Improvement of the Volatile Components of Cork from *Quercus suber* L. by an Autoclaving Procedure

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The volatiles of cork slabs submitted to a novel industrial treatment, autoclaving, were compared with the volatiles of the untreated slabs to establish the usefulness of this treatment as a cleanup procedure. The condensed steams corresponding to the first and a second autoclaving process were also analyzed. The volatile compounds of cork and of autoclaved condensed steam were extracted by simultaneous distillation−extraction and analyzed by gas chromatography and combined gas chromatography−high-resolution mass spectrometry. Cork slabs treated with steam under pressure showed smaller amounts of the volatile components with musty, moldy, and related odors, such as 3-methyl-1-butanol, 1-octanol, and guaiacol and other mold metabolites. The condensed steam contained compounds corresponding to those extracted from the cork. The autoclaving process has potential for industrial use to clean the cork and to reduce volatiles of microbial origin in cork samples.

Keywords: *Quercus suber L.; volatiles; autoclaving process; condensed steam; musty and moldy odors*

INTRODUCTION

In recent years considerable efforts have been made to improve quality control in the manufacture of cork stoppers. There is an increasing interest in knowing how changes in the composition of the cork during the different manufacturing steps can affect the quality of the final product, so that the process can be suitably modified.

Traditionally, after being stripped from the tree, the cork slabs are aged for some months in the forest. At the factory the slabs are boiled for about 1 h and, in general, left to stand in piles for 3 weeks to become flat. As soon as the water content is optimal, the slabs are ready to be processed to cork stoppers. It is mainly during this period of 3 weeks that molds may grow on the slabs. Daly et al. (1984) have demonstrated that the moisture content of the cork slab is a major factor in controlling the growth of inherent mold contamination. Under suitable moisture, temperature, and ventilation conditions several molds are able to grow on cork slabs. As a consequence of this growth, some species may produce volatile metabolites which taint the wine in contact with this cork.

A new treatment that enables processing of the cork slab directly after the boiling stage (without the traditional 3 weeks of storage of the wet slabs) has been introduced. This process disinfects the cork and extracts volatile compounds of microbial origin associated with the "cork taint" (Champcork, 1992). The process consists of autoclaving the cork slabs with water vapor, for 18−20 min, at 130 °C and at a pressure of 180 kPa. After being exposed to this procedure, the cork slabs can be used without delay in the normal line of the cork stopper production.

The purpose of this study was to examine the effect of the autoclaving process on the volatiles content of the cork and to determine whether these changes occur due to chemical transformation or by vapor phase transport of the volatile components. Normally, factory production uses only one autoclaving step. To examine the resistance of compounds to removal, a second autoclaving step was used in this work.

EXPERIMENTAL PROCEDURES

Cork and Autoclave Condensed Steam Samples. Three cork slabs (120 cm × 60 cm × 5 cm) were chosen from the cork factory. Each of them was divided in three sections. For each slab one section was not introduced into the autoclave (sample BA). The other two sections were introduced into an industrial autoclave containing about 200 other cork slabs (ca. 400 kg/batch). Steam from this autoclave was collected as follows: a water-cooled condenser was attached to a lateral tap of the autoclave. About 5 L of autoclave condensed steam was collected (VP1). Collection of condensates began when the internal temperature of the autoclave reached 100 °C and continued until the end of the process.

One of the sections from each of the chosen three slabs was removed after the normal industrial process (sample 1A). The other sections were left in for a second autoclaving process (sample 2A). The steam from this autoclave procedure was collected as well (VP2).

Extraction of the Cork and Autoclave Condensed Steam. The samples of cork and autoclave condensed steam were submitted to a process of simultaneous distillation−extraction (SDE) in a modified Likens−Nickerson apparatus (Schultz et al., 1977).

Cork. The cork volatiles were extracted as reported before (Rocha et al., 1996).

