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Antioxidant Content of and Migration from Commercial Polyethylene, Polypropylene, and Polyvinyl Chloride Packages

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Antioxidants commonly used in polyolefins were studied in commercial food packages made of low- and high-density polyethylene (LDPE and HDPE), polypropylene (PP), polyvinylchloride (PVC), and polyethylene terephtalate (PET) and in a LDPE film extruded at the laboratory. The phenolic antioxidants BHA, BHT, AO 2246, AO 425, Ethanox 330, Irganox 1010, and Irganox 1076 were studied together with the phosphite Irgafos 168 and their two degradation products, phosphate and DBP. Antioxidants were extracted from polyolefins using microwave energy and analyzed using high-performance liquid chromatography (HPLC) to determine the antioxidant content in the diverse commercial films. Irganox 1010 and Irganox 1076 were found in the majority of the samples generally together with the phosphite Irgafos 168 and its oxidized product (phosphate). Specific migration levels of each antioxidant were determined by HPLC after pretreatment with solid-phase extraction (SPE) in aqueous food simulants and after their dilution with tetrahydrofuran (THF) in fatty food simulant. These levels were much lower than limits allowed by legislation.

KEYWORDS: Antioxidants; food package; HPLC; migration

INTRODUCTION

Packages protect food against external pollution, although it is necessary to have in mind that food packaging is not completely inert (*1*). In this way, additives and other substances present in plastic packages that can migrate into the food and pollute it are regulated by legislation through composition limits in the plastic or migration limits into food (*2*, *3*). However, one of the problems for controlling the migration of substances into food is the fact that the substances that can be transferred to food from each plastic package are unknown (*4*, *5*). Therefore, different studies have been made on commercial plastic packages to screen their potential migrants (*6*−*8*) or to determine their migration levels in food simulants (*6*, *9*−*11*).

Antioxidants are key ingredients in the compounding of polyethylene and polypropylene due to the limited stability of polyolefins to high temperatures and ultraviolet (UV) light (*12*). Determination of antioxidant levels in polyolefinic material gives information about their potential migration and at the same time a measurement of plastic quality. Although there are many studies on the migration of antioxidants from polyolefinic materials, in general, they are focused on a few antioxidants, of which the phenolic antioxidants Irganox 1010 or Irganox 1076 and the phosphite Irgafos 168 (*13*−*15*) are the most usual.

Because antioxidants in polyolefinic packages are widely used and taking into account the unknown plastic composition, a greater range of antioxidants of different molecular weights, commonly used in polyolefins, will be studied here. These are several phenolic antioxidants, two lower molecular weight compounds, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); two medium molecular weight compounds, AO 2246 and AO 425; and several high molecular weight compounds, Ethanox 330, Irganox 1010, and Irganox 1076. Besides these phenolic compounds a phosphite antioxidant, Irgafos 168, is considered together with two degradation products: its hydrolysis product, DBP, and its oxidation product, phosphate.

The objective is to study the presence of these phenolic and phosphite antioxidants in different commercial packages and their migration levels into the four food simulants allowed by legislation (*16*): simulant A (distilled water), simulant B (acetic acid 3%), simulant C (ethanol 10%), and simulant D (olive oil). Additionally, a low-density polyethylene (LDPE) film extruded in our own laboratory is studied to investigate the possible relationship between matrix properties and migration levels.

The technique of extraction assisted by microwave energy has been used to extract additives from plastic materials by different authors with good results in short times (*17*−*21*). For this reason, the extraction of antioxidants from the polymeric matrix will be performed using microwave energy and followed by their quantification using high-performance liquid chromatography−ultraviolet (HPLC-UV) diode array according to the conditions obtained in a previous study (*22*).

Specific migration tests will be carried out with HPLC-UV diode array determination after a preconcentration step with SPE

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Table 1. Selected Antioxidants

	chemical name	CAS Registry No.	M_w	SML[a] (mg kg^{-1})	source
AO 2246	2,2′-methylenebis(4-methyl-6-*tert*-butylphenol)	119-47-1	340.5	1.5[b]	
AO 425	2,2′-methylenebis(4-ethyl-6-*tert*-butylphenol)	88-24-4	368.6		
BHA	butylated hydroxyanisole (mixed isomers 2[3]-*tert*-butyl-4-hydroxyanisole; 2[3]-*tert*-butylhydroquinone monomethyl ether, minimun 90% 3 isomer/9% 2-isomer)	25013-16-5	180.2	30	Sigma-Aldrich (Steihheim, Germany)
BHT	2,6-di-*tert*-butyl-*p*-cresol	128-37-0	220.4	3.0	
DBP	2,4-bis(*tert*-butyl)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		
Irganox 1010	pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate	6683-19-8	1178		Ciba (Basel, Switzerland)
Irganox 1076	octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate	2082-79-3	531	6.0	

[a] Allowed by Commisson Directives 2002/72/EC (*2*) and 2004/19/EC (*29*). [b] Limit allowed by legislation for the sum of both compounds.

