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Evaluation of transfer of wine aroma compounds through PET bottles

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ABSTRACT: Aroma compound could be lost during food storage in polymer packaging by transfers through material. The aim of this study was to develop a method that allows evaluating wine aroma compounds permeation through active PET bottles. A semiquantitative method has been adapted to the system. Results showed a regular permeation of all studied aroma compounds but a high variation between replicates. In active PET bottle containing 1% of oxygen scavenger, amount lost by permeation after 12 months storage at 20°C was about 6.13 ± 0.37 μgbottle^{-1}. When 50% of recycled PET was added to active PET bottle, permeation rate was increased about 15%. The study of sorption of aroma compounds in both polymer matrices did not allow to explain this difference of permeation, but the structure of recycled PET seemed to induce modifications in aroma compound diffusion through active PET which could increase transfer. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41784.

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INTRODUCTION

During storage in packaging with low barrier properties, the aromatic profile of the food may be altered by the transfer of gas or organic volatile compounds, such as aroma molecules. The significance of those phenomena is estimated through the measurement of the solubility and permeability coefficients of the compound penetrating through the material. Unlike steam and oxygen, there is no standard method for measuring the solubility and permeability of organic volatile compounds. Those two coefficients are estimated through methods inspired from those developed for steam or gas, which can vary according to the available tools.[1–3] Those methods usually involve high concentrations or partial pressures, which do not reflect those found in food. The coefficients found using those methods allow comparison between different materials and between different aroma compounds without, however, being able to predict their behavior in real conditions.

It is necessary to measure sorption and permeation phenomena in more realistic conditions, matching those found in food packaging, and to develop methods suitable for low amounts of organic volatile compounds. Regarding sorption, methods conforming to the concentrations found in food products have already been proposed. The most common methods use solvents or supercritical CO_2 for the extraction of aroma compounds in the material after contact with diluted solutions or products.[4–8] Other authors proposed a more inventive methodology based on the extraction of headspace optionally combined with solid-phase microextraction (SPME) of amounts released by sorption through the film after submitting it to very low values of partial pressure of aroma compound.[9]

The evaluation of the permeation of organic volatile compounds in realistic conditions was made possible by trapping and concentrating them using solid-phase microextraction and then analyzing them using gas chromatography.[4,6] This technique was mostly used for the characterization of transfers through films in contact with sponge cakes or liquid solutions. To our knowledge, only one method has been developed for measuring the permeation of organic volatile compound in a filled bottle.[10] SPME coupled with gas chromatography seems to be suitable for this purpose, and we propose to test this technique on a real case, the packaging of rosé wine in a PET bottle. The aromatic profile of wine is characterized by many aroma compounds, such as esters, alcohols, acids, aldehydes, and terpenes, some of them in concentrations as low as a few parts per billion. Moreover, PET is known for its inertia regarding aroma compounds, further limiting the permeation.[8,11–13] However, losses of volatile compounds by permeation through PET were clearly evidenced for orange juice.[10] In a study described earlier, using saturated vapor pressure and a semidynamic method, the permeation of both aroma compounds, hexanol and isoamyl acetate, through PET was determined, and relatively, strong values of permeability were found.[14] It was also proved that the

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presence of oxygen scavenger or recycled PET impacted the value of permeability.

The objectives of this study were to test the method of SPME for the permeation evaluation of wine aroma and to develop a way to quantify them. In addition, sorption of aroma compounds during wine storage was also evaluated. Aroma transfers through both PET bottles with or without recycled PET were investigated and compared.

MATERIAL AND METHODS

Chemicals

Dichloromethane and internal standard, 4-nonanol, were provided by Sigma-Aldrich, France. Isoamyl acetate, isoamyl alcohol, octanoic acid, ethyl octanoate, hexanoic acid, hexyle acetate, ethyl hexanoate, isobutanol, methionol, 2-phenylethanol were purchased from Sigma-Aldrich, France and hexanol from Prolabo, France. Anhydrous sodium sulfate was provided by Merck, France.

Packaging, Filling, and Storage Conditions of Wine

Wine used was a Rosé Cinsault from South of France supplied by UCCOAR – Val d'Orbieu (Carcassonne, France). Wine pH was 3.23, its ethanol content was around 11.9% (v/v). After bottling, total oxygen content was around 4.9 mgL^{-1} and free and total SO_2 around 33 and 130 mgL^{-1}, respectively. These data resulted from experimental analyses carried out by UCCOAR (Carcassonne, France) and Experimental Unit of Pech Rouge (UEPR, INRA, Gruissan, France).

Wine was packed in 75-cL bordelaise glass bottles (BSN Glasspack SA, Villeurbanne, France) and in two kinds of 75-cL polyethylene terephtalate (PET)-active bottles supplied by SIDEL Blowing Service (Le Havre, France). Both of the active PET contained 1% of oxygen scavenger, and only one contained 50% of recycled PET (namely osPET and osRPET, respectively). The weight of both PET bottles was 38 g, thickness around 350 μm for bottle body, increasing until 470 μm in the lower part and 700 μm in the top of the bottle shoulder. A polypropylene cap with a multilayer connective joint was used as closure (Novatwist™ from Novembal, Chateaubriant, France).

Filling was performed by the Experimental Unit of Pech Rouge (UEPR, INRA, Gruissan, France). Both bottles were filled using a "Perrier filler" equipped with a WineBrane® filtration system (INOXPA) (membrane porosity 1 μm for prefiltration and 0.65 μm for final filtration). The bottles were capped by using a Zalkin TM3 machine. The wine bottles were stored for 12 months at 20°C and under a 400 lux light to mimic storage conditions in supermarket.

