Effect of Temperature and Type of Food Simulant on Antioxidant Stability

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ABSTRACT: Additive migration levels in food simulants from polimeric materials that are intended to be into contact with food can be affected by additive stability under the migration test conditions. In this work, the stability of some phenolic antioxidants and one oxidized phosphite antioxidant was studied in four food simulants: distilled water, 3% (w/v) acetic acid, 10% (v/v) ethanol, and the fatty food simulant olive oil, under different temperatures 5, 40, and 70°C, during ~20 days. Samples were analyzed by reversed-phase high performance liquid chromatography (HPLC) with UV diode-array detector. In general, antioxidants appeared to be more stable in olive oil than in the aqueous simulants. Among aqueous simulants, water and 10% ethanol allowed the highest stability of antioxidants at low temperatures. The 3% acetic acid allowed good stability for the lowest phenolic compounds even at high temperatures, but the highest molecular weight compounds decomposed very fast even at low temperatures. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 656–663, 2006

Key words: antioxidants; degradation; food simulants; high performance liquid chromatography (HPLC); migration

INTRODUCTION

An incomplete list of additives that can be used in the manufacture of plastic materials and articles that are intended to come into contact with foodstuffs is established by Directive 2002/72/EEC,[1] which also lays out specific migration levels (SML) according to their individual toxicity that must not be exceeded in the migration tests. Four types of food simulants are stabilized to perform the migration test by Directive 82/711/EEC,[2] three of them are aqueous simulants A (distilled water), B (3% w/v acetic acid), and C (10% v/v ethanol) and the other is a fatty food simulant, simulant D, rectified olive oil that could be replaced for a synthetic triglyceride mixture, by sunflower oil or by corn oil. If these fatty simulants cannot be used for technical reasons connected with the method of analysis, simulant D could be substituted by isooctane, 95% ethanol, or modified polyphenylene oxide. To carry out the specific migration test, plastic material is put in contact with food stimulant, under determined temperature and time conditions. However, it must be considered that this value will not be representative of the real migration level from the packaging if the analyte is not stable during the test; therefore, a stability study of antioxidants in food simulants is necessary to know the way the analyte is affected by heat exposure in each simulant during the test.

While considerable researches have been focused on the stability of plastic additives during processing, there is a lack of studies on the stability of additives in food simulants.[3,4] There is not much information on the types of additives that may undergo degradation in food simulants and about their degradation products, as well as on the type of media or heat exposure to which they are more sensitive.[5] Several works that studied stability of different additives in the food simulants employed in migration tests have been reviewed. The stability of phosphite antioxidant Ultranox 626 in aqueous simulants, 95% ethanol, and isooctane under different conditions of time and temperature, has been studied by Pérez-Lamela et al.[6] The stability of two antioxidants, Irganox 245 and Irganox 1035, one ultraviolet absorber, Chimmasorb 81, and one optical brightening agent, Uvitex OB, in olive oil has been the aim of the work of Quinto-Fernández et al.[4] The stability of antioxidants Irganox 3114, Irganox 1035, Irganox 245, Irganox 1098, and Irgafos P-EPQ in the three aqueous simulants and 95% ethanol has been studied by Demertzis and Franz.[3] A research about specific migration of four additives determining the stability of BEA (antiestatic agent), DEHA (plasticizer), Irganox 1076 (antioxidant), and alkyl (C_8–C_{22}) sulfonic acid (sodium salts) (an aid to polymerization), under the test migration conditions, has been carried

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TABLE I
Selected Antioxidants

	Chemical name	CAS No.	M_w	SML (mg/kg^{-1})[a]	Source
AO 2246	2,2′-Methylene bis(4-methyl-6-*tert*-butylphenol)	119–47-1	340.5	1.5[b]	Sigma-Aldrich (Steinheim, Germany)
AO 425	2,2′-Methylene bis(4-ethyl-6-*tert*-butylphenol)	88–24-4	368.6		
BHA	Butylated hydroxyanisole (mixed isomers 2[3]-*t*-butyl-4-hydroxyanisole; 2[3]-*t*-butylhydroquinone monomethyl ether, minimum 90%-3 isomer/9%-2 isomer)	25013–16-5	180.2	30	
BHT	2, 6 Di-*tert*-butyl-*p*-cresol	128–37-0	220.4	3.0	
DBP	2,4-Bis (1, 1-dimethylethyl)-phenol	96–76-4	206.3		Fluka (Buchs, Switzerland)
Ethanox 330 (E330)	1.3,5-Trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) benzene	1709–70-2	775.2		Sigma-Aldrich (Steinheim, Germany)
Irgafos 168	Tris(2, 4-di-*tert*-butylphenyl) phosphite	31570-04–4	646.9		Ciba (Basel, Switzerland)
Irganox 1010	Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate	6683–19-8	1178		
Irganox 1076	Octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate	2082–79-3	531	6.0	

