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Migration Studies To Assess the Safety in Use of a New Antioxidant Active Packaging

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Both specific and overall migration tests have been applied to new experimental food packaging-active plastic films with antioxidant properties, including in its composition a natural rosemary extract. Determination of volatile and semivolatile compounds migrating from plastic to the four established simulants showed that both specific and overall migration was very low. The results obtained gave values 20 times lower than the established limits in the worst case. So, from the point of view of health risk, the new active packaging can be considered as safe. Analytical procedure used provided the necessary information about the migration behavior, with good analytical characteristics and detection limits in the sub μg kg^{-1} range. Besides, no significant difference was found between laboratory and factory-made samples, which is an important issue for industrial production, the next step in the development of the new antioxidant active film.

KEYWORDS: Active packaging; migration; antioxidant; rosemary; polypropylene film

INTRODUCTION

Most foods deteriorate in quality and safety during transport, processing, and storage, oxidative reactions being those having the greatest impact in limiting the shelf life of perishable foods (*1*−*4*). In the food industry, deoxygenating and special packaging or treatments have solved some of these problems (*5*), but it must be pointed out that the simple reduction of oxygen contents has only a partial effect on oxidation control. The almost total control of oxidation is currently achieved by the use of antioxidants, which are able to scavenge the radical species and then inhibit the oxidation process. In this sense, some authorized antioxidants are commonly used in food (*6*). However, there are a wide variety of foodstuffs such as fresh food, nuts, and many others that cannot be protected, as they are fresh or raw foodstuffs in which the addition of other substances is not permitted.

One of the most promising and exciting systems to protect the foods against oxidation without these limitations is to use an antioxidant active packaging (*7*−*10*). This consists of introducing the antioxidants into the packaging instead of adding them directly to the food. This system can be theoretically applied to any kind of food.

A wide series of compounds are used as antioxidants in food packaging materials, and most of them are synthetic molecules (*11*−*15*), although natural compounds such as tocopherol (*10*, *16*, *17*) or rosemary extracts (*18*) have been also used. Most of them act as scavenger, but the role of volatile compounds present in natural extracts and the synergic effect with the nonvolatile ones is not clear yet (*19*).

To accomplish the legislation of food contact materials, any packaging material has to follow the general established limits of migration, which means both the overall and the specific migration limits (*20*).

The overall migration (OM) means the total mass of compounds transferred from the packaging material to the food or food simulant, while the specific migration limit (SML) is the mass of each individual compound transferred to the food or food simulant (liquid with a simple chemical composition which emulates the behavior of real food, e.g., water or aqueous acetic acid) in contact with the packaging. In both cases, OM and SML have to be obtained for each packaging material intended to be in contact with food. For this purpose, migration tests in which the packaging material is in contact with some food or food simulant for a fixed period of time under controlled temperature have been developed (*21*−*24*). The food or food simulant then has to be analyzed, and the low concentration of migrants in such a media is found.

This is the case of the new antioxidant active packaging developed in our research group during the last 3 years. The new active packaging consists of a plastic layer that can be polypropylene or polyethylene in which some natural antioxidants are immobilized (*25*, *26*). This way, the active plastic layer acts as antioxidant and protects the food inside the package. Among the advantages of this developed system, which extends the shelf life of packaged food, the noncontact plastic-food requirements can be emphasized. This paper shows the migration study carried out with the new packaging material. As very low concentration of compounds, if any, is expected in the food simulants, very sensitive analytical procedures are required for the migration study. Three aqueous simulants, distilled water,

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3% acetic acid, and 10% ethanol in water and isooctane as fat simulant, have been used in this case.

To concentrate the sample and based on previous experience (*27*−*29*), solid-phase microextraction (SPME) has been applied, and further analysis by high selective and sensitive gas chromatography−mass spectrometry (GC−MS) was used. The results obtained are shown and discussed.

MATERIALS AND METHODS

Chemicals. α-Pinene (98%, CAS number 7785-26-4), camphene (95%, 79-92-5), β-pinene (99%, 18172-67-3), α-phellandrene (>95%, 4221-98-1), α-terpinene (>95%, 99-86-5), limonene (97%, 5989-27-5), 1,8-cineole (99%, 470-82-6), γ-terpinene (97%, 99-85-4), terpinolene (>97%, 586-62-9), linalool (97%, 78-70-6), camphor (96%, 76-22-2), isoborneol (95% 124-76-5), and 1-borneol (99%, 464-45-9) were purchased from Aldrich (Madrid, Spain); α-humulene (>98%, 6753-98-6) and β-caryophyllene (>98.5%, 87-44-5) were from Fluka (Madrid, Spain). Amexol, a commercially available natural extract of rosemary (*Rosmarinus officinalis L.*) registered like spice, was provided by Laboratorios Amerex S. A. (Madrid, Spain). Ethanol absolute (for analysis ACS ISO), glacial acetic acid (for analysis ACS ISO), and isooctane (for analysis ACS) were from Scharlab (Barcelona, Spain).