C_{18} for aqueous simulants or after dilution of the sample with tetrahydrofuran (THF) for olive oil. In this study a solid-phase extraction (SPE) method (*23*) will be used to determine migration levels of antioxidants in aqueous simulants instead of the liquid−liquid extraction method developed previously (*24*) because this method produces the best recoveries and precision results. Typically, for the analysis of antioxidants in olive oil, HPLC after dilution of sample with THF or acetone is used (*25*−*27*) because of great difficulties to achieve their extraction employing another analytical methodology. In fact, in the consulted references, liquid−liquid extraction allowed the recovery of only BHT and AO 2246 from sunflower oil (*10*), whereas Irganox 1076 could not be determined in olive oil (*28*).

EXPERIMENTAL PROCEDURES

Chemicals. The studied antioxidants were obtained from the sources presented in **Table 1**. It is noted that the antioxidant DBP is a hydrolysis product of the phosphite Irgafos 168 (*30*).

Methanol, ethanol, and THF of HPLC gradient grade were supplied by Merck (Darmstadt, Germany). Acetonitrile and dichloromethane of ultragradient HPLC grade were supplied by J. T. Baker (Deventer, The Netherlands). Water was purified on a Milli-RO system (Millipore, Bedford, MA). Glacial acetic acid (HPLC) was supplied by Panreac Quimica (Barcelona, Spain). Olive oil was used as fatty food simulant.

Filter papers 40 ashless, circles of 125 mm, were from Whatman (Maidstone, U.K.). Filters 45 μm, 4 mm in diameter, were from Waters (Milford, MA).

HPLC. A Waters 2695 instrument (Waters) with a gradient pump and an automatic injector was used. The nine analytes were completely separated using a stainless steel column, 3.0 × 150 mm, packed with Symmetry C_{18}, 3.5 μm particle size (Waters). The detection system was a model 996 UV photodiode array (Waters). The signal acquired from the detector was recorded by a personal computer operating under the Millenium^{32} software v. 3.20 (Waters). The conditions of chromatographic method are shown in **Table 2**.

In determining the migration levels of antioxidants in the olive oil matrix a 100% THF step was included in the chromatographic program to avoid interferences of peaks corresponding to olive oil and to clean the analytical column.

The aqueous food simulant samples were analyzed after their SPE pretreatment, whereas fatty food simulant samples were diluted with THF before their chromatographic analysis such that 2 ± 0.01 g of sample was diluted to 5 mL with THF.

Each compound was identified by comparison of its retention time with the corresponding peak in the standard solution and its UV spectrum. The quantification of the analytes was carried out using a calibration plot of an external standard. Antioxidant response in simulant D was corrected by subtracting the olive oil blank response.

Table 2. Chromatographic Method Conditions

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

elution for aqueous simulant samples and microwave extracts
wavelength = 276 nm

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 fatty food simulant (olive oil) samples
wavelength = 220 nm

time (min)	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

Polymer Samples. *Commercial Package.* The studied commercial samples were polymeric packages used for different kinds of foods from the supermarket. Laboratory sample code, specific commercial use, and type of material are given for each sample (**Table 3**). Materials at first unknown were identified by Fourier transform infrared spectrometry (FTIR) analysis using a Broker system (Ettlingen, Germany).

Polymer Samples Extruded in the Laboratory. A nonstabilized LDPE Lupolen 1840H from Basell was selected in this study. LDPE film was extruded in the laboratory by using a Brabender DSE 20 double-screw extruder with five heating zones with the following zone temperature settings: 210/200/200/200/200/200 °C. Initially, a LDPE master batch containing 0.2% (w/w) Irgafos 168 and 0.4% (w/w) Irganox 1076 was produced by mixing the LDPE granules free of additives with the suitable amount of antioxidants. This mixture was re-extruded several times (n = 5) to obtain granules with a homogeneous distribution of antioxidants.