[a] Established by Directives 2002/72/EC[1] and 2004/19/EC.[9]
[b] Limit established by legislation for the sum of both compounds

out by O'Brien et al.[7] The specific migration of antioxidant Irganox 1076 and plasticizer DEHA in the alternative solvents to olive oil, isooctane and 95% ethanol, has been studied by Cooper et al.[8] In these reviewed works, stability tests were carried out spiking the additives at their SML into the simulants that were submitted to time–temperature conditions used in the migration test. Test samples were analyzed at the end of the assay against fresh samples of the additives spiked at the same concentration.

However, the present stability study intends to know the curve of decomposition of several antioxidants along time instead of only measuring their recovery at the end of the test because, in the migration test, additives diffuse gradually into simulant during all the exposure and so, molecules of the same antioxidants are present in food simulants at different time periods, whereas, in the stability experiments, the additive is exposed to simulant for the full time. Therefore, the aim of this work is to study the stability of some phenolic antioxidants and the oxidation product of one phosphite antioxidant in the four food simulants at different temperatures and time intervals.

EXPERIMENTAL

Materials

The studied antioxidants were obtained from the sources presented in Table I. It is noted that the antioxidant DBP is a hydrolysis product of the phosphite Irgafos 168.[10]

Methanol, ethanol, and tetrahydrofuran (HPLC-gradient grade) were supplied by Merck (Darmstadt, Germany). Acetonitrile (ultra-gradient HPLC grade) was supplied by J.T. Baker (Deventer, Holland). Water was purified on a Milli-RO system (Millipore, Bedford, MA). Acetic acid glacial (HPLC-gradient grade) was supplied by Panreac Quimica (Barcelona, Spain). Olive oil, complying with the specifications given in EU Directives, was used as fatty food simulant.

High performance liquid chromatography

The chromatographic experiments for the analysis of aqueous simulant samples, were carried out following the method developed in a previous work.[11] A Waters 2695 equipment (Waters, Milford, MA) with a gradient pump and automatic injector was used. The nine analytes were completely separated using a stainless steel column of dimensions (3.0×150 mm^2) packed with Symmetry C_{18} and 3.5 μm particle size (Waters, Milford, MA). The detection system was a model 996 UV photodiode array (Waters, Milford, MA). The signal acquired from detector was recorded by a personal computer operated using the Millenium 32 software V. 3.20 (Waters). The conditions of chromatographic method are shown in Table II. The aqueous simulants were analyzed directly, whereas, the fatty stimulant

TABLE II
Chromatographic Method Conditions

Elution for aqueous simulants[a]			
Time (min)	Methanol (%)	Water (%)	Curve
0	50	50	
5	100	0	Linear
22	100	0	Linear
23	50	50	Convex
25	50	50	Linear

Elution for olive oil[b]					
Time (minutes)	Methanol (%)	Acetonitrile (%)	THF (%)	Water (%)	Curve
0	10	10	0	80	Linear
5	35	35	0	30	Linear
10	100	0	0	0	Linear
20	100	0	0	0	Linear
22	0	0	100	0	Linear
30	0	0	100	0	Linear
32	50	50	0	0	Linear
34	10	10	0	80	Convex
39	10	10	0	80	Linear

Column symmetry C_{18} 3.0 × 150 mm × 3.5 μm, flow = 0.5 mL min^{-1}, column oven temperature = 30 °C, injection volume = 20 μL.
[a] Wavelength = 276 nm.
[b] Wavelength = 220 nm.

was diluted with tetrahydrofuran (THF) before chromatographic analysis, such that 2 ± 0.01 g of sample was diluted until 5 mL with THF.

This method had to be modified to determine the antioxidants in the olive oil matrix to avoid interferences of peaks corresponding to olive oil and to clean the analytical column with THF after each injection (Table II).

Each compound was identified by comparison of its retention time with corresponding peak in the standard solution and its UV spectrum. Quantification was carried out using a calibration plot of external standard. In the stability test in simulant D, some antioxidants were eluted with small chromatographic peaks corresponding to olive oil; so, antioxidant response was corrected by subtracting the olive oil blank response.

