Microwave and Conventional Heating Effects on Thermoxidative Degradation of Edible Fats

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The effects of microwave treatments on the thermoxidative degradation of five edible fats and oils (sunflower oil, high oleic sunflower oil, virgin olive oil, olive oil, and lard) were determined. The samples were subjected to the following three well-controlled treatments: (a) microwave heating, (b) heating in a conventional electric oven, and (c) exposure to microwave energy without heating. A comparative study was carried out on the deterioration of the oils as a result of microwave and conventional oven heating. Degradations were quantified by means of a combination of chromatographic techniques and related analytical index. Data analysis showed greater alterations in microwave-heated samples than in corresponding samples heated in a conventional oven. Finally, microwave energy, without heating (temperature lower than 40 °C), produced no oil alterations.

Keywords: *Microwave energy; conventional oven; heating effect; edible fats and oils; thermoxidative alteration*

INTRODUCTION

The application of the microwave oven (MO) heating to culinary techniques and food processing is a more recent development (Tsuyuki, 1982) than other traditional cooking techniques such as roasting, boiling, or frying. Advantages of microwave cooking include savings in time and energy and ease of use. These advantages make it one of the most attractive cooking methods.

The heating of food in a MO is caused by interaction of an electromagnetic field with the chemical constituents of foods (Kamel and Stauffer, 1993; Decareau, 1995). These interactions immediately generate heat because of molecular friction and excitation.

To date, there is insufficient information available on the consequences of microwave heating on the composition and nutritional quality of the food. There has been speculation on the ease of free radical formation when fatty foods are exposed to microwave energy (Lie Ken Jie and Yan-Kit, 1988).

Since oil oxidation involves free radicals, this heating process could accelerate all of the oxidative reactions at a higher rate than conventional oven heating or frying processes. These processes have been widely studied in recent decades by many researchers who have established the importance of oil composition, surface/volume ratio, and time of heating on the compounds formed (Dobarganes and Pérez-Camino, 1987; Perkins and Printer, 1988; Christie et al., 1993). Analytical methodologies have advanced as has knowledge on the novel compounds and, nowadays, in many countries there is legislation to control the quality of oils and fats used in heating or frying processess. The permitted limits for polar compounds formed during heating of oils have been established by column chromatography [*Official State Bulletin (Spain)*, 1989]. In contrast, few studies have been published on the alterations that take place during MO heating. Recent works that examined such effects (Yoshida et al., 1992, 1993; Ruíz-López et al., 1995) did not look at the oxidative compounds produced as a consequence of the temperature.

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The present study aimed to elucidate the influence of microwave heating on the thermoxidative stability of common oils and fats in household use. Changes in the polar glyceridic compounds are quantified by a combination of solid-phase extraction and liquid chromatography (SPE-HPSEC). Variations in the related analytical index with the alterations produced were determined as well as losses of natural antioxidants. Furthermore, comparisons were made between the changes occurring in a MO and those in a conventional oven at the same temperature, time, and surface/volume ratio of oil. Finally, the effect on oils of microwave energy without heating was evaluated.

EXPERIMENTAL PROCEDURES

Samples. Five different edible fats and oils were used in this study. Commercial sunflower oil (SO), high oleic sunflower oil (HOSO), olive oil (OO), and lard (LA) were purchased at a local market. Virgin olive oil (VOO) (EEC, 1991), extracted from the Pajarera olive variety, was obtained from an industrial plant (Cano e hijos, Córdoba, Spain). The samples were treated in microwave and conventional ovens as follows:

(1) SO-MH. One hundred milliliters of sunflower oil was divided into five open Sovirel flasks (20 mL each; 4 cm diameter), which were placed at equal distances on the rotatory plate of the microwave oven. They were heated for 120 min at half-power (i.e. they were subjected to total microwave energy for 60 min). The oil temperature was determined every 30 min with a chromel−alumel thermocouple and remained at 170 ± 10 °C. The five samples were combined after oven treatment and before analysis.

(2) SO-CH. These samples were prepared as in the preceding paragraph, but they were heated to 180 ± 2 °C by air convection in an electric conventional oven for 120 min.

(3) SO-M. These samples were distributed as described for SO-MH and were subsequently exposed to microwave energy for 120 min, at intervals of 50 s without allowing the temperature to exceed 40 °C.

