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Haloanisoles and new barrels: highlighting the role played by molds

>>> The aging of wine in oak barrels has enjoyed renewed interest with the advent of modern enology and the demonstration of its importance in giving expression to the finest wines. With a standard capacity of 225 L, potentially 300 bottles of 75 cL, any untoward incident involving a barrel can be highly detrimental to the producer. For this reason, preventing the accidental presence of 2,4,6-trichloroanisole (TCA) in barrels has been a major technical and economic issue for the cooperage sector for several years. <<<

LEC laboratory is closely involved with this sector, and since 2012 has been offering a haloanisole control system for new barrels based on the principle of dynamic sampling of volatile organic compounds (VOCs)[1]. In 2015 in the United States, a wine aged in a French oak barrel was found to have a concentration of 11 ng/L of TCA (2,4,6-trichloroanisole), whereas it had tested negative when leaving production. The wine also contained 82 ng/L of TCP (2,4,6-trichlorophenol), a TCA precursor. This very unusual concentration suggested a possible natural conversion of TCP to TCA, either during vinification or during container transport. Malolactic fermentation trials in the presence of halophenols did not confirm their transformation into their respective haloanisoles during MLF. The existence of molds likely to develop inside new barrels has been considered. This phenomenon has been well documented in premises containing structural timber elements treated with pentachlorophenol preparations which, over time and under the action of mold, end up contaminating the atmosphere with haloanisoles[2, 3, 4, 5].

Purpose of this study

Assess the ability of common molds to transform halophenols into haloanisoles inside new barrels. The observations led LEC laboratory to develop its barrel control system, to analyze for the presence of all these compounds.

■ First phase of the experiment

A blend of 20 % untoasted oak chips and 80 % with medium toast were contaminated by maceration in an ethanol solution containing the main halophenols (TeCP, TCP, PCP, TBP). Maceration was for 24 hours in a solution containing 5 µg/L of each compound with a ratio of 100 g of oak per liter of solution.

After drying for 12 hours, 10 g of contaminated chips were placed in 100 mL flasks and treated according to 3 different procedures:
➔ No. 1: Control.

➔ No. 2: Addition of 10 mL of ultrapure water to maintain an environment conducive to microbial growth.
➔ No. 3: Addition of 10 mL of ultra-pure water and 100 mg of wheat flour: this is used in cooperage to make a paste to seal the heads of the barrel.

There were three replicates for each procedure. The flasks were hermetically sealed and placed in a temperature-controlled chamber at 20 °C and then analyzed using a thermal desorption system for the concentration and injection of the VOCs, a gas chromatograph equipped with a suitable column for their separation and a universal detection system with a Flame Ionization Detector (FID) and a Mass Spectrometer (MS).

■ Results of the first phase

After 3 weeks, the oak from the 3 replicates of each procedure was recovered, dried overnight in the open air and then analyzed according to a COFRAC accredited internal method. The results obtained are summarized in Table 1.

Table 1. Results of oak analyses expressed in µg/kg. *N.D. = Not detected, i.e. below the detection limit for the method.

	Procedure No. 1			Procedure No. 2			Procedure No. 3		
	Trial 1/3	Trial 2/3	Trial 3/3	Trial 1/3	Trial 2/3	Trial 3/3	Trial 1/3	Trial 2/3	Trial 3/3
TeCA	N.D.*	N.D.	N.D.	0.8	0.9	0.6	2.2	1.7	2.2
TeCP	17.9	18.5	17.1	13.1	12.8	13.5	9.7	7.7	8.2
TCA	N.D.	N.D.	N.D.	2.2	2.6	1.8	3.7	2.6	1.8
TCP	10.2	10.5	9.5	5.7	4.6	6.5	2.7	2.5	3.2
PCA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	< 0.3	< 0.3
PCP	15.9	16.5	15.6	11.7	11.8	12.8	11.0	10.0	9.6
TBA	N.D.	N.D.	N.D.	1.8	2.3	1.4	4.3	3.3	2.9
TBP	14.5	14.6	13.9	10.4	9.8	10.9	6.1	5.1	6.2

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A significant decrease in halophenol concentrations compared with the control is observed in the wet oak, while there are substantial concentrations of haloanisoles. These observations are even more marked in the presence of flour.

Presence of well-known molds

Identification by a specialist laboratory of the microorganisms in the wet oak made it possible to identify the presence of *Aspergillus flavus* and *Aspergillus oryzae*, well-known molds commonly found in the agri-food industry[6] (Figure 2). It seemed relevant to check if this phenomenon could also take place in barrels under export conditions.

■ Second phase of the experiment

Three small new barrels of 28 L were hydrated according to the following procedure: addition of 200 mL of ultrapure water, left to rest for 1 hour on each head and 1 hour on each side. The barrels were then drained overnight, upside-down through the bunghole. They were then subject to three different procedures with the following characteristics:

➔ No. 1: Ultrapure rinsing water, with addition of 1 g of flour after draining.

➔ No. 2: Rinsing water, with addition of halophenols.

➔ No. 3: Rinsing water, with addition of halophenols and addition of 1 g of flour after draining.

