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Removal of 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) from wine

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In four test series different wines were contaminated with varying amounts of TCA and TBA and then filtered with special filter sheets ('Fibrafix TX-R'). The objective of the tests was to determine if there is an effect of this filtration on the content of TCA, on TBA aroma compounds and aluminium in the wines in comparison to conventional filter sheets. The data obtained from the sensory and analytical tests showed, that a successful reduction of TCA and TBA below the detection threshold value could be achieved, whereas the wine flavour was not affected negatively. Furthermore the tests regarding the absorbance limit corroborated the data obtained by Filtrox in internal test series of about 2000 l wine per m^2 filtration surface. In contrast no TCA-reducing effect could be noted for a filtration of the spoiled wines with a conventional filter sheet. Regarding the migration of aluminium ions from the filter sheet into the wine, only a slight increase of 0.4 mg/l at the maximum was detected after filtration with 'Fibrafix TX-R' filter sheets.
Keywords: wine, filtration, filter sheets, TCA, TBA

Entfernung von 2,4,6-Trichloroanisol (TCA) und 2,4,6-Tribromoanisol (TBA) aus Wein. *In vier Testserien wurden verschiedene Weine mit unterschiedlichen Mengen von TCA und TBA kontaminiert und dann durch spezielle Filterschichten ('Fibrafix TX-R') filtriert. Ziel der Tests war es zu bestimmen, ob es im Vergleich zu konventionellen Filterschichten einen Effekt dieser Filtration auf die TCA-Gehalte, die TBA-Aromakomponenten und den Aluminiumeintrag in die Weine gab. Die Daten aus den sensorischen und den analytischen Untersuchungen zeigten, dass eine Reduktion von TCA und TBA unter die Nachweisgrenze erreicht werden konnte, ohne das Flavour des Weines zu beeinträchtigen. Weiters bestätigten die Tests hinsichtlich des Absorptionslimits die von Filtrox in internen Testserien erhaltenen Daten von ca. 2000 l Wein pro m^2 Filteroberfläche. Im Gegensatz dazu konnte bei Filtration der kontaminierten Weine mittels konventioneller Filterschichten kein TCA-reduzierender Effekt festgestellt werden. Bezüglich des Eintrags von Aluminiumionen aus den Filterschichten in den Wein wurde nach der Filtration mit 'Fibrafix TX-R'-Filterschichten nur eine leichte Erhöhung von maximal 0,4 mg/l festgestellt.*
Schlagwörter: Wein, Filtration, Filterschichten, TCA, TBA

L'élimination du 2,4,6-trichloroanisole (TCA) et du 2,4,6-tribromoanisole (TBA) du vin. *Au cours de quatre séries test, différents vins ont été contaminés par des quantités différentes de TCA et de TBA et ensuite filtrés à travers des couches filtrantes spéciales ('Fibrafix TX-R'). L'objectif des tests a été de déterminer si cette filtration, par rapport aux couches filtrantes habituelles, exerce un effet sur les teneurs en TCA, sur les composantes aromatiques TBA et sur l'apport d'aluminium dans les vins. Les données obtenues des examens sensoriels et analytiques ont montré qu'il était possible d'obtenir une réduction du TCA et du TBA au-dessous de la limite de détection sans porter atteinte à la saveur du vin. En outre, les tests ont confirmé les données obtenues par Filtrox dans des séries test internes quant à la limite d'absorption, soit près de 2000 l de vin par m^2 de surface filtrante. En revanche, aucun effet réducteur de TCA n'a pu être constaté lors de la filtration des vins contaminés au moyen des couches filtrantes habituelles. Quant à l'apport d'ions d'aluminium provenant des couches filtrantes dans le vin, on n'a constaté qu'une faible augmentation de 0,4 mg/l au maximum après la filtration au moyen des couches filtrantes 'Fibrafix TX-R'*
Mots clés : vin, filtration, couches filtrantes, TCA, TBA

At the beginning of the eighties the compound 2,4,6-trichloroanisole (TCA) was identified as a main cause for cork taint in wine (Tanner et al., 1981; Buser et al., 1982). In the last years a number of further compounds with similar negative flavour attributes have been discovered in mouldy and musty smelling wines (e.g. geosmin, 2-methylisoborneol, octane-3-one, pyrazines, etc.) (Mazzoleni et al., 1994; Kugler und Rapp, 1997; Hesford und Schneider, 2002; Sefton and Simpson, 2005). The main attention in research, however, is still focused on the halogenated anisoles, which is due to their very low perception threshold values.
In technical literature the range concerning the perception threshold value of TCA varies relatively widely. The perception threshold value for drinking water is indicated to be below 1 ng/l (Griffith, 1974), whereas the threshold value for wine is given to be at about 2 to 5 ng/l (Table 1). Threshold values in wine depend strongly on the kind of wine, the wine style and the experience of the panelist (Mazzoleni and Maggi, 2007; Prescott et al., 2005).