Autoclave Condensed Steam. Condensed steam (1.5 L, with 5.8 μL of internal standard) was placed in a modified Likens−Nickerson apparatus and extracted for 3 h with 60 mL of pentane. Three successive samples of condensed steam (total 4.5 L) were extracted with the original 60 mL of pentane.

The pentane extracts from cork and autoclave condensed steam were cooled to −10 °C to separate the frozen water from the organic phase by decantation. The excess of low-boiling solvent was removed by distillation using a Vigreux microfractionating column. The concentrate (about 800 μL) was stored in a glass screw-top vial at −10 °C.

Gas Chromatography (GC) and Gas Chromatography−Mass Spectrometry (GC−MS). Experimental details of the chromatographic and mass spectrometric conditions are reported elsewere (Rocha et al., 1996).

Identification of components was achieved by comparison of the GC retention times and mass spectra with those, when

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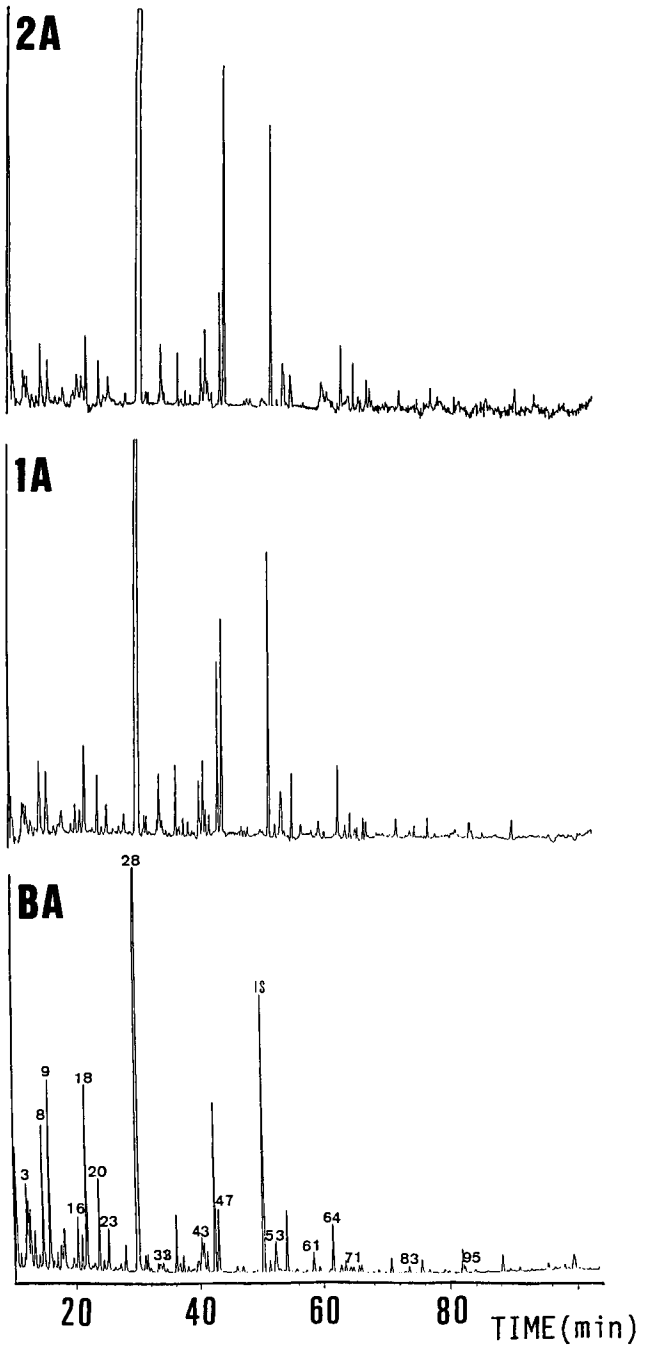

Figure 1. Total ion chromatograms of cork before autoclaving process (BA) and of cork submitted to one (1A) or two (2A) autoclaving processes (IS, internal standard). See Table 1 for peak assignments.

available, of the pure standard compounds. All mass spectra were also compared with those of the data system library (NIHS Library) and other published spectra (*Eight Peak Index of Mass Spectra*, 1974).