Table 3. Studied Commercial Package Samples

sample code	type of material	food packaged	reference no.[a]
CL01	HDPE	yogurt	07, milk products
CL02	HDPE	frozen vegetables/paste	08, miscellaneous products
CL03	LDPE	biscuits	02, cereals and bakers' wares
CL04	LDPE	bread	02, cereals and bakers' wares
CL05	LDPE	bread	02, cereals and bakers' wares
CL06	LDPE	bread	02, cereals and bakers' wares
CL07	LDPE	integral rice	02, cereals and bakers' wares
CL08	LDPE	pasta	02, cereals and bakers' wares
CL09	LDPE	fresh vegetables	04, fruit and vegetables
CL10	LDPE	fresh vegetables	04, fruit and vegetables
CL11	LDPE	grated cheese	07, milk products
CL12	LDPE	whole milk	07, milk products
CL13	LDPE	whole milk	07, milk products
CL14	LDPE	frozen vegetables	08, miscellaneous products
CL15	LDPE	frozen vegetables	08, miscellaneous products
CL16	LDPE	frozen prawn	08, miscellaneous products
CL17	LDPE	ice cube	08, miscellaneous products
CL18	PET	cheese	07, milk products
CL19	PP	biscuits	02, cereals and bakers' wares
CL20	PP	bread	02, cereals and bakers' wares
CL21	PP	bread	02, cereals and bakers' wares
CL22	PP	bread	02, cereals and bakers' wares
CL23	PP	toast bread	02, cereals and bakers' wares
CL24	PP	quince jelly	04, fruit and vegetables
CL25	PP	cheese	07, milk products
CL26	PP	not determined[b]	
CL27	PP	not determined[b]	
CL28	PP	not determined[b]	
CL29	PVC	small stick bread	02, cereals and bakers' wares

[a] Reference number given by Council Directive 85/572 CEE (*16*). [b] Material before making the package.

Table 4. Concentration of Studied Antioxidants in Studied Commercial Packages

μg g^{-1}	DBP	BHT	AO 2246	AO 425	Irganox 1010	E330	Irgafos 168 ox	Irganox 1076	Irgafos 168
CL01	nd[a]	nd	nd	nd	nd	nd	nd	nd	28
CL02	nd	nd	nd	nd	102	nd	219	190	143
CL03	nd	nd	nd	nd	376	nd	369	100	429
CL04	nd	nd	nd	nd	nd	nd	1490	125	56
CL05	nd	nd	nd	nd	nd	nd	129	nd	nd
CL06	nd	nd	nd	nd	nd	nd	21	nd	221
CL07	nd	nd	nd	nd	369	nd	386	nd	252
CL08	nd	nd	nd	nd	215	nd	399	44	358
CL09	nd	nd	nd	nd	228	nd	427	nd	123
CL10	nd	nd	nd	nd	556	nd	815	nd	483
CL11	nd	nd	nd	nd	nd	nd	nd	nd	nd
CL12	nd	nd	nd	nd	nd	nd	224	96	34
CL13	nd	nd	nd	nd	80	nd	252	129	343
CL14	nd	nd	nd	nd	nd	nd	342	105	343
CL15	nd	nd	nd	nd	143	nd	407	138	216
CL16	nd	nd	nd	nd	26	nd	175	326	428
CL17	nd	nd	nd	nd	nd	nd	nd	nd	nd
CL18	nd	nd	nd	nd	53	nd	214	100	147
CL19	nd	nd	nd	nd	1216	nd	2416	60	526
CL20	nd	nd	nd	nd	244	nd	498	73	355
CL21	nd	nd	nd	nd	315	nd	607	nd	303
CL22	nd	nd	nd	nd	391	nd	714	66	154
CL23	nd	nd	nd	nd	299	62	404	nd	240
CL24	nd	nd	nd	nd	119	nd	55	nd	173
CL25	nd	nd	nd	nd	204	nd	172	nd	183
CL26	3	14	6	5	162	nd	340	132	229
CL27	28	32	nd	61	360	nd	880	nd	315
CL28	nd	nd	nd	nd	274	nd	728	nd	nd
CL29	nd	nd	nd	nd	497	nd	1523	nd	220

[a] Not detected (<DL).

Part of this initial master batch was extruded directly to obtain the first film sample, CL30, which was to contain approximately 1000 μg g^{-1} of Irgafos 168 and 2000 μg g^{-1} of Irganox 1076. Another part of the initial master batch was mixed with virgin LDPE granules in the ratio of 1:4 and was again extruded to obtain the second film sample, CL31, at concentrations of approximately 1500 μg g^{-1} of Irgafos 168 and 3000 μg g^{-1} of Irganox 1076.

Due to the characteristics of the extrusion process the exact concentrations in the polymer are not known; the theoretical values may have decreased through thermal degradation or handling.

Extraction of Antioxidants from Polymeric Film. Extraction by microwave energy was performed using a Milestone microwave laboratory system ETHOS TC (Sorisde, Italy) equipped with a 10-vessel position carousel; the instrument is temperature controlled.