Table 1: Perception and recognition thresholds

TCA (ng/l)	TCA (ng/l)	medium	literature
4.0		dry white wine	Ribereau-Gayon et al., 2006
1.4 - 4.6	4.2 - 10	wine	Sefton and Simpson, 2005
210 (a);17.4 (b)		Sauvignon blanc	Suprenant and Butzke, 1997
10.0; 60.0; 100		white wine red wine	Pfeifer, 2002
22.0		red wine	Alvarez-Rodriguez et al., 2002

(a) unexperienced panel; (b) experienced panel

Regarding the formation of TCA, it is well-known that TCA results from the microbiological methylation of chlorophenoles, which derives from the hypochloride treatment of the cork raw material during the production process.
It can also be formed by the dismantling of chloric chemicals like for example pentachlorophenol (e.g. wood-preserver 'Raco', pesticides 'Dowicide') (Rudy und Scholten, 2007; Simpson and Sefton, 2007; Daniels-Lake et al., 2007). A third source for trichloroanisole is the microbiological formation of TCA by mould fungi such as *Penecillium spec.,* whereby phenol is formed due to the reactions of the pentosephosphate pathway with the preliminary stage of shikimic acid (Pfeifer, 2002). Afterwards this phenol is chlorinated chemically in the presence of hypochloride.
After the use of pentachlorophenolic agents had been forbidden at the end of the eighties, this compound was replaced by tribromophenol (TBP) in fungicides and wood preservatives, especially in the sector of the packaging industry. At the same time the use of TBP as a flame protection agent for wood (e.g. pallets) and in synthetic materials increased. The use of tribromophenol in the food sector also implicated problems as like trichlorophenol, an anisole (2,4,6-tribromoanisole) can be formed by chemical dismantling of tribromophenol (Rudy and Scholten, 2007; Whitfield and Shaw, 1997). It has already been proven in various studies that musty off-flavours in tainted food and wines were not due to TCA but were caused by TBA (Chatonnet et al., 2004).
Polyethylene (PE) packaging in particular, showed a high permeability for TBA (Whitfield and Shaw, 1997; Chatonnet et al., 2004). Thus in different investigations TBA contamination was proven from plastic wine stoppers, the plastic sealing compound of crown and screw caps, from natural corks as well as from filter layers, wooden pallets, cardboard boxes and plastic packaging (Hesford und Schneider, 2002; Rudy and Scholten, 2007). As a perception threshold value for TBA, a value of 5 ng/l is generally indicated. Up to now different approaches were made regarding the removal of TCA and TBA from tainted wines; either tainted wines were fined with activated charcoal and filtered afterwards, or polyethylene was added as an adsorbent to the wine. Yeast cell wall preparations were also tested for the removal of chlorinated anisoles and TBA (http://www.keller-mannheim.de/fileadmin/pdf/getraenke_deutsch/downloads/DSM-Artikel_Hochadsorptive_Hefezellwaende.pdf).

Filter sheet 'Fibrafix TX-R'

During the Intervitis/Interfructa 2007 in Stuttgart the Filtrox company presented a depth filter sheet, which was claimed to be able to remove TCA and TBA from wine, without negatively affecting the wine flavour. The outward appearance and handling of the filter sheets 'Fibrafix TX-R' is very similar to conventional filter sheets: they have to be rinsed before filtration, sterilization can be conducted with hot water (85 °C) or steam (125 °C), and a flow rate of 350 l/m^2/h is recommended by the manufacturer. The filter layer consists of refined and bleached cellulose as well as a small quantity of polyamidamine (below 3 %) to increase the

wet-strength.
The active component in the sheets is an inorganic substance called 'TRIEX'.
'TRIEX' serves as an absorbent within the filter sheets, binding TCA and/or TBA, and hereby removes those compounds from the wine. In accordance with the FDA (Food and Drug Administration) TRIEX is classified as an aluminium silicate and therefore equated with bentonite and kaoline by US law, meaning, the sheets can be used in US wine industry. In Europe the sheets are not yet certified for TCA removal in wine, although this issue will be examined by the Organisation Internationale de la Vigne et du Vin (O.I.V.) soon.
To test the filtering effect and capacity of the filter sheets different test trials were conducted in the Section of 'Enology and Wine Technology' of the Geisenheim Research Center, Germany.
Analysis of TCA and TBA was carried out in the Section of 'Microbiology and Biochemistry', aroma analysis was supported by the Section of 'Wine Analysis and Beverage Technology'.

Material and methods

Filtration

All filtration trials were performed with 20 x 20 cm filter sheets in a Seitz stainless-steel sheet filter. In order to be able to draw a comparison, Seitz EK filter sheets were used besides the 'Fibrafix TX-R' filter sheets. The filtration surface in all tests was 0.2 m^2, the filtration rate was about 60 litres per hour. The wines were filled into 20 litre kegs and pressed through the filter sytem by compressed air.
For the trial a 2006 'Müller Thurgau' QbA dry from Rheinhessen and a 2006 Rosé Cuvee QbA dry from the Rheingau region were used ('Müller Thurgau': 12,2 %vol.; residual sugar 0.7 g/l; total acidity 4.4 g/l; pH 3.7; sulphurous acid: free 56.0 mg/l, total 113.0 mg/l. Rosé: 13.6 %vol.; residual sugar 9.5 g/l; total acidity 6.1 g/l; pH 3.6; sulphourus acid: free 30.0 mg/l, total 134.0 mg/l).

Sensory analysis

Fifteen trained panelists were chosen from the staff of the Section of 'Enology and Wine Technology' of the Geisenheim Research Center for sensory tests of the variants. The sensory analyses were performed as triangular tests according to DIN 10951 (Liptay-Reuter und Ptach, 1998). The test design and statistical evaluation of the data was conducted by using the computerized system 'Fizz' provided by Biosystemes, France. An interpretation of the preference was done only in case of a correct identification of the differing sample. Results with $\alpha \leqslant 0.05$ are considered to be significant.

