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ARTICLE

Changes in the Sorption of Diverse Volatiles by *Saccharomyces cerevisiae* Lees during Sparkling Wine Aging

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The volatile profile of sparkling wine is influenced by the retention and release of volatile compounds by lees during the aging process. Here we attempted to identify the volatiles that are most retained by lees in aging conditions and to study how their sorption varies during aging. We estimated the lees sorption capacity for several representative volatile compounds in sparkling wine samples at a range of time points during aging by assessing the volatiles sorbed on the lees surface and those present in the corresponding wines. The sorption of volatiles was proportional to their hydrophobicity, and their retention by the lees surface changed during aging. The sorption of less hydrophobic compounds decreased after the first 2 months of aging, while that of the most hydrophobic volatiles increased until 18 months, and decreased dramatically thereafter. These results indicate that the length of aging on lees determines the type and the amount of wine volatiles removed with lees in the disgorging step. While most polar aromas seem to be released from the lees surface at the earliest stages of aging, highly hydrophobic compounds and esters in general are progressively retained and subsequently desorbed into wine. Changes in the physicochemical properties of the lees cell surface were monitored during aging, but these could explain only the decrease in the sorption of less hydrophobic compounds.

KEYWORDS: Sparkling wine; lees; aging; volatiles; sorption; cell surface; SPME

INTRODUCTION

The traditional method of making sparkling wines includes a second fermentation in a closed bottle and extensive aging on lees. This period ranges from a minimum of 9−12 months to several years. This practice influences the sparkling wine "bouquet", increasing its complexity and providing toasty, lactic, sweet, and yeasty notes, which increase the longer the aging period (*1*). These sensory attributes reflect the composition of the volatile fraction of sparkling wines. Although there is no full consensus between studies, it appears that, during the second fermentation and subsequent aging in contact with lees, a number of compounds, such as acetate and ethyl esters, decrease in amount, while others, such as norisoprenoids, acetal, diacetyl, and furans, increase over time (*1*−*4*). In addition to enzymatic and chemical reactions that lead to the formation and degradation of volatiles, the interaction between aroma compounds and lees cell walls may affect the aroma of sparkling wines as a result of sorption phenomena (*5*,*6*). In fact, the capacity of wine yeast to adsorb volatile compounds (*7*−*11*) can modify the concentration of several of these compounds once lees are removed, as reported in white wines after contact with lees (*12*, *13*).

The sorption capacity of yeast cell walls has been studied using viable or dry yeasts (*14*, *15*), yeast cell walls (*7*), and yeast cell wall components (*8*, *16*, *17*), and only in a few cases have artificially autolysed yeasts been examined (*10*, *18*); however, this issue has not been addressed in lees from aged wine. Likewise, studies have been carried out in model systems but not in enological aging conditions. Although the nature of the interactions between volatile compounds and yeasts is not fully understood, the results of the above-mentioned studies suggest that yeast sorption is a balance between hydrophobic, Lewis acid/base and electrostatic interactions, and that the physicochemical characteristics of the yeast surface influence yeast sorption of some volatile compounds (*14*, *15*). Nonetheless, the physicochemical properties of lees cell surfaces were recently shown to be strongly modified during sparkling wine aging (*19*), thus possibly influencing sorption capacity.

When considering lees−wine interactions at a molecular level, cell wall mannoproteins are believed to play a major role in volatile adsorption (*7*,*8*,*14*). Nevertheless, not all mannoproteins contribute to this process, and the presence of specific binding

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sites has recently been proposed (*7*). Those same authors reported that distinct specific proteins on yeast walls have different effects on the sorption of 4-ethylphenol. In addition, it has been postulated that plasma membrane phospholipids of autolysed cells participate in the sorption of geosmin by hydrophobic interactions (*18*).

The volatile profile of sparkling wine is expected to be influenced by the retention and release of volatile compounds by lees during aging. Furthermore, it has been demonstrated that lees cells modify their physicochemical surface properties as a result of cell structure modifications induced by autolysis during sparkling wine aging.

The aim of the present study was to investigate the volatile compounds most retained by lees in enological aging conditions and examine how their sorption varies during aging on lees. A greater understanding of the physicochemical interactions between lees and wine volatiles may contribute to clarifying the contribution of aging on lees to the aromatic features of sparkling wine, and thus facilitate modulation of these features by optimizing the length of aging.