Water was obtained from an Elix 5 water purification system from Millipore (Madrid Spain). The gases used in this study (helium and nitrogen, both C-50 quality) were supplied by Carburos Metálicos (Zaragoza, Spain).

Stock solutions in ethanol (∼1 mg g^{-1} of each compound) were prepared. From these solutions, appropriate dilutions were made directly with the simulants for calibration and analytical characteristics determination purposes. All of the solutions used in this study have been systematically controlled by weight to improve accuracy.

Samples. Base polymer for the manufacturing of plastic films intended for use in active packaging consisted of 20 μm thickness coextruded bioriented polypropylene film made by Poligal, S. A. (Narón, Spain), being a three-layer structure as follows: external layers (5%) of poly(propylene)−3% ethylene copolymer from BP (Lillo, Belgium) including silica at ppm level as antiblocking agent, and inner layer (95%) isotactic polypropylene homopolymer containing erucamide and quaternary amines at ppm level from Repsol (Tarragona, Spain). Four different experimental samples were prepared, containing the natural rosemary extract Amexol (concentration between 0.1 and 8.0% w/w in plastic film) via an innovative process protected by a European Patent. Two of the samples (designed as AR1 and AR2, the latter containing double concentration of rosemary extract than the first) were produced in a pilot plant, whereas the other two (designed as EN1 and EN2, with the same concentration of extract as AR1 and AR2 respectively) were obtained at industrial scale to evaluate the real production process. Polypropylene film identical to that used to prepare the active film was also used as blank.

Analysis of Aqueous Simulants by SPME. SPME procedure with an apolar, 100 μm thickness poly(dimethylsiloxane) fiber was carried out by means of a manual reusable syringe assembly from Supelco (Bellefonte, PA). Before their first use, fibers were conditioned according to specifications supplied by the manufacturer.

Based on a previous work of our research group (*27*), SPME studies were made by total immersion mode in the three aqueous simulants, and 20 mL was selected as sample volume, contained in 20 mL glass vials. A magnetic stirrer was then added, and the vial was crimped with a PTFE faced septum. After 2 min stirring (600 rpm), the fiber was immersed into the sample for 20 min at room temperature (22 ± 2 °C). Magnetic stirring was kept during the sampling for improving homogeneity and reproducibility. After extraction, fiber was thermally desorbed in the split/splitless injection port of the gas chromatograph for 10 min to ensure both the quantitative transfer of analytes and the adequate cleaning of the fiber. Besides, fiber was conditioned daily at recommended manufacturer temperature for 15 min, previously to the first extraction. To check interferences, routine blank analyses with purified water were also carried out.

Analysis of Fatty Simulant. Due to the similarity of polarity between PDMS fiber and isooctane, SPME was not used with this simulant. In this case, after finishing migration test, isooctane samples were concentrated up to 2.5 g under nitrogen stream, and then injected into the gas chromatograph for their analysis, because isooctane is a suitable solvent for use in gas chromatography.

Calibration. Seven calibration points, each one analyzed by quadruplicate in the range under study, were carried out. The standards above-described were used to obtain the calibration plots.

Instruments. Chromatographic analysis was performed on a Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph with an HP 5971A mass selective detector. A Varian (Palo Alto, CA) Factor Four VF-5ms capillary column of 60 m × 0.25 mm i.d. and 0.25 μm film thickness was used. The oven program was as follows: initial temperature 50 °C hold for 3 min; raised at 15 °C min^{-1} to 300 °C hold for 5 min. Injector temperature: 250 °C. Detector temperature: 280 °C. Injection mode: splitless (3 min). Carrier gas: helium, constant flow mode at 1 mL min^{-1}. For identification, SCAN mode was selected (45−520 amu). To improve sensitivity and decrease detection limits, SIM (selective ion monitoring) was used in migration studies for quantitative purposes, by choosing the two most sensitive *m*/*z* ratios for each compound, as follows: α-Pinene (93, 92), camphene (93, 121), β-pinene (93, 41), α-phellandrene (93, 91), α-terpinene (121, 93), limonene (68, 67), 1,8-cineole (43, 81), γ-terpinene (93, 136), terpinolene (121, 93), linalool (71, 93), camphor (95, 81), isoborneol (95, 41), and 1-borneol (95, 41), α-humulene (93, 80), and β-caryophyllene (93, 80).

