JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Degradation of Edible Oil during Food Processing by Ultrasound: Electron Paramagnetic Resonance, Physicochemical, and Sensory Appreciation

Daniella Pingret,[†] Grégory Durand,[‡,§] Anne-Sylvie Fabiano-Tixier,[†] Antal Rockenbauer,[⊥] Christian Ginies,[†] and Farid Chemat*[,†]

[†]Sécurité et Qualité des Produits d'Origine Végétale, Université d'Avignon et des Pays de Vaucluse, INRA, UMR408, F-84000 Avignon, France

[‡]Equipe Chimie Bioorganique et Systèmes Amphiphiles, Université d'Avignon et des Pays de Vaucluse, 33 rue Louis Pasteur, F-84000 Avignon, France

[§]Unité Mixte de Recherche 5247, Centre National de la Recherche Scientifique and Universités de Montpellier 1&2, Institut des Biomolécules Max Mousseron, Faculté de Pharmacie, 15 avenue Charles Flahault, F 34093 Montpellier Cedex 05, France

[⊥]Molecular Pharmacology, Institute of Natural Sciences, Pusztaszeri 59, H-1025 Budapest, Hungary

**ABSTRACT:** During ultrasound processing of lipid-containing food, some off-flavors can be detected, which can incite depreciation by consumers. The impacts of ultrasound treatment on sunflower oil using two different ultrasound horns (titanium and pyrex) were evaluated. An electron paramagnetic resonance study was performed to identify and quantify the formed radicals, along with the assessment of classical physicochemical parameters such as peroxide value, acid value, anisidine value, conjugated dienes, polar compounds, water content, polymer quantification, fatty acid composition, and volatiles profile. The study shows an increase of formed radicals in sonicated oils, as well as the modification of physicochemical parameters evidencing an oxidation of treated oils.

**KEYWORDS:** *ultrasound, sunflower oil, degradation, electron paramagnetic resonance, spin trapping*

## INTRODUCTION

The increasing demands of higher quality and quantity of products in the food industry brought attention to the use of ultrasound (US) as a food-processing technique of interest as a technological benefit and/or as a technique to alter product functionalities. This technique presents several advantages over conventional methods in terms of energy consumption, time, and higher throughput. Ultrasound methods are used in the food industry for numerous processes on high lipid containing food products such as milk, yogurt, and cheese, presenting great results in cooking, cutting, emulsification/homogenization, and microbial inactivation. Interest in applying ultrasound methods in food processing lies in the fact that power ultrasounds are able to induce modifications (chemical, functional, physical, and structural, etc.) in some of food properties.[1]

Although power ultrasounds present numerous technological benefits,[2] some undesirable changes in food composition or characteristics have been reported after ultrasound treatment in the past few years.[3−5] Despite the observation of these phenomena, the potential restrictions and/or disadvantages of chemical effects have often been overlooked. Acoustic cavitation might be responsible for initiating the formation of degradation products, which can trigger radical chain reactions and provoke substantial quality impairments in those products. The potential restrictions and/or uses of chemical effects generated by cavitation phenomena are shown in Figure 1.

The particle displacement caused by the application of ultrasounds in liquid media induces the formation of cavitation bubbles that provoke extreme conditions of pressure and temperature that can generate violent physical forces by collapse that include microjets, shear forces, and shock waves, influencing the bulk liquid surrounding the bubble or inside the bubble itself.[2,6]

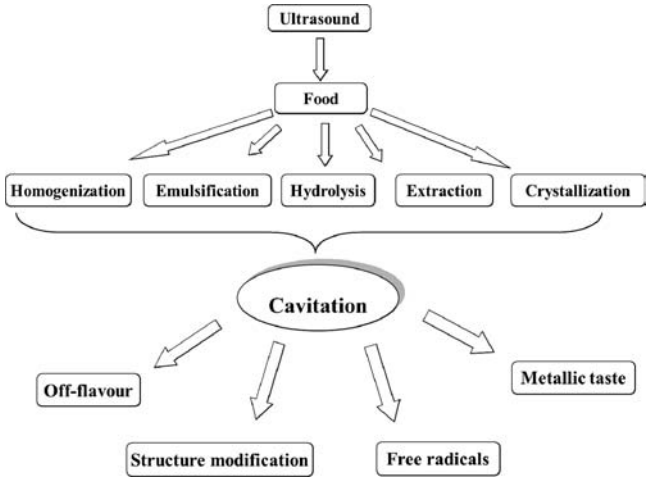


**Figure 1.** Chemical effects generated by cavitation phenomena.

**Received:** April 10, 2012
**Revised:** July 10, 2012
**Accepted:** July 17, 2012
**Published:** July 17, 2012





dx.doi.org/10.1021/jf301286f | J. Agric. Food Chem. 2012, 60, 7761−7768

Within the collapsing cavitation bubble, the extreme temperature and pressure conditions can induce the dissociation of water into hydroxyl radicals and hydrogen atoms, which can trigger chain reactions at the interface of the bubble or in the surrounding liquid. The formation of free radicals in both aqueous and nonaqueous media has been evidenced by electron spin resonance.[7]

Some studies have shown the appearance of off-flavors in some food products containing lipids when submitted to ultrasounds.[4,8−13] Besides flavor impairments, degradation of fats in food might decrease the nutritional quality and safety of those products.[14] Chemat et al.[10] verified oxidation in oil emulsions after sonication even when in indirect contact with the ultrasound source. Although the use of ultrasounds is disseminated in the food industry, the potential restrictions induced by the effects of sonication on food matrices have not been extensively examined.

To the best of our of knowledge, only four papers on the study of lipid degradation in oil samples using electron paramagentic resonance (EPR) spectroscopy have been published.[15−18] In this work, sunflower oil was used as a model of high lipid containing food products and thus was submitted to a treatment with two different ultrasonic probes to better understand the phenomena of oil degradation by cavitation or the shear forces induced by ultrasounds. A spin-trapping study coupled to EPR spectroscopy as well as the assessment of the classical physicochemical parameters of oxidation was performed.