RESULTS AND DISCUSSION

Figure 1 represents gas chromatograms of cork samples BA, 1A, and 2A, and Figure 2 shows gas chromatograms of autoclave condensed steam (VP1 and VP2). Table 1 lists the compounds identified in the pentane extracts of cork and of condensed steam samples. The percentage figure given represents, for cork, the area of the peak with respect to the internal standard normalized to 1 g of cork and, for the condensed steam, with respect to 1 L of condensate. The data in Table 1

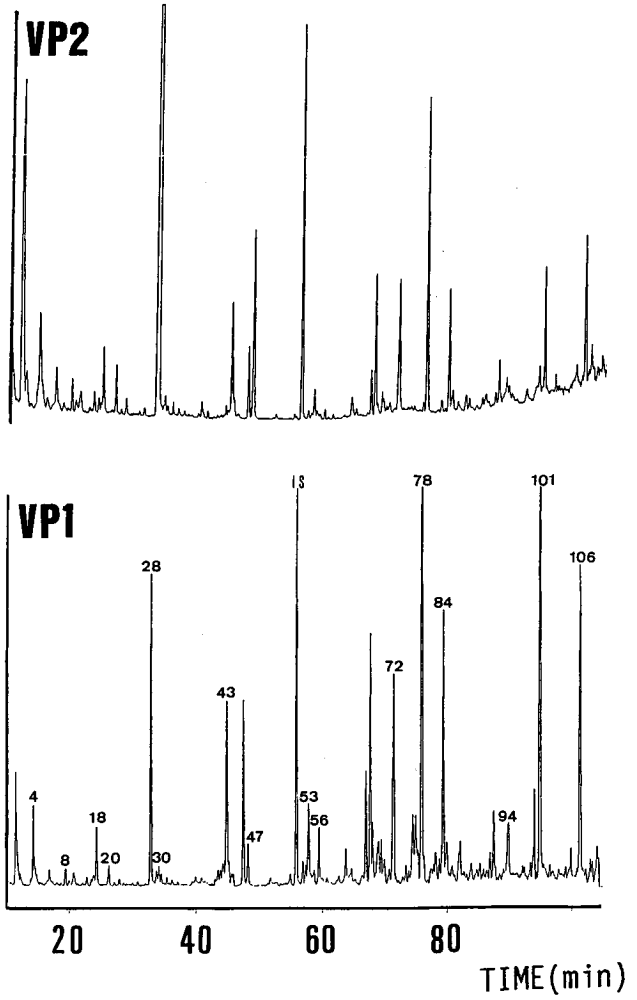

Figure 2. Total ion chromatograms corresponding to the first (VP1) and a second (VP2) autoclave condensed steam (IS, internal standard). See Table 1 for peak assignments.

represent the average of 12 GC analyses for each cork sample (duplicates of 6 samples) and 8 for the condensed steam (duplicates of 4 samples).

The volatile compounds found in cork and in autoclave condensed steam extracts were grouped by chemical class. The major compounds in extracts were aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, alkanes, aromatic compounds, cycloalkanes, furans, terpenoids, and lignin-related compounds. In a previous paper the origin and the possible potential contribution of these volatile components to cork flavor have been discussed (Rocha et al., 1996).

Table 1 shows that, in general, the first (1A) and second (2A) autoclaving processes decrease the volatiles content of the cork when compared with the cork before autoclaving (BA). The condensed steam contains the volatile compounds that have been extracted from cork slabs during the autoclaving process. Some of the more volatile components may be partially lost at the safety valve of the autoclave and may not have been recovered.

The presence of the compounds on cork slabs resulting from fatty acid oxidation or microbial degradation such as aliphatic aldehydes, aliphatic alcohols, aliphatic ketones, alkanes, and alkenes decreased in the course of the autoclaving processes. The increase in 1-octanol after the second autoclaving process can be attributed to the thermal degradation of some fatty acids under long thermal treatment.