Specific Migration Tests. Water, 10% aqueous ethanol (v/v), and 3% aqueous acetic acid (w/v) as aqueous simulants were selected, and isooctane as fatty simulant to obtain the maximum possible information.

Double sided, total immersion migration tests were performed as follows: a 12 cm^2 piece of each plastic sample and 20 mL of the studied simulant (area-to-volume ratio 6 dm^2/1 L) were placed in a 20 mL glass vial. Samples (plastic + simulant) were then introduced for 10 days in a thermostatic oven set at 40 °C according to Directive 97/48/CE (*20*). These conditions were more aggressive than the expected for the intended applications in this case with the idea of representing the worst case for such a food packaging. Immediately after the test, the plastic was removed and the simulant was extracted by SPME (in the case of aqueous samples) or concentrated (isooctane) according to the procedure described above. Four replicates were tested and analyzed for each individual sample, to assess the homogeneity of plastic films.

Overall Migration Tests. All of the conditions were the same as for specific migration, but in this case, after the exposure of plastic samples to simulants, the polymer was removed, simulants evaporated to dryness, and solid residue was gravimetrically determined. Six independent replicates were analyzed for each plastic sample and each simulant.

RESULTS AND DISCUSSION

As the packaging material contains natural rosemary extract, which is a complex mixture of volatile and nonvolatile compounds, some of these would likely be identified in the food simulants. As was already commented in the introductory section, it must be stated that the goal of this work is the characterization of both volatile and semivolatile substances migrated, which means those transferred from the plastic film to the simulants during the migration tests. In fact, the presence of a determined substance in plastic at high concentrations per se does not mean necessarily high migration values, or vice versa. In this sense, it should not be surprising that typical compounds usually present in rosemary extracts, like α- and β-pinene, camphene, myrcene, or limonene (*30*, *31*), have not been detected. Although these compounds are more volatile than those found in the samples, its absence in the chromatograms can be attributed to the high efficiency of the immobilizing system used to fix the rosemary extract into the plastic. This can be confirmed in **Figures 1** and **2**, in which the GC−MS chromatograms obtained when aqueous solutions of a mixture of standards and rosemary extract, respectively, extracted by SPME in both cases are shown. Several authors confirm the high degree of variation of rosemary extracts, depending on the way of preparation and the origin itself of plants (*32*).