Permeation Method

SPME Fiber Characteristic. All solid-phase microextractions (SPME) were performed using a 50/30 μm, 2 mm divinylbenzene (DVB)/carboxen (CAR)/polydimethylsiloxane (PDMS) fiber (Supelco, Bellafonte, USA). This tripolar fiber allows the extraction of a wide range of volatile and semivolatile compounds with different polarities and molecular weights. In addition, the layered coating of fiber with both DVB and CAR authorizes the trapping of larger analytes in DVB layers and smaller analytes in CAR, thus expanding the molecular weight of analytes that can be extracted and enabling extraction of analytes at trace levels. This fiber has been already used for the analysis of wine aroma and appears to be the best choice for permeation measurement.[15–19]

Preliminary Assays with a Model Solution. To evaluate the feasibility of the SPME method, assays were carried out using a model solution containing 12% ethanol (v/v), salts, and the 11 selected aroma compounds at the concentration found in wine. A determined volume of solution was put in a hermetic glass reactor equipped with a cap closed with a septum and allowing sampling. The volume of solution used respected the ratio between the volume occupied by a bottle and the volume of the headspace. After 30 min of equilibrium period under stirring at 20°C, the selected SPME fiber was put in contact with the headspace during 30 min. The aroma compounds extracted with the fiber were desorbed in the gas chromatography (GC) injector at 250°C for 10 min and analyzed by GC.

Loading of Internal Standard onto the SPME Fiber. The main objective of this study was to quantify the amount of aroma compound permeating through polymer bottles. For this, an internal standard, 4-nonanol, was used. It was loaded onto the SPME fiber before the sample extraction step. A vial containing 20 μL of the internal standard solution (1 $mgmL^{-1}$ in ethanol) and 1 mL of ultrapure water as solvent was stirred during 5 min at 20°C to volatilize 4-nonanol. The SPME fiber was put in contact with the headspace of the vial during 1 min under stirring.

The 4-nonanol concentration trapped on the fiber was determined using an external calibration curve performed on the same GC and by injecting five solutions of 4-nonanol in ethanol at different concentration. The R^2 was about 99.8.

Extraction of Aroma Compounds During Permeation Experiment. The permeation system developed is presented in Figure 1. A PET bottle closed by a multilayer cap, and full of the studied wine, was placed in a hermetic glass reactor (volume = 4.25 L) equipped with a cap closed with a septum and allowing sampling. The reactor was placed in a climatic chamber to maintain constant temperature (20°C) and under 400 lux. After 3, 7, 9, and 12 months of storage, the selected SPME fiber was put in contact with the headspace around the bottle by the way of the septum of cap. The fiber was exposed during 1 h in order to trap and to concentrate the permeated aroma compounds. The fiber was then introduced in the GC injector and compounds directly desorbed at 250°C for 10 min and analyzed by GC following the protocol detailed below.

Each experiment was performed in duplicate or triplicate. Eleven compounds of different polarities and volatilities (Table I) were selected from the studied wine. The target peaks were assigned, automatic integration was done, inspected and manually re-integrated if necessary. Before each use of SPME fiber, this one was heated twice to eliminate all traces of compounds during 30 min at 250°C.

Chromatographic and Detection Conditions. Aroma compounds desorbed from the fiber were analyzed by gas chromatography (GC), using a Varian 3800 GC equipped with a DB-WAX®

Figure 1. System used to evaluate permeation of aroma compounds through PET bottles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

column (30 m × 0.25 mm, 0.25 μm) and a flame ionization detector (FID; hydrogen = 30 $mLmin^{-1}$, air = 300 $mLmin^{-1}$, nitrogen= 0 $mLmin^{-1}$). Hydrogen was used as carrier gas with a flow rate of 2 $mLmin^{-1}$. The oven temperature stayed for 3 min at 60°C, and then, it was raised by 3°$Cmin^{-1}$ up to 245°C and was kept at 245°C for 20 min. Injector and detector temperatures were 250°C and 300°C, respectively. The splitless injection mode was applied. For the quantification, the response factors of each compound toward the internal standard were determined.

Extraction and Analysis of Aroma Compounds Sorbed in PET Bottles. The amount of sorbed aroma compounds in the PET bottle and in the cap were measured before filling to check the presence of volatile compounds in PET and after 3, 7, 9, and 12 months of storage. Wine aroma compounds trapped into PET bottle and into connective joint of cap were extracted with dichloromethane by contact during 12 h under magnetic stirring (250 rpm). A known quantity of internal standard (10 μL of a 6.81 $mgmL^{-1}$ of 4-nonanol) in ethanol was added at the beginning of the extraction. The resulting organic phase was dried using anhydrous sodium sulfate and concentrated under a nitrogen flow to approximately 2 mL. Extracts were analyzed by gas chromatography (GC), using a Varian 3800 GC equipped with a DB-WAX column (30 m × 0.25 mm, 0.25 μm) and a flame ionization detector (FID; hydrogen = 30 $mLmin^{-1}$, air- = 300 $mLmin^{-1}$, nitrogen = 30 $mLmin^{-1}$). Hydrogen was used as carrier gas with a flow rate of 2 $mLmin^{-1}$. The oven temperature stayed 3 min at 60°C, and then, it was raised by 3°$Cmin^{-1}$ up to 245°C and was kept at 245°C for 20 min. Injector and detector temperatures were 250°C and 300°C, respectively. Injection was done in split mode with a 1:20 ratio. Three replicates were made for each experiment. Selected aroma compounds were identified using known standards, and the quantification was performed using the internal standard for which the response coefficient of each compound was determined.