Analysis of 2,4,6-trichloroanisole and 2,4,6-tribromoansiole in wine

Analysis of the halogen-anisoles was carried out according to Sponholz et al. (2001) modified by Fischer and Rauhut (2007) as follows.

Sample preparation by Stir Bar Sorptive Extraction (SBSE). 3.5 g of sodium chloride (Roth, Germany) were weighed into a 10 ml glass vial and 10 ml of sample was added.
After appending the two deuterated internal standards 2,4,6-trichloroansiole-d_5 (29.9 ng/l, CDN Isotopes, Canada) and 2,4,6-tribromoanisole-d_5 (31.4 ng/l, CDN Isotopes, Canada) a stir bar coated with polydimethylsiloxane (Twister^{TM}, dimensions: length: 10 mm, film thickness: 0.5 mm, Gerstel, Germany) was added to the sample. The vial was crimped and the sample extracted for 60 min at 1000 rpm.
After extraction the Twister was rinsed with bidest. water, wiped with a lint-free tissue and put into a desorption tube.

Analysis by Gas Phase Chromatography and Selected Ion Monitoring (GC-MS/SIM)

The loaded Twister was thermodesorped into a HP5 (5 % phenylmethylsiloxanes) capillary column 60 m x 320 µm x 0,25 µm installed on an Agilent 6890 chromatograph equipped with a Thermodesorption System TDS A (Gerstel, Germany) operating in splitless mode (initial temperature = 20 °C, rate 60 °C/min to 280 °C, hold for 2.5 min) coupled with a Cooled Injection System CIS 4 (Gerstel, Germany) operating in solvent vent mode (initial temperature = -150 °C, rate 12 °C/s to 280 °C, hold for 5 min).
Helium was used as carrier gas at constant flow rate (1.1 l/min). The GC temperature was programmed from 50 °C to 115 °C at a rate of 15 °C/min, then up to 150 °C (hold for 10 min) at a rate of 3 °C/min and finally up to 230 °C (hold for 5 min) at a rate of 15 °C/min. An Agilent 5973 N quadrupole mass detector operating in electron impact mode was used for detection (source temperature = 230 °C, quadrupole temperature = 150 °C) in single ion monitoring (SIM mode): 2,4,6-TCA-d_5: 215, 217; 2,4,6-TCA: 210, 212; 2,4,6-TBA-d_5: 349, 351; 2,4,6-TBA: 344, 346.

Analysis of volatile compounds in wine and model wine

Analysis of the minor and major aroma compounds was carried out according to Lopez et al. (2002) and Ortega et al. (2001) with slight modifications. Major wine volatiles were analysed after dichlormethan microextraction (Ortega et al., 2001), minor and trace volatile compounds were isolated by solid-phase extraction with LiChrolut EN (Lopez et al., 2002).

GC/MS analysis of the extracts was performed using a GC (GC 6890, Agilent Technologies, Little Falls, USA) equipped with a ZB-WAX column (30.0 m x 250 µm x 0.25 µm Phenomenex, Aschaffenburg, Germany) and a mass selective detector (MSD 5973, Agilent Technologies). GC/MS conditions were as follows: temperature programme GC 40 °C, 4 °C/min, 250 °C (10 min); carrier gas helium with a constant flow of 1.3 ml/min; split-less time 3 min; transfer line temperature 250 °C; MS source temperature 230 °C; MS Quad temperature 150 °C.

Preliminary test phase

To test the general removal effect of the filter sheets 'Fibrafix TX-R', two preliminary tests were conducted, followed by two main test series and one additional test. In a first preliminary test about 60 litres of wine were filtered and bottled. The aim of this trial was to examine the change of the non-contaminated wines throughout filtration, by sensory tests and chemical analysis of the aroma compounds. In the context of the second preliminary test phase in each case 20 litres of the wines were contaminated with 10 ng/l TCA, mixed and filtered afterwards. During filtration samples were taken in numbered 0.75 l-screw-cap bottles. This trial was firstly aimed to see whether there was a removal effect of TCA at all, and secondly, if there could be noticed a change of this effect during the process of filtration.

During the filtration eleven bottles were filled. The bottles no. 1 (beginning of filtration), 4 and 7 (middle of filtration), as well as no. 10 and 11 (end of filtration) were analyzed by GC-MS. The analysis showed, that only traces of TCA could be found in bottle no. 11. Due to the fact that TCA could be found in bottle no. 11 even though the filter sheets should not have reached their limit of capacity, the second preliminary test was expanded. Therefore 40 litres of wine (rosé) were contaminated with 20 ng/l TCA, another 40 litres of