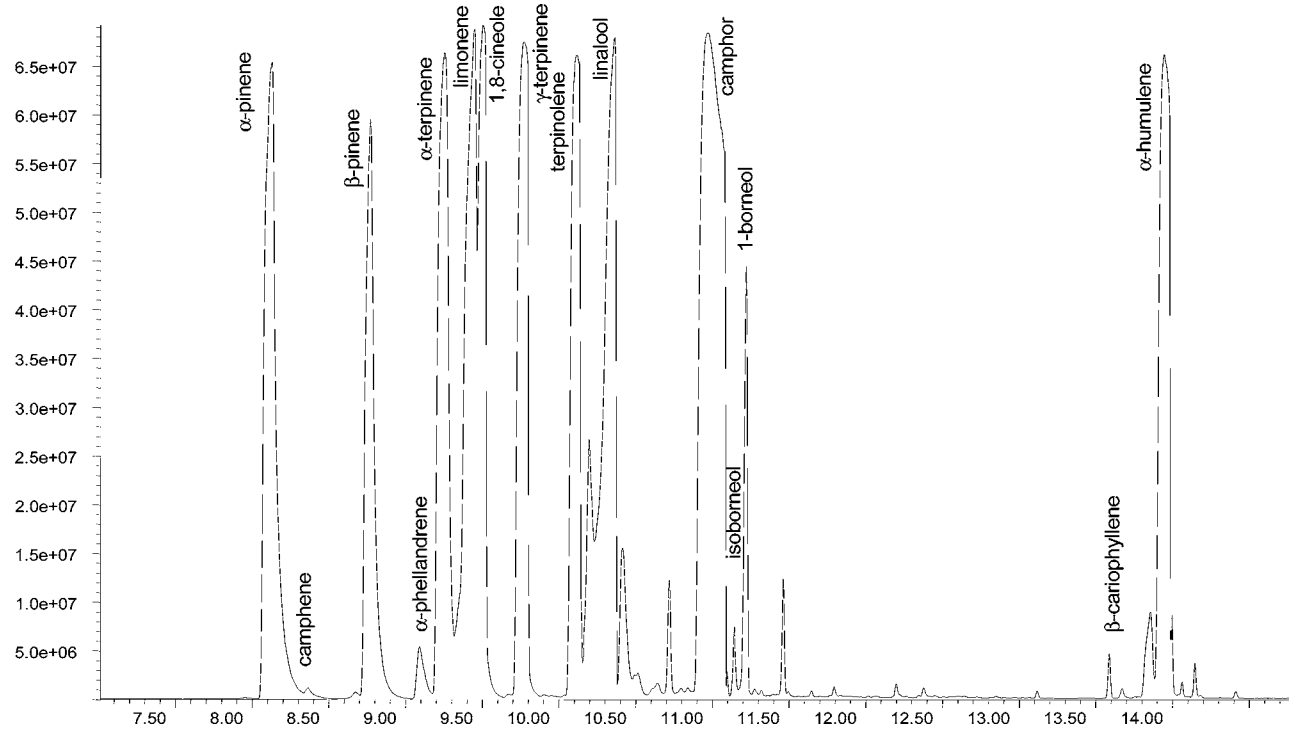

Figure 1. GC–MS chromatogram from an aqueous standard solution of compounds extracted by SPME.