The treated HOSO, OO, VOO, and LA samples were obtained in the same way as for SO as described in *1*, *2*, and *3*, to give HOSO-MH, HOSO-CH, HOSO-M, OO-MH, OO-CH, OO-M, VOO-MH, VOO-CH, VOO-M, LA-MH, LA-CH, and LA-M, respectively. All samples were completed in duplicates.

The study was completed a second time using the methods described. Each reported value is the mean of two measurements from two replicates.

S0021-8561(97)00181-7 CCC: $14.00

Reagents and Solvents. All of the reagents and solvents used were of analytical reagent grade, except HPLC eluents, which were of HPLC grade supplied by Merck (Darmstadt, Germany). The solid-phase extraction (SPE) columns packed with 1 g of activated silica were from Supelco (Bellefonte, PA).

Equipment. The MO was a Moulinex Micro Chef, FM B745 AGS (France). The full power reached was 1 kW, and the frequency of the radiation emitted was 2450 MHz. A Beckman DU 640 spectrophotometer (Palo Alto, CA) was used to determine absorbance at 232 and 270 nm.

A Rancimat (Metrohm CH 9100) was used to determine oxidative stability of the samples. A food oil sensor (NI-20, Northern Instruments Co., Lino Lakes, MN) was used to measure the dielectric constant. A high-performance liquid chromatograph, HP-1050, equipped with a refractive index detector (Hewlett-Packard HP-1047A, Avondale, PA) was used to determine distribution of glyceridic polar compounds.

Analytical Procedures. *Glyceridic Polar Compounds.* Isolation and quantitation of glyceridic polar compounds was performed by SPE followed by HPSEC (Albi et al., 1995). The amount of the sample introduced into the column was 50 mg, in hexane (500 μL). Two hundred microliters of 1-monooleyl-*rac*-glycerol (5 mg/mL) in hexane was used as an internal standard since a previous experiment had established that this compound is not present in any of the samples studied.

The SPE columns placed in a vacuum system were first eluted with 15 mL of *n*-hexane/diethyl ether (90:10, v/v). A second fraction which made up the polar compounds was collected by eluting 5 mL of chloroform/methanol (2:1, v/v) through the column. Separations were checked by silica TLC. The second fraction was then evaporated, redissolved in 2 mL of tetrahydrofuran, and analyzed by HPSEC using two columns (Hewlett-Packard, PL-gel particle size 5 μm, 30 cm × 0.75 cm i.d.) in series: the first with a pore size of 500 Å and the second of 100 Å. The eluent was tetrahydrofuran at a constant flow rate of 1 mL/min. Detection was done with a refractive index detector.

Determinations of acid value, peroxide value, and α-tocopherol content were carried out following the analytical methods described in Regulations of the European Union Commission EEC/2568/91 and EEC/656/95 (European Union Commission, 1991, 1995) and by IUPAC (1992). Phenolic compounds were quantified by a spectrophotometric method. They were isolated by extraction three times of a solution of oil in hexane (50%, v/v) with 20 mL of methanol/water (60:40, v/v). Results are given as miiligrams of caffeic acid per kilogram of oil (Vázquez et al., 1973; Gutfinger, 1981). Oxidative stability was determined by the Rancimat method (Läubli and Bruttel, 1986), programmed at 100 °C, and changes in dielectric constant were measured with a food oil sensor (Graziano, 1979), the instrument being calibrated with the untreated oils.

Statistical Analysis of Experimental Data. Each reported value is the mean of two measurements from two replicates. Duncan's multiple range test (Duncan, 1955) was applied to determine significance between means.

RESULTS AND DICUSSION

Physicochemical characteristics of the initial untreated and treated samples used in this study are shown in Table 1. For all samples each value is the mean of four data corresponding to two measurements from two replicates.

The results obtained for acid and peroxide values show in both cases a slight increase during the treatments except for the microwave treatments without heating, for which they were equal ($p < 0.05$) to the original ones. The data indicate that hydrolytic and oxidative degradations took place but not in a very clear or marked way, in contrast to data found by other researchers who reported severe hydrolytic changes occurred during microwave heating. Moreover, our results are consistent with the diacylglycerol (DG) and free fatty acid (FFA) determinations by SPE-HPSEC described below.

Another measurement of interest related to total degradations is the dielectric constant (Table 1). For all cases, the greatest increases ($p < 0.01$) correspond to the samples heated in a MO (MH), these being the samples that suffer the greatest degradations. These increases correspond to a considerable decrease ($p < 0.01$) in the tocopherol content (Table 2), which in the case of olive oils reaches 100%. In the same way, losses in the phenolic compounds present in olive oils VOO and OO are very marked (96 and 85%, respectively). No significant differences ($p < 0.01$) for samples exposed to microwave energy (temperature <40 °C) were observed.