Table 1. Volatile Components Identified in Cork before the Autoclaving Process (BA), in Cork Submitted to One (1A) or Two (2A) Autoclaving Processes, and in the First (VP1) and Second (VP2) Autoclave Condensed Steams, Grouped by Chemical Classes

peak no.	compound	reliability of ID[a]	%[b]				
			BA	1A	2A	VP1	VP2
		Aliphatic Alcohols					
1	1-butanol	A, B, C	0.47	0.39	tr[c]	tr	ND[d]
16	3-methyl-1-butanol	A, B, C	1.55	0.77	0.61	0.32	0.23
27	1-octen-3-ol	A, B, C	ND	ND	ND	0.21	tr
53	1-octanol	A, B, C	1.57	0.43	0.70	1.85	0.63
76	2-decen-1-ol	B	ND	ND	ND	0.02	ND
101	1-undecen-4-ol	B	tr	ND	ND	15.85	4.83
104	2,7-dimethyl-2,6-octadien-1-ol	B	0.12	ND	ND	ND	ND
106	5,9-dimethyl-9-decen-3-ol	B	0.06	ND	ND	7.18	2.90
		Aliphatic Aldehydes					
2	3-methylbutanal	B, C	0.38	0.14	tr	0.24	0.09
10	2-methylpentanal	B, C	0.33	0.20	tr	tr	ND
18	hexanal	A, B, C	5.46	1.59	1.53	2.01	1.27
31	heptanal	A, B, C	0.58	0.41	0.21	0.16	0.09
40	2-heptenal	A, B	tr	tr	ND	0.28	0.31
45	octanal	A, B, C	0.60	0.51	0.04	0.26	ND
52	2-octenal	A, B	0.43	0.35	0.14	0.31	tr
56	nonanal	A, B, C	2.00	2.00	0.76	1.08	0.26
68	2-nonenal	B, C	0.27	0.23	tr	ND	ND
71	decanal	B, C	0.22	0.34	0.29	0.42	ND
79	2,4-nonadienal	A, B, C	0.53	0.48	0.39	ND	ND
87	2,4-decadienal	A, B, C	0.12	ND	ND	0.59	ND
92	2,4-dodecadienal	B	ND	ND	ND	0.59	ND
98	2-tridecenal	B	tr	tr	tr	ND	ND
		Aliphatic Ketones					
2	3-methyl-2-pentanone	B, C	0.41	0.21	0.18	0.12	0.03
30	2-heptanone	A, B, C	0.50	0.40	0.02	0.33	0.19
44	6-methyl-5-hepten-2-one	A, B, C	0.71	0.61	0.04	0.47	ND
62	6-methyl-3,5-heptadien-2-one	B	0.18	0.17	ND	ND	ND
89	2-undecanone	A, B, C	ND	ND	ND	tr	ND
81	6,10-dimethyl-5,9-undecadien-2-one	B	0.46	tr	tr	ND	ND
		Alkanes					
3	2-methylheptane	A, B, C	2.05	1.41	0.51	0.02	tr
5	3-methylheptane	A, B, C	1.78	1.64	0.42	tr	tr
8	octane	A, B, C	4.51	3.58	0.91	0.67	0.26
11	?-dimethylheptane[e]	B, C	0.98	0.77	0.16	ND	ND
15	?-ethylmethylhexane	B, C	0.81	0.62	0.05	0.14	0.39
17	3-methyloctane	B, C	1.06	0.73	0.50	ND	ND
20	nonane	A, B, C	2.01	0.53	ND	0.50	0.32
		Alkenes					
48	3-ethyl-2-methyl-1,3-hexadiene	B	0.20	0.22	0.08	tr	ND
49	3,5-dimethyl-1,6-octadiene	B	0.11	0.11	0.08	ND	ND
		Alkynes					
55	1-octyne	B, C	0.11	tr	ND	ND	ND
		Aromatic Compounds					
9	toluene	A, B, C	4.32	2.86	0.96	tr	tr
22	1,4-dimethylbenzene	A, B, C	0.86	0.69	tr	0.11	0.36
23	1,3-dimethylbenzene	A, B, C	1.52	1.15	0.44	0.09	0.19
25	1,2-dimethylbenzene	A, B, C	0.96	0.85	0.24	ND	ND
29	propylbenzene	A, B, C	0.27	0.86	0.31	0.26	0.12
33	1,3,5-trimethylbenzene	A, B, C	0.23	0.19	0.14	0.08	0.10
37	1,2,4-trimethylbenzene	A, B, C	0.53	0.39	0.25	0.09	0.10
38	1,2,3-trimethylbenzene	A, B, C	0.15	tr	tr	0.13	0.18
41	1-methyl-4-isopropylbenzene	B, C	tr	tr	tr	ND	ND
		Cycloalkanes					
6	1,3-dimethylcyclohexane	B, C	1.32	1.29	tr	ND	ND
7	1,2-dimethylcyclohexane	B, C	0.44	tr	tr	ND	ND
13	ethylcyclohexane	A, B, C	1.92	1.73	0.04	0.39	0.84
19	1-ethyl-3-methylcyclohexane	B, C	tr	tr	tr	tr	tr
24	isopropylcyclohexane	B, C	0.27	0.11	tr	ND	ND
26	1-ethyl-2-methylcyclohexane	B, C	tr	tr	tr	ND	ND
		Furans					
28	furfural	A, B, C	36.92	49.37	71.88	5.59	47.43
35	2-acetylfuran	A, B, C	1.65	1.71	1.72	0.17	0.77
36	2-pentylfuran	B, C	0.40	0.44	0.51	ND	ND
47	5-methylfurfural	A, B, C	2.13	6.24	7.48	0.87	4.75
63	3,4-dimethyl-2,5-furandione	B	0.10	0.13	0.17	ND	ND
86	5-methyl-2,5-dihydro-2(3*H*)-furanone	B	0.12	0.18	0.26	ND	ND