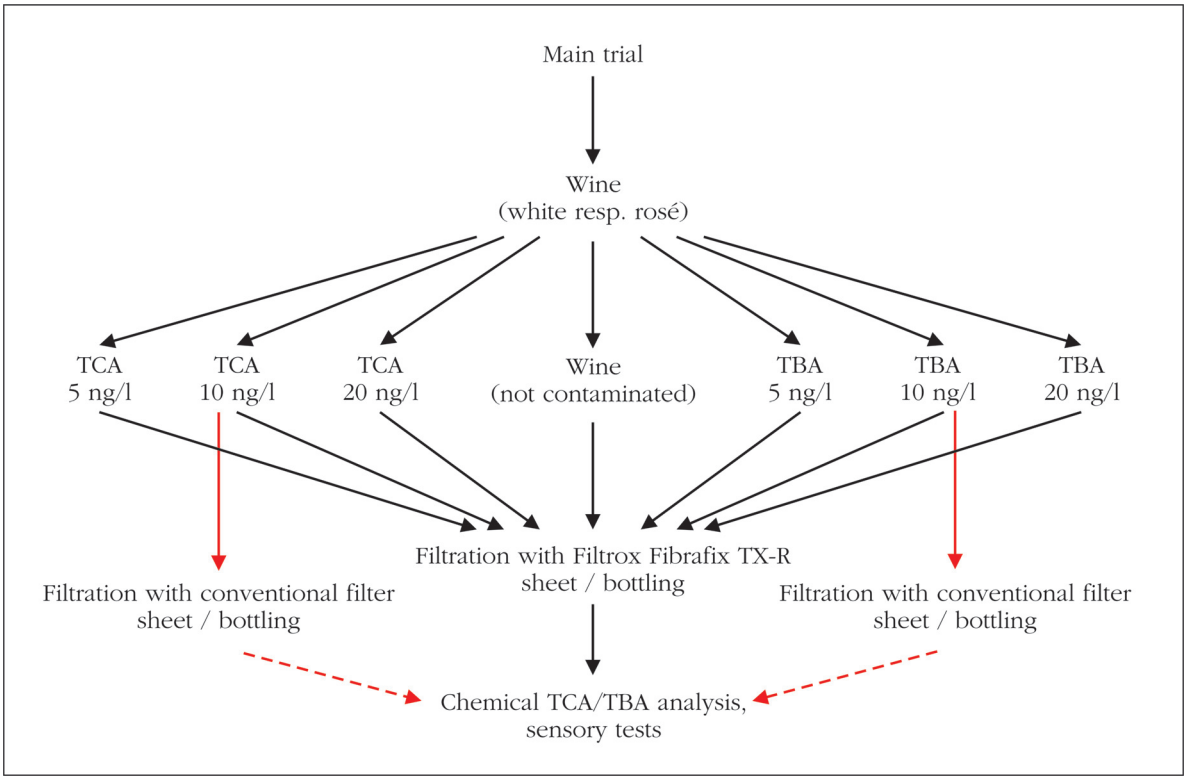

Figure 1: Experimental design

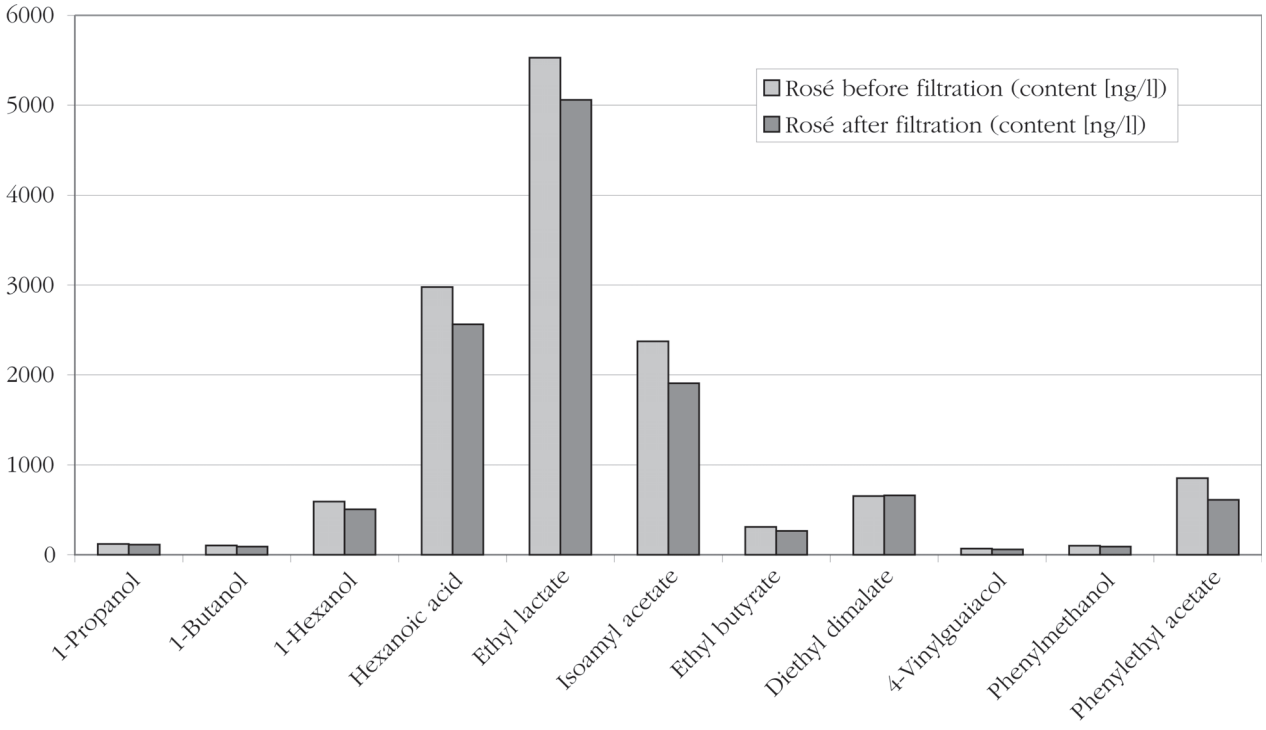

Figure 2: Flavour compounds in rosé wine before and after filtration

the same wine were contaminated with 20 ng/l TBA. Both batches were filtered and samples were taken in intervals of 10, 20, 30 and 38 litre in 0.75 l-screw-cap bottles.