MATERIALS AND METHODS

Chemicals. Sodium acetate trihydrate, acetic acid glacial, hydrochloric acid, NaCl and $CaCl_2$ were from Panreac (Barcelona, Spain). Ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-methylbutyl octanoate, phenylethyl acetate, diethyl succinate, 3-methylbutan-1-ol and β-damascenone were from Sigma-Aldrich (St. Louis, MO, USA).

Lees and Sparkling Wine Samples. Lees of the same strain of *Saccharomyces cerevisiae* belonging to the private collection of the winery Freixenet S.A. were from industrial sparkling wines obtained from two distinct *coupages* of base wines (A and B) produced in distinct crop years from Xarel·lo, Parellada and Macabeo grapes. Lees and the corresponding wines were collected and separated after 2, 10, 18, and 40 months of aging. Lees and wines from two bottles were analyzed for each sampling point (total of 16 wine samples and the 16 corresponding lees samples). Each lees and wine sample was analyzed in duplicate. Sampling points corresponded to the aging periods of cava sparkling wine categories: Cava ($\geq$9 months), Reserva ($\geq$15 months) and Gran Reserva ($\geq$30 months) (*20*), plus one sampling point in the initial stages of the aging (2 months).

Lees were isolated from wines as follows: the content of 1 bottle of sparkling wine over lees was centrifuged for 15 min at 1410*g* and 4 °C (Rotina 48CR, Tuttlingen, Germany). The supernatant was then placed in a 250 mL amber flask and stored at −20 °C until analysis. The pellet was then washed three times with 10 mL of sodium acetate buffer (pH 3.6, 0.3 M). Lees were resuspended in 5 mL of the same sodium acetate buffer for the determination of cell surface properties and cell counts, or in 1 mL of phosphate buffer in NaCl 0.9% (pH 7, 10 mM) for the analysis of volatiles. Lees were kept refrigerated and under nitrogen atmosphere until analysis.

Cell Counts. The number of cells in each lees suspension sample was measured by using a Coulter counter (Multisizer II, with a 70 μm aperture tube): for which 1.0 mL of cell suspension was added to 49.0 mL of NaCl 0.9% solution (Panreac, Barcelona, Spain). In order to prevent flock formation, the stock solution was properly shaken during measurements. A narrow and sharp Gaussian-type distribution curve of cell size was obtained, indicating a satisfactory cell separation in the stock solution. The number of cells/mL was established by dividing the number of events by the volume of the loop (500 μL). For each suspension the measure was made in triplicate.

Sorption Capacity for Wine Volatiles. *Headspace Solid Phase Microextraction (HS-SPME) of Lees and Wines.* The analysis of volatiles sorbed on the lees surface during sparkling wine aging was carried out as previously reported (*11*). In this case, 15 mg of lees placed in a 10 mL vial and suspended in 1 mL of 10 mM phosphate buffer in 0.9% NaCl (pH 7) was maintained at 50 °C under magnetic stirring (700 rpm). A divinylbenzene/Carboxen/polydimethylsiloxane SPME fiber (DVB/CAR/PDMS) 50/30 μm, 2 cm long, from Supelco (Bellefonte, PA, USA), was exposed to the sample headspace during 40 min, then immediately desorbed in the GC injection port.

Four milliliter samples of the corresponding sparkling wines were analyzed in the same extraction conditions.

Gas Chromatography/Mass Spectrometry (GC/MS). Identification and quantification of compounds was performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a Supelcowax-10, 30 m × 0.25 mm i.d., 0.25 μm film thickness. The column temperature was held at 60 °C for 3 min, increased to 75 at 4 °C/min, then to 260 at 8 °C/min and held for 5 min. The injector temperature was 260 °C, and the time of desorption of the fiber into the injection port was fixed at 5 min. Helium was used as carrier gas, at a linear velocity of 38 cm/s. The temperature of the ion source was 175 °C, and the transfer line, 280 °C.

GC−MS analysis in the complete scanning mode (SCAN) was performed in the 40−300 au mass range.