Table 1. Chemical and Physical Characteristics of the Fat and Oils before and after Treatments

sample	acid value[a,b] (% oleic acid)	peroxide value[a,b] (mequiv of O_2/kg)	dielectric constant[a,b]	α-tocopherol[a,c] (ppm)	polyphenols[a,b] (ppm)
SO	0.09	6.1	1.89	731	nd[d]
SO-CH	0.11	7.3	0.32[e]	445	
SO-MH	0.15	8.0	1.60[e]	206	
SO-M	0.10	6.4	0.04[e]	575	
HOSO	0.09	6.5	0.82	695	nd
HOSO-CH	0.13	7.6	0.08[e]	376	
HOSO-MH	0.17	9.0	1.10[e]	106	
HOSO-M	0.10	6.6	0.05[e]	572	
VOO	0.26	16.1	0.79	176	284
VOO-CH	0.26	11.7	0.07[e]	95	206
VOO-MH	0.29	19.8	0.91[e]	0	12
VOO-M	0.25	17.5	0.03[e]	139	261
OO	0.36	11.2	0.08	170	103
OO-CH	0.40	11.0	0.21[e]	48	35
OO-MH	0.42	10.4	1.21[e]	0	16
OO-M	0.40	11.6	0.04[e]	160	101
LA	1.63	2.9	0.13	nd	nd
LA-CH	1.65	4.5	0.47[e]		
LA-MH	1.82	9.4	0.91[e]		
LA-M	1.62	3.2	0.04[e]		

[a] Mean value of four determinations. [b] CV ≤ 10%. [c] CV ≤ 5%. [d] nd, not detected. [e] Reference value their untreated sample.

Table 2. Natural Antioxidant Losses and Oxidative Stability of the Fat and Oils before and after Treatments

sample	stability[a] (h)	α-tocopherol[b]	polyphenols[b]
SO	9.5		
SO-CH	7.4	39	
SO-MH	3.4	72	
SO-M	8.7	21	
HOSO	27.8		
HOSO-CH	22.2	46	
HOSO-MH	4.3	85	
HOSO-M	26.8	18	
VOO	51.0		
VOO-CH	46.2	46	10
VOO-MH	8.1	100	96
VOO-M	43.8	21	8
OO	37.8		
OO-CH	34.7	72	64
OO-MH	3.4	100	85
OO-M	37.8	6	3
LA	12.1		
LA-CH	5.0		
LA-MH	1.6		
LA-M	12.4		

[a] Mean value of four determinations, CV ≤ 8%. [b] Percent of initial values.

Table 3. Distribution of Polar Compounds (PC) Determined by SPE-HPLC of the Fat and Oils before and after Treatments

	polar compounds[a]					
		distribution (mg/g of fat)				
sample	total PC (%)	TG-P[b]	TG-D[b]	ox-TG[b]	DG[b]	FFA[b]
SO	5.2	nd[c]	7.5	22.5	16.9	5.0
SO-CH	7.5	2.7	20.8	29.5	17.1	4.8
SO-MH	16.2	17.6	63.7	59.3	16.5	4.4
SO-M	5.2	nd	8.7	21.2	17.2	5.2
HOSO	4.2	nd	3.9	15.2	18.2	5.0
HOSO-CH	5.2	nd	10.8	18.8	17.7	4.9
HOSO-MH	15.1	6.9	63.7	59.3	16.5	4.4
HOSO-M	5.2	nd	8.7	21.2	17.2	5.2
VOO	2.6	nd	nd	10.1	11.6	4.3
VOO-CH	3.9	nd	8.5	12.2	13.4	4.7
VOO-MH	8.8	5.3	27.2	37.0	13.7	4.6
VOO-M	2.8	nd	nd	9.6	13.6	4.7
OO	3.1	nd	1.4	6.6	17.1	5.8
OO-CH	4.8	nd	8.6	13.0	19.3	6.7
OO-MH	11.3	7.1	31.7	47.9	20.1	6.4
OO-M	3.7	nd	1.6	8.5	17.9	6.7
LA	5.3	nd	1.2	14.8	18.3	19.1
LA-CH	7.1	1.2	6.8	20.8	21.1	21.6
LA-MH	10.6	4.2	21.3	42.6	19.0	19.2
LA-M	5.0	nd	1.0	13.2	16.8	18.9

[a] Mean value of four determinations, CV ≤ 7%. [b] Abbreviations: TG-P, triglyceride polymers; TG-D, triglyceride dimers; ox-TG, oxidized tryglyceride monomers; DG, diglycerides; FFA, free fatty acids. [c] nd, not detected.