## MATERIALS AND METHODS

**Materials and Reagents.** High-oleic sunflower oil was purchased at a local supermarket. α-Phenylbutylnitrone phenyl-*N*-*tert*-butylnitrone (PBN) was synthesized according to the procedure of Huie and Cherry[19] and purified by two successive recrystallizations from ethyl acetate/diethyl ether at the laboratory. 2,2,6,6-Tetramethyl-1-piperidine-*N*-oxyl (Tempo) and *p*-anisidine were purchased from Sigma-Aldrich Srl (France), and *p*-anisidine was purified according to the AOCS analytical method. All other reagents were of analytical grade. Ultrasonic devices used were a Pyrex horn of 17 mm tip (Milestone, Italy) operating at 24 kHz (maximum power of 180 W) and a titanium alloy microprobe of 6 mm tip (Ultrasonic Processor, Fisher Bioblock Scientific, France) operating at 20 kHz (maximum power of 140 W).

**Ultrasonic Treatment of Samples.** All ultrasound treatments (US)were performed in 50 mL of sample for 15 min in double-mantle glass vessels at 25 °C. All experiments were carried out in triplicates. US were performed with two different ultrasonic probes; to obtain the same ultrasonic energy in samples with both probes, their power and amplitude were calculated and adjusted to obtain the same ultrasonic intensity. Because the actual input power from each device is converted to heat, which is dissipated in the medium, the actual ultrasound power was determined by calorimetry, calculated as shown in eq 1

$$P = m \times C_p \times \frac{dT}{dt} \quad (1)$$

where $C_p$ is the heat capacity of the solvent at constant pressure (J $g^{-1}$ $K^{-1}$), $m$ is the mass of solvent (g), and $dT/dt$ is the temperature rise per second. The $C_p$ was calculated from the slope of the curve from internal energy variation as a function of temperature at constant volume using a calorimeter. The consequent ultrasonic intensity (UI) was calculated for each ultrasonic probe using the calculated power (from eq 1) as shown in eq 2

$$\mathrm{UI} = \frac{4P}{\pi D^2} \quad (2)$$

where UI is the ultrasonic intensity (W $cm^{-2}$), $P$ is the ultrasound power (W) as calculated by eq 1, and $D$ is the internal diameter (cm) at the tip of the probe. The UI of 3.49 W $cm^{-2}$ was used for both ultrasonic treatments. The temperatures at the beginning and end of ultrasound treatment for each probe were assessed for further comparison.

**Physicochemical Analysis.** The determination of the following physicochemical parameters in samples was carried out according to the analytical methods described by AOCS: FFA or free fatty acids (AOCS official method Ca 5a-40), conjugated diene level by UV spectrophotometric method (AOCS Cd 7-58), polar compounds (AOCS Cd 20-91), peroxide value (AOCS Cd 8-53), anisidine value (AOCS Cd 18-90), and fatty acid composition by gas chromatography (AOCS Ce 1-62 and Ce 2-66). The total oxidation value (TOTOX) value was calculated by the sum of 2PV and AV. The determination of polymerized triglycerides was performed by high-performance size exclusion chromatography (ISO 16931), and the water content in the sunflower oil samples was determined according to the Karl Fischer method.

**Electron Paramagnetic Resonance Analysis.** EPR measurements were carried out on an MS 300 benchtop EPR spectrometer from Magnettech GmbH. The instrument settings were as follows: $B_0$ field, 3347.80 G; sweep width, 198.8 G; modulation amplitude, 2 G; microwave power, 10 mW; receiver gain, $9 \times 10^2$; scan time, 40 s; number of scans, 40. After ultrasound treatment, the oil samples were cooled in a freezer, and a solid amount of PBN was immediately dissolved into each sample to obtain a 20 mM solution. The PBN−oil mixture was left to equilibrate under agitation on an RT-10 magnetic stirrer plate (IKAMAG, Germany) over 24 h in the dark. A quartz flat cell fixed in the EPR cavity was filled by the oil mixture and immediately tested by EPR. Toluene solutions of PBN were tested as a control, and no paramagnetic signal was detected, demonstrating the high purity of the PBN used in this study. The absolute concentrations of nitroxides were calculated by comparing the intensities of the EPR spectra of oil solutions with TEMPO solutions in toluene in a concentration range. Assays were performed in triplicates. The EPR spectra were simulated by an automatic fitting program.[20] The $g$ factor, the nitrogen ($a_N$) and proton ($a_H$) couplings, and the three-line parameters were adjusted until convergence was achieved. The total radical concentration was calculated from the adjusted spectral amplitude of the adduct spectra compared to the TEMPO spectrum.

**Fatty Acid Methyl Ester (FAME) Derivatives Analysis.** Fatty acid contents of samples were determined using a modified FAME method.[21] Twenty milligrams of samples was weighed to the nearest milligram into a Pyrex tube fitted with a Teflon-lined cap. Nonadecanoic acid (400 μL of a 1 g/L solution) in dichloromethane was added as internal standard. Methylation was performed using boron trifluoride (10%) in methanol (1 mL), which was added to the samples followed by dichloromethane (1 mL), and the mixture was vortex-mixed. The tubes were placed in a Stuart SBH200D block heater from Bibby Sterilin Ltd. (Stone, Staffordshire, UK) at 100 °C for 30 min. Then, the tubes were removed and cooled to room temperature before the addition of dichloromethane (1 mL) to extract FAMEs. Hydrogenocarbonate 0.5 M (2 mL) was added; the mixture was shaken to allow phase separation, and the supernatant was discarded. This step was realized twice. Anhydrous sodium sulfate was then added to bind any residual water.