Main test phase

Regarding the results of the preliminary tests, showing an obvious diminishing of TCA and TBA concentration, respectively, further tests were carried out. The experimental design of the main tests (Fig. 1) included three different degrees of contamination with TCA and TBA. In a first step three batches with 19 litres of wine each (white wine and rosé wine, respectively) were contaminated with 5 ng/l, 10 ng/l and 20 ng/l TCA and filtered after a steam sterilization of the filter. After disposing 10 litres the wine was directly filled into sterilized screw-cap bottles. In a second step a trial analogous to the first one was proceeded by using TBA instead of TCA in the same concentrations as mentioned above. Additionally 50 litres rosé of wine and white wine were contaminated with 10 ng/l TCA and filtered with a conventional filter sheet to check, if there is an effect on the TCA concentration by a conventional filtration. Additionally to the tests to determine the level of the TCA removal from wine a further test was carried out to check the filtration limit of the filter sheets, for keeping back the contaminants TCA and TBA.

Therefore 500 litres of Müller Thurgau white wine were contaminated with 20 ng/l TCA and filtered through the Fibrafix filter sheets. The bottling took place in 40 litre intervals.

Results

Preliminary test phase

The sensory tests of the wines of the first preliminary test in comparison to the same wines filtered with conventional filter sheets showed a significant difference between the wines while no significant preference could be observed (Table 2). The analysis of the aromatic compounds of these wines revealed only slight changes. Figure 2 and Figure 3 show exemplarily some analysed compounds in the wine before and after filtration, all changes within the range of variation of the measurement inaccuracy.

In the first part of the second preliminary test phase the analysis of TCA and TBA showed that in the wine of the bottles no. 1, 4, 7 and 10 no TCA could be detected

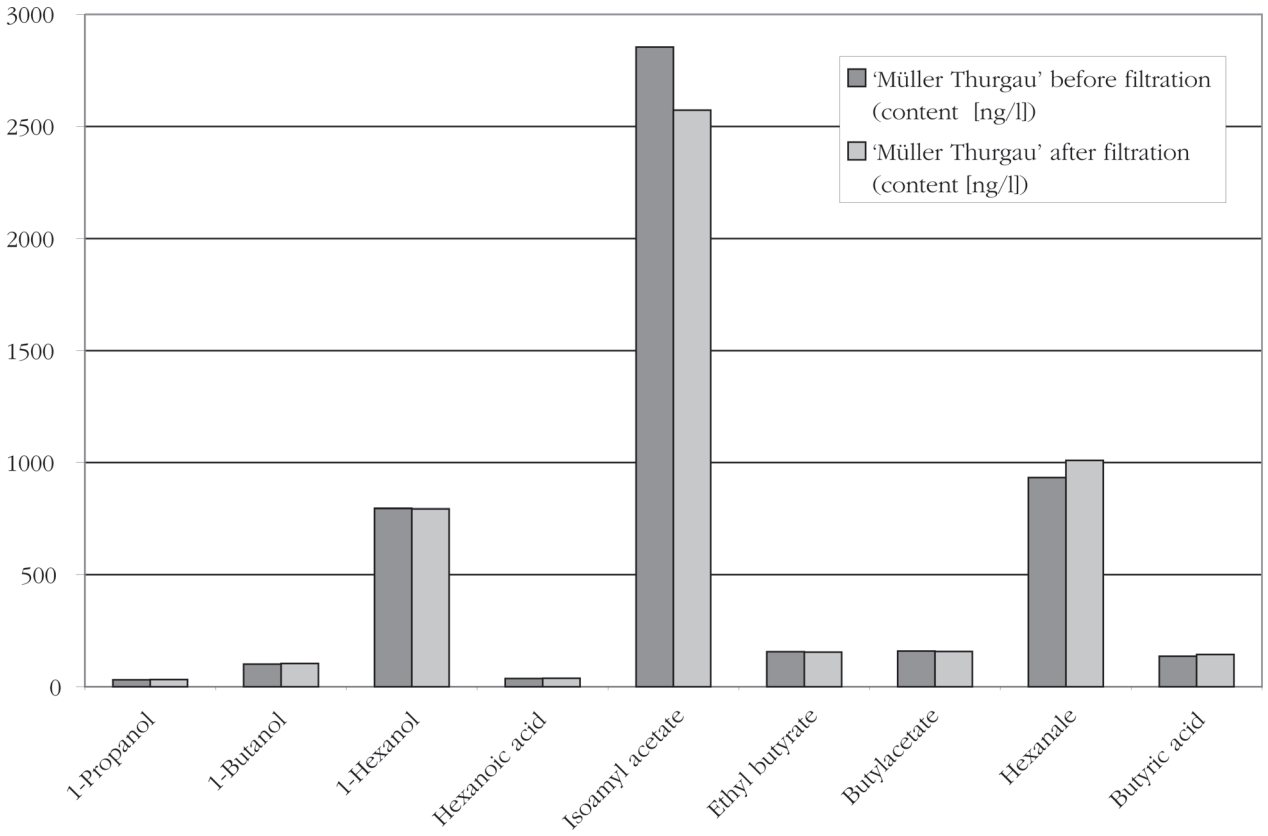

Figure 3: Flavour compounds in 'Müller Thurgau' wine before and after filtration

after filtration, whereas the wine in bottle no. 11 contained 1.2 ng/l TCA, which made an extension of this preliminary test phase necessary. The analysis of the samples taken during the additional tests revealed that after the filtration of 10 litres of contaminated wine the amount of TCA in the wine remained at a constant level of 1.2 ng/l until the end of filtration. Especially remarkable was the effect of the filtration process on the content of TBA in wine, as no TBA was detected in the analyzed samples.