RESULTS

Permeation Method Development

The first step of the permeation method development was to evaluate the feasibility of the use of SPME to trap and concentrate wine aroma compound. This study focused on 11 aroma compounds selected because of their physicochemical characteristics (Table I) and concentration in wine (Table III). These aromas have various water solubility, vapor pressures, and hydrophobicity. The hydrophobicity is evaluated by the octanol/water partition coefficient (Log *P*), with *P* is the ratio between the concentration of a compound between octanol and water phase. When a compound has a Log $P > 2$, it is considered as apolar and having a weak solubility in water (Table I). The solubility of aroma compounds and consequently their volatilities

Table I. Physicochemical Characteristics of the 11 Selected Aroma Compounds Found in the Studied Wine

Code	Aroma compounds	Chemical formula	Molar mass ($gmol^{-1}$)	Density (gcm^{-3})	Vapor pressure at 25°C [a] (Pa)	Log *P*	Solubility in water [b] (gL^{-1})
2PE	2-Phenylethanol	$C_8H_{10}O$	122	1.017	10	1.36	22
EH	Ethyl hexanoate	$C_8H_{16}O_2$	144	0.869	221	2.83	0.308
EO	Ethyl octanoate	$C_{10}H_{20}O_2$	172	0.867	30	3.90	0.0334
Hac	Hexanoic acid	$C_6H_{12}O_2$	116	0.927	21	1.72	5.898
H	Hexanol	$C_6H_{14}O$	102	0.812	126	1.86	6.885
HA	Hexyl acetate	$C_8H_{16}O_2$	144	0.87	185	2.83	0.308
IA	Isoamyl acetate	$C_7H_{14}O_2$	130	0.876	747	2.26	1.100
IAO	Isoamyl alcohol	$C_5H_{12}O$	88	0.809	635	1.22	0.44
I	Isobutanol	$C_4H_{10}O$	74	0.803	1200	0.76	1.1
M	Methionol	$C_4H_{10}OS$	106	1.03	21	0.40	0.49
Oac	Octanoic acid	$C_8H_{16}O_2$	144	0.91	3	2.74	0.789

[a] http://www.thegoodscentscompany.com/.
[b] Handbook of Chemistry and Physic, CRC Press.

Table II. Repeatability of the Method Detailed for Three Experiments, Evaluation of the Efficiency of the Fiber to Catch Wine Aroma Compounds

Aroma compounds	Areas			Average	Standard deviation	Variation (%)	Amount in initial solution (%)	Amount of aroma sorbed in SPME fiber (%)
	1	2	3					
2-Phenylethanol	1,751,385	1,677,792	2,174,941	1,868,039	268,320	14	24.1	37.1
Ethyl hexanoate	2,515,409	2,132,244	1,697,343	2,114,999	409,306	19	0.5	42.0
Ethyl octanoate	451	691	511	551	125	23	0.8	0.0
Hexanol	228,496	188,950	201,275	206,240	20,235	10	0.7	4.1
Hexyle acetate	7249	5,808	7,613	6,890	955	14	0.2	0.1
Hexanoic acid	38,661	77,915	68,785	61,787	20,541	33	2.1	1.2
Isobutanol	295,211	291,915	283,863	290,330	5,838	2	6.5	5.8
Isoamyl acetate	26,118	21,529	23,188	23,612	2,324	10	3.6	0.5
Isoamyl alcohol	9,526	10,474	11,002	10,334	748	7	57.4	0.2
Methionol	20,826	16,578	21,120	19,508	2,542	13	1.2	0.4
Octanoic acid	393,209	471,363	420,738	428,437	39,642	9	2.9	8.5

can be modified by the presence of ethanol. This had been previously demonstrated by comparing the gas–liquid partition coefficients of compounds in water or in 12% v/v ethanolic solution[6]. For example, 2-phenylethanol, in high amount in wine, is characterized by a strong solubility in alcoholic solution and low volatility (reduction of K by 93% in the presence of ethanol). Conversely, the amount of ethyl hexanoate in wine was low, but it is 20 times more volatile and less soluble in ethanolic solution (reduction of K by 29% in the presence of ethanol).[6] The volatility can affect the permeation.

To evaluate the method feasibility, we used a model solution directly put in the reactor and containing these 11 aroma compounds at wine concentration. Results are reported in Table II, and tests were performed after 30 min of equilibrium period under stirring and 30 min of contact between fiber and headspace. All studied aroma compounds were detected with this method.

Reproducibility of the method was evaluated by repeating the same experiment 3 times. Standard deviations varied depending on the aroma compound. It could be noticed that three compounds (hexanoic acid, ethyl hexanoate, and ethyl octanoate) showed a standard deviation close or superior to 20%. These three aroma compounds were in low concentration in model solution ($< 2 mgL^{-1}$) and two of them (ethyl octanoate and hexanoic acid) are characterized by a low vapor pressure. Bonino *et al.* (2003) had reported that variation of standard deviation depended on aroma compounds and nature of the SPME fiber.[15]

Furthermore, different percentages were found between initial concentration in model solution and the amount sorbed in SPME fiber. It could be explained by the different volatilities of the studied aroma compounds, which depend on vapor pressure and affinity for alcoholic solution. In a previous work, the affinity was determined for 2-phenylethanol and for ethyl hexanoate by studying their partition coefficient between a model solution with 12% ethanol (v/v) and the headspace.[6] Knowing the coefficient partition and the concentration in the liquid solution, the concentration in the gas phase can be evaluated. Then, the concentration in headspace for 2-phenylethanol was about 3.21 μgL^{-1} and for ethyl hexanoate equal to 16.8 μgL^{-1} (Table III). The amount of ethyl hexanoate should be 5 times higher in the headspace than 2-phenylethanol, but the calculated percentage did not allow to verify this hypothesis (Table II). It could be evidenced that the amount of ethyl hexanoate was higher than 2-phenylethanol. Using K described by Morakul *et al.*[20] for other compounds reported in Table III, similar conclusion was obtained. For instance, a weak sorption of isoamyl acetate on the fiber was