FAMEs were analyzed on a HP 5890 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with a FID detector and autosampler, attached to a DB-225 capillary column (30 m × 0.25 mm × 0.5 μm film thickness). One microliter of sample was injected in split mode at 250 °C. The carrier gas was used at the velocity 35 cm $s^{-1}$. The oven temperature program was as follows: initial temperature, 50 °C, raised from 50 to 180 °C at 20 °C $min^{-1}$ and from 180 to 220 °C at 3 °C $min^{-1}$, and then held at 220 °C for 10 min. Identification of fatty acids was performed by comparison with 37 FAME standards (Supelco). FAMEs were quantified as percentages of the total methyl ester peak areas. Analyses were performed at least three times, and the mean values were reported.

Table 1. Characterization of Sunflower Oil (SO) for Untreated and Sonicated Samples

| sample | peroxide value (mequiv $O_2$/kg) | $E_{233}$ | $E_{268}$ | anisidine value | TOTOX | free fatty acids (mg KOH/g) | polar compounds (%) | nitroxides ($\mu$M) |
|---|---|---|---|---|---|---|---|---|
| SO untreated | 2.12 ± 0.29 | 0.217 ± 0.001 | 0.106 ± 0.001 | 0.060 ± 0.001 | 4.3 | 0.02 ± 0.01 | 6.5 ± 0.10 | 1.09 ± 0.10 |
| SO titanium | 3.55 ± 0.01 | 0.273 ± 0.001 | 0.164 ± 0.001 | 1.210 ± 0.010 | 8.31 | 0.04 ± 0.01 | 5.5 ± 0.10 | 0.95 ± 0.18 |
| SO Pyrex | 6.27 ± 0.23 | 0.268 ± 0.001 | 0.170 ± 0.001 | 2.620 ± 0.001 | 15.16 | 0.12 ± 0.01 | 5.5 ± 0.10 | 2.39 ± 0.18 |

**Volatile and Off-Flavor Analysis.** The headspace solid-phase microextraction (HS-SPME) procedures were performed using an A0C 5000 (Shimadzu, Kyoto, Japan). The silica fibers and automatic SPME holder were purchased from Supelco (Bellefonte, PA, USA), and a carboxen−polydimethylsiloxane (CAR/PDMS, 75 $\mu$m) fiber was used. For each extraction, 5 g of oil was hermetically sealed in 20 mL screw-top clear vials with aluminum seals and PTFE/silicone septa (Supelco). The samples were equilibrated during the incubation time at 40 °C for 10 min. Subsequently, the SPME device was automatically inserted into the sealed vial through the septum, and the fiber was exposed to the sample headspace at 40 °C for 35 min. The agitator tray was turned on during the incubation and extraction procedure. Following the sampling procedure, the SPME fiber was immediately inserted into the gas chromatography−mass spectrometry (GC-MS) injector, and the fiber was thermally desorbed for 3 min at 250 °C.

GC-MS analyses were performed on a QP2010 (Shimadzu) equipped with a UBWAX capillary column (30 m, 0.25 mm i.d., 0.5 $\mu$m film thickness). The injection port (250 °C) operated in split mode with a ratio of 10. The carrier gas was He at the constant velocity of 35 cm $s^{-1}$. The initial oven temperature of 35 °C was held for 3 min, ramped at 3 °C $min^{-1}$ to 150 °C, and then ramped at 10 °C $min^{-1}$ to 230 °C. This final temperature was held for 10 min. The mass spectrometer operated in the electron impact mode at 70 eV with continuous scans (every 0.5 s) over the mass to charge ratio ($m/z$) from 29 to 300. Data were collected with GC-MS Solution software.

**Sensory Appreciation.** The influence of US on the sensory characteristics of the samples was evaluated. The qualitative sensory evaluation was conducted by a panel consisting of 18 graduate students and staff members from the University of Avignon, France. The subjects were seated in sensory booths with appropriate ventilation and lighting. Randomly coded samples were presented to each panelist, and the sensory attributes were evaluated. Tap water was supplied to the panelists for rinsing between samples.

**Statistical Analysis.** One-way analysis of variance (ANOVA) was conducted to determine the effect of the two ultrasound treatments on sunflower oils' physicochemical parameters using Statgraphics V software (Statistical Graphics Corp., Rockville, MD, USA). Each measurement was replicated three times. To determine which means are significantly different from each other, Tukey's multiple-range test method was used. Trends were considered to be significant when means of compared parameters differed at the $P < 0.05$ significance level.

## RESULTS AND DISCUSSION

**Physicochemical Analysis.** In the case of lipid degradation, the analytical methods for estimating oxidation include the quantification of some primary and secondary oxidation products by direct or indirect methods and the assessment of several oxidation stages. All combined provide more detailed information on the oxidative pathway. In the radical oxidation process, the formed peroxides can become initiators at certain settings, increasing the rate of oxygen consumption as they are formed. A significant ($P < 0.05$) general increase in all oxidation measurement parameters of ultrasound-treated oil is observed with a marked difference between treatments. Indeed, a higher oxidation was observed in most cases for the Pyrex horn compared to the titanium alloy one (Table 1).

During the formation of hydroperoxides in polyunsaturated fatty acids oxidation, a rearrangement of the double bond can occur, resulting in the formation of conjugated dienes (CD) with an intense absorption at 233 nm. The formation of conjugated trienes (CT) can also take place, with a typical absorption at 268 nm. Although the increases in CD and CT values reflect the formation primary oxidation product in oils, these parameters are not easily correlated to the extent of the degradation, because the dienes participate in additional oxidative reactions. Nevertheless, the CD and CT increases are proportional to the oxygen uptake and generation of peroxides, being well correlated to peroxide value.[22] When compared to the pure untreated oil, the measurement of primary oxidation products presented increases of 23% for CD and 55% for CT in the case of treatment by the titanium alloy horn against 23 and 60% for the Pyrex horn for the same parameters ($P < 0.05$). A greater difference was observed in the peroxide value (PV) from the oil treated by the titanium alloy horn, with an increase of 67% compared to the untreated oil, whereas the Pyrex treatment presented an increase of 76% compared to titanium treatment, which represents 3 times the value of the untreated oil ($P < 0.05$).