A film sample is cut into small pieces of approximately 0.5 × 1 cm and extracted by microwave energy under following conditions: 2 g of sample, 30 mL of dichloromethane extraction solvent, a 2 min heating time, a 1 min extraction time, and a temperature of 55 °C. After extraction, the vessels were allowed to cool to ambient temperature, and the liquid phase was filtered (through ashless filters) and directly analyzed by HPLC-UV diode array using the chromatographic conditions shown in **Table 2** for aqueous samples.

Migration Test. *Commercial Samples.* Migration tests were performed using cells with a single side contact. One film of sample, approximately 1 dm^2, was put in contact with 165 mL of simulant. The conditions of the migration tests were 10 days at 40 °C using simulants of distilled water (simulant A), acetic acid at 3% (simulant B), ethanol at 10% (simulant C), and olive oil (simulant D) (*2*). These conditions (40 °C and 10 days) are the most severe conditions indicated by European legislation (*31*) for plastic materials intended to come into contact with foodstuff at room temperature or below for an unspecified period.

Blanks with migration cells were made for all procedures to check for interferences.

Extruded Samples. Thermally sealed bags were used to study specific migration from samples CL30 and CL31. As the studied samples were too narrow to use the migration cells, tubes of approximately 25 cm × 4 cm were made to mantain the ratio of 100 mL of simulant to 2 dm^2 of surface recommended by legislation (*32*). The sealed bags were filled with simulants A−D and were stored at 40 °C for a period of 10 days.

Extraction of Antioxidants from Simulant. *Aqueous Simulants.* The samples were pretreated with C_{18} cartridges Sep-Pak Plus 360 mg purchased from Waters. A Büchi (Flawil, Switzerland) V-500 vacuum system equipped with a pressure controller Büchi B-721 was used to force the sample through the cartridge.

An amount of 100 mL of aqueous food simulant samples (A, B, or C) was modified by adding acetic acid and/or ethanol until a concentration of 3% (v/v) of acetic acid and 10% (v/v) of ethanol was reached for all samples. Before extraction, silica C_{18} cartridges were conditioned first with 4 mL of methanol and then with 4 mL of distilled water. The aqueous food simulant samples were percolated at a flow rate of approximately 1−2 mL min^{-1}, and the vacuum system was adjusted to 970 mbar or less to maintain this flow rate. Elution of the retained antioxidants was carried out with 5 mL of methanol and 3 mL of THF. Both solvents were passed sequentially and collected together. The final extract was analyzed by HPLC (**Table 2**).

Fatty Food Simulant (Olive Oil) Samples. An amount of 2 ± 0.01 g of olive oil was diluted with THF to a volume of 5 mL before the quantification of antioxidants by HPLC (**Table 2**).

RESULTS AND DISCUSSION

Polymeric Commercial Package. First, the concentration of the selected antioxidants was determined for each package. Then, for a selected number of samples the specific migration levels were determined in the aqueous food simulants A−C and in the fatty food simulant (olive oil).

Determination of Antioxidants with MAE-HPLC. The extraction method employed in this study was optimized in a previous study (*22*) to quantify Irganox 1076, Irgafos 168, and oxidized Irgafos 168 in a LDPE matrix. However, in this study it has also been used for other polymeric matrices: HDPE, LDPE, PP, and PVC.

Table 4 shows antioxidant levels determined in the studied commercial packages. The majority of analyzed commercial

Table 5. Comparison between Antioxidant Concentration in Samples and Levels Allowed by Legislation

			max concn allowed (%)	
antioxidant	sample	max concn found (%)	FDA Title 21 178.2010 (*3*)	British Standard 1992 (*33*)
AO 2246	CL26	0.0006	0.1	
AO 425	CL27	0.006		0.5
Irganox 1010	CL19	0.12	0.5	0.5
Ethanox 330	CL23	0.006	0.5	
Irgafos 168[a]	CL19	0.29	0.2 or 0.25	
Irganox 1076	CL16	0.03	0.25	0.5

[a] It has been considered the sum of phosphite and phosphate.

samples contain a number of the studied antioxidants, mainly the high molecular weight compounds. Only two LDPE samples did not show the presence of any of the studied antioxidants in their formulations.

Only two PP samples, CL26 and CL27, contained the lowest molecular weight antioxidants, BHT, AO 2246, and AO 425, at levels in the range of 5−61 μg g^{-1}. This is much lower than the highest molecular weight antioxidants, which were also determined in these samples. Only sample CL23, intended to pack toasted bread, showed the presence of Ethanox 330, in addition to the other three high molecular weight antioxidants. On the other hand, the highest molecular weight compounds were determined in the majority of samples (93%) at levels between 26 and 2416 μg g^{-1}: the phenolic compounds Irganox 1010 and Irganox 1076 were determined in 72 and 48% of the samples, respectively. Irgafos 168 was determined as phosphite in 86% of the samples, phosphate (oxidized product of Irgafos 168) in 90% of the samples, and DBP in 7% of the samples. Irgafos antioxidant appeared as expected with its oxidized product (83% of the samples) because this compound is used as a processing antioxidant. Both compounds have been quantified separately because the extraction method avoids degradation of Irgafos 168. The presence of Irgafos 168 in most of the samples is indicative that the initial concentration was adequate to protect the polymer during packaging manufacture.