Table III. Value Coefficient Partition of Gas/Ethanolic Solution and Concentrations in the Model Solutions in the Headspace Calculated from the Value of $K_{gas/ethanolic}$ Solution or from Internal Standard Fixed on SPME Fiber

Aroma compounds	$K_{gas/ethanol}$ at 20°C	C model solution (mgL^{-1})	C headspace calculated from the K (μgL^{-1})	C headspace calculated from IS (μgL^{-1})
2-Phenylethanol	0.000086[a]	37.336	3.2	12.8
Ethyl hexanoate	0.0229[a]	0.733	16.8	16.5
Isoamyl acetate	0.0113[b]	5.6	63	0.195
Isoamyl alcohol	0.00033[b]	88.8	29.3	0.064
isobutanol	0.00019[b]	10.08	1.9	1.6

[a] Peychès-bach *et al.* (2011)[6] (ethanolic solution at 12%v/v).
[b] Morakul *et al.* (2010)[20] (ethanolic solution at 13%v/v).

observed, whereas its estimated concentration in the headspace is quite high compared to the other compounds such as 2-phenylethanol. The method developed was well effective to detect the aroma compounds of wine, but quantification is needed to avoid under or over estimation.

Development of a Quantification Method. Measurement of permeation of aroma compounds through PET bottles should be quantitative to evaluate global losses of aroma compounds due to the transfer phenomena. Then, a quantification method has been adapted to the system. A known amount of internal standard (4-nonanol) was fixed on SPME fiber, and the fiber was put in contact with glass reactor headspace (Figure 1).

To fix internal standard onto the SPME fiber, the same principle was always applied, using a known amount of internal standard diluted with a solvent. This solution was put in a hermetic flask closed by a cap equipped with a septum. After reaching equilibrium between solution and headspace, a SPME fiber was inserted, through the septum, in the flask and get in contact with the headspace. After a given time, SPME fiber was removed and placed in contact with glass reactor headspace. Several conditions were tested, such as solvent nature, equilibrium period, and contact duration, between fiber and internal standard solution.

Solvent choice was important, because it should not interfere with internal standard or fiber and should not saturate the polymer constituting the fiber, which exclude pure ethanol. A first experiment was carried out with miglyol, a fatty substance, permitting a good dilution of internal standard. But this solvent was not very easy to use because of its viscosity. It was replaced by pump oil, based on work from Setkova *et al.* (2007) who had used this method to quantify ice wine aroma compounds.[18,21,22] This solvent seems to be ideal because it allows an easy dilution of internal standard, but the pump oil used in our experiment was not adapted. Indeed, chromatographic results showed a background noise, probably due to oil compounds, and this noise blurred the analysis because studied aroma compounds were in small amount. Water was finally chosen as the solvent. The internal standard had a low solubility in water, so it was firstly diluted in absolute ethanol to increase its solubility. A very concentrated solution was made to limit the amount of ethanol in the internal standard solution and therefore avoid fiber saturation.

However, the amount fixed on fiber should be small and close to the amount found in wine for the least concentrated aroma compound. To reach this objective, two couples of temperature/contact time have been tested: 45°C and 30 s as suggested by Setkova *et al*[21] and 20°C and 1 min. Similar results have been obtained, and the final procedure has been selected. Assays have been repeated 4 times with the same internal standard solution and the same fiber, and the standard variation was inferior to 6%.

The response factor of each aroma compound for 4-nonanol was also evaluated and used for quantification.

Finally, the last step is used to calculate the concentration in the headspace from the internal standard and to compare with the value found using the partition coefficient (Table III).

The predictive amount calculated with the partition coefficient was very close for ethyl hexanoate and isobutanol and did not match very well for 2-phenylethanol. For isoamyl acetate and isoamyl alcohol, the results matched very badly. It could be due to inappropriate value of K in relation to the presence of other compounds but also to the specific affinity of fiber for some compounds[17]. As it was not possible to conclude, the method can be considered as semiquantitative only.

Optimization of Contact Duration Between SPME Fiber and Reactor Headspace. The optimization of time contact between SPME fiber and the headspace was evaluated with full wine PET bottles stored in a reactor. A first experiment showed that after 30 min of contact, the amounts extracted are too low to be exploited due to the weak transfer of aroma compounds, that is, the contact duration needed to be increased. One hour of time contact was experimented and allowed to sorb the 11 studied aroma compounds, including those which permeate in a small amount. To verify whether aroma compounds, which had permeated, had been well extracted by the SPME fiber, a second extraction during 1 h was carried out, and aroma compounds were analyzed by GC. This experiment showed that majority (95%) of aroma compounds present in the reactor headspace were sorbed during the first extraction by the fiber and a contact of 1 h was enough.

During storage the method is adopted to measure the amount of permeation of aroma compounds through PET bottles.

Evaluation of Wine Aroma Compound Permeation Through PET Bottles

Permeation Through the System Cap/PET Bottles. PET bottles containing wine were capped and stored in the glass reactor. The evaluated permeation matched both transfers through bottle matrix and cap.

Permeation kinetics of aroma compounds through PET bottles containing 1% of oxygen scavenger was followed during 12 months. The Figure 2 shows the amount loss by permeation for each aroma compound during storage. The 11 studied aroma compounds passed through PET bottles and caps in a sufficient amount to be detected after 3 months of storage at 20°C. However, the amount of aroma compounds was low, and it allowed concluding that there was no saturation in the headspace, and therefore, permeation was not slowed down by a saturation phenomenon.