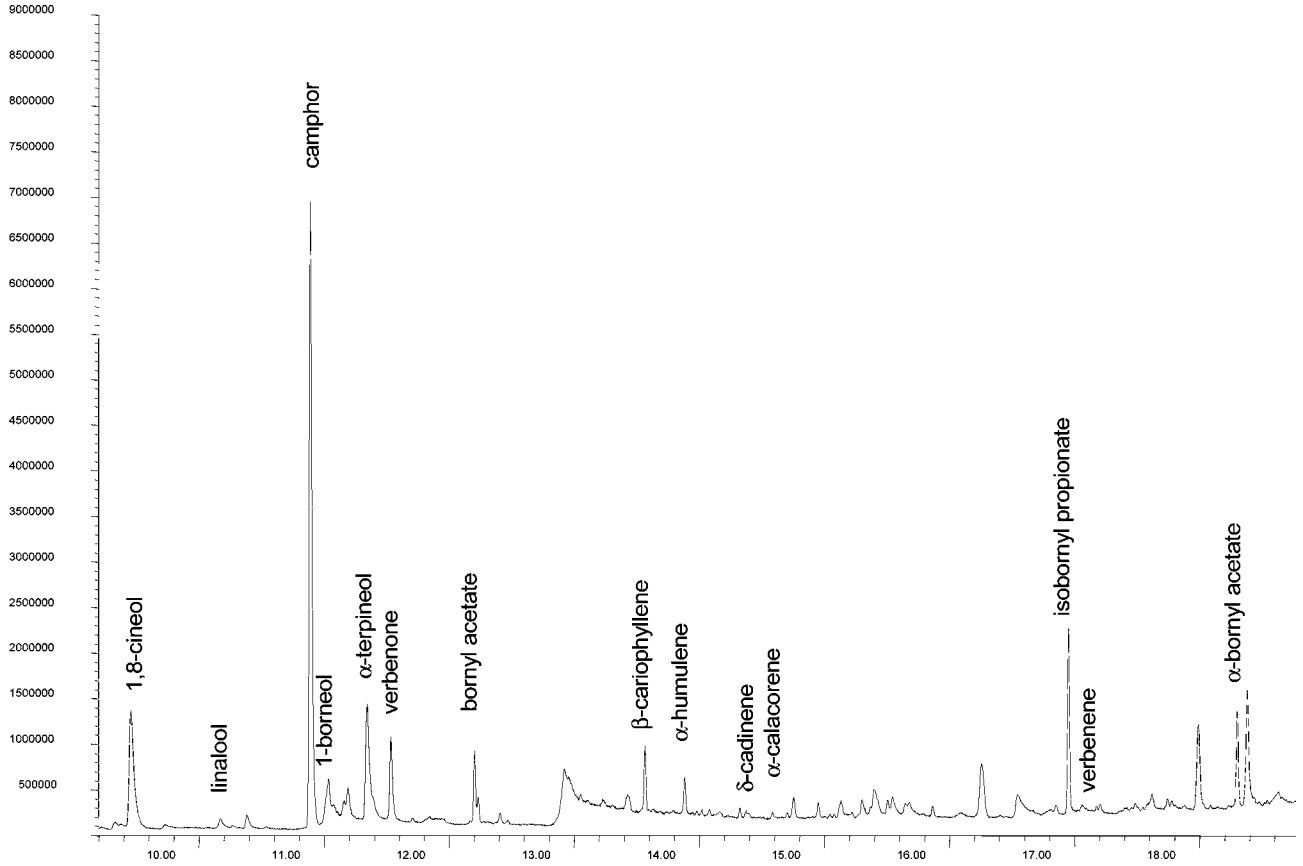

Figure 2. GC–MS chromatogram from an aqueous solution of rosemary extract (Amexol), extracted by SPME.

The main detected compound in the simulants, in terms of peak area, was camphor as can be seen in **Figure 2**. Because this compound is listed in Directive 2002/72/CE (*33*) and it is subjected to restriction due to the risk of deterioration of organoleptic characteristics of food, it was selected for the determination of analytical characteristics of the proposed SPME method. **Table 1** shows the analytical characteristics, calculated by preparing standard solutions of camphor in the four simulants, then processing them by SPME in the same way as migration samples according to the procedure described in the experimental section. Linear ranges, from sub μg kg^{-1} (0.03 in isooctane, 0.11 in water, or 0.58 in 3% acetic acid, with the only exception of 1.22 in 10% ethanol) to at least 2 orders of magnitude more, the least favorable case in 10% ethanol (104.53

Table 1. Analytical Characteristics of the Proposed SPME Method (Referred to Camphor[a] as Model Compound)

simulant	linear range (μg kg^{-1})	linearity (R^2)	det. lim. (μg kg^{-1})	quant. lim. (μg kg^{-1})	% RSD, $n = 4$ (@ μg kg^{-1})
water	0.11–80.32	0.9965	0.03	0.11	25.3 (3.24)
3% acetic acid	0.58–92.67	0.9968	0.17	0.58	28.2 (1.57)
10% ethanol	1.22–104.53	0.9999	0.37	1.22	29.8 (1.43)
isooctane	0.03–63.51	0.9996	0.01	0.03	5.1 (34.48)

[a] Only compound listed (#41680) in Directive 2002/72/CE and 1st amendment (13/02/2003); Restriction: compliance with note 9 in annex VI (risk of deterioration of organoleptic characteristics of food).

μg kg^{-1}), or about 3 orders in the other simulants (63.51 μg kg^{-1} for isooctane, 80.32 and 92.67 for water and 3% acetic acid, respectively) were achieved. Isooctane gave the best performance, with 4 orders of magnitude, but in this case it must be taken into account that sample handling was completely different for the rest of simulants and SPME was not used. Linearity was higher than 0.9965 in all cases, with values near to one in the case of 10% ethanol and isooctane. Very low detection limits, in the sub μg kg^{-1} range, were achieved in all of the cases, slightly better for water and isooctane, thus allowing the determination of trace concentrations of migrants. Finally, reproducibility expressed as RSD was much better for isooctane (5.1%) than for aqueous simulants (25−30%), but two reasons for this must be taken into account: first, that aqueous samples were evaluated at concentrations considerably lower than isooctane; second, that aqueous simulants were analyzed by SPME, which introduces an additional variability source when compared with isooctane samples, simply concentrated under nitrogen then directly injected into the GC system. In any case, for aqueous samples at μg kg^{-1} level, the RSD values found are quite common and acceptable.

Up to 11 compounds were detected in simulants. Because most of them show a similar terpene chemical structure, their unequivocal identification was difficult in several cases, because the available spectrum libraries (Wiley, NIST) gave both low quality of matching and several times, the same compound at different retention times, which is not possible. Thus, considerable additional work has been done by combining three data sources, mass spectra, tables of typical rosemary extract composition, and chromatographic retention index data, to achieve this goal. Finally, the compounds shown in **Table 2** were identified with a 99% confidence level. In several cases, the identification was especially difficult, such as estragol, which appears only in one of the aqueous samples, and at a very low concentration level. **Figure 3** shows the chromatogram obtained from a migration test in a water sample extracted by SPME, with seven compounds ranging from camphor to cariophyllene oxide.