The barrels were then shrink-wrapped and stored upright for 4 weeks under temperate conditions (10 to 15 °C, relative humidity of about 40 %).

■ Results of the second phase

The first observations were as follows:

➔ Continuous monitoring of the interior of one of the barrels made it possible to determine that the humidity remained stable at around 90 % (in the case of six-month monitoring of a standard 225 L barrel previously rinsed and drained, the humidity inside never dropped below 80 %).

➔ After two weeks, the presence of haloanisoles was observed in the atmosphere inside barrels 2 and 3.

➔ After 4 weeks, mold spots were clearly visible on the head of barrel 2 (Figure 1). It was therefore necessary to dismantle barrels 2 and 3 for observation and analysis of the oak.

In barrel 3 (Figure 2), the molds completely colonized the lower head, on which the flour had been deposited.

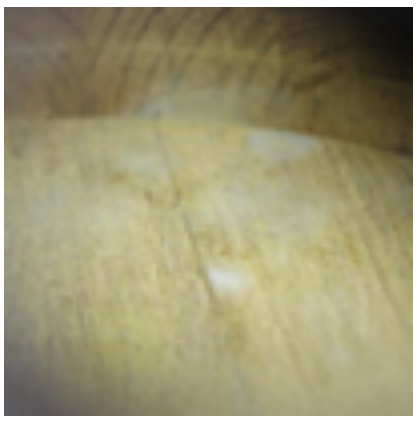

Figure 1. Mold spots on the head of barrel 2.

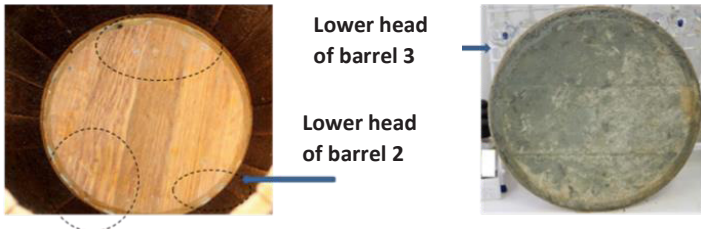

Figure 2. State of the heads of barrels 2 and 3 after dismantling.

Samples of oak were taken from the upper and lower heads and the staves, with the results summarized in Table 2.

Table 2. Results of oak analysis of the barrels expressed in µg/kg.

	BARREL 2 (+ HALOPHENOLS)			BARREL 3 (+ HALOPHENOLS & FLOUR)		
	Upper head	Lower head	Staves	Upper head	Lower head	Staves
TeCA	< 0.3	0.5	0.8	1.7	26.1	2.3
TeCP	2.5	9.7	5.1	2.7	12.9	1.7
TCA	< 0.3	< 0.3	0.5	0.0	6.6	1.0
TCP	0.8	2.2	2.0	0.9	3.1	0.5
PCA	N.D.	0.6	< 0.3	0.3	N.D.	0.6
PCP	3.3	21.7	9.4	3.4	79.6	3.4
TBA	0.5	0.8	1.4	1.9	41.0	3.1
TBP	2.9	7.9	7.7	1.9	4.7	0.7

■ Conclusions

➔ Wet oak is a potential substrate for common molds, favored by the presence of residues from the flour used to seal the heads.

➔ The ability of these microorganisms to convert halophenol precursors into bad-smelling haloanisoles in a few days was demonstrated by this study.

➔ It is therefore strongly recommended to ensure the absence of these precursors in barrels, especially those intended for export. ■

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1 List of haloanisoles/phenols subject to routine analysis: TCA (2,4,6-trichloroanisole), TCP (2,4,6-trichlorophenol), TBA (2,4,6-tribromoanisole), TBP (2,4,6-tribromophenol), TeCA (2,3,4,6-tetrachloroanisole), TeCP (2,3,4,6-tetrachlorophenol), PCA (pentachloroanisole), PCP (pentachlorophenol).

2 McAllister, K.A., Lee, H. & Trevors, J.T. (1996). Microbial degradation of pentachlorophenol. *Biodegradation* https://doi.org/10.1007/BF00056556

3 Allard, A. S., Remberger, M., & Neilson, A. H. (1987). Bacterial O-methylation of halogen-substituted phenols. *Applied and environmental microbiology*, 53(4), 839–845.

4 Chatonnet, P., Fleury, A., and Boutou, S. (2010). Identification of a New Source of Contamination of *Quercus* sp. Oak Wood by 2,4,6-Trichloroanisole and Its Impact on the Contamination of Barrel-Aged Wines. *J. Agric. Food Chem.*, 58 (19), pp 10528–10538. https://doi.org/10.1021/jf102571v

5 McNally, K.J., and Harper, D.B. (1991). Methylation of phenol by chloromethane in the fungus *Phellinus pomaceus*. *Microbiology*. https://doi.org/10.1099/00221287-137-5-1029

6 Tindale, C. R., Whitfield, F. B., Levingston, S. D. and Nguyen, TH. (1989). Fungi isolated from packaging materials: Their role in the production of 2, 4, 6-trichloroanisole. *J. Sci. Food Agric.*, 49: 437-447. https://doi.org/10.1002/jsfa.2740490406