Results obtained showed high deviation (between 9 and 34% of variation depending on the aroma compound) between two replicates for the same analysis. These high standard deviations could be explained firstly by the variation obtained with SPME method (Table II) and secondly by an eventual heterogeneity between bottles due to the structure of material that they were composed. In fact, PET bottles used in this experiment contained 1% of oxygen scavenger, which was a very weak amount, difficult to distribute homogeneously into the PET matrix during the preform manufacturing and after, during the bottle blowing. The resulting heterogeneity could induce some preferential way and affect the diffusion and the permeation

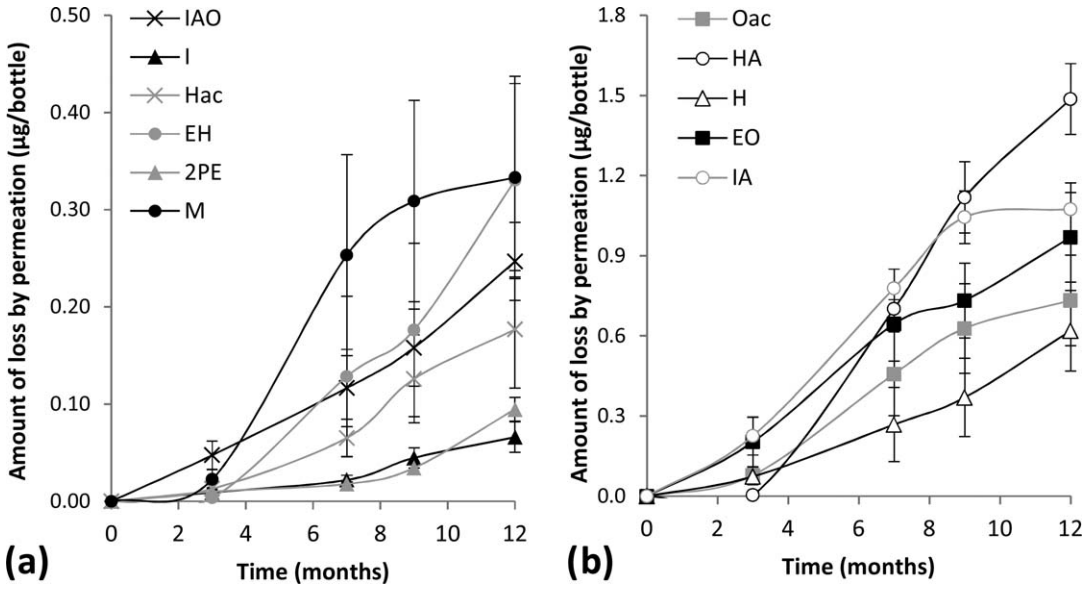

Figure 2. Amount of loss by permeation through osPET material (µgbottle^{-1}). (2a) ●, methionol (M); ●, ethyl hexanoate (EH); ×, isoamyl alcohol (IAO); ×, hexanoic acid (HA); ▲, isobutanol (I); ▲, 2-phenylethanol (2PE); (2b) ○, hexyl acetate (HA); ○, isoamyl acetate (IA); ■, ethyl octanoate (EO); ■, octanoic acid (Oac); △, hexanol (H).

explaining the variable results. The heterogeneity of the tested material would have required an evaluation on more replicates.

Permeation seemed to increase regularly during time of storage for most aroma compounds (Figure 2a). However, for some of them, such as methionol or isoamyl acetate, permeation after 9 months of storage was slowing down (Figure 2b). For each studied aroma compound, the permeation rate was calculated from the slope obtained from the curve of amount of losses during storage time (Figure 2) expressed in µgbottle^{-1}months^{-1}. When permeation slowed down during time, the permeation rate was defined during the faster phase (Table IV). Calculated permeation rates varied from 4.10×10^{-3} µgbottle^{-1}months^{-1} for isoamyl alcohol to 1.16×10^{-1} µgbottle^{-1}months^{-1} for hexyle acetate, thus 30 times more for the aroma which had the higher permeation rate. It was observed that the nature of the aroma compounds strongly impacted the amount which could permeate during time. Indeed, isoamyl alcohol and 2-phenylethanol, despite their high amount in wine, permeate lower than other aroma compounds such as hexyle acetate, for example (Table IV).

Moreover, it should be noted that the three aroma compounds that had the higher permeation after 12 months storage belong to the most apolar studied compounds: hexyle acetate (1.49 µgbottle^{-1}), isoamyl acetate (1.07 µgbottle^{-1}), and ethyl octanoate (0.97 µgbottle^{-1}), while the most polar aroma compounds, methionol and isobutanol were part of the less permeating aroma compounds (0.33 and 0.07 µgbottle^{-1}, respectively) (Table I and V). Transfer rate depends not only on sorption of the molecule in the material but also on diffusion of the molecule through the matrix. It seemed that the packaging as whole (bottle and cap) had an important affinity for apolar aroma compounds, which could increase the transfer rate. The amount of each aroma compounds sorbed in the polymeric matrix after 12 months storage is presented in Table VI. Sorption took place in two polymers with different natures, an apolar PE constitutive of the layer of the cap in contact with the wine headspace and a more polar PET constitutive of the bottle. It was difficult to directly link observed sorption with polarity of aroma compounds. However, as reported for a contact with an apolar material as PE,[8] the most apolar compound was the most sorbed (ethyl octanoate), followed by ethyl hexanoate and octanoic acid which are relatively apolar compounds. In contrast, hexyl acetate and isoamyl acetate relatively apolar compounds showed a weak sorption. Moreover, a high sorption was also observed for the two major aroma compounds of wine, 2-phenylethanol and isoamyl alcohol. These results showed that in contradiction to results reported in the literature for simplified systems,[4] the amount lost by permeation could not be directly linked to the amount sorbed by the bottle and cap.