Stability test procedure

Individual standard solutions of each antioxidant were prepared in all aqueous simulants (A, B, and C) and in olive oil at concentrations around their SML and were submitted to different temperatures (5, 40, and 70°C) during an interval of 20 days. Periodically, the solutions were analyzed by HPLC-UV diode-array to determine the percentage of each antioxidant that remains stable along time. The studied antioxidants are shown in Table I.

The phosphite antioxidant Irgafos 168 is a secondary antioxidant that protects the polymer during processing,[12] so its oxidation product is the main form of this antioxidant in food packagings and it is the analyte determined in this study. On the other hand, the analytical methodology that is used to determine the stability of antioxidants causes *per se* the oxidation of phosphate[13]; therefore, it is not possible to quantify the Irgafos 168 levels.

Preparation of samples

Directive 2002/72/EEC[1] establishes SML for six of the studied antioxidants (Table I); therefore, stability of antioxidants in each simulant was studied for the following concentrations: 1.5 mg kg^{-1} for AO 2246 and AO 425 and 5 mg kg^{-1} for the others.

For stability test in aqueous simulants, individual stock standard solutions of the antioxidants (1000 mg L^{-1}) were prepared in acetonitrile for BHA, DBP, BHT, and Irganox 1010, in a mixture of methanol:THF (75:25) for Ethanox 330 and in THF for Irganox 1076 and Irgafos 168. These stock solutions were diluted with acetonitrile to obtain individual working solutions at 100 mg L^{-1}; then, these working solutions were used to spike individual samples solutions of 10 mL of each antioxidant in distilled water, 3% acetic acid, and 10% ethanol at 5 mg L^{-1} for all the antioxidants except for AO 2246 and AO425 that were prepared at 1.5 mg L^{-1}. Dissolution of all antioxidants, quite poor at ambient temperature, was ensured with this way of preparation.

For stability experiments in olive oil, individual samples solutions of 25 mL of olive oil at 1.5 mg kg^{-1} for AO 2246 and AO 425 were prepared using individual stock standard solutions of 250 mg L^{-1} prepared in THF and for the other antioxidants, individual samples solutions in olive oil at 5 mg kg^{-1} were prepared using individual stock standard solutions at 1000 mg L^{-1} prepared in THF. The stability of Irganox 1076 could not be determined in olive oil as the performed analytical method was not suitable. Besides solutions of antioxidants in olive oil, a blank of olive oil was submitted to the same temperature and time conditions of stability test to be able to correct the chromatographic signal of antioxidants.

According to Garde et al.,[13] fully oxidized Irgafos 168 is obtained after 24 h of dissolution in THF. Therefore, this antioxidant is detected as its oxidized product because THF is used in stock standard solution preparation, as it has been indicated in the Experimental.

The sample solutions of the four simulants spiked with studied antioxidants were kept into individual transparent glass vials and stored for 20 days at test

TABLE III
Temperature Conditions Selected for Stability Assays[2]

Contact temperature during use	Assay temperature (°C)
$T \leq 5°C$	5
$20°C < T \leq 40°C$	40
$40°C < T \leq 70°C$	70

migration conditions. At fixed intervals of time, portions of them were analyzed by HPLC.

Test migration conditions

The tests were carried out at 5, 40, and 70°C, according to temperature conditions established by Directive 82/711/EEC[2] that sets the migration test temperature depending on contact temperature during use (Table III). The longest time indicated by the legislation for the migration test is 10 days; therefore, several determinations of antioxidant stability were performed during the first 10 days of assay; besides, one final determination at 20 days was realized.

RESULTS

Figures 1–3 show the results obtained in the stability test of each antioxidant in simulants A, B, C, and D in a time interval of 20 days at 5, 40, and 70°C, respectively. The percent of recovery of each antioxidant has been calculated as the ratio between concentration of each antioxidant in simulant at a fixed time and the concentration of that antioxidant at the beginning of the test. It was considered that, a decrease >50% of the initially added amount of each antioxidant in a food stimulant shows that the substance is not stable in that food simulant at specified test conditions.[6]