Table 2 shows the concentration of migrants detected in the four food simulants, expressed in μg kg^{-1} of simulant. If data expressed in mass per surface of plastic are needed, the conversion is very easy, by considering that the correction factor from μg dm^{-2} to μg kg^{-1} is 6, with the standard volume-to-surface ratio used (it assumes 1 kg dm^{-3} as density of simulant, then 1 kg is contained in a cube of six faces of 1 dm^2 each). According to the general rules of migration and food safety considerations, it is assumed that 1 kg of food is in direct contact with 6 dm^2 of packaging material. It must be notified that not all of the compounds finally identified were available as standards. In these cases, a relevant compound present in sample can be used as representative standard for the rest. Here, camphor was chosen for two main reasons: first, is the only compound cited in Legislation, and, second, its chemical structure is very close to the rest of compounds, so, it can be assumed that both extraction behavior by SPME and detector response by GC−MS are similar to the other analytes.

From qualitative (number of detected compounds) and quantitative points of view, taking into account migration values, it seems that migration took place in a major extension in water and isooctane. Nevertheless, and considering that all of the compounds exhibit behavior similar to camphor, the most representative compound, by taking into account both detection limits (0.17 μg kg^{-1} in 3% acetic acid and 0.37 μg kg^{-1} in 10% ethanol, respectively) and quantitative migration values (in most cases about 0.6 μg kg^{-1}), it could be also perfectly possible that similar migration occurs in all of the simulants, but for both acetic and ethanol, these values are near the experimental limit of the method. In fact, in 10% ethanol, none of the compounds was found above the detection limit, and only 1,8-cineole, β-cariophyllene, and α-humulene were detected in four cases in 3% acetic acid. Several hypotheses can be proposed to explain these behaviors, such as the lower sensitivity of the SPME in these simulants (see **Table 1**), lower migration in these conditions, greater saturation of the fiber, or even the presence of interferences in case of acetic acid and ethanol with respect to water as simulant.

It must be also pointed out that migrant compounds are not the same in each case, showing every simulant a characteristic profile, as it could be expected because polarities, pH, viscosity, ... of simulants are quite different from one another. These differences condition the rate of migration of the compounds in the considered simulants. 1,8-Cineole was mainly detected in isooctane, with no significant differences among samples. On the other hand, camphor, verbenone, bornyl acetate, β-cariophyllene, and cariophyllene oxide were the most characteristic compounds in water, showing trace concentrations in isooctane, whereas α-humulene showed reasonable values in this simulant and was not detected in water.

Table 2. Migration Values for Detected Compounds, Expressed in μg kg^{-1} Food Simulant ($n = 4$, Confidence Interval: 95%)[a]

	water				3% acetic acid				10% ethanol				isooctane			
compound	AR1	AR2	EN1	EN2	AR1	AR2	EN1	EN2	AR1	AR2	EN1	EN2	AR1	AR2	EN1	EN2
1,8-cineole	–	–	–	–	–	0.61 ± 0.28	–	–	–	–	–	–	4.21 ± 0.68	4.17 ± 0.67	4.80 ± 0.78	4.23 ± 0.70
camphor	3.62 ± 1.47	3.04 ± 1.23	3.57 ± 1.45	2.42 ± 0.98	–	–	–	–	–	–	–	–	0.58 ± 0.10	0.62 ± 0.11	1.22 ± 0.20	0.64 ± 0.11
α-terpineol[b]	–	–	–	–	–	–	–	–	–	–	–	–	0.60 ± 0.10	0.61 ± 0.10	0.58 ± 0.10	0.57 ± 0.10
1-borneol	0.58 ± 0.23	0.61 ± 0.25	0.57 ± 0.23	0.62 ± 0.25	–	–	–	–	–	–	–	–	0.58 ± 0.10	0.62 ± 0.11	0.60 ± 0.10	0.59 ± 0.10
verbenone[b]	2.97 ± 1.20	2.96 ± 1.20	3.04 ± 1.23	2.98 ± 1.21	–	–	–	–	–	–	–	–	0.55 ± 0.09	0.61 ± 0.10	0.64 ± 0.11	0.58 ± 0.10
cinnamaldehyde[b]	–	–	–	–	–	–	–	–	–	–	–	–	0.61 ± 0.10	–	–	–
bornyl acetate[b]	3.02 ± 1.22	2.37 ± 0.96	2.97 ± 1.20	2.43 ± 0.98	–	–	–	–	–	–	–	–	–	–	–	–
β-cariophyllene	2.95 ± 1.19	3.01 ± 1.22	2.44 ± 0.99	2.36 ± 0.96	–	0.58 ± 0.26	–	–	–	–	–	–	–	–	–	–
α-humulene	–	–	–	–	–	1.80 ± 0.81	–	3.02 ± 1.36	–	–	–	–	1.23 ± 0.20	1.19 ± 0.20	2.40 ± 0.39	0.57 ± 0.10
estragol[b]	–	0.62 ± 0.25	–	–	–	–	–	–	–	–	–	–	–	–	–	–
cariophyllene oxide[b]	3.01 ± 1.22	2.43 ± 0.98	3.57 ± 1.45	2.98 ± 1.21	–	–	–	–	–	–	–	–	–	0.62 ± 0.11	1.16 ± 0.19	0.58 ± 0.10