In the lipid oxidation, an initial increase in the PV is observed, with a subsequent decrease as the secondary volatile products are formed.[22] Due to their low sensory threshold value, aldehydes are considered to be mainly responsible for the appearance of off-flavors subsequent to lipid degradation. The anisidine value (AV) is often used for the assessment of secondary oxidation products of unsaturated fatty acids, especially dienals and 2-alkenals, and even though it lacks of specificity, associated with PV, the AV can be a useful indicator of oil quality, particularly for oils presenting low peroxide values.[23]

The measurement of secondary oxidation products by the AV presented a similar behavior to that of the primary oxidation products. The most substantial increase is represented by the AV, which increased by 43 and 20 times when compared to the value of the pure untreated oil for treatment with the Pyrex and titanium alloy horns, respectively ($P < 0.05$). It has been found that a high rate of hydroperoxide generation (high PV) does not always involve a high rate of generation of secondary oxidation products (low AV).[23] Similarly, AV determination is useful for assessing the quality of products such as frying oils that often have low PV values.[24] Indeed, some interference might occur in the primary oxidation products measurement in samples containing CT, because some secondary products such as ethylenic diketones and conjugated ketodienes and dienals also absorb at 268 nm.[23] The sum of 2PV and AV is known as the TOTOX, and this parameter provides a better representation of the overall quality status and a better estimation of the progressive deterioration of the oil.[22,24] This general increase in oxidation evaluation parameters can be confirmed by the TOTOX value, which increased 2 times when compared to the untreated sample for

the titanium alloy horn, against an increase of 6 times for the Pyrex horn when compared to this same value.

The same behavior was observed for the FFA, with increases of 2 times the untreated oil value for the titanium alloy horn and 6 times for the Pyrex horn ($P < 0.05$). FFA are usually already present in minor quantities in edible oils, are more susceptible to oxidation than esterified fatty acids, and can be formed by hydrolysis and pyrolysis from the cleavage of triacylglycerol (TAG). Because FFA decrease the surface tension, an increase of the diffusion rate of oxygen from the headspace into the oil with subsequent acceleration of oxidation is observed.[25] The augmentation of those compounds suggests a consequent increase in the oil degradation. Therefore, a noticeable oxidation is evidenced for both ultrasound treatments, with a pronounced oxidation issued from the treatment with the Pyrex horn, especially for the secondary oxidation products. Formed hydroperoxides present conjugated double bonds, resulting from 1,4-pentadiene (dienes) or from 1,4,7-octatriene (trienes) units present in linoleic or in linolenic acyl groups, which can be measured by a direct UV method at 232−234 and 268−270 nm, respectively. Water content quantification by the Karl Fischer method showed both treated and untreated samples presented 0.13 ± 0.0065% of water, which can also play a role in the oxidation process. During prolonged heating of edible oils, a polymerization of triglycerides and an increase of the polar compounds can take place and result in quality impairments of the food product.[22,25] The polymerized triglycerides were quantified to 0.2 ± 0.014% in both treated and untreated samples, which is lower than the threshold of 3% proposed by the analytical method (ISO 16931). Those compounds are formed exclusively by frying conditions; therefore, a time factor is intrinsically related to this type of degradation product. Despite the extreme temperature microconditions in the cavitation bubbles, the temperature conditions during the short time of ultrasonic treatment did not cause polymerization of triglycerides or changes in the polar compounds.

The fatty acids composition (Table 2) of the sunflower oil before and after both ultrasound treatments was verified, and the results show no significant difference between these three samples. From these results, it is possible to observe the sample is rich in monounsaturated fatty acids (MUFA), having the oleic acid as major fatty acid, accounting for 60%, followed by linoleic acid (30%), whereas the total saturated fatty acids (SFA) account for 11%. Sunflower oil is a significant source of long-chain unsaturated fatty acids, especially linoleic acids. However, for some food applications, high-oleic sunflower oil is preferable to high-linoleic sunflower oil, because it is supposed to be more resistant to oxidative degradation under frying and storage conditions.[26] Also, sunflower oil rich in MUFA might help decrease the risk of coronary heart diseases, emphasizing the interest in food applications of this type of oil.

**Table 2. Fatty Acid Composition of both Untreated and Sonicated Sunflower Oils (SO)**

| | SO untreated (%) | SO Pyrex (%) | SO titanium (%) |
|---|---|---|---|
| *C16:0* | 4.5 | 4.4 | 4.4 |
| CC16:1 | 0.1 | 0.1 | 0.1 |
| *C18:0* | 5.8 | 5.4 | 6.1 |
| *C18:1* | 59.0 | 59.2 | 59.3 |
| *C18:2* | 29.6 | 30.4 | 29.3 |
| C18:3 | 0.1 | 0.1 | 0.1 |
| C20:0 | 0.2 | 0.2 | 0.2 |
| C20:1 | 0.1 | 0.2 | 0.1 |
| C22:0 | 0.6 | 0.5 | 0.6 |
| ∑*SFAs* | *11.0* | *10.5* | *11.3* |
| ∑*MUFAs* | *59.3* | *59.5* | *59.5* |
| ∑*PUFAs* | *29.7* | *30.5* | *29.4* |
| C18:1/C18:2 | *2.0* | *1.9* | *2.0* |
| MUFA/SFA | *5.4* | *5.6* | *5.3* |
| MUFA/PUFA | *2.0* | *2.0* | *2.0* |

**Electron Paramagnetic Resonance Analysis.** The spin-trapping technique is based on the reaction of a transient radical with a spin trap leading to a stable and persistent spin adduct that is detectable by EPR (Figure 2). Over the past four decades, the spin-trapping technique has been widely employed for the detection and characterization of transient radicals that are undetectable under normal conditions.[27,28] More recently, it has found interest in food processing and has been successfully used to investigate the oxidative stability of oil.[15−18,29] Among several classes of spin traps available, nitrone compounds are the molecules of choice due to their specificity and ability to quantify radicals.