The highest value obtained for each antioxidant in the analyzed samples was compared (**Table 5**) with the levels allowed by the FDA (*3*) and British Standard 1992 (*33*). Only one of the analyzed samples, CL19, showed a level of Irgafos 168 higher than the limits allowed by FDA, whereas the rest of the values obtained are noticeably lower.

The composition of the mixtures of antioxidants used in the samples was checked. It should be noted that Irganox 1010 or Irganox 1076 was always found together with Irgafos 168 or some of its degradation products. As can be seen in **Figure 1**, the most usual mixtures were Irgafos 168 with one of the phenolic compounds, Irganox 1076 (10%), Irganox 1010 (28%), or both (34%), showing their widespread joint use.

The possible relationship between the composition of the mixture of antioxidants and the type of material or the type of packaged food was also studied.

Figure 2 shows the composition of the antioxidant mixture determined for the two main types of materials studied: PP and LDPE. Irgafos 168 considered as the sum of the phosphite compound and its two degradation products, phosphate and DBP, was the most abundant antioxidant for both LDPE (63−100%) and PP (63−73%). The percentage of degraded antioxidant was different for each individual sample. On the other hand, the content of Irganox varied depending on the type of material. Whereas Irganox 1010 appeared more often than

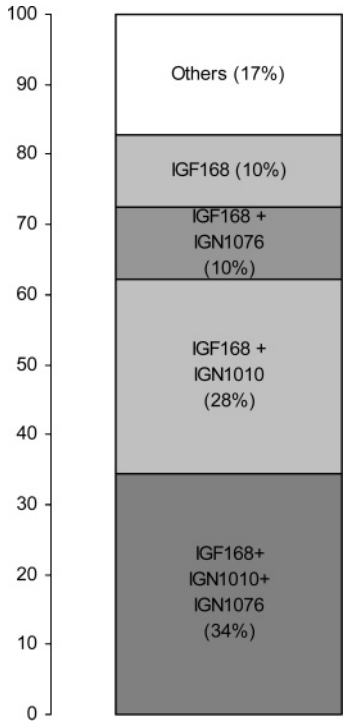

Figure 1. Percentages of commercial package samples (shown in **Table 4**) that contain different mixtures of the studied antioxidants.

Irganox 1076 in the PP samples, both Irganox 1010 and Irganox 1076 appeared in the same number of samples of LDPE. No relationship could be established between the composition of the sample and the type of food.

In summary, in the studied antioxidants that cover a molecular weight range from 180 to 1178 uma, the highest molecular weight compounds were ones that were determined the most frequently. Additionally, it was found that the combinations of Irgafos 168 with a phenolic antioxidant of Irganox 1010 or Irganox 1076 are commonly used in the fabrication of commercial food packaging, in different percentages according to the type of material used. Finally, the simultaneous presence of Irgafos 168 and its oxidized product in the majority of studied samples confirms the interest of microwave extraction as a tool to control the plastic material quality.

Determination of Specific Migration Levels. Four samples were chosen to carry out specific migration tests: CL04, CL19, CL27, and CL28.

For SPE C_{18}-HPLC-UV an analytical method (aqueous samples) repeatability assay was performed using samples (n = 6) of 100 mL spiked with 0.5 mg L^{-1} of each antioxidant (recoveries between 84 and 103%), obtaining a relative standard deviation between 1.6 and 8.3%. For the dilution HPLC method (olive oil) a sample of oil spiked with 5 mg L^{-1} of each antioxidant was analyzed on different days (n = 6), obtaining a relative standard deviation between 1.3 and 13%. **Table 6** shows detection and quantification limits of the analytical methods used, SPE-HPLC for aqueous simulants and dilution HPLC for olive oil, together with specific migration limits of each antioxidant in the contact conditions.

Potential migration levels expected for each matrix were calculated by considering that 100% mass transfer of antioxidant to simulant occurred from the films, that is, the worst case for the migration test. For this calculation, initial antioxidant concentration levels in the film (determined by microwave study) and mass of the film in contact with the simulant were considered.