Table 1 (Continued)

peak no.	compound	reliability of ID[a]	% [b] BA	1A	2A	VP1	VP2
		Terpenoids					
42	1,8-cineole	A, B, C	0.33	0.24	0.16	0.69	0.51
51	geranial	B, C	ND	ND	ND	tr	ND.
60	fenchyl alcohol	A, B, C	tr	ND	ND	ND	ND
64	camphor	A, B, C	1.61	1.04	0.56	2.39	0.94
70	isoborneol	A, B, C	0.19	0.09	ND	0.75	tr
73	α-terpineol	A, B, C	0.23	0.20	0.14	0.42	ND
81	geraniol	A, B, C	0.12	0.10	ND	tr	ND
83	copaene	A, B, C	0.52	0.45	0.30	tr	ND
		Phenols					
59	phenol	A, B, C	0.07	0.06	ND	tr	ND
65	2-methylphenol	A, B	ND	ND	ND	5.06	2.81
67	3-methylphenol	A, B	ND	ND	ND	0.64	0.37
72	4-methylphenol	A, B	ND	ND	ND	5.76	3.41
77	2,5-dimethylphenol	A, C	ND	ND	ND	1.92	0.15
78	2,3-dimethylphenol	A, C	ND	ND	ND	15.48	7.58
84	3,5-dimethylphenol	A, C	ND	ND	ND	5.10	3.97
85	3,4-dimethylphenol	A, C	ND	ND	ND	1.09	0.81
91	?-ethylmethylphenol	A	ND	ND	ND	tr	tr
93	?-ethylmethylphenol	A	ND	ND	ND	0.64	1.00
94	1,1-dimethylethylphenol	A	ND	ND	ND	1.28	tr
102	2,6-di-*tert*-butyl-4-methylphenol	B, C	0.25	0.95	1.69	ND	ND
		Chlorinated Compounds					
96	2,3-dichloroanisole	A, B	ND	ND	ND	tr	ND
		Lignin-Related Compounds					
43	benzaldehyde	A, B, C	0.14	0.54	0.82	4.29	3.39
57	phenylethanone (acetophenone)	A, B, C	0.11	0.07	ND	ND	ND
58	benzenomethanol (benzyl alcohol)	A, B, C	0.16	1.02	0.76	0.04	ND
61	4-methoxyphenol (guaiacol)	A, B, C	0.83	0.56	0.23	0.65	0.49
66	1,2-dimethoxybenzene (veratrole)	A, B, C	0.29	0.31	tr	1.26	tr
69	1,3-dimethoxybenzene (resorcinol)	A, B, C	0.21	0.19	0.07	0.68	ND
74	2-methoxy-4-methyl-1-propylbenzene	B	tr	tr	tr	ND	ND
75	1-(4-methylphenyl)ethanone (4-methylacetophenone)	A, B, C	tr	tr	ND	0.39	ND
95	1-(2-hydroxy-5-methylphenyl)ethanone (2-hydroxy-5-methylacetophenone)	A, B	0.26	0.20	tr	tr	tr
105	4-hydroxy-3-methoxybenzaldehyde (vanillin)	A, B, C	tr	0.05	0.07	ND	ND
		Others					
4	1,1-diethoxyethane	B, C	1.74	0.98	0.30	2.04	2.33
32	2-butoxyethanol	B, C	0.22	0.18	0.11	ND	ND
39	2-hydroxy-2-methyl propanoic acid	B	ND	ND	ND	0.37	ND
80	benzothiazole	A, B, C	tr	ND	ND	tr	ND
88	methyl 2-hydroxy-5-methylbenzoate	B, C	0.07	tr	tr	ND	ND
109	3,8-dihydroxy-3,4-dihydronaphthalen-1-one	B	0.12	ND	ND	ND	ND