Compounds in the headspace of lees and wine samples were identified by comparison of their mass spectra and retention times with those of standard compounds, or by comparison with those of the Wiley mass spectral library, 6th ed. Kovats retention indices were determined with reference to a homologous series of linear alkanes and compared with those available in the literature. Chromatographic areas of the volatiles selected for the study were determined in lees and wines by extracting and analyzing the following mass spectral fragments: *m*/*z* 88 (for ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl 9-decenoate, 2-methylbutyl octanoate); *m*/*z* 91 (phenylethyl acetate); *m*/*z* 101 (diethyl succinate), *m*/*z* 70 (2- and 3-methylbutan-1-ol), *m*/*z* 192 (vitispirane, sum of isomers); *m*/*z* 157 (trimethyl dihydronaphthalene, TDN) and *m*/*z* 190 (β-damascenone).

Semiquantitative Determination of Volatile Compounds. As the amount of volatiles on the lees surface depends on their concentration in wine, and given that wine volatile profile may change over time, lees sorption capacity for each compound was estimated as the relative ratio between the chromatographic response of volatiles extracted from the headspace of lees (chromatographic areas/no. of cells per bottle) and of the corresponding wines (chromatographic areas/mL of wine per bottle), following eq 1:

$$R_r = \frac{\frac{A_{cell}}{N} \times n}{\frac{A_{wine}}{V} \times v} \quad (1)$$

where A_{cell} and A_{wine} are the chromatographic area counts of volatiles from lees and wine headspace analysis, respectively; N is the number of cells analyzed for each lees sample; V is the volume of wine analyzed; n is the approximate number of cells contained in a bottle (3×10^8 cells); and v is the volume of wine contained in a bottle (750 mL).

Given that the volatilization of compounds from suspended lees and from wine varies, these ratios are not absolute, but they are suitable for monitoring the volatile sorption differences as a function of the characteristics of the compounds and the length of aging on lees.

Cell Surface Characteristics. The possible correlation between the sorption of lees volatiles and the physicochemical properties of the lees surface was evaluated. With this aim, cell surface hydrophobicity, electron donor/acceptor properties and zeta potential of each lees sample were determined as reported in a previous study (*19*). In brief, the electron donor character (EDC) and the electron acceptor character (EAC) were calculated as the difference between the % affinity of lees for chloroform and hexadecane, and for ethylacetate and decane, respectively, following Bellon-Fontaine et al. (*21*). Cell surface hydrophobicity (CSH) was determined as the % affinity of lees for hexadecane, while the lees surface charge was quantified by measurement of the electrophoretic mobility of cells on laser Zetacompact equipment (CAD Instrumentation, Limours, France).

Statistical Analysis. The Statgraphics Plus (1999) packages were used for the statistical analysis of data. Factorial analysis of variance was carried out to assess the influence of length of aging, Log *P* and wine *coupage* on the retention of volatiles. Moreover, we performed stepwise multiple regression to evaluate possible correlations between cell surface physicochemical properties and the retention of volatile compounds.

RESULTS AND DISCUSSION

The sorption of volatiles on the lees surface during sparkling wine aging was evaluated for several compounds that differ in their physicochemical characteristics and that are representative

Table 1. Relative Ratios of Volatile Compounds between Lees and Wine Headspace (Mean ± Standard Deviation), and Their Hydrophobicity (Log *P*)