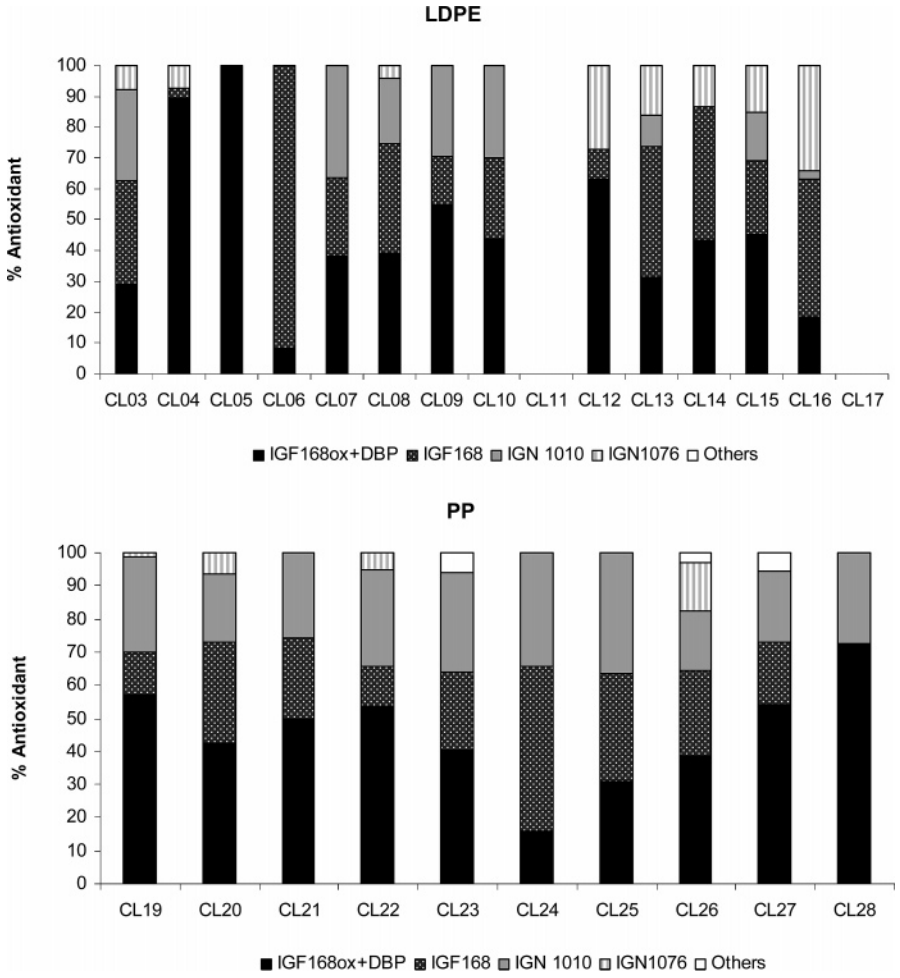

Figure 2. Percentages of antioxidants Irganox 1010, Irganox 1076, and Irgafos 168 (as sum of phosphite, phosphate, and DBP) in LDPE and PP commercial package samples.

Table 6. Detection and Quantification Limits for Proposed Analytical Methods of Specific Migration and Specific Migration Limits (SML) Allowed by Legislation

cells with rate 165 mL: 1 dm^2 (simulant volume: plastic surface)					
		aqueous simulants (SPE)		simulant D (dilution)	
	SML (mg dm^{-2})	DL (mg dm^{-2})	QL (mg dm^{-2})	DL (mg dm^{-2})	QL (mg dm^{-2})
BHA	5.0[a]	4.2×10^{-3}	1.4×10^{-2}	4.4×10^{-2}	1.5×10^{-1}
DBP		4.4×10^{-3}	1.5×10^{-2}	6.3×10^{-2}	2.1×10^{-1}
BHT	0.50[a]	5.1×10^{-3}	1.7×10^{-2}	7.7×10^{-2}	2.6×10^{-1}
AO 2246	0.25[b,c]	4.6×10^{-3}	1.5×10^{-2}	5.7×10^{-2}	1.9×10^{-1}
AO 425		4.3×10^{-3}	1.4×10^{-2}	5.9×10^{-2}	2.0×10^{-1}
Irganox 1010	without SML[b]	3.6×10^{-3}	1.2×10^{-2}	1.2×10^{-1}	3.9×10^{-1}
Ethanox 330	without SML[b]	3.7×10^{-3}	1.2×10^{-2}	1.3×10^{-1}	4.5×10^{-1}
Irgafos 168 ox	without SML[b]	4.7×10^{-3}	1.6×10^{-2}	1.6×10^{-1}	5.4×10^{-1}
Irganox 1076	0.99[b]	4.5×10^{-3}	1.5×10^{-2}	not determined	

[a] Commission Directive 2004/19/CE of 1 March 2004 (*29*). [b] Corrigendum OJ L39 13/2/2003, p 1 Directive 2002/72/CE (*2*). [c] Specific migration limit total allowed for the sum of both compounds.

