Investigations on the removal of 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) from wine

[1]R. Jung & [1]V. Schaefer, [1]M. Christmann, [2]M. Hey, [3]S. Fischer, [3]D. Rauhut

Forschungsanstalt Geisenheim, [1]Fachgebiete Kellerwirtschaft, [2]Weinanalytik und Getränkeforschung, [3]Mikrobiologie und Biochemie
Von-Lade-Straße 1, D-65366 Geisenheim
Tel. 06722-502 177, Fax. 06722-502 170, email: r.jung@fa-gm.de & v.schaefer@fa-gm.de

1. Introduction

At the beginning of the eighties the compound 2,4,6- trichloroanisole (TCA) was identified as a main cause of cork taint in wine [1;2].
In the last years a number of further compounds with similar negative flavour attributes were discovered in mouldy and musty smelling wines (e.g. geosmine, 2-methylisoborneol, octane-3-one, pyrazines, etc.) [3;4;5;6].

The main attention in research, however, is still focussed 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 strong.
The perception threshold value for drinking water is indicated to be below 1 ng/L [7], whereas the threshold value for wine is given to be at about 2-5 ng/L (see table 1).
Threshold values in wine depend strongly on the kind of wine, the wine style and the experience of the panelist [8;9].

Table 1:

Perception threshold TCA (ng/L):	Recognition threshold TCA (ng/L):	Medium:	Literature:
4.0		dry white wine	[10]
2.0	6.0	dry white wine	[11]
1.4 – 4.6	4.2 - 10	wine	[6]
210 (a); 17.4 (b)		Sauvignon blanc	[12]
10.0 60.0-100		white wine red wine	[13]
22.0		red wine	[14]

(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. woodpreserver “Raco”, pesticides “Dowicide”) [15;16;17].
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 [13]. Afterwards this phenol is chlorinated chemically in the presence of hypochloride.

After the use of pentachlorophenolic agents was 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 [18;15].

It has already been proven in various studies musty off-flavours in tainted food and wines were not due to TCA but were caused by TBA [19].

Polyethylene (PE) packaging in particular, showed a high permeability for TBA [18]. 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 [5;15].
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 [20].

1.1. Filter sheet "Fibrafix TX-R"

During the Intervitis/Interfructa 2007 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 an 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 certified yet for TCA removal in wine, although this issue will be examined by the OIV shortly.

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.
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”.

2. Material and methods

2.1. 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 TXR” filter sheets.
The filtration surface in all tests was 0,2 m^2, the filtration rate was around 60 litres per hour.
The wines were filled in 20 litre KEG barrels 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.

2.2. 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 triangle tests according to DIN 10951 [23].
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 $\alpha \leq 0.05$ are considered to be significant.

2.3. 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. [21] modified by Fischer and Rauhut [22] as follows:

Sample preparation by Stir Bar Sorptive Extraction (SBSE):
3,5 g of sodium chloride (Roth, Germany) was weight 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 Isoptopes, Canada) and 2,4,6-tribromoansiole-d_5 (31,4 ng/L, CDN Isoptopes, 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 in 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.

2.4. 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. [24] and Ortega et al. [25] with slightly modifications. Major wine volatiles were analysed after dichlormethan microextraction [25], minor and trace volatile compounds were isolated by solid-phase extraction with LiChrolut EN [24].
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.

3. Preliminary tests 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 the 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 the same wine were contaminated with 20 ng/L TBA.

Both batches were filtered and samples were taken in intervals of 10, 20, 30 an 38 litre in 0.75 L screwcaped bottles.

4. 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 (figure 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 in sterilized screwcaped bottles.

In a second step a trial analogue to the first one was proceeded by using TBA instead of TCA in the same concentrations as mentioned above.

Additionally 50 litres rosé 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.

Besides 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.

