of rancidity, a sensory attribute indicating the degree of fat oxidation, which showed values slightly lower (around 0.4) for irradiated than for control samples. The panel of tasters did not find perceptible differences among the samples regarding the rancid flavor. Although the use of PV analysis for such long time periods is not so meaningful as for shorter time periods, the PV found in this study (Fig. 2) suggest that the fat in almonds stored for 4 mon at 20°C is not affected by ionization with accelerated electrons at doses of up to 5 kGy, since after 4 mon the PV were similar to those found in nonirradiated control fruits. In no case were significant statistical differences ($P < 0.05$) found between the applied radiation doses. These results are in agreement with those obtained by other authors for the ionization of dried fruits (15). Likewise, although it is known that lipids are sensitive to ionization and that the presence of oxygen accelerates autoxidation, some authors have argued that, after long storage periods, final oxidation products do not differ from those found in nonirradiated lipids (16).

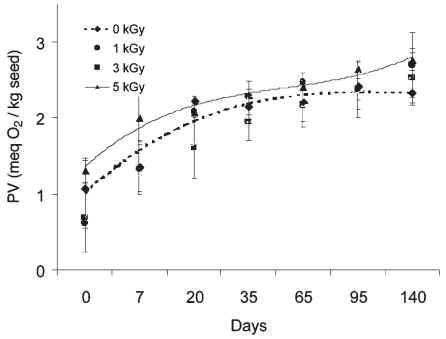
The lipid fraction of the almond seed ranges from 50 to 60%, and within this fraction, around 80–90% are monounsaturated FA or PUFA (17). The degree of unsaturation of FA is a very significant factor in the oxidation speed, since PUFA oxidize during the storage period, even at freezing points, whereas saturated FA oxidize only at temperatures over 60°C (18). Thus, the lipid fractions of plants, such as almonds, can easily be oxidized when ionized due to their high degree of unsaturation.

ESR spectroscopy. Under ESR spectroscopy, the almond samples showed a symmetrical signal with a *g* value of 2.007



ATTRIBUTE	*F* (ANOVA)	SIGNIFANCE
Sweetness	2.774	ns
Color	2.333	ns
Bitterness	1.748	ns
Texture	2.222	ns
Rancidity	1.552	ns
Global quality	3.328	ns

FIG. 1. Scores given by the sensory taste panel for overall quality attributes after 5 mon of storage, and ANOVA analysis (*F* values) of the organoleptic parameters (ns = not significant).



STORAGE (days)	*F* (ANOVA)	SIGNIFICANCE
0	1.13	ns
7	0.86	ns
20	4.38	ns
35	0.54	ns
65	0.66	ns
95	0.12	ns
140	0.13	ns

FIG. 2. PV (meq O_2 per kg of seed) at different ionization doses and their ANOVA analysis (*F* values: ns = not significant)

at room temperature. Although the signal intensity increased with the dose rate of the previous irradiation (Fig. 3), this signal was also already present in nonirradiated samples. In spite of the low significance of the *g* value, from the point of view of a structural determination, the observed values are higher than those expected for organic radicals centered on carbon atoms, but in agreement with reported values for peroxyl organic radicals or free radicals in nucleophilic heterocyclic compounds (19). The band width (ΔH_{PP}) was 8.25 ± 0.05 G, except for the sample irradiated at 5 kGy, which gave ΔH_{PP} = 8.75 ± 0.05 G. Time exerted a leveling effect on the content of free radicals: 12 mon after the first ESR analysis, the samples (stored at 2°C under N_2) irradiated at lower dose rates showed an increased intensity and ΔH_{PP} reaching the values of the 5 kGy sample, the latter remaining constant over time (see Fig. 3B); the values of the 5 kGy sample thus seem to be plateau values. This suggests an acceleration of the aging process during irradiation or for a period of time immediately after irradiation. The final free radicals of the autoxidation process are not necessarily located in FA residues and may be present as inert free radicals of other organic substrates, e.g., derivatives from the radical hydroxylation of aromatic rings (7).

NMR spectroscopy. The recording of HRMAS NMR spectra of triturated almond samples, in the ^1H and ^{13}C mode, gave high-resolution spectra (see Figs. 3, 4). ^1H–^{13}C heteronuclear correlation spectra were also easily obtained (Fig. 5). The recorded spectra correspond to the glyceride fraction as inferred by comparison with previously reported spectra of pure edible oils (1,20). Comparison of the NMR spectra of irradiated vs. nonirradiated samples showed no significant dif-

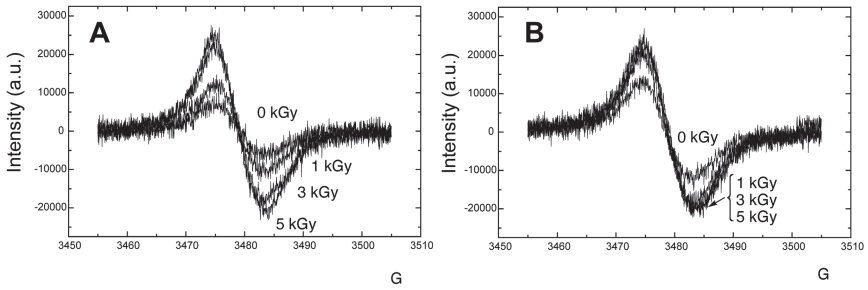


FIG. 3. ESR spectra for samples of almonds irradiated by accelerated electrons: (A) initial samples (see Experimental Procedures section); (B) the same samples after 12 mon of storage at 2°C in a N_2 atmosphere.