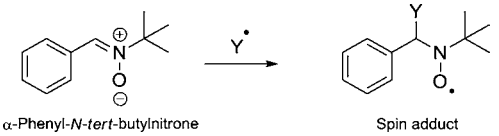


**Figure 2.** Spin-trapping mechanism of a free radical (Y•) by the α-phenyl-*N*-*tert*-butylnitrone.

The addition of α-phenyl-*N*-*tert*-butylnitrone (20 mM) to nontreated sunflower oil led, after 24 h, to an EPR signal resulting from the trapping of transient radicals by the nitrone function. The same spectrum was observed with the ultrasound-treated oil with a higher spin-adduct concentration (Figure 3). In both cases, that is, nontreated and treated oils, the spectra were characterized by three hyperfine lines from the coupling between the electron spin and the nitrogen atom ($a_N$ = 15.0 G). Another splitting from the β-hydrogen was also observed with a coupling constant $a_H$ = 1.85 G. This is in agreement with values of the literature for an oxygen-centered adduct in apolar media. Indeed, Ottaviani et al.[15] observed a nitrogen hyperfine splitting constant of 15.3 G in olive oil that was attributed to a hydroxyl spin adduct. Similarly, Szterk et al.[17] reported that POBN, a PBN derivative, was able to form two spin adducts with vegetable oils having splitting constants that were, respectively, $a_N$ = 13.78 G and $a_H$ = 1.80 G for the first spin adduct and $a_N$ = 15.54 G and $a_H$ = 2.00 G for the second one. On the basis of these hyperfine splitting constant values, they concluded that the former species came from the trapping of the superoxide anion radical spin, whereas the latter one originated from the trapping of the hydroxyl radical. The large β and γ relaxation parameters obtained using the program ROCKY[20] indicate a slow rotation of the nitroxide in a medium of high viscosity (Table 3). The good quality of spectral fit indicates the presence of a single radical adduct.

In the absence of ultrasonic treatment, the concentration of radicals after 24 h was found to be 1.09 ± 0.10 μM (Table 1). This confirms that even in the absence of ultrasound treatment, residual free radicals are present in sunflower oil as it was also demonstrated by indirect techniques (this work). When using the titanium horn, no significant change in the free radical

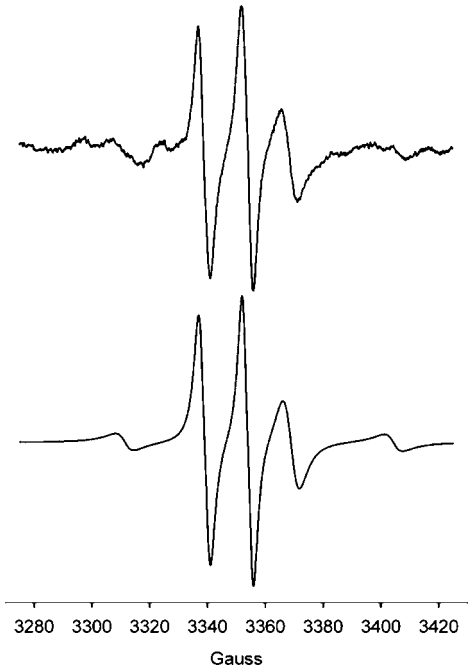


**Figure 3.** Experimental (top) and simulated (bottom) EPR spectra of ultrasound-treated sunflower oil.

**Table 3. EPR Parameters of the PBN−Spin Adduct Observed in Sunflower Oil**

| Parameters of the Spin Adduct (Gauss) | |
|---|---|
| $a_N$ | 15 |
| $a_H$ | 1.85 |
| $\alpha$ | 2.2 |
| $\beta$ | −1 |
| $\gamma$ | 1.2 |

concentration was observed, whereas a significant increase by ∼2.2 times was measured with the Pyrex horn. Such an increase is in full agreement with the data obtained by the other techniques. Indeed, except for polar compounds and polymeric triglycerides, all of the other physicochemical parameters in the oil samples treated by the Pyrex horn presented a more prominent increase, denouncing that oxidation is more accentuated by the sonication with this type horn when compared to the titanium one. The results from spin-trapping analysis suggest the increased degradation observed for the Pyrex horn is due to the formation of radicals in the treated oil, in opposition to the consequent degradation observed in the oils treated by the titanium horn, which shows no evidence of radical formation. Therefore, in the case of the Pyrex horn, the data suggest the consequent degradation verified by the other physicochemical parameters (peroxide value, free fatty acids, anisidine value, etc.) might be due to the radicals formed during sonication using this type of ultrasonic horn, which might not be the case for the titanium horn.

**Volatiles, Sensory, and Off-Flavors Analysis.** The off-flavors observed in the samples treated by ultrasounds corresponding to the formation of volatile secondary compounds characteristic of oil oxidation such as pentanal, hexanal, heptanal, 2-heptenal, and 1-octen-3-ol were confirmed by SPME analysis. Because these compounds present a very low odor threshold, their presence even at low concentrations prejudice the sensory quality of the oil.[30] The results show an increase of 2 times the concentration of pentanal and hexanal in the sonicated oil for the titanium horn compared to untreated oil, against 1.5 times for the Pyrex horn. The heptanal concentration increased 3 times for the titanium horn and 2 times for the Pyrex horn. The 2-*Z*-heptenal increased 11 times for the titanium horn and 5 times for the Pyrex horn. The concentration of 1-octen-3-ol increased 6 times for the titanium horn against 2 times for the Pyrex one.

From the volatiles analysis it is possible to note the appearance of degradation compounds on both ultrasound treatments. Whereas treatment with the Pyrex horn induced the production of a greater amount of degradation compounds, the titanium alloy horn treatment induced a significantly lower amount of those compounds, which was reflected in the sensory appreciation of samples. The panel described the presence of bitter, rancid, and metallic tastes and odors on both sonicated samples, and those off-flavors were more pronounced in oils sonicated with the titanium horn in comparison to the Pyrex one.