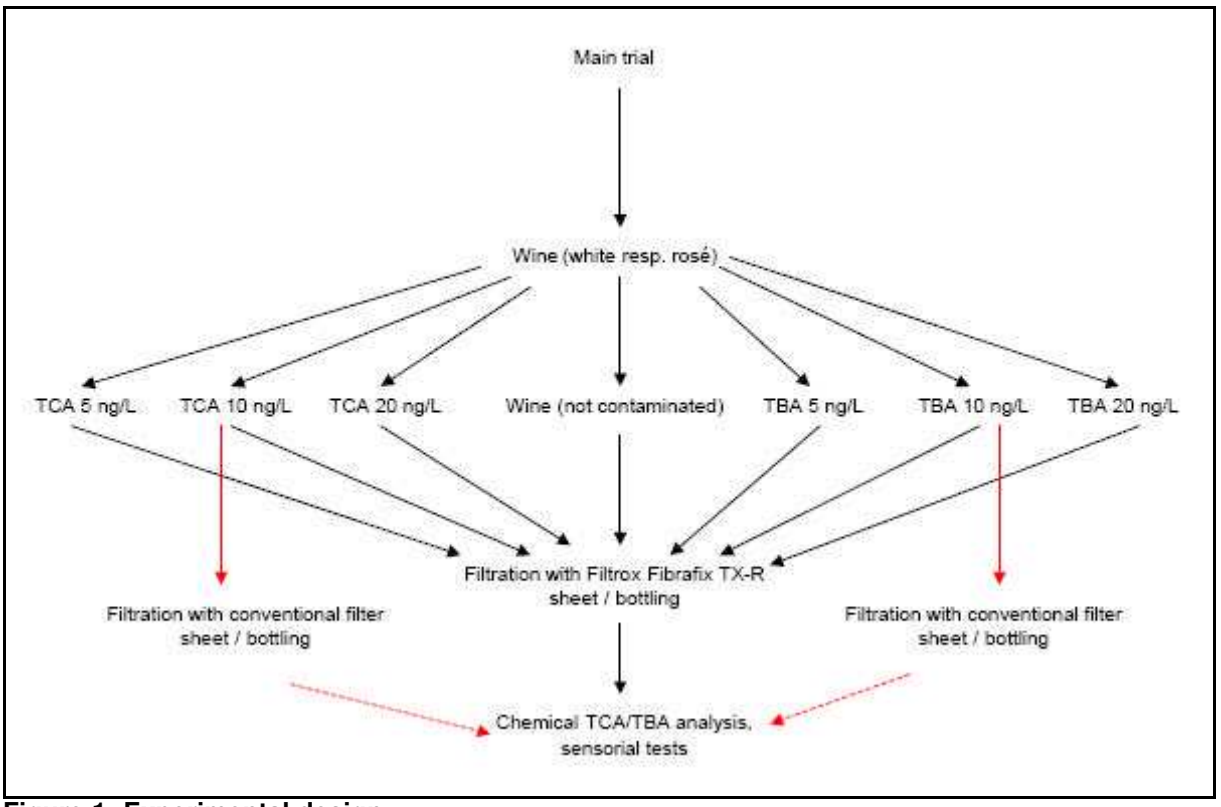

Figure 1: Experimental design

5. Results

5.1. 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 (see 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.

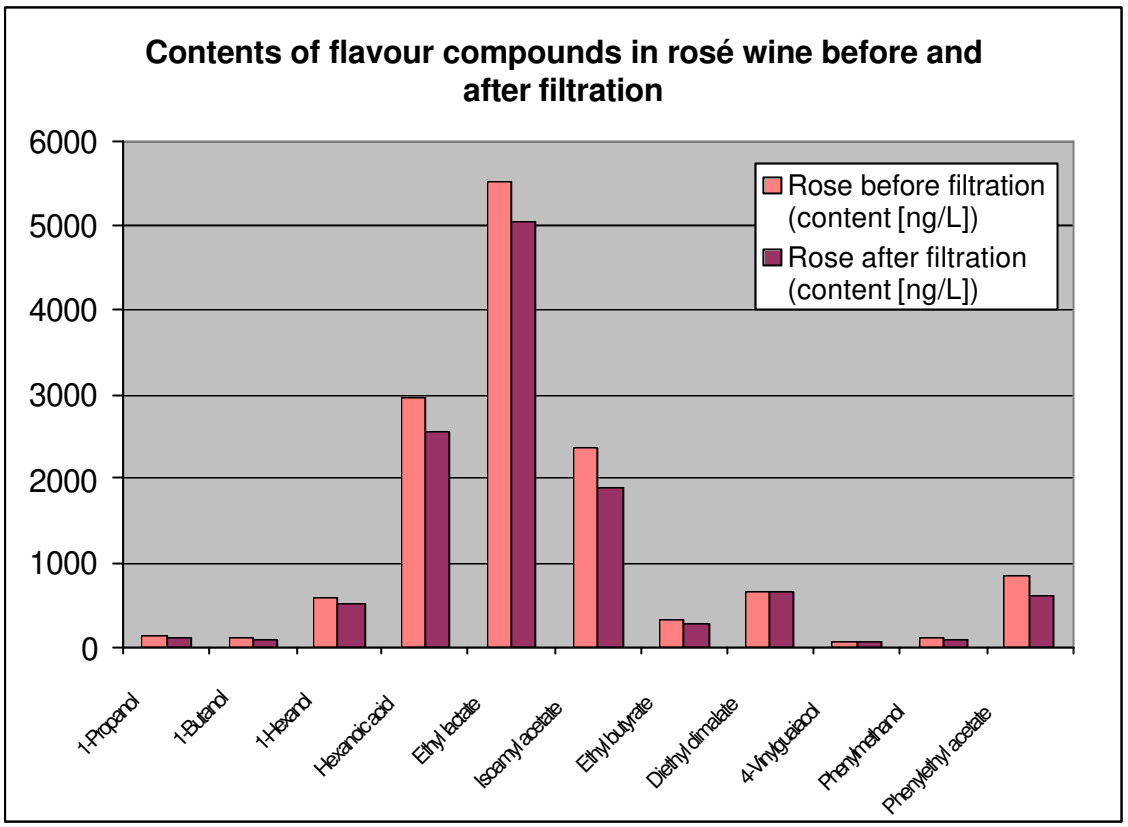

Figure 2: Flavour compounds in rosé wine before and after filtration