The total amount of the 11 studied aroma compounds stored in osPET bottles and lost by permeation after 12 months storage was about 6.13 ± 0.37 µgbottle^{-1} which represent almost 0.003% of the initial and final amount cumulated of these eleven aroma compounds, permeation in the studied case was then very low.

Comparison of Permeation Between osPET and osRPET Bottles. The developed permeation method is used to observe whether the presence of recycled PET in the bottle matrix modified permeation of aroma compounds as it was demonstrated in a previous publication[14]. The two bottles were closed with the same cap, so variation could be directly assimilated to differences between materials constitutive of the bottle body: one was constituted with virgin PET and 1% of oxygen scavenger (osPET) and the other contained 50% recycled PET and 1% of the same oxygen scavenger (osRPET).

Table IV. Concentration in Wine (mg.L^{-1}) and Permation Rate (μg.bottle^{-1}.month^{-1}) of Aroma Compounds in osPET Bottle

Aroma compounds	Concentration in wine (mg.L^{-1})		Permeation rate (μgbottle^{-1}month^{-1})
	t_0	t_{12}	
2-Phenylethanol	21.94 ± 0.92	28.40 ± 0.51	5.66E-03
Ethyl hexanoate	0.40 ± 0.03	0.68 ± 0.03	2.28E-02
Ethyl octanoate	0.63 ± 0.04	1.20 ± 0.06	8.25E-02
Hexanol	0.77 ± 0.06	0.99 ± 0.03	4.54E-02
Hexyl acetate	0.13 ± 0.01	0.02 ± 0.004	1.16E-01
Hexanoic acid	1.95 ± 0.06	2.39 ± 0.06	1.33E-02
Isobutanol	3.95 ± 0.16	3.55 ± 0.21	4.86E-03
Isoamyl acetate	3.32 ± 0.31	0.95 ± 0.06	1.12E-01
Isoamyl alcohol	62.80 ± 2.42	59.61 ± 2.40	4.10E-03
Methionol	0.23 ± 0.02	0.24 ± 0.01	3.32E-02
Octanoic acid	4.28 ± 0.18	5.31 ± 0.19	6.32E-02

Evaluation of permeation during storage in the case of the osRPET bottle highlighted for some aroma compounds the same slowing down phenomenon of the permeation rate as it was observed for the osPET bottle in Figure 2. This deceleration was observed not only for isoamyl acetate and methionol, as in the osPET bottle, but also for isoamyl alcohol and isobutanol, which had a slowing down after 3 months of storage, and for ethyl hexanoate, which slowed down after 7 months of storage. Others aroma compounds had a regular permeation rate during the whole storage time. Permeation rates can be calculated for the osRPET bottle and varied, depending on the nature of the aroma, between 5.12×10^{-3} μgbottle^{-1}months^{-1} for 2-phenylethanol and 1.64×10^{-1} μgbottle^{-1}months^{-1} for ethyl hexanoate. The total amount of the 11 studied aroma compounds of wine, stored in osRPET bottles, and lost by permeation after 12 months of storage was about 7.06 ± 0.47 μgbottle^{-1}. This kind of bottle induced a permeation rate of about 15% more than osPET bottles.

Comparison of the amount lost by permeation through the bottle and cap after 12 months storage is presented in Table V. Only four aroma compounds showed a different behavior between both bottles. The amount of permeated ethyl octanoate was higher through osPET bottles than through recycled bottles. In contrast, ethyl hexanoate, isobutanol, and isoamyl alcohol had a greater permeation through the recycled PET bottle than through the osPET bottle. Similar behavior was observed for isoamyl acetate. Moreover, it could be noted that, as previously observed for osPET bottles, the most apolar aroma compounds had a higher permeation (except for ethyl octanoate), and the most polar compounds were part of the less permeating aroma compounds.

Permeation is a global transfer phenomenon, including sorption of aroma compounds at the surface of the polymer, diffusion through polymeric chains and desorption outward. Differences observed between both bottles could be due to several phenomena. To better understand these phenomena, the concentration of aroma compounds in the wine after contact with materials and sorption in the matrix "bottle" and "cap" was studied.

The concentration of each aroma compound in wine stored in both bottles was studied after 12 months storage. Concentration of aroma compounds that were in different amount after 12 months storage in osPET and osRPET is presented in Figure 3. The two major alcohols, isoamyl acetate and 2-phenylethanol, were found in a similar concentration in both PET bottles. A higher concentration in one of the studied bottles could explain a higher permeation. A significant difference was observed for some aroma compounds (hexyle acetate, methionol, hexanol, hexanoic acid, octanoic acid) but did not concern aroma compounds for which permeation were different in the presence and absence of recycled PET, that is, it did not explain the higher or lower permeation of these aroma compounds. These differences could be linked to oxidation phenomenon, since some compounds are sensitive to oxidation and to a slightly different oxygen permeability of the two materials (1.51 et 1.47×10^{-17} molm^{-1}s^{-1}Pa^{-1} for PET et RPET, respectively)[14].