[a] The symbol – means not detectable according to the analytical characteristics shown in **Table 1**. [b] Quantitated by using camphor as standard.

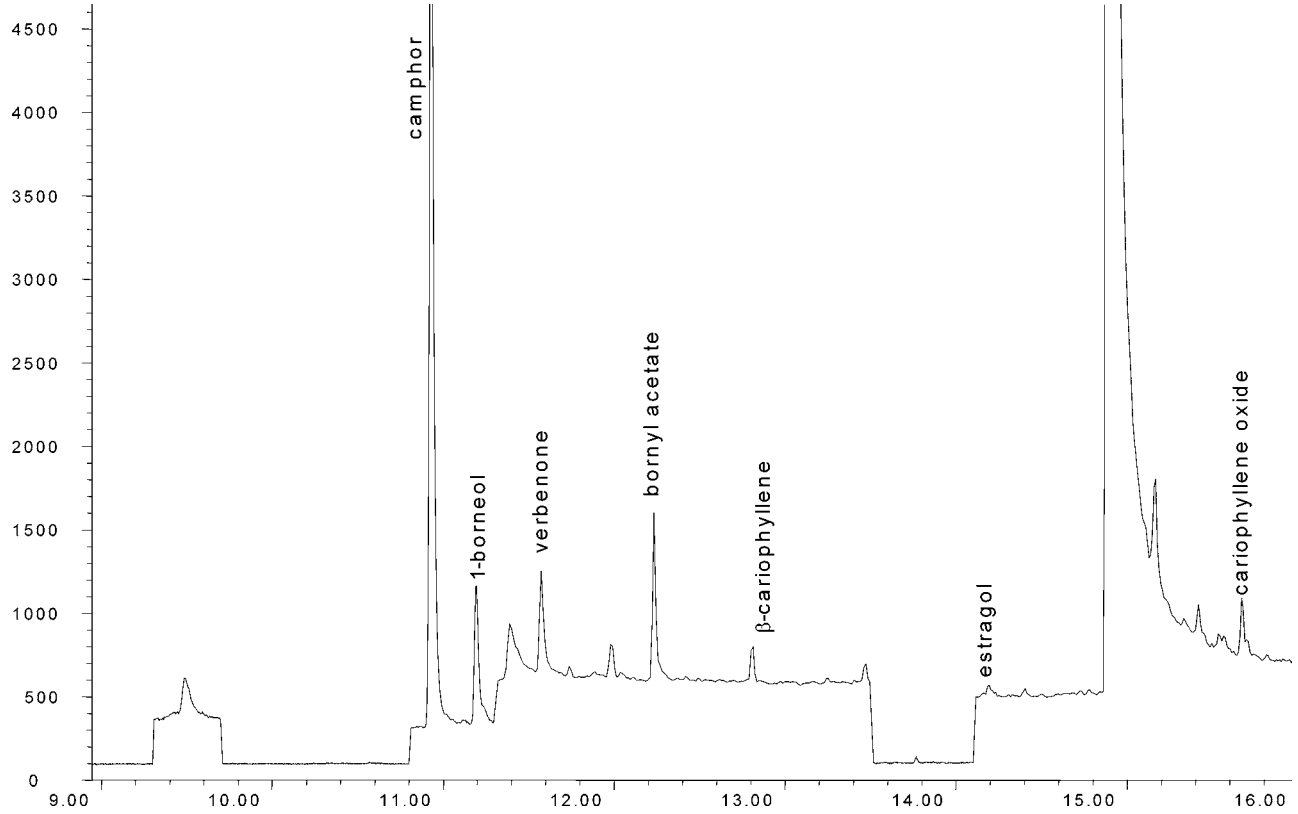

Figure 3. GC–MS chromatogram obtained from a migration test in water used as simulant extracted by SPME.