Glyceridic Polar Compounds. *Recovery and Repeatability of SPE-HPSEC.* The analytical technique had good repeatability (RSD 3%), and the recovery was complete (105%). The separation obtained with the SPE silica columns was adequate (Albi et al., 1995).

The repeatability of the method was studied by using a heated sunflower oil sample, a new SPE column for each replicate ($n = 6$), and another sample of an untreated VOO. In the latter case, the replicates ($n = 6$) were done with reused columns. The SPE columns were washed with methanol and acetone consecutively and then dried by passing nitrogen so that they could be reused. Results (data not shown) indicate good repeatability for both assays (RSD 7 and 4%, respectively).

Table 3 summarizes mean values of the total glyceridic polar compounds (PC) and their distribution. In all cases, the coefficients of variation (CV %) were <7%. Samples heated in a conventional oven had fewer changes than those heated in a MO, for all fats and oils studied. Because the temperature was the same in both cases, the microwave energy was considered to have a greater effect on sample alteration. We thought that the microwave energy potentiates the effects of the temperature, because the heating is made as a consequence, mainly, of the internal friction of the molecules (Schiffmann, 1993) and could lead to some zonal overheating in the oil, which is different from conventional heating that is carried out for convection and conduction. To determine the importance of the microwave energy at low temperature on the oils, a new experiment was designed. A set of samples was subjected to microwave energy with the temperature maintained below 40 °C (resulting samples M). Data from this experiment are also shown in Tables 1 and 2. Total polar compounds as well as their distributions in the five original samples and their corresponding samples treated with microwave energy (M) showed no significant differences ($p < 0.01$). Similar findings were recorded for the other analytical index, indicating that the microwave energy alone had no effect on the oils but that the effects were produced as a result of the combination of energy and temperature.

Data on the distribution of polar compounds showed that thermic and oxidative degradations took place but that thermic alterations were more abundant than oxidative ones. This effect can be easily demonstrated in the case of SO heated in a conventional oven. As was observed, compounds related to oxidative alteration of oxidized triacylglycerol monomers (ox-TG) increased 7 mg/g (31%) after the samples had been subjected to 2 h of the heating process, but triacylglycerol dimers (TG-D), mainly related to thermic degradations, increased 13 mg/g (177%) and, moreover, triacylglycerol polymers (TG-P) appeared. This effect was more marked in the case of the SO-MH (ox-TG and TG-D increases of 163 and 749%, respectively). The same behavior was observed in all of the fats studied. These results agree with the possible appearance of zonal overheating in the samples (MH).

There were no significant ($p \leq 0.05$) differences in the diglycerides (DG) and free fatty acids (FFA) of the untreated and treated samples (data shown in Table 3). This was true for all five fats studied and for all treatments. These data confirm the findings for the acidity value. We cannot ensure that hydrolytic alterations take place to any marked degree during a microwave heating process. For this reason a new experiment was designed to detect hydrolytic degradation. A new set of samples ($n = 6$) was saturated with humidity and subjected to the action of the microwaves. After 120 min to 170 ± 10 °C, FFA were measured by titration as well as by SPE-HPSEC (Albi et al., 1995). The results showed that there were no modifications in comparison to the original samples ($p < 0.01$%). Again, this confirms that in contrast to oxidative and thermic degradations hydrolytic degradation is not relevant in this process.

Finally, SO and HOSO showed the greatest variations in the PC, whereas, as expected, olive oils and lard showed the least changes. This is due in part to their fatty acid composition but, in olive oils, mainly, also to their polyphenol contents, which have been demonstrated to be closely related to the resistance of oils to oxidative alterations (Blekas et al., 1995).

ACKNOWLEDGMENT

We are grateful to Ms. C. Arévalo and Ms. P. García-Rodríguez de Quesada for technical assistance.

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Received for review March 5, 1997. Revised manuscript recieved July 8, 1997. Accepted July 14, 1997.[⊗] CICYT provided financial support (ALI94-0819).

JF970181X

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1997.