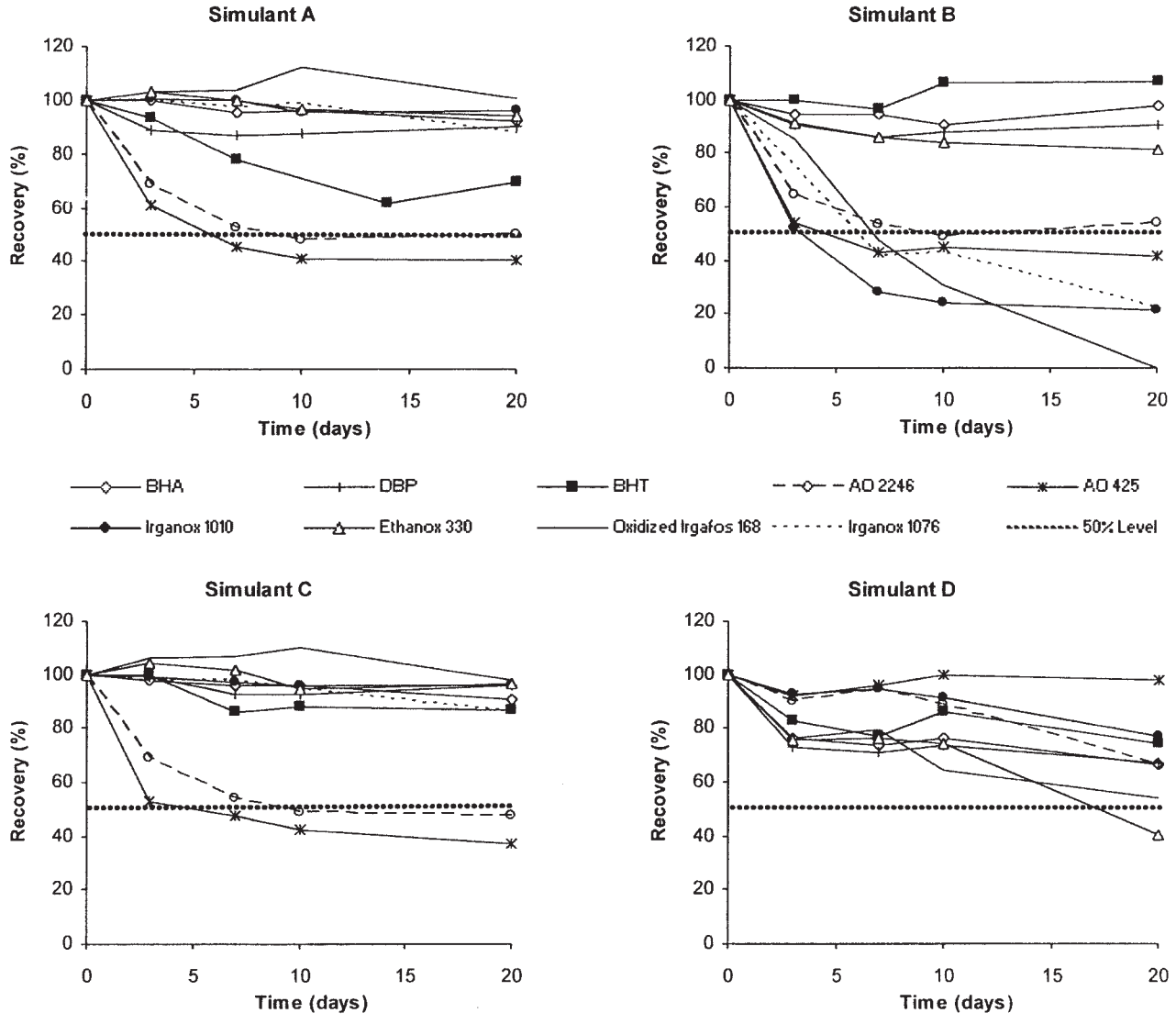

Figure 1 Stability test for antioxidants in food simulants at 5°C.