Behavior of Industrial Samples. To check the robustness of the new antioxidant packaging material in the migration tests, several industrial samples were obtained and the migration tests were carried out in the experimental conditions above-described. Migration values were similar in all samples, independently of their origin (pilot plant or industrial scale) and initial concentration (% rosemary extract). The first point is highly positive, because the scaling from pilot plant to real production is feasible with minimum differences in quality. It is only remarkable the fact that industrial production shows higher differences between EN1 and EN2, with different rosemary extract concentration, as previously mentioned. As an example, α-humulene is not detected in EN1, but shows 3.02 μg kg^{-1} in EN2 when using 3% acetic acid as simulant, whereas in isooctane the behavior is just the opposite. In general, the initial concentration of rosemary extract seems to not be relevant for migration purposes, and similar values have been found in all cases within the tested range. The most likely reason is that extract is well immobilized in the polymeric matrix, and the differences in concentration are not high enough to show a clear difference in the release rate. Besides, it must be pointed out that the immobilization process is highly effective, because the absolute quantities of analytes released to the simulants are very low in all cases.

From the food safety point of view, the main objective of this work, all of the values are well below the established specific migration limits (SMLs) for most of the regulated substances present in plastics intended for food contact applications. In fact, all of these substances are present in a natural extract, profusely used as flavoring food additive without any risk for health. So, according to the results of specific migration, the studied active packaging can be classified without any doubt as safe.

Overall Migration Studies. To take into account the total release of possible compounds present in the initial composition (*34*, *35*), overall migration tests were performed with the samples AR1 and AR2 made at the pilot plant. **Table 3** summarizes the results obtained. As can be seen, in all cases, these results are very low, even in the case of isooctane (up to 1.26 mg kg^{-1}), and far below the established limit (60 mg kg^{-1}). Besides, film production offers an extra protection against migration in several cases, such as in the case of 3% acetic acid, where the polypropylene film itself without active compounds shows up to 9 times higher migration values than the active film samples. This fact could be attributed to an additional chemical resistance effect that the active agents exert on the PP film. So, overall migration tests reinforce the safety-in-use hypothesis already deduced from the specific migration study.

Table 3. Overall Migration Values for Laboratory-Made Samples (AR1 and AR2); $n = 6$ Samples, Confidence Interval 95%

simulant	PP blank (mg kg^{-1})	AR1 (mg kg^{-1})	AR2 (mg kg^{-1})
water	0.12 ± 0.60	0.06 ± 0.00	0.60 ± 0.06
3% acetic acid	0.54 ± 3.30	0.06 ± 0.00	0.06 ± 0.00
10% ethanol	0.00 ± 0.00	0.12 ± 0.06	0.06 ± 0.00
isooctane	0.78 ± 0.06	0.84 ± 0.00	1.26 ± 0.00

SAFETY

Most of the studied terpene-like compounds are flammable or highly flammable and incompatible with strong oxidizing agents. Some of them (α-pinene, camphene, β-pinene, α-terpinene, limonene, γ-terpinene, linalool, camphor, isoborneol, 1-borneol, α-humulene) are irritating to eyes, respiratory system, and skin. α-Terpinene and limonene are toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Toxicity levels (expressed as LD50 in rats, oral) range from 1310 mg kg^{-1} for camphor to 5800 mg kg^{-1} for 1-borneol (typical values from 3000 to 5000 mg kg^{-1} for the rest of them). Amexol is registered like spice, accepted as food additive, and no special care is required. Ethanol is highly

flammable, and contact with alkali metals, ammonia, oxidizing agents, and peroxides must be avoided; LD50 (human, oral) is 1400 mg kg^{-1}. Acetic acid is flammable and causes severe burns; its LD50 (rat, oral) is 3310 mg kg^{-1}. Isooctane is highly flammable, incompatible with both oxidizing and reducing agents, irritating to skin, very toxic to aquatic organisms, and may cause long-term adverse effects in the aquatic environment, may cause lung damage if swallowed, and the vapors may cause drowsiness and dizziness; LD50 (rat, oral) is 500 mg kg^{-1}.

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