**Comprehension of the Mechanism of Ultrasound Lipid Degradation.** Generally, lipid degradation arises from hydrolysis or oxidation, which can occur by autoxidation, photo-oxidation, or enzymatic oxidation and depends on multiple factors that include photosensibilization, fatty acids composition, and types of oxygen, as well as the presence of minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds, and antioxidants.[31] Degradation of fats in food products can not only prejudice acceptance by consumers but also result in a decrease in safety by the formation of molecules that are able to couple to intracellular nucleophiles. The binding to proteins and DNA results in their denaturation or impairment of normal physiological function by induction of mutation.[14]

Lipid oxidation follows a radical chain reaction mechanism through initiation, propagation, and termination stages. However, multiple factors can be involved in the initiation step, resulting in a faster or slower degradation of the lipid sample. This autocatalytic reaction generally starts with the formation of $L^{\bullet}$ (lipid alkyl radical) in the presence of an initiator. Because the spin angular momentum needs imperatively to be conserved during reactions and C═C is usually in the singlet state and O−O in the triplet state, to overcome the spin barrier, initiators or catalysts are required either to remove an electron from the lipid or oxygen or to change the electron spin of the oxygen to start the oxidation process.[25,31] The known catalysts (or initiators) of lipid oxidation are metals, light, heat, enzymes, ozone, and free radicals.[31−33] Due to exposure to one or more initiators, the processing method has great influence in the oxidative stability of the oil. In our studies, the initiation pathways of light, enzymes, and ozone were not considered, because no addition of compounds or light was made in the short time of treatment. Therefore, only intrinsic minor constituents were considered to be susceptible to co-initiate the oxidation process.

In the case of exposure to high temperatures, the breakage of the covalent bond C−C or C−H bond is observed as well as the formation of a variety of lipid alkyl radicals that initiate the radical chain reactions of lipid oxidation. However, in moderate temperatures, preferably the covalent bonds of O−O are broken in existing traces of ROOH or LOOH that generate, by means of intermediate reactions, the $L^{\bullet}$ that initiates the chain reaction.[25] Therefore, after the sonication treatment, the

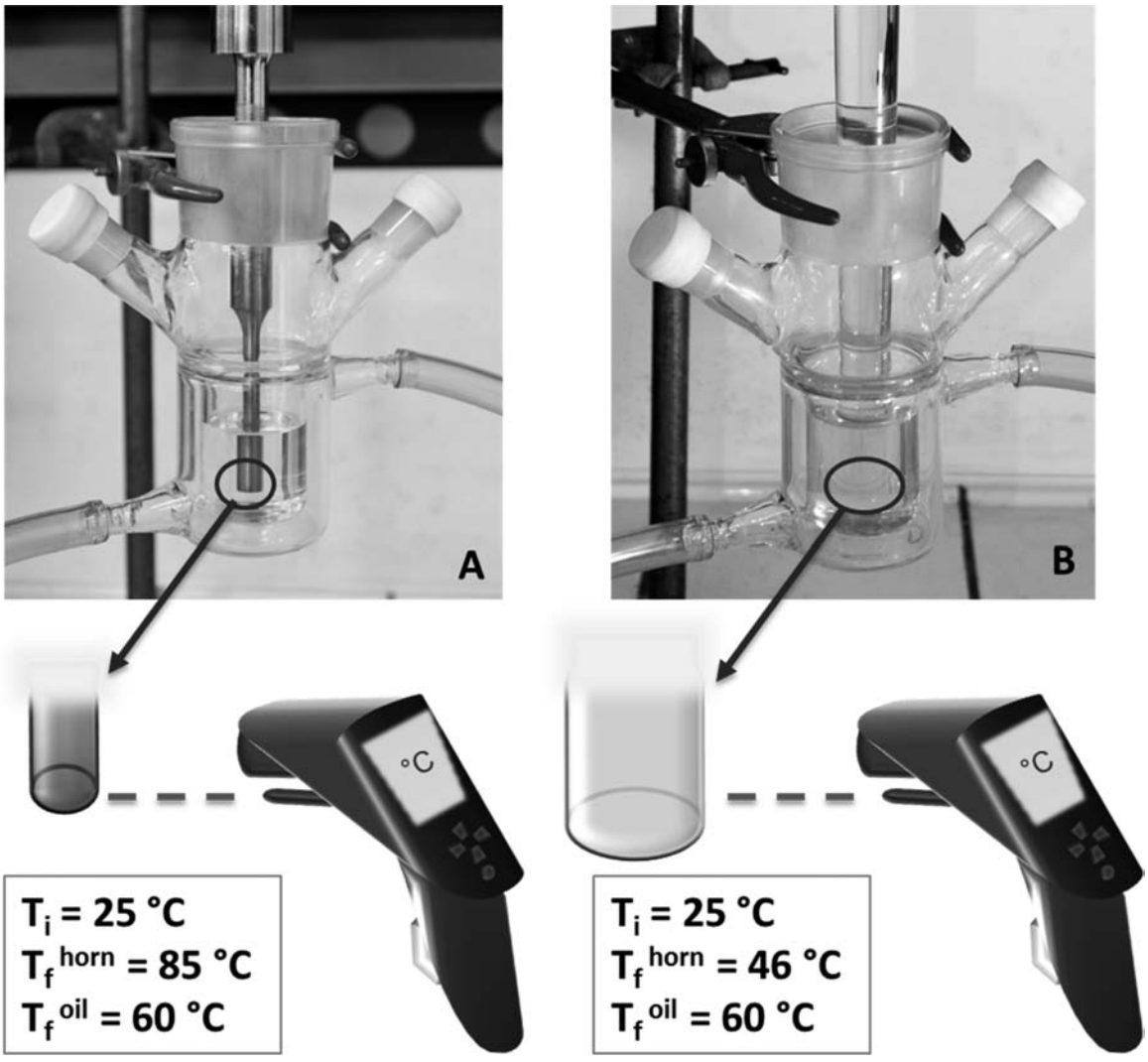


**Figure 4.** Temperature assessment of both ultrasonic horns after sonication ($T_i$, initial temperature; $T_f$, final temperature).