[a] The reliability of the identification or structural proposal is indicated by the following: A, mass spectrum and retention time consistent with those of an authentic standard; B, structural proposals are given on the basis of mass spectral data (NIHS Library); C, mass spectrum consistent with spectra found in the literature. [b] Estimated concentrations for all compounds were made by peak area comparisons to the area of a known amount of internal standard (ethyl pentanoate) with no correction for individual detector response factors. [c] Trace, peak area percent less than 0.02 [d] Not detected. [e] ?, unidentified isomer.

Table 2. Determination of Furfural and 5-Methylfurfural by the Derivative Spectrophotometric Method in Cork before the Autoclaving Process (BA) and in Cork Submitted to One (1A) or Two (2A) Autoclaving Processes

sample	furfural[a]	5-Me-furfural[a]
BA	0.11	0.00
1A	10.24	0.96
2A	32.98	3.53

[a] Concentration expressed in mg/g of cork.

The amounts of aromatic compounds, cycloalkanes, and terpenoids decrease in corks 1A and 2A when compared with cork BA. This decrease can be explained by an effective extraction in the autoclave, due to the combined effect of temperature and high pressure.

Two processes appear to be operating with respect to lignin-related compounds. Acetophenone, guaiacol, resorcinol, 4-methylacetophenone, and 2-hydroxy-5-methylacetophenone, which are reported to be formed by microbiological lignin degradation in cork BA (Rocha et al., 1996), decrease with the autoclaving process. On the other hand, the autoclaving process itself appears to cause some increase in the levels of lignin-related compounds, such as benzaldehyde and benzyl alcohol, probably through thermal degradation.