Table V. Amount Lost by Permeation After 12 Months of Storage in osPET and osRPET Bottles (μgbottle^{-1})

Aroma compounds	osPET	osRPET
2-Phenylethanol	0.09 ± 0.01^a	0.08 ± 0.03^a
Ethyl hexanoate	0.33 ± 0.10^b	1.35 ± 0.12^a
Ethyl octanoate	0.97 ± 0.17^a	0.53 ± 0.15^b
Hexanol	0.62 ± 0.15^a	0.52 ± 0.09^a
Hexyl acetate	1.49 ± 0.13 ^{a}	1.57 ± 0.24^a
Hexanoic acid	0.18 ± 0.06^a	0.15 ± 0.04^a
Isobutanol	0.07 ± 0.02^b	0.26 ± 0.07^a
Isoamyl acetate	1.07 ± 0.10^a	1.21 ± 0.27^a
Isoamyl alcohol	0.25 ± 0.04^b	0.57 ± 0.06^a
Methionol	0.33 ± 0.10^a	0.18 ± 0.07^a
Octanoic acid	0.73 ± 0.17^a	0.65 ± 0.17^a
Total amount sorbed	4.21 ± 0.36	5.49 ± 0.47

^{a,b}signified significant difference between osPET and osRPET.

Table VI. Amount Lost by Sorption (bottle + cap) After 12 Months of Storage in osPET and osRPET Bottles (μgbottle^{-1})

Aroma compounds	osPET	osRPET
2-Phenylethanol	28.87 ± 1.20	28.55 ± 2.14
Ethyl hexanoate	15.35 ± 1.53	15.06 ± 1.54
Ethyl octanoate	53.73 ± 7.16	51.86 ± 7.00
Hexanol	1.15 ± 0.11	1.16 ± 0.09
Hexyl acetate	2.98 ± 0.25	2.72 ± 0.23
Hexanoic acid	3.72 ± 0.28	3.81 ± 0.39
Isobutanol	1.26 ± 0.04	1.26 ± 0.13
Isoamyl acetate	3.37 ± 0.52	3.24 ± 0.35
Isoamyl alcohol	15.91 ± 1.07	14.77 ± 0.55
Methionol	5.54 ± 0.60	5.81 ± 0.59
Octanoic acid	26.44 ± 1.16	25.24 ± 2.93

Moreover, we have already observed that after contact with wine, the oxygen permeability of virgin and recycled PET decreased and slightly more for recycled than for virgin.[14]

The total sorption of aroma compounds both in the cap and in each different PET bottles is presented in Table VI. None significant difference between the two systems were observed. Then, the difference of permeation could not be explained by sorption.

A last hypothesis could be highlighted. Diffusion might be the leading phenomenon in permeation transfer. Diffusion depends on the structure of the polymer, nature and chain length, crystallinity, nature but also on sterical volume of diffusing molecule. The presence of recycled PET could explain the higher permeation. Indeed, during the recycling process, the polymer could lose some properties, such as optical, physical, and barrier properties.[22] Recycled PET chains could be shorter because of the mechanical action of the process and these shorter chains could facilitate molecular movement into the matrix. It has been observed that spherulite repartition was not the same in virgin and recycled PET.[14,23] Spherulites in recycled PET were smaller and more heterogeneous, which was explained by the presence of impurities in recycled PET, which play a role of nucleating agent and could lead to the formation of numerous sites of spherulites formation. Smaller and more numerous spherulites might create preferential paths for aroma compound diffusion. Recycled PET could be favorable to aroma compound transfer by facilitating diffusion through the matrix. According to the measurement of isoamyl acetate and hexanol permeabilities done by Dombre *et al.* (2014), it had been demonstrated that both aroma compounds tend to permeate twice higher in 100% recycled PET than in standard PET.[14] In case of studied bottles that contain only 50% of recycled PET, only three aroma compounds showed a higher transfer rate in recycled than in virgin osPET: ethyl hexanoate, isobutanol, and isoamyl alcohol.

Evaluation of Losses by Transfer. Figure 4 presents the repartition of losses of aroma compounds due to sorption and permeation phenomena in osRPET bottles. The repartition of losses for osPET is not reported but is close to one obtained for osRPET. Sorption was the main source of losses by transfer for the aroma compounds studied (up to 97%). Permeation only reached a maximum of 34% of these losses (for hexyle acetate). It could be highlighted that, depending on the aroma compound, the amount sorbed in the cap matrix was higher than the amount sorbed in the bottle material and conversely. As already pointed, the cap was made from an apolar polymer (PE) and the bottle body from a more polar polymer (PET) and the affinity of the aroma compounds for these polymers are different.

Moreover, the volatility and concentration of the aroma compounds in liquid phase and in the headspace impacted the sorbed amount. In the joint cap, sorption occurred by contact with the aroma vapors and in the case of the bottle body, sorption occurred by liquid and vapor contact. Moreover, apolar or polar compounds such as octanoic acid, isoamylic alcohol, 2-phenylethanol, and isobutanol were preferentially sorbed in the

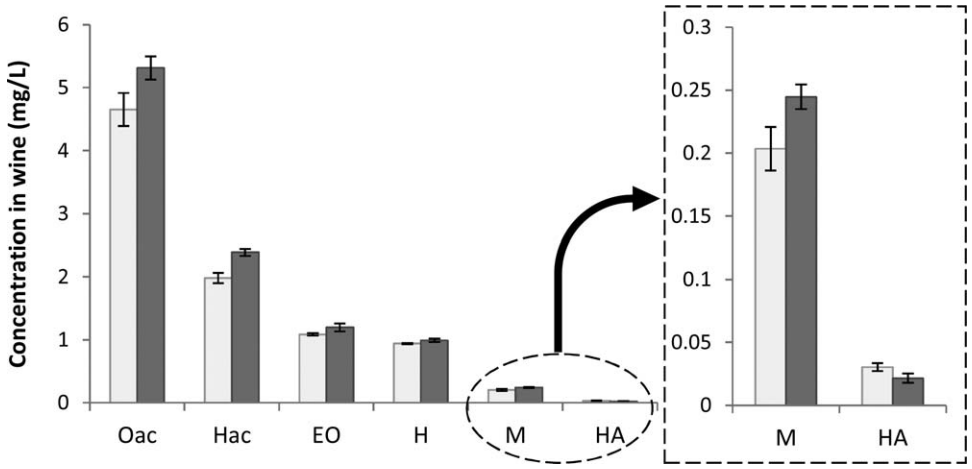

Figure 3. Concentrations (mgL^{-1}) of aroma compounds in wine after 12 months of storage for aroma compound with significantly different amount between osPET (□) and osRPET (■) bottles. Oac: octanoic acid; Hac: hexanoic acid; EO: ethyl octanoate; H: hexanol; M: methionol; HA: hexyle acetate.