		relative ratio							
compound	Log *P*[a]	2[b] A[c]	2 B[d]	10 A	10 B	18 A	18 B	40 A	40 B
sum of 2-[e] and 3-methylbutan-1-ol[f]	1.2	0.011 ± 0.005	0.008 ± 0.002	0.009 ± 0.001	0.007 ± 0.000	0.004 ± 0.001	0.005 ± 0.000	0.004 ± 0.001	0.004 ± 0.001
diethyl succinate [f]	1.2	0.017 ± 0.000	0.011 ± 0.001	0.002 ± 0.001	0.003 ± 0.000	0.003 ± 0.001	0.004 ± 0.001	0.001 ± 0.000	0.001 ± 0.000
phenylethyl acetate [f]	2.3	0.008 ± 0.005	0.003 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.000 ± 0.000	0.003 ± 0.001	0.002 ± 0.000	0.000 ± 0.000
ethyl hexanoate [f]	2.4	0.009 ± 0.001	0.015 ± 0.015	0.002 ± 0.000	0.004 ± 0.001	0.004 ± 0.001	0.008 ± 0.002	0.001 ± 0.000	0.003 ± 0.002
vitispirane[e]	3.1	0.031 ± 0.001	0.040 ± 0.002	0.018 ± 0.000	0.015 ± 0.002	0.015 ± 0.001	0.022 ± 0.000	0.017 ± 0.001	0.019 ± 0.002
β-damascenone [f]	3.2	0.019 ± 0.004	0.016 ± 0.003	0.006 ± 0.004	0.005 ± 0.004	0.006 ± 0.006	0.013 ± 0.001	0.006 ± 0.000	0.009 ± 0.001
ethyl octanoate [f]	3.5	0.082 ± 0.004	0.051 ± 0.003	0.118 ± 0.002	0.093 ± 0.010	0.100 ± 0.010	0.116 ± 0.004	0.055 ± 0.001	0.058 ± 0.006
ethyl-9-decenoate [e]	4.0	0.46 ± 0.05	0.14 ± 0.03	0.61 ± 0.03	0.34 ± 0.03	0.69 ± 0.07	0.88 ± 0.02	0.41 ± 0.03	0.34 ± 0.02
TDN isomers [e]	4.2	0.63 ± 0.09	0.70 ± 0.01	0.79 ± 0.02	0.72 ± 0.15	0.99 ± 0.14	1.56 ± 0.11	0.56 ± 0.02	0.34 ± 0.02
ethyl decanoate [f]	4.6	1.14 ± 0.19	0.63 ± 0.01	1.50 ± 0.02	1.42 ± 0.09	1.88 ± 0.32	2.07 ± 0.09	0.78 ± 0.04	0.56 ± 0.03
2-methylbutyl octanoate[f]	4.8	3.08 ± 0.04	3.00 ± 0.10	4.46 ± 0.44	3.30 ± 0.47	4.06 ± 0.77	4.90 ± 0.46	1.98 ± 0.22	1.14 ± 0.08

[a] Hydrophobicity of compounds expressed as Log *P* value (*23*). [b] Aging period (months). [c] Base wine *coupage* "A". [d] Base wine *coupage* "B". [e] Tentatively identified. [f] Identified by comparison with reference compounds.

Table 2. Effect of Different Factors and Interactions on the Relative Ratio of Volatiles between Lees and Wine, Calculated by Factorial Analysis of Variance

factor	%[a]	*p*[b]
month (*A*)	3.8	<0.0001
Log *P* (*B*)	85.0	<0.0001
base wine *coupage* (*C*)	0.1	<0.05
interactions		
AB	10.5	<0.0001
AC	ns	>0.05
BC	ns	>0.05

[a] Percent of explained variance by each factor and interaction. [b] Significance of the effect.

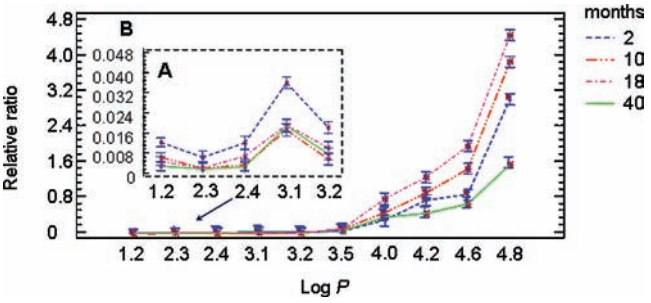

Figure 1. Effect of the interaction between aging time (months) and compound hydrophobicity (Log *P*) on the sorption of wine volatiles by lees (relative ratio), as obtained by factorial analysis of variance. **A**: Changes in the sorption of compounds with Log *P* values from 1.2 to 3.2 during aging. **B**: Changes in the sorption of compounds with Log *P* values from 3.5 to 4.8 during aging. Log *P* 1.2: 3-methylbutan-1-ol, diethyl succinate. Log *P* 2.3: phenylethyl acetate. Log *P* 2.4: ethyl hexanoate. Log *P* 3.1: vitispirane. Log *P* 3.2: β-damascenone. Log *P* 3.5: ethyl octanoate. Log *P* 4.0: ethyl-9-decenoate. Log *P* 4.2: TDN isomers. Log *P* 4.6: ethyl decanoate. Log *P* 4.8: 2-methylbutyl octanoate.