It is important to note the progressive increase of the amount of furans such as furfural, 2-pentylfuran, 2-acetylfuran, 5-methylfurfural, and 5,5-dimethyl-2,5-dihydro-2-furanone with the thermal treatment in the autoclave. Accumulation of these compounds indicates that Maillard reactions occur (Mansilla et al., 1992; Tu et al., 1992). As it is known that the SDE method may increase furan formation, during the sample preparation for GC analysis, the levels of furfural and 5-methylfurfural were determined by the derivative spectrophotometric method, which does not involve thermal treatment (Rocha et al., 1994). The results in Table 2 confirm that the SDE method produces some furans as

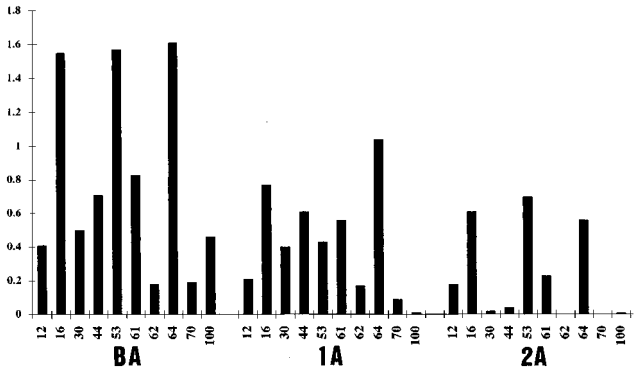

Figure 3. Profile of musty, moldy, or related odor compounds (3-methyl-1-butanol, 1-octanol, and guaiacol) and other mold metabolites (methyl ketones, camphor, and isoborneol) present in cork before autoclaving process (BA) and in cork submitted to one (1A) or two (2A) autoclaving processes.

artifacts (for example, cork BA is shown not to contain 5-methylfurfural when the spectrophotometric method is used, but the GC analysis incorrectly indicates its presence). Although the amounts of furfural and 5-methylfurfural determined by GC may be overestimated, the trends can be considered to be correct. Thus, it is correct to conclude that the autoclaving process increases furan formation, especially during the second autoclaving process.

The volatiles that are removed from the cork are found in the vapor. The autoclave condensed steam shows a correspondence with the volatiles that were extracted from the cork samples. In general, the condensed steam of the second autoclaving process showed lower levels of the compounds when compared to the condensed steam of the first autoclaving process (Table 1), although the aromatic compounds, ethylcyclohexane, and furans show an increase from VP1 to VP2. The increase of furans in the vapor phase is the logical consequence of their formation in cork during the autoclaving process.

Some compounds, such as 1-octen-3-ol, 2-decin-1-ol, 2-undecanone, geranial, 2,4-dichloroanisole, 2-hydroxy-2-methyl propanoic acid, and several phenols (2-methylphenol, 3-methylphenol, 4-methylphenol, dimethylphenol isomers, ethylmethylphenol isomers, and 1,1-dimethylethylphenol), are present only in the condensed steam. The enrichment in the vapor phase of these components may be related to their high solubility in water and to the high quantity of cork (400 kg/batch) that originated each sample of condensate.

The volatile components with musty, moldy, or related odors, such as 3-methyl-1-butanol, 1-octanol, guaiacol, and other mold metabolites (methyl ketones, camphor, and isoborneol), were found in smaller quantities in cork slabs submitted to the autoclaving process, as can be seen in Figure 3. The autoclave processing is highly effective for cleaning cork since 46.44% of those volatile components are removed after the first treatment. Moreover, 1-octen-3-ol, 2-nonanone, geraniol, 2,4-dichloroanisole, and other odor-active compounds are present only in the first vapor, showing that one process is sufficient to extract them. In spite of the fact that the second autoclaving process reduces by 70.66% the amount of volatile compounds with musty and moldy odors and mold metabolites, this procedure is not recommended because it increases the formation of furans, 1-octanol, and the lignin-related compounds benzaldehyde and benzyl alcohol. The long treatment may cause chemical modifications of the cork polymers.

The short autoclaving treatment offers significant advantages, some of which are of important practical interest for the cork industry: it allows the processing of the cork slabs directly after the boiling stage, avoiding the 3 weeks of wet storage and consequently reducing the probability of microbial growth; it also removes or reduces volatiles of microbial origin or arising from the cork.

The present results show that the autoclaving process appears to be a powerful industrial process to clean cork. The changes in volatiles content after one treatment are mainly due to vapor phase transport and improved the quality of cork slabs used to produce stoppers.

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