Main test phase

The main test trials confirmed the TCA and TBA reduction which was noted in the preliminary test phase. Figure 4 shows that the TCA content of all samples, independent from the degree of contamination stabilized at about 1.2 ng/l after filtration. This value means an enormous TCA reduction even below the perception threshold of TCA in all samples. These results confirm the effective TCA (and geosmin) reduction of these filter material reported by EDER et al. (2008). Like in the preliminary tests no TBA could be detected in the samples of the main trial after filtration (Fig. 5). (detection limit 1 ng/l).

The data in Figure 6 show that the additionally tested filtration of spoiled wine with conventional filter sheets had no reducing effect on the TCA concentration of the tainted wine.

Limit of TCA absorbance

Based on technical data provided by the company FILTROX (St. Gallen, Switzerland) claiming a possible volume of 2000 l/m^2 to be filtered, it was calculated, that when working with a filtration surface of 0.2 m^2, the point, where the TCA and TBA absorbance of the sheet should be depleted, had to be after about 400 litres wine. The result of our test filtration of 500 litres of 'Müller Thurgau' wine, contaminated with 20 ng/l TCA (Fig. 7) showed that up to 120 litres of filtration volume no TCA could be detected in the wine samples. The samples taken between 160 and 240 litres filtration volume contained constantly about 1.5 ng/l. Afterwards