of the volatile profile of both wine and lees. These compounds and their hydrophobicity (Log *P*, the logarithm of the octanol/water partition coefficient) are reported in **Table 1**. They were taken into consideration for the following reasons: esters such as ethyl octanoate, ethyl decanoate, 2-methylbutyl octanoate and ethyl-9-decenoate are the major volatiles recovered from lees headspace, accounting for up to 80% of the total chromatographic area (*11*); methylbutan-1-ol is the major higher alcohol in wine, while phenylethyl acetate, diethyl succinate, vitispirane, TDN isomers and β-damascenone are related to the length of aging on lees of sparkling wine (*1*, *4*, *22*). Moreover, most of these volatiles are described as contributors to wine aroma (*1*, *24*, *25*). Other volatile compounds, namely, methylbutyl acetate, hexanol, β-ionone and ethyl hexanoate, were previously studied in model systems for their interactions with yeast cell wall constituents (*7*, *8*, *16*, *17*, *26*). In the present study of real wine and lees samples, these compounds, with the exception of ethyl hexanoate, were not quantifiable in lees headspace after their isolation from wine. Their poor recovery could be attributed to the low content in sparkling wine, or to their minor interaction with the lees surface.

In order to evaluate lees sorption capacity during aging, independently from the modifications that occur in the volatile profile of sparkling wine, we estimated the retention of each compound by lees as the relative ratio between the chromatographic responses of compounds extracted from the headspace of lees and of their corresponding wines, following eq 1 (**Table 1**).

The factorial analysis of variance showed that the sorption of wine volatiles (relative ratio) was significantly influenced by the hydrophobicity of compounds, by length of aging and by the wine *coupage* (**Table 2**). Hydrophobicity of compounds was the main factor affecting their sorption by lees, as indicated by the observation that this parameter showed the highest percent of explained variability. For the base wines sampled, the type of *coupage* had a significant but small effect on sorption, while the length of aging on lees, and in particular the interaction between length of aging and compound hydrophobicity, showed a considerable effect on this process. This interaction is illustrated in **Figure 1**, in which the sorption of volatiles by lees (relative ratio) at a range of time points during aging is reported as a function of compound hydrophobicity (Log *P*). Although some authors postulate that the sorption of compounds on the yeast wall is unrelated to polarity (*27*, *28*), these data obtained in enological conditions prove that the highest lees sorption capacity is achieved for volatiles with high hydrophobicity (Log *P* values above 4, namely ethyl 9-decenoate, TDN isomers, ethyl decanoate and 2-methylbutyl octanoate). This finding is consistent with results obtained in a model system (*7*). Moreover, the affinity of lees for wine volatiles changed during aging depending on compound hydrophobicity. Relative ratios indicate that lees affinity for less hydrophobic compounds (Log *P* from 1.2 to 3.2, see **Table 1**) dropped after two months of aging (**Figure 1A**), while for more hydrophobic compounds (Log *P* from 3.5 to 4.8, see **Table 1**) lees sorption progressively increased until certain stages of aging (18 months) and decreased at relatively long periods (40 months) (**Figure 1B**), when almost complete degradation of cell wall structure is supposed to occur (*29*). As relative ratios were considered, these differences do not depend on the changes in

Table 3. Significant Correlations ($p < 0.05$) between Relative Ratios of Wine Volatiles and Lees Surface Properties, Obtained by Stepwise Multiple Regression

	CSH[a]		Z[b]	
compound	m[c]	%[d]	m	%
2-[e] and 3-methylbutan-1-ol[f]			−0.031	37.9
diethyl succinate[f]	0.021	73.5		
phenylethyl acetate[f]	0.007	27.4		
vitispirane isomers[e]	0.027	50.1		
ethyl hexanoate[f]	0.021	31.8		
β-damascenone[f]	0.016	37.5		