These potential migration levels considering 100% migration (**Table 7**) are considerably lower than SMLs allowed by legislation. For aqueous simulants the quantification limits achieved with the SPE method allowed the determination of the studied compounds, except for DBP and BHT in sample CL27. However, for simulant D, the dilution method has higher detection and quantification limits, so that it allows quantification of only the sum of Irgafos 168 (as oxidized compound) and the detection of Irganox 1010, whereas Irganox 1076 cannot be quantified.

Experimental migration values for each sample are also shown in **Table 7**. Experimental migration levels of the studied antioxidants were extremely low and much lower than the potential values, so that only traces of the studied antioxidants were detected in the aqueous simulants from samples CL04, C27, and CL28 at levels detectable but not quantifiable, whereas Irgafos 168 oxidized was the most frequently detected antioxidant.

Important differences were found by comparison of the experimental and potential (100% mass transfer) migration

Table 7. Results of Specific Migration in the Four Simulants A–D from Commercial Samples

sample	antioxidant	concn in the film (μg g^{-1})	specific migration level (mg dm^{-2}) potential 100%	experimental A	B	C	D
CL04 (LDPE) 54 μm	Irgafos 168 ox	1490	6.8×10^{-1}	*a*	*a*	nd[b]	nd
	Irganox 1076	125	5.7×10^{-2}	nd	nd	nd	nd
	Irgafos 168	56	2.5×10^{-2}	nd	nd	nd	nd
	sum of Irgafos 168	1545	7.0×10^{-1}	nd	nd	nd	nd
CL19 (PP) 28 μm	Irganox 1010	1216	4.4×10^{-1}	nd	nd	nd	nd
	Irgafos 168 ox	2416	8.7×10^{-1}	nd	nd	nd	nd
	Irgafos 168	526	1.9×10^{-1}	nd	nd	nd	nd
	sum of Irgafos 168	2941	1.1	nd	nd	nd	nd
CL27 (PP) 24 μm	DBP	28	9.3×10^{-3}	nd	nd	nd	nd
	BHT	32	1.1×10^{-2}	nd	nd	nd	nd
	AO 425	61	2.0×10^{-2}	nd	nd	nd	nd
	Irganox 1010	360	1.2×10^{-1}	*a*	nd	nd	nd
	Irgafos 168 ox	880	2.9×10^{-1}	*a*	*a*	*a*	nd
	Irgafos 168	315	1.0×10^{-1}	nd	nd	nd	nd
	sum of Irgafos 168	1195	4.0×10^{-1}	nd	nd	nd	nd
CL28 (PP) 48 μm	Irganox 1010	274	1.6×10^{-1}	nd	nd	nd	nd
	Irgafos 168 ox	728	4.2×10^{-1}	*a*	nd	*a*	nd
	sum of Irgafos 168	728	4.2×10^{-1}	nd	nd	nd	nd

[a] Detected but not quantified (DL < concentration < QL). [b] Not detected (<DL).

Table 8. Specific Migration Results in the Four Simulants A–D from Samples Extruded at the Laboratory

sample	compound	film composition (μg g^{-1})	specific migration level (mg dm^{-2}) potential 100%	experimental A	B	C	D
CL30 (LDPE) 75–77 μm	Irgafos 168 ox	243	1.7×10^{-1}	nd[a]	nd	nd	3.6×10^{-1}
	Irganox 1076	1323	9.2×10^{-1}	nd	nd	nd	nd
	Irgafos 168	334	2.3×10^{-1}	nd	nd	nd	nd
	sum of Irgafos 168	577	4.0×10^{-1}	nd	nd	nd	nd
CL31 (LDPE) 27–45 μm	Irgafos 168 ox	327	1.1×10^{-1}	nd	nd	nd	2.9×10^{-1}
	Irganox 1076	2311	7.7×10^{-1}	nd	nd	nd	nd
	Irgafos 168	668	2.2×10^{-1}	nd	nd	nd	nd
	sum of Irgafos 168	995	3.3×10^{-1}	nd	nd	nd	nd

[a] Not detectable (<DL).

levels. For instance, Irgafos 168 oxidized was detected experimentally in the aqueous simulants for samples CL04, CL27, and CL28, whereas it was not detected in any simulant for sample CL19, for which the expected potential migration level was the highest. At the same time it is important to note that this same sample, CL19, with a content of Irgafos 168 over the limit specified by the FDA (**Table 5**), does not present a detectable migration.