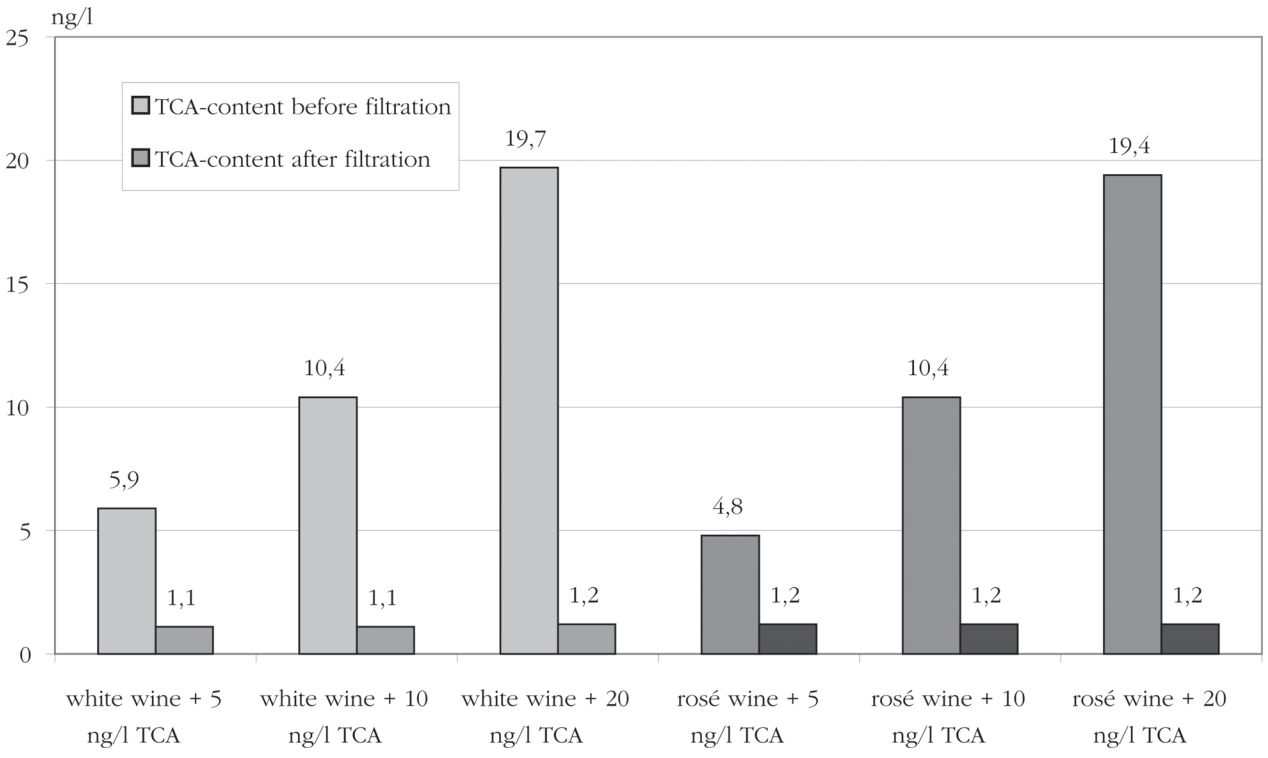

Figure 4: Removal of TCA

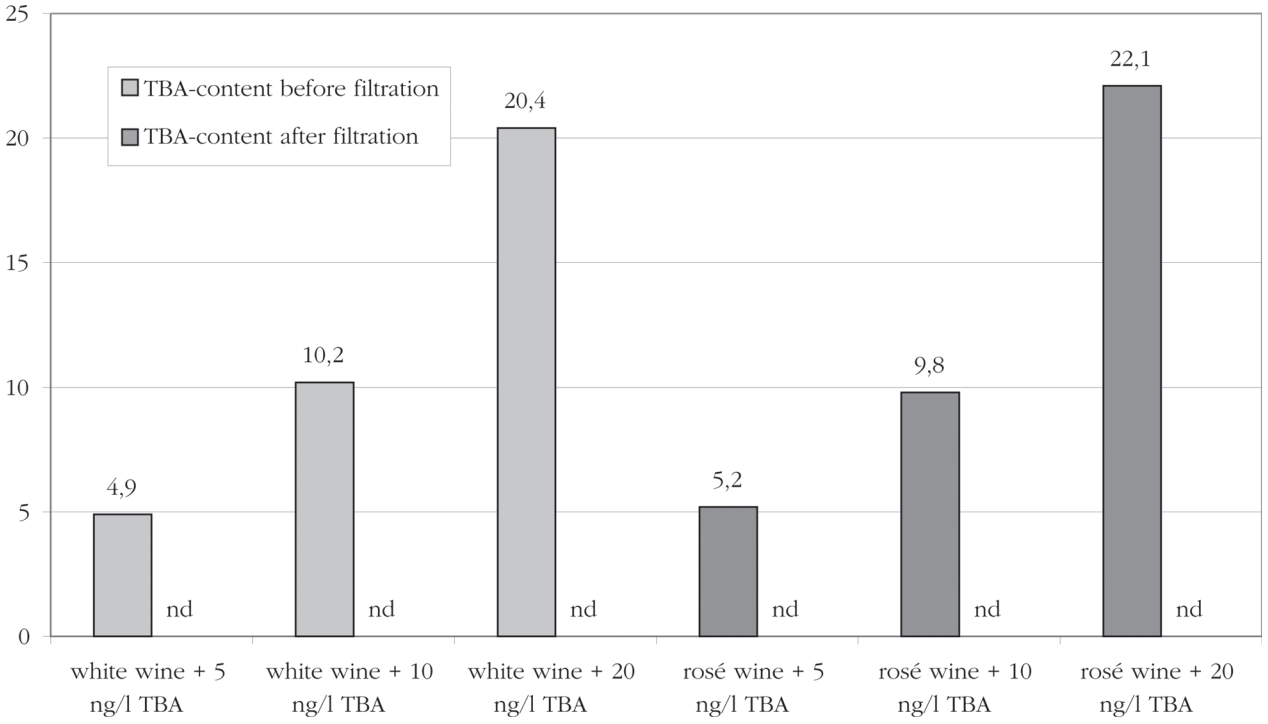

Figure 5: Removal of TBA (nd - not detected)

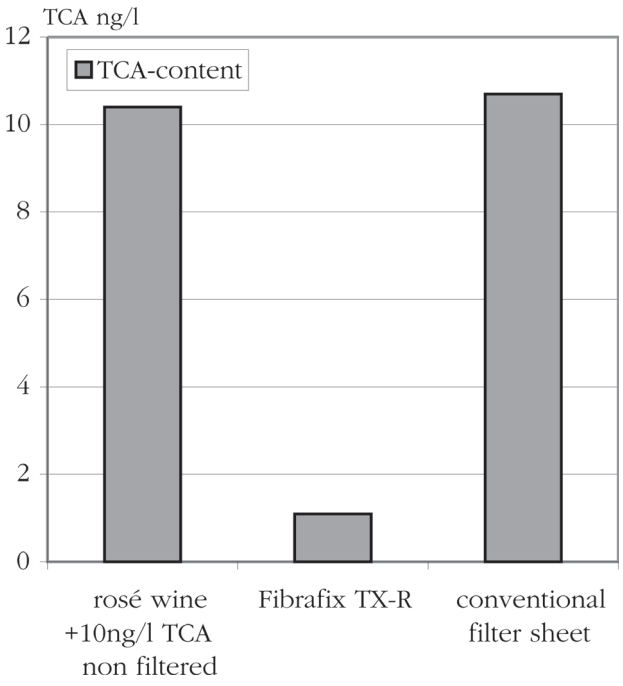

Figure 6: Comparison of TCA removal

an increase of the TCA concentration of about 0.8 ng/l with each sampling (40 litres interval) could be observed until after 360 litres the wine contained 4.0 ng/l TCA. During the step to 400 litres the TCA concentration actually increased by 1.6 ng/l whereby the TCA concentration rose to 5.6 ng/l. The last two samplings showed a further increase of the TCA concentration, whereon the filtration was stopped after 480 litres also due to the fact, that the wine was already sensorily described as being 'musty-mouldy'.

Sensory analysis

All sensory tests confirmed a reduction of the off-flavour produced by TCA through filtration. Regarding the TCA sensibility of the panelists one test was conducted which proved generally that 13 out of 15 were able to detect TCA at a level of 2.4 ng/l (Table 2). The panelists were unable to differentiate between a wine contaminated with 20 ng/l TCA filtered with Fibrafix TX-R and a non-contaminated wine filtered with Fibrafix TX-R in triangle testing. A comparison of a non-contaminated wine filtered with a conventional filter sheet and the same wine filtered with Fibrafix TX-R showed, that the wines could be differentiated by the panelists, but no preference could be ascertained (data not shown).