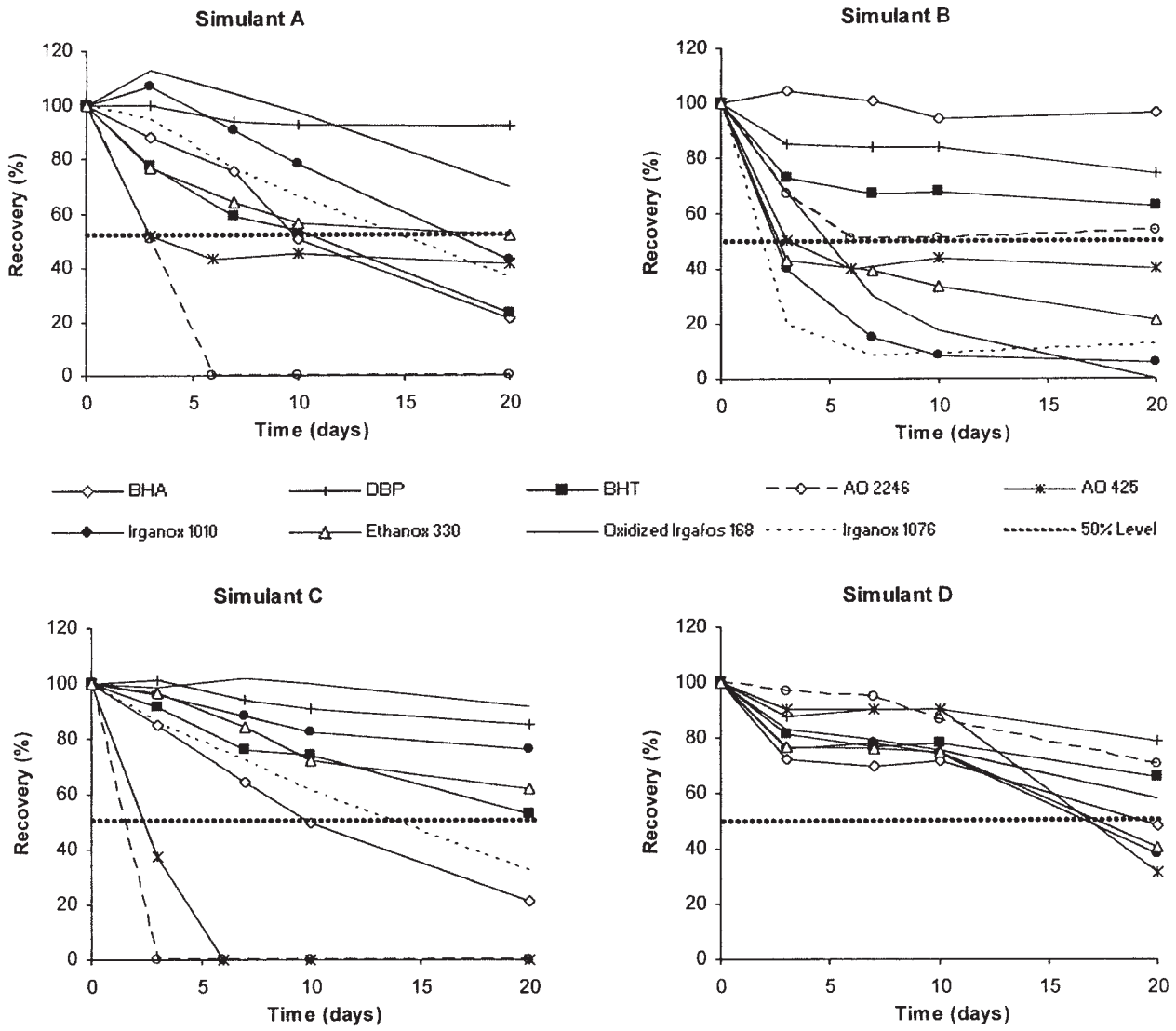

Figure 2 Stability test for antioxidants in food simulants at 40°C.

As it has been indicated, the performed chromatographic method to determine the antioxidants in olive oil was not suitable for Irganox 1076; so, its stability study in olive oil could not be carried out. Other extraction methods tested by liquid extraction (LLE) or solid phase extraction (SPE) did not allow its determination either. In this way, in the consulted references, other authors were not able to recover Irganox 1076 by SPE or LLE[5,7] from olive oil. Only O'Brien et al.[7] determined Irganox 1076 in this fat simulant by a HPLC method, where a fluorescence detector was necessary to decrease the olive oil response, maintaining the response of the additive. This method was used again in later works.[8,14]

Figures of each simulant at studied temperatures are compared to carry out the analysis of results. As it can be seen in Figure 1, at 5°C, stability of antioxidants is quite good. The curves of the figures in simulants A and C are very similar. Most of the compounds appear to be stable during the entire assay without decreasing their recoveries, except for AO 2246 and AO 425. These compounds were partially decomposed during the first days of assay and then their recoveries were kept constant around 40–50% until the end of the test.