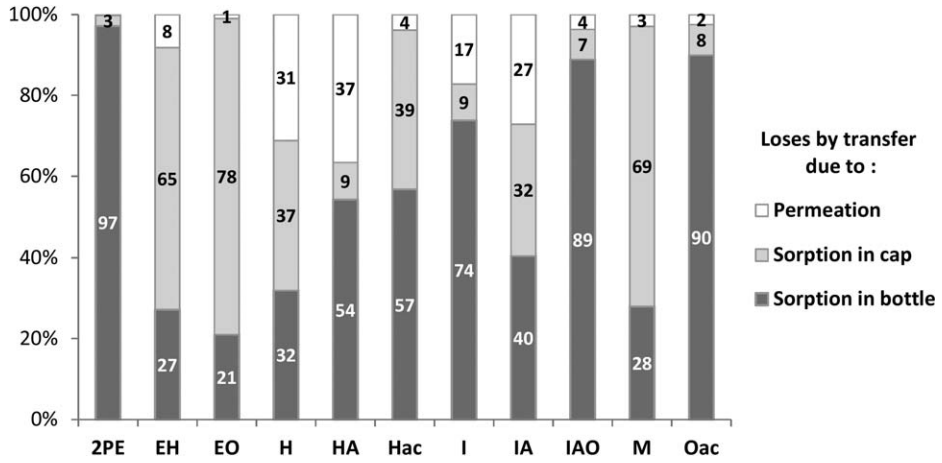

Figure 4. Repartition of losses of aroma compounds due to transfer through osRPET bottle. 2PE: 2-phenylethanol; EH: ethyl hexanoate; EO: ethyl octanoate; H: hexanol; HA: hexyle acetate; Hac: hexanoic acid; I: isobutanol; IA: isoamyl acetate; IAO: isoamyl alcohol; M: methionol; Oac: octanoic acid.

bottles. Conversely, ethyl octanoate and ethyl hexanoate, apolar compounds, were more sorbed in the cap. A higher sorption of methionol in the cap compared to the bottle was observed and was difficult to explain. Methionol is a polar aroma compound with low volatility and was in low concentration in wine. The cap joint is made of multilayers with the PE layer in contact with the headspace but also with an inner EVOH layer, which is well known for its high polarity and could explain the affinity of methionol for the joint cap.

Considering global losses of aroma compounds when wine was stored in RPET bottles, losses by transfer had to be taken into account, but also losses by chemical phenomena such as oxidation, acido-catalyzed reaction (hydrolysis, esterification) or acetalization. The global losses were evaluated by the analysis of the amount of each studied aroma compounds after 12 months storage compared to the initial amount. The losses by transfers were subtracted to the global losses to estimate the amount of aroma compounds lost by chemical reactions. Table VII presents losses for four aroma compounds only. Indeed, among the studied aroma compounds, these four were only with a decreasing concentration during wine storage. The percentage of losses due to transfers represent a very small amount for isobutanol, isoamyl acetate, and isoamyl alcohol (< 0.3%). However, 6% of losses of hexyle acetate were allocated to transfers. Indeed, it was demonstrated previously that permeation for this aroma compound was very high. Nevertheless, there was a low amount of this compound in the wine (Table II), and it had a very small impact on the aromatic profile because of its important detection threshold, 1.5 mgL^{-1}.[24] The amount lost by transfer in the studied case was negligible on the aromatic profile of the wine but could be important if the initial amount of this ester in the wine was higher. Only 11 aroma compounds were studied in these experiments. It could be conceivable that, among several aroma compounds which are constitutive of wine, some of them with a lower detection threshold could permeate or sorb in a great enough amount to impact the aromatic profile of the wine.

CONCLUSION

The method developed in this study was able to follow the permeation of aroma compounds of a wine stored in PET bottles and to allow a semiquantification. It could be applied in the case of other foods or packaging to comparison purpose. This study allows to conclude on the impact of the use of recycled PET on aroma transfer through PET-based packaging: the structure of recycled PET, that is, shorter chains and spherulite repartition, seems to induce a stronger diffusion through the matrix and in consequence to increase the transfer. The general study of aroma compound losses by transfer through PET bottles evidences the weak impact of this phenomenon (< 10%) in contrast with losses due to chemical reactions such as oxidation and esterification of hydrolysis. However, depending on the nature of

Table VII. Losses Repartition Between Transfers and Chemical Reactions for Four Aroma Compounds After 12 Months of Storage in osRPET Bottle

	Amount lost ($\mu gbottle^{-1}$)				
	By sorption				
Aroma compounds	In bottle	In cap	By permeation	By chemical reactions	% of losses due to transfers
Hexyl acetate	2.33	0.39	1.57	71.3	5.67
Isobutanol	1.12	0.14	0.26	616.1	0.25
Isoamyl acetate	1.80	1.44	1.21	1556.6	0.29
Isoamyl alcohol	13.64	1.13	0.57	5861.8	0.26

the aroma compound and its detection threshold, these losses, added to others, could be detrimental and should be taken into account to limit impact on the aromatic profile of wine.

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