temperatures of both horns were measured by an infrared thermometer (Testo845 - Testo, France), and an increase of 20 °C was observed for the pyrex horn, whereas the titanium one presented an increase of 60 °C, although the temperature of the oil remained the same (60 °C) in both conditions (Figure 4). For that reason, it is possible that the moderate temperatures generated by the Pyrex horn induce the formation of numerous subproducts that, with the addition of ultrasonic energy, might then follow the free radical initiation reaction. Some studies were carried out to determine the temperature attained in cavitation bubbles using molecular emission of diatomics ($C_2$) or emission from metal atoms (Fe, Cr, Mo) originating from volatile organometallics, and the order of magnitude of the temperatures attained reached 5000 K in some cases.[34] On the other hand, in studies using radioactive collision processes in a weakly ionized gas, temperatures near 20000 K have been proposed.[6]

Metals can also initiate the oxidation process, and sunflower oil may contain from 2.2 to 8.5 ppb copper and from 0.22 to 0.31 ppm iron,[31] knowing the Codex Alimentarius recognizes the maximum level for those metals not to exceed 0.1 and 1.5 ppm, respectively. Therefore, this small amount of metal, associated with other initiators such as high temperature or radicals, could help co-initiate the oil oxidation process, producing lipid alkyl radicals. It is possible that water contained in those samples has also a role in the oxidation pathway. The initial concerns at the time of first appearances of degradation in samples treated by a metallic ultrasonic horn were that metal particles of the probe would co-initiate the oxidation reaction. However, Chemat et al.[10] demonstrated that oxidation also occurs in a glass vessel immersed in an ultrasonic bath, discounting the hypothesis of the sole oxidation by metals from ultrasonic apparatus. Our study corroborates those findings by evidencing degradation by a Pyrex horn in oils contained in glass vessels; however, both laboratory glasses and the horn are made of Pyrex, which contains 14% boron, 51% oxygen, 0.3% sodium, 1% aluminum, 38% silicon, and <1% potassium according to the National Institute of Standards (USA). Therefore, the treatment by ultrasound is not performed in metal-free conditions, and those metal compounds contained in the glass structures might co-initiate lipid oxidation during sonication. However, the type of metal compound has a major importance in the oxidation induction rate, because different metals catalyze different reactions; whereas some accelerate hydrogen peroxide decomposition, others will accelerate hydroperoxide decomposition.[31] In our study, the metals present in the two sonication settings are different for the

titanium and Pyrex horns, which might explain the differences in the extent of oxidation produced by each horn.

According to the overall results, it is possible to conclude that there is an increase in the formation of degradation products due to the treatment performed with the titanium horn. This comportment is also verified for the Pyrex horn, but with a more marked formation of those compounds, suggesting therefore a more pronounced degradation. Indeed, the EPR analysis showed a significant increase of radicals in the oil after only 15 min of treatment for the Pyrex horn, which was not the case for the titanium one. The temperature analysis of both horns allowed the observation that the titanium horn presents a higher increase of temperature when compared to the Pyrex horn.

For those reasons, it is possible to infer that the oil degradation mechanism for the titanium horn might be mainly thermal, whereas the one for the Pyrex horn might be mainly radical. Because the treatment with the Pyrex horn stimulates the production of radicals, when the temperature parameter is included, the degradation occurs more rapidly than for the treatment by the titanium horn, which does not stimulate the formation of radicals. This is the probable reason why the increase is not that remarkable for the other physicochemical parameters.

Ultrasounds are used in emerging technologies that have been applied to numerous food industry domains alone or coupled with other techniques with advantages over other conventional techniques. Nevertheless, the effects of sonicated foods are often overlooked and, in high fat content foods, the appearance of off-flavors is observed, suggesting lipid degradation. This work demonstrates, by means of EPR analysis and other degradation parameters, the role of ultrasounds as an initiator in the oxidation of lipids with the increase of free radicals and oxidative products in sonicated oils when compared to untreated samples. Further studies should be carried out to better elucidate the degradation mechanism in sonicated edible oils.

## AUTHOR INFORMATION

### Corresponding Author

*E-mail: farid.chemat@univ-avignon.fr. Phone:+33(0) 4.90.14.44.65. Fax:+33(0)4.90.14.44.41.

### Funding

G.D. acknowledges financial support from Région PACA (APO2009 PhotoMolEnergie) for the purchase of the EPR instrument used in this study.

### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Chemat, F.; Zill-e-Huma; Khan, M. K. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813−835.

(2) Mason, T. J.; Paniwnyk, L.; Lorimer, J. P. The uses of ultrasound in food technology. *Ultrason. Sonochem.* **1996**, *3*, S253−S260.

(3) Tiwari, B. K.; O'Donnell, C. P.; Patras, A.; Cullen, P. J. Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *J. Agric. Food Chem.* **2008**, *56*, 10071−10077.

(4) Chouliara, E.; Georgogianni, K. G.; Kanellopoulou, N.; Kontominas, M. G. Effect of ultrasonication on microbiological, chemical and sensory properties of raw, thermized and pasteurized milk. *Int. Dairy J.* **2010**, *20*, 307−313.

(5) Rawson, A.; Tiwari, B. K.; Patras, A.; Brunton, N.; Brennan, C.; Cullen, P. J.; O'Donnell, C. Effect of thermosonication on bioactive compounds in watermelon juice. *Food Res. Int.* **2011**, *44*, 1168−1173.

(6) Brenner, M. P.; Hilgenfeldt, S.; Lohse, D. Single-bubble sonoluminescence. *Rev. Mod. Phys.* **2002**, *74*, 425.

(7) Riesz, P.; Berdahl, D.; Christman, C. L. Free radical generation by ultrasound in aqueous and nonaqueous solutions. *Environ. Health Perspect.* **1985**, *64*, 233−252.

(8) Riener, J.; Noci, F.; Cronin, D. A.; Morgan, D. J.; Lyng, J. G. A comparison of selected quality characteristics of yoghurts prepared from thermosonicated and conventionally heated milks. *Food Chem.* **2010**, *119*, 1108−1113.