In simulant B, BHA, BHT, and DBP (phenolic compounds of lower molecular weight) and E330 appeared to be stable during the entire assay, similar to the way they showed in simulants A and C. The recoveries of medium molecular weight compounds AO 2246 and AO 425 were decreasing in the first days of assay until levels of 40–50% that kept constant until the end of the test, similar to the way they showed in simulants A and C. On the other hand, compounds of higher molecular weight Irganox 1010, Irganox 1076, and oxidized Irgafos 168 were unstable at 10 days.

Simulant D allowed the highest stability for all the antioxidants at 5°C, only at the end of test, the recov-

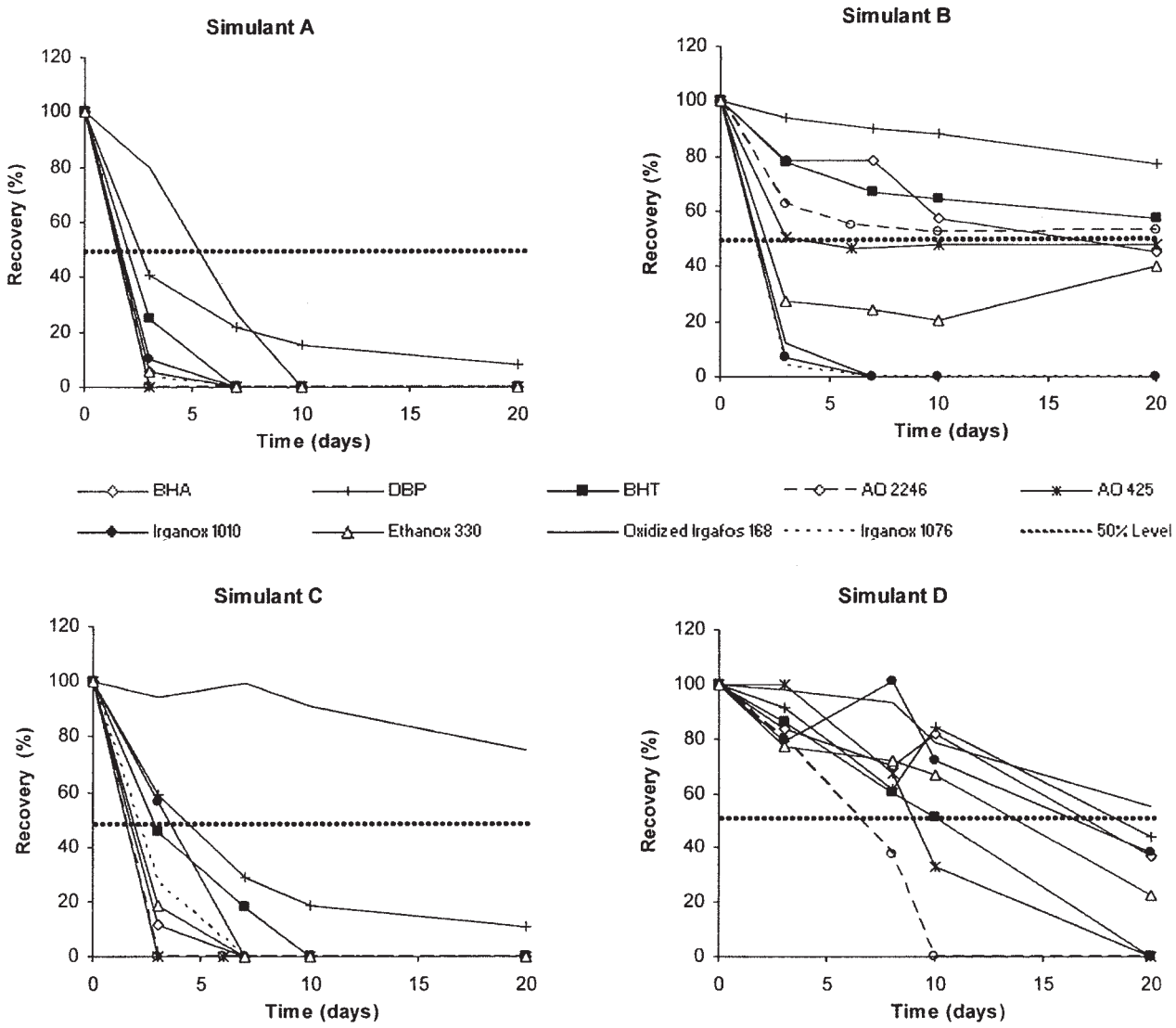

Figure 3 Stability test for antioxidants in food simulants at 70°C.

eries of compounds decreased making E330 unstable at 20 days with a recovery lower than 50%.