Aluminium ion migration

In consideration of the fact that the active ingredient (TRIEX) in the filter sheets belongs to the aluminium silicates the question was raised, if there is a migration of aluminium ions into the wine during the filtration

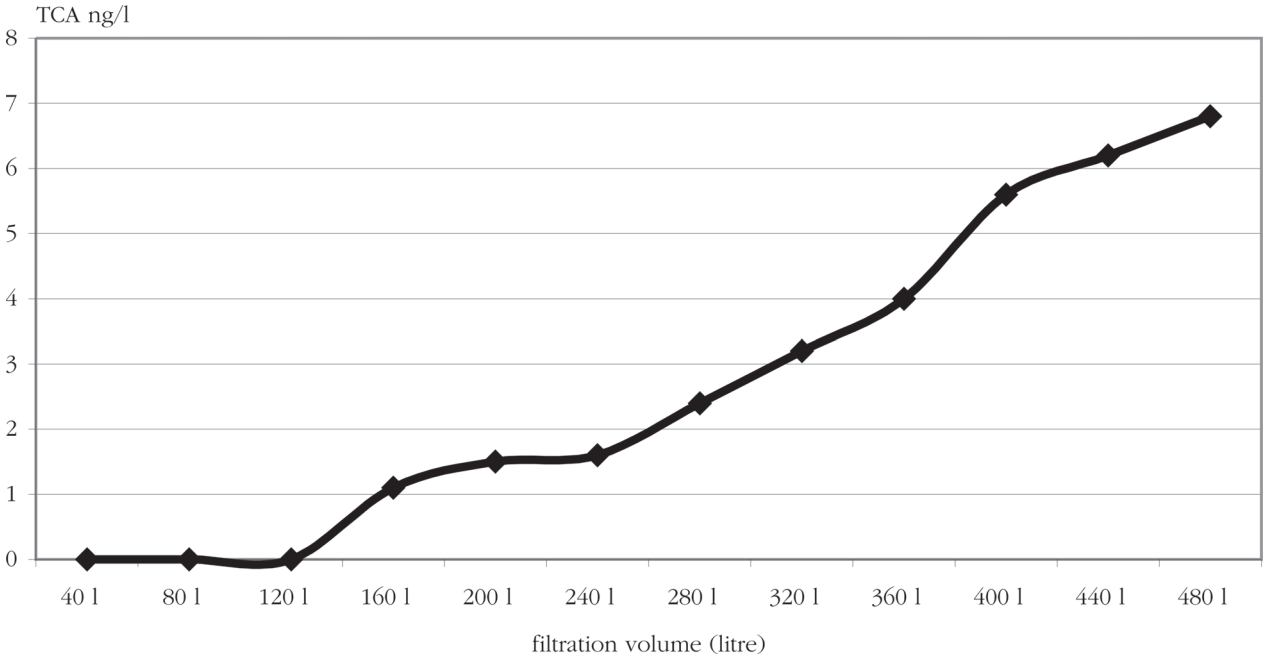

Figure 7: Efficiency/Capacity of Fibrafix TX-R filter sheets (Contamination 20ng/l TCA; filter-surface 0.2 m^2; rate 60l/h)

Table 2: Results of sensory evaluation by means of triangular tests

Results of tests (α-risk)	Answers		α
	No.	Correct	
TX-R filtered wine (no TCA)/TX-R filtered wine 20 ng/l TCA before filtration	15	7	0,2030
TX-R filtered wine (no TCA)/EK filtered wine (no TCA)	15	9	0,0308
TX-R filtered wine (no TCA)/TX-R filtered wine (2,4 ng/l TCA)	15	13	<0.0001

process. This is especially important when a wine already contains an extended amount of aluminium ions for example due to a treatment with bentonite. To answer this question four samples out of the last test trial (filtration limit test) along with an unfiltered sample were analyzed by a certified analytical laboratory. The results of the analysis showed an aluminium content of 2.3 mg/l in the non-filtered wine. The remaining samples were taken after 40, 160, 240 and 480 litres filtration volume. The results of the aluminium analysis are shown in Figure 8.

Obviously the elution of aluminium ions is a little bit higher at the beginning of the filtration since the aluminium content in the 40 litre sample is 0.4 mg/l higher than in the non-filtered wine, whereas in all the other samples the aluminium content was only raised by 0.1 mg/l compared to the non-filtered wine.

Regarding the very low changes of the aluminium contents and considering the range of variation of the measurement inaccuracy, the observed changes are negligible. This means that the product safety in our trial was not affected by the migration of aluminium ions from the filter sheets into the wine regarding a legally permitted maximum of 8 mg/l (Weinverordnung, 2002).

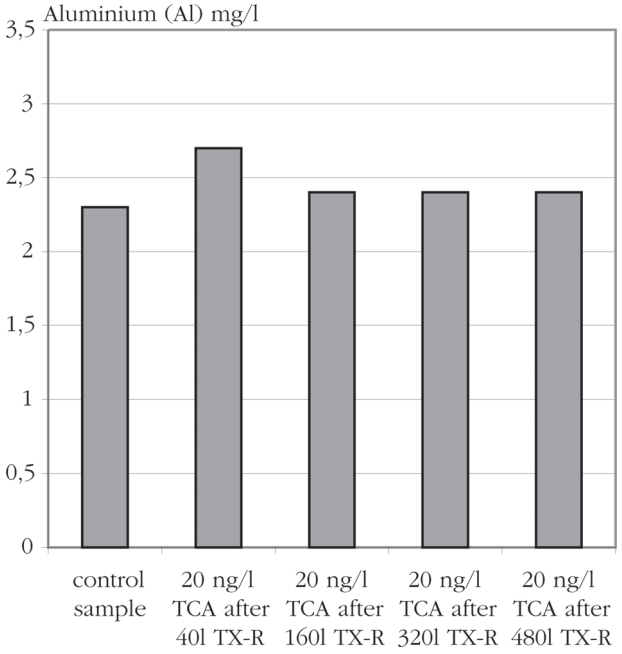

Figure 8: Migration of aluminium ions into the wine after filtration with "Fibrafix TX-R"

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