(9) Pingret, D.; Fabiano-Tixier, A.-S.; Petitcolas, E.; Canselier, J.-P.; Chemat, F. First investigation on ultrasound-assisted preparation of food products: sensory and physicochemical characteristics. *J. Food Sci.* **2011**, *76*, C287−C292.

(10) Chemat, F.; Grondin, I.; Costes, P.; Moutoussamy, L.; Sing, A. S. C.; Smadja, J. High power ultrasound effects on lipid oxidation of refined sunflower oil. *Ultrason. Sonochem.* **2004**, *11*, 281−285.

(11) Chemat, F.; Grondin, I.; Shum Cheong Sing, A.; Smadja, J. Deterioration of edible oils during food processing by ultrasound. *Ultrason. Sonochem.* **2004**, *11*, 13−15.

(12) Jahouach-Rabai, W.; Trabelsi, M.; Van Hoed, V.; Adams, A.; Verhé, R.; De Kimpe, N.; Frikha, M. H. Influence of bleaching by ultrasound on fatty acids and minor compounds of olive oil. Qualitative and quantitative analysis of volatile compounds (by SPME coupled to GC/MS). *Ultrason. Sonochem.* **2008**, *15*, 590−597.

(13) Patrick, M.; Blindt, R.; Janssen, J. The effect of ultrasonic intensity on the crystal structure of palm oil. *Ultrason. Sonochem.* **2004**, *11*, 251−255.

(14) Kasai, H.; Maekawa, M.; Kawai, K.; Hachisuka, K.; Takahashi, Y.; Nakamura, H.; Sawa, R.; Matsui, S.; Matsuda, T. 4-Oxo-2-hexenal, a mutagen formed by omega-3 fat peroxidation, causes DNA adduct formation in mouse organs. *Ind. Health* **2005**, *43*, 699−701.

(15) Ottaviani, M. F.; Spallaci, M.; Cangiotti, M.; Bacchiocca, M.; Ninfali, P. Electron paramagnetic resonance investigations of free radicals in extra virgin olive oils. *J. Agric. Food Chem.* **2001**, *49*, 3691−3696.

(16) Papadimitriou, V.; Sotiroudis, T.; Xenakis, A.; Sofikiti, N.; Stavyiannoudaki, V.; Chaniotakis, N. Oxidative stability and radical scavenging activity of extra virgin olive oils: an electron paramagnetic resonance spectroscopy study. *Anal. Chim. Acta* **2006**, *573*−*574*, 453−458.

(17) Szterk, A.; Stefaniuk, I.; Waszkiewicz-Robak, B.; Roszko, M. Oxidative stability of lipids by means of EPR spectroscopy and chemiluminescence. *J. Am. Oil Chem. Soc.* **2011**, *88*, 611−618.

(18) Valavanidis, A.; Nisiotou, C.; Papageorgiou, Y.; Kremli, I.; Satravelas, N.; Zinieris, N.; Zygalaki, H. Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. *J. Agric. Food Chem.* **2004**, *52*, 2358−2365.

(19) Huie, R.; Cherry, W. R. Facile one-step synthesis of phenyl-tert-butylnitrone (PBN) and its derivatives. *J. Org. Chem.* **1985**, *50*, 1531−1532.

(20) Rockenbauer, A.; Korecz, L. Automatic computer simulations of ESR spectra. *Appl. Magn. Reson.* **1996**, *10*, 29−43.

(21) Morrison, W. R.; Smith, L. M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride−methanol. *J. Lipid Res.* **1964**, *5*, 600−608.

(22) Shahidi, F.; Zhong, Y. Lipid oxidation: measurement methods. In *Bailey's Industrial Oil and Fat Products*; Wiley and Blackwell: Hoboken, NJ, 2005; Vol. *1*, pp 357−385.

(23) Guillén, M. D.; Cabo, N. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chem.* **2002**, *77*, 503−510.

(24) Wai, W. T.; Saad, B.; Lim, B. P. Determination of TOTOX value in palm oleins using a FI-potentiometric analyzer. *Food Chem.* **2009**, *113*, 285−290.

(25) Schaich, K. M. Lipid oxidation: theoretical aspects. In *Bailey's Industrial Oil and Fat Products*; Wiley and Blackwell: Hoboken, NJ, 2005; Vol. *1*, pp 269−355.
(26) Smith, S. A.; King, R. E.; Min, D. B. Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. *Food Chem.* **2007**, *102*, 1208−1213.
(27) Villamena, F. A.; Zweier, J. L. Detection of reactive oxygen and nitrogen species by EPR spin trapping. *Antioxid. Redox Signal.* **2004**, *6*, 619−629.
(28) Janzen, E. G.; Haire, D. L. *Advances in Free Radical Chemistry*; JAI Press: Greenwich, CT, 1990.
(29) Velasco, J.; Andersen, M. L.; Skibsted, L. H. Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. *Food Chem.* **2004**, *85*, 623−632.
(30) Jeleń, H. H.; Obuchowska, M.; Zawirska-Wojtasiak, R.; Wąsowicz, E. Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J. Agric. Food Chem.* **2000**, *48*, 2360−2367.
(31) Choe, E.; Min, D. B. Mechanisms and factors for edible oil oxidation. *Compr. Rev. Food Sci. Food Saf.* **2006**, *5*, 169−186.
(32) Hamilton, R. J.; Kalu, C.; Prisk, E.; Padley, F. B.; Pierce, H. Chemistry of free radicals in lipids. *Food Chem.* **1997**, *60*, 193−199.
(33) Yin, H.; Xu, L.; Porter, N. A. Free radical lipid peroxidation: mechanisms and analysis. *Chem. Rev.* **2011**, *111*, 5944−5972.
(34) McNamara, W. B.; Didenko, Y. T.; Suslick, K. S. Sonoluminescence temperatures during multi-bubble cavitation. *Nature* **1999**, *401*, 772−775.