Figure 2 shows the results obtained at 40°C. In simulant A, the most stable compounds during the assay were DBP and oxidized Irgafos 168, hydrolysis and oxidation products of Irgafos 168, respectively. Regarding the rest of the compounds, at 10 days, AO2246 was not stable, while, AO425, BHT, BHA, and E330 showed a recovery around 40–60%, and Irganox 1010 and Irganox 1076 appeared to be stable. At 20 days, BHA and BHT were also unstable as AO2246. E330 and AO 425 maintained a recovery around 40–60%, while, Irganox 1010 and Irganox 1076 levels decreased until this level. Therefore, in simulant A, antioxidants suffered a fast decomposition by increasing the temperature from 5 to 40°C. AO 2246 became unstable during the first days of test, and at 20 days, the most stable compounds were DBP and Irgafos 168, degradation products of Irgafos 168.

In simulant C, at 10 days, AO 2246 and AO 425 were not stable, while, BHA showed a recovery around 50%. At 20 days, the number of unstable compounds grew up, with BHA and Irganox 1076 being unstable in addition to AO 2246 and AO 425. Also, BHT and E330 showed recoveries around 50–60%. Therefore, if temperature increases from 5 to 40°C, instability of AO 2246 and AO 425 increases in a short time. Besides, this increase of temperature makes BHA and Irganox 1076 become unstable in a longer time.

In simulant B, the lower size phenolic compounds BHA, BHT, and DBP appeared to be stable during the entire experiment. The medium molecular weight compounds AO 2246 and AO 425 remained more stable in simulant B than in the other aqueous simulants, showing the same curve of stability to that at 5°C. In contrast, high molecular weight compounds, Irganox 1010, Irganox 1076, oxidized Irgafos 168, and E330, suffered faster degradation in simulant B

than in A or C, and at 5 days, they were not stable, yet. In this way, the high molecular size compounds are easily decomposed in simulant B at 40°C than at 5°C.

At 5°C, simulant D allowed the best stability for all studied antioxidants along the time. Only at the end of the assay, at 20 days, it can be seen that recoveries have decreased, being lower than 50% for AO 425, E330, and Irganox 1010 and around 50% for BHA. Therefore, the increase of temperature from 5 to 40°C affects the stability of the studied antioxidants in long intervals of time, but not in a shorter time.

Considering the results at 70°C (Fig. 3), it can be seen that curves of simulant A and C are very similar, with a faster degradation for most of the antioxidants than that at lower temperatures. The only compound that remains stable during the entire test is oxidized Irgafos 168 in simulant C. Rest of the antioxidants showed a very low recovery from the start of assay in both simulants.

At 70°C, stability is better in simulant B than in other aqueous simulants for all antioxidants, except for oxidized Irgafos 168. The low molecular weight antioxidants DBP and BHT appeared to be stable during the entire assay and BHA until 10 days. Stability of AO 2246 and AO 425 was similar to the stability in the low temperature assays (5 and 40°C) in simulant B. Once again, the compounds that suffered more degradation in simulant B were the high molecular weight antioxidants E330, Irganox 1010, Irganox 1076, and oxidized Irgafos 168 as happened at 5 and 40°C; so, these compounds were unstable from the first days of assay at 70°C.

In simulant D, at 70°C, antioxidants were not stable during the entire assay, in contrast to that observed at lower temperatures, but recoveries were decreasing gradually. At 10 days from the beginning of the assay, all compounds appeared to be stable, except AO 2246 and AO 425. At 20 days, recoveries of all compounds have decreased substantially and only oxidized Irgafos 168 could be considered stable. It must be noted that compound quantification in the assay at 70°C was difficult, especially for analysis at 20 days, because chromatographic signal corresponding to olive oil was suffering changes along the assay, showing new chromatographic peaks. Quantification was corrected by subtracting the signal due to a blank of olive oil submitted to the stability assay temperature-time conditions.

Observing the temperature effects on the antioxidants, it can be seen that, in general, the rise in temperature causes an increase of degradation, although there are differences between simulants. So, degradation increases substantially with the temperature in simulants A and C, whereas, antioxidants are hardly affected in simulants B and D. In simulant B, antioxidant stability of low size phenolic antioxidants BHA, BHT, and DBP is quite good even at high temperatures, although recoveries are low when temperature increases. They are stable at 10 days even in the test at 70°C and recovery only decreases upto 50% at 20 days. The medium molecular weight compounds AO 2246 and AO 425 are not affected by the increase in temperature, showing recoveries very similar at all tested temperatures. The high molecular weight antioxidants Irganox 1010, Irganox 1076, and oxidized Irgafos 168 are the most degradated compounds at 70°C and their recoveries are lower when temperature increases.