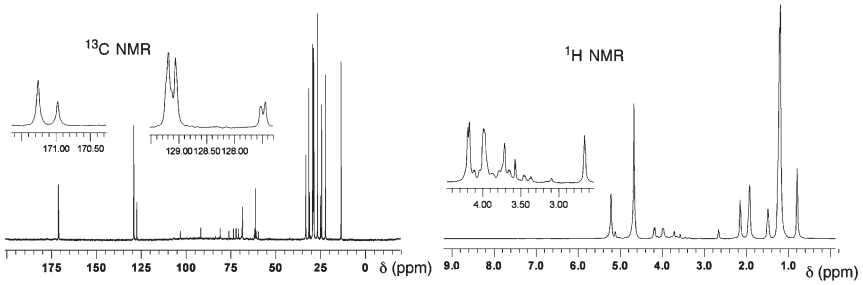


FIG. 4. ^1H (left) and ^{13}C NMR (right) high-resolution magic angle spinning (HRMAS) spectra (500 MHz instrument) of triturated peeled almonds from nonirradiated samples (0 kGy).

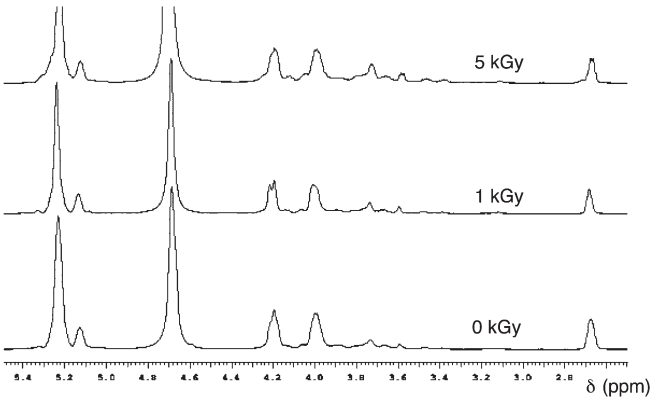


FIG. 5. ^{1}H NMR HRMAS spectra (500 MHz) of a coarse triturated almond from nonirradiated (0 kGy) and electron beam-irradiated samples (1 and 5 kGy). The small differences in the regions 3.5–4.2 and 5.2–5.4 ppm (δ) correspond to different contents of 1,2- and 1,3-DG (see text). For abbreviation see Figure 4.

ferences. For example, in the range of the method's sensitivity, the ratio of the unsaturated/saturated FA was the same. However, the low-intensity ^{1}H NMR signals in the regions of 3.5–3.7 and 4.0–4.2 ppm show a small, but detectable, intensity increase for samples incurring a radiation dose (Fig. 4). These signals correspond to the protons –C$\underline{H}_2$OH, –C$\underline{H}_2$OCOR, –C$\underline{H}$OH, and –C$\underline{H}$OCOR of 1,2- and 1,3-DG. Consequently, a transformation of TG to DG due to the irradiation probably occurs. Twelve months after the NMR experiments, the DG levels for irradiated and nonirradiated samples showed no detectable differences, which is consistent with the leveling effect detected in the ESR results discussed above.

One explanation for the increased DG fraction produced by the electron beam irradiation is as follows. As a result of electron capture by the O_2 present in the sample, electron beam irradiation in contact with air results in the formation of superoxide ($O_2^{-\cdot}$). In the presence of water or a proton donor (e.g., an organic hydroxyl group), this radical anion disproportionates (21) to H_2O_2 and O_2 (see Eqs. 1–3). The reaction of $O_2^{-\cdot}$ with water, disproportionating to hydrogen peroxide and dioxygen, leads to highly basic local media due to the generation of hydroxide anions (or alkoxy anions). These anions would saponify acyl glycerides (see Eqs. 1–3), leading to an increase in DG, as detected. In this respect, a different effect should be expected in the case of γ-ray or electron-beam irradiation, because superoxide anion should be obtained in higher ratios in the last case, and in lesser degree for deaerated samples.

$$O_2 + e^- \rightarrow O_2^{-\cdot} \quad [1]$$

$$2\ O_2^{-\cdot} + 2\ H_2O \rightarrow O_2 + H_2O_2 + 2\ OH^- \quad [2]$$

$$R\text{-}COOR' + OH^- \rightarrow RCOO^- + R'OH \quad [3]$$

The results show that use of the HRMAS NMR probe in solid almond samples, avoiding cross-polarization techniques, enables the direct analysis of the more mobile part of the lipid fraction (i.e., that contained in vacuoles). The use of adequate NMR magic-angle probes could extend the direct analysis of nut and seed oils to ^{1}H NMR spectroscopy.

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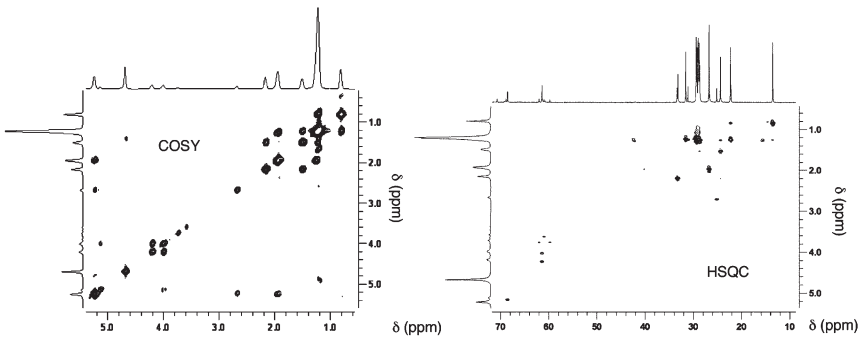

FIG. 6. HRMAS 2-D COSY and heteronuclear single quantum correlation (HSQC) spectra (500 MHz) of a coarse triturated almond sample. For other abbreviation see Figure 4.

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