Polyolefins Extruded in the Laboratory. *Determination of Antioxidants by MAE-HPLC.* Samples were analyzed using the extraction method by microwave energy and HPLC. The obtained concentration values for antioxidants Irgafos 168, Irganox 1076, and Irgafos 168 oxidized are shown in **Table 8**.

Determination of Specific Migration. The potential migration levels (migration 100%) for extruded LDPE samples were calculated by considering the initial concentration of antioxidants and the mass of the sample in contact with the simulant (**Table 8**). Because the ratio of plastic surface in contact with simulant to volume of simulant for the specific migration test is bigger using sealed bags than using cells, SML, DL, and QL were calculated again. As can be seen, quantification limits of analytical methods either in aqueous simulants or in olive oil

Table 9. SML Allowed by Legislation and DL and QL of Methods for Determination of Specific Migration in Aqueous Simulant and Olive Oil Using Sealed Bag

	sealed bag with ratio 100 mL: 2 dm^2 (simulant: area)				
		aqueous simulant (SPE)		olive oil (dilution)	
	SML (mg dm^{-2})	DL (mg dm^{-2})	QL (mg dm^{-2})	DL (mg dm^{-2})	QL (mg dm^{-2})
Irgafos 168 ox	without SML	1.4×10^{-3}	4.7×10^{-3}	4.9×10^{-2}	1.6×10^{-1}
Irganox 1076	0.30[a]	1.4×10^{-3}	4.6×10^{-3}	not determined	

[a] Corrigendum OJ L39 13/2/2003, p 1 Directive 2002/72/CE (*2*).

(**Table 9**) would allow the quantification of the sum of Irgafos 168 for a migration of 100% (phosphite and phosphate).

Table 8 also shows the experimental specific migration levels obtained. When experimental and potential migration values are compared, the following conclusions can be drawn:

In aqueous simulants experimental values were much lower than the potential values (100% migration), whereas in olive oil potential and experimental levels of Irgafos 168 (as the sum of both compounds phosphite and phosphate) were quite close. This fact confirms the high capacity of migration for antioxidants in olive oil.

If the results of both Irgafos 168 compounds, phosphite and phosphate, are observed separately, it can be seen that the experimental migration of Irgafos 168 oxidized from samples CL30 and CL31 in the simulant D was higher than its potential level (100% migration). However, when potential migration levels of both compounds are summed, the experimental value is lower than this theoretical value. To explain this fact it must be taken into account that in the polymer Irgafos 168 has been quantified separately as phosphite and phosphate, whereas in olive oil, it could be quantified only as phosphate. This was expected as according to Riquet et al. (*27*) Irgafos 168 in olive oil converts into phosphate by an oxidation process.

By comparison of migration values from samples CL30 and CL31 in olive oil with the sample characteristics of antioxidant concentration and thickness of the plastic film, it was observed that the sample with the lowest concentration of antioxidants had the highest migration level of Irgafos 168 oxidized. This can be explained as the result of the higher thickness of sample CL30 used for the migration test with olive oil, 75−77 μm, as opposed to 27−45 μm corresponding to sample CL31. These results show the importance of the thickness of the plastic film and its antioxidant concentration for migration. This result shows that there is a total extraction of Irgafos 168, and therefore the specific migration depends upon the mass of sample.

Finally, if the results obtained in the migration tests for samples extruded in the laboratory and commercial samples are compared, it can be seen that for commercial samples antioxidants could be detected in only aqueous simulants, whereas for extruded samples they could be quantified in olive oil. It must be taken into account that DL and QL were lower for tests using sealed bags (**Table 9**) than for those using cells (**Table 6**) because of their higher ratio of sample surface to simulant volume. Therefore, tests with bags were stricter than tests with cells, although it is limited by low antioxidant solubility in the simulant, especially in aqueous simulants.

ABBREVIATIONS USED

DL, detection limit; FDA, U.S. Food and Drug Administration; FTIR, Fourier transform infrared spectrometry; HDPE, high-density polyethylene; HPLC, high-performance liquid chromatography; LDPE, low-density polyethylene; MAE, mi-

crowave-assisted extraction; PE, polyethylene; PP, polypropylene; PVC, polyvinylchloride; QL, quantification limit; RP, reversed phase; SML, specific migration limit; SPE, solid-phase extraction; THF, tetrahydrofuran; UV, ultraviolet.

SAFETY

Categories of danger of used solvents are the following: acetic acid glacial, flammable, corrosive; acetonitrile, highly flammable, harmful, and irritant; dichloromethane, carcinogenic; ethanol, highly flammable; methanol, highly flammable and toxic; tetrahydrofuran, highly flammable and irritant.

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