Similarly, simulant B (3% acetic acid, w/v) would be protecting low molecular weight phenolic compounds against high temperatures. This agrees with published results; decomposition of phenolic compounds is substantially slower in acid solvents than in neutral or basic. However, high molecular weight compounds are very unstable in this simulant, even at low temperatures.

In simulant D, effects on the stability are not observed until the highest temperature of the assay, i.e., 70°C. Stability for the antioxidants in olive oil is very good at all tested temperatures during the first 10 days of experiment, although recoveries go decreasing when temperature is increased.

Comparing the obtained results with other published works, it has been seen that consulted references do not show many data about stability of the antioxidants that have been studied in this work, only some data about BHT, Irganox 1010, AO 2246, and Irganox 1076 have been found. Migration studies of BHT[15] and Irganox 1010[15,16] from low density polyethylene (LDPE) or copolymers of ethylene and vinyl acetate films into food simulants or foods were performed and it was found that both antioxidants were degraded in 8% ethanol solution or 50% ethanol after some days at 49°C. This agrees with the results that have been obtained in our assays at 70°C, where both antioxidants are completely degraded during the assay either in simulant A (distilled water) or in simulant C (10% ethanol, v/v), as shown in the Figure 3.

However, results obtained for Irganox 1076 (Fig. 2) disagree with the work of Simoneau and Hannaert (1999).[5] An Irganox 1076 recovery of 97% in 15% ethanol and 103% in 3% acetic acid have been obtained for a stability assay of 10 days and 40°C by these authors. For 1h/100°C, they achieved 104% in 15% ethanol and 105% in 3% acetic acid. Their recoveries were higher than obtained in our work. On the other hand, the stability of AO 2246 has been studied by Spyropoulos,[17] but he did not determine in 3% acetic acid and 15% ethanol at 40°C because AO 2246 was not soluble at its SML at that temperature. They obtained a 100% of recovery for 1 h at reflux in 15% ethanol and 83% for 1 h at 100°C in 3% acetic acid. Observed differences could be caused by the preparation conditions of spiked simulants. Simoneau and

Hannaert[5] spiked simulant with a ratio stock solution volume/simulant volume of 1 μL/mL, while, in the present work, Irganox 1076 solution was prepared with a ratio of 50 μL/mL. Spyropoulos[17] spiked simulant with AO 2246 with a ratio of 2 μL/1 mL, while, in this work, it was prepared with a ratio of 15 μL/mL, that ensures solubility of all compounds at ambient temperature.

In olive oil, there are only bibliographic data (as far as we know) about Irganox 1076 stability,[8,14] which could not be studied in this work, and AO 2246. So, Spyropoulos [17] determined stability of AO 2246 in olive oil at a concentration twice its SML and a recovery of 72% was achieved for an assay of 10 days/40°C. In the same conditions of time and temperature, the recovery obtained in present assay was 87% (Fig. 2). The stability test was carried out at higher temperature: 1 h at 100°C in olive oil obtaining 100% recovery and 1 h at 175°C where recovery was 85%. In the assay performed in this study at 70°C, AO 2246 appeared to be stable at 3 days with a recovery of 80%, but at the next measurement, at 8 days, showed a recovery of 37% (Fig. 3).

CONCLUSIONS

The following conclusions can be drawn from the present study:

- Comparing the results for each simulant when temperature is increased, it can be seen that, in general, antioxidants are less stable at high temperatures. In simulants A and C, this temperature effect is higher than that in B and D.
- Comparing the effect of each simulant on antioxidant stability at a fixed temperature, it is noted that olive oil is the simulant that allows the highest stability of the studied compounds. All considered antioxidants are stable in olive oil during 10 days at 5, 40, and 70°C, except AO 2246 and AO 425 at 70°C.
- The low molecular weight phenolic compounds BHA, BHT and DBP are stable in simulant B even at high temperatures, whereas they are stable in the other aqueous simulants only at low temperatures.
- Medium molecular weight phenolic compounds AO 2246 and AO 425 show a behavior independent of temperature in simulant B.
- The high molecular weight antioxidants are very unstable in simulant B even at low temperatures, while, they are stable at low temperatures in simulants A and C.
- It is noted that oxidized Irgafos 168 is stable in simulant C even at 70°C.

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