[a] Cell surface hydrophobicity. [b] Cell surface zeta potential. [c] Slope of the linear regression prediction model. [d] Percent of the sorption variability explained by each cell surface property. [e] Tentatively identified. [f] Identified by comparison with reference compounds.

wine characteristics but rather reflect the changes that occur on the lees surface, thus indicating that the retention of volatiles by lees depends on physicochemical properties of aroma compounds and the cell surface. The structural changes reported to occur in the yeast cell wall as a result of the autolysis (*30*−*32*) would explain the variation observed in lees affinity for the same compound during aging. In order to evaluate the possible correlation between lees sorption of volatiles and the physicochemical properties of the lees surface during wine aging, cell surface hydrophobicity, electron donor/acceptor properties and zeta potential of each lees sample were assessed. The physicochemical surface properties of lees samples considered in the present study (data not shown) were equivalent to those obtained from analogous samples from the same batches reported in a previous study by our group (*19*), and were proved to be significantly dependent on the time that the sparkling wine was aged on lees. The influence of these cell surface characteristics on the sorption of each volatile compound during aging was evaluated by a multiple regression analysis (**Table 3**). A significant influence of lees surface characteristics on relative ratios was detected only for the less hydrophobic volatiles (Log $P \leq 3.2$, see **Table 1**), while no correlation was found between lees surface properties and sorption of the most hydrophobic esters and TDN isomers (Log $P \geq 3.5$, see **Table 1**). These observations are consistent with the different behavior observed in the sorption of these two groups of compounds during aging (**Figures 1A** and **1B**). It can be presumed that interactions of distinct nature or involving distinct parts of the yeast cell take place, depending on the characteristics of volatile compounds. Cell surface hydrophobicity was the main parameter influencing the sorption of less hydrophobic volatiles, while zeta potential appeared to explain the most methylbutan-1-ol results (**Table 3**). Like the sorption of the above-mentioned compounds, both lees surface hydrophobicity and negative charge decreased during aging, possibly due to a loss of mannopropeins (*19*). Nevertheless, some of these correlations explained a low percentage of data variability, thereby indicating that these surface properties do not fully account for the variation observed in sorption.

The lack of correlation between the sorption of the most hydrophobic wine volatiles and the physicochemical characteristics of lees along aging (**Table 3**) could be explained in several ways. The modification of yeast cell wall structure induced by autolysis could imply changes in the availability or the accessibility of those proteins proposed by Pradelles et al. (*14*) to have the capacity to provide or decrease potential specific binding sites. In contrast, Chalier et al. (*17*) observed that compounds such as ethyl hexanoate have a higher affinity for the glycosidic part of mannoprotein than for the proteic part, and Boido et al. (*33*) reported a stable linkage of wine aromas to the external polysaccharides of bacterial cell walls. The increase in exposure of the glucan layer resulting from the hydrolysis of bonds connecting β→1−3 glucans to mannoproteins (*32*) could increase sorption of the ethyl esters of higher alcohols during some stages of wine aging (**Figure 1**). Finally, the participation of the inner plasma membrane in the sorption of the most hydrophobic compounds, as suggested by Pradelles et al. (*18*) for geosmin (Log *P* 3.3), could explain their increased sorption until certain stages of aging. The participation of lees plasma membrane in the sorption of volatiles would be unrelated to the cell surface physicochemical characteristics, but would be associated with the increased porosity of lees cell wall during autolysis (*30*).

In conclusion, the study of the retention of diverse volatiles by the lees surface in wine indicates that sorption depends on the physicochemical characteristics of aromas and lees cell wall. Sorption was proportional to the hydrophobicity of compounds. However, the retention of volatiles by the lees surface changed during the aging period: the sorption of less hydrophobic compounds appeared decreased after the first 2 months of aging, while that of the most hydrophobic volatiles increased until 18 months, to decrease dramatically thereafter. These results suggest that the wine aroma profile resulting from aging on lees is influenced by interaction with the lees surface. The length of aging would determine the type and amount of wine volatiles removed with lees in the disgorging step. While most polar aromas would be released from the lees surface at the earliest stages of aging, highly hydrophobic compounds and esters in general would be progressively retained until approximately 18 months and subsequently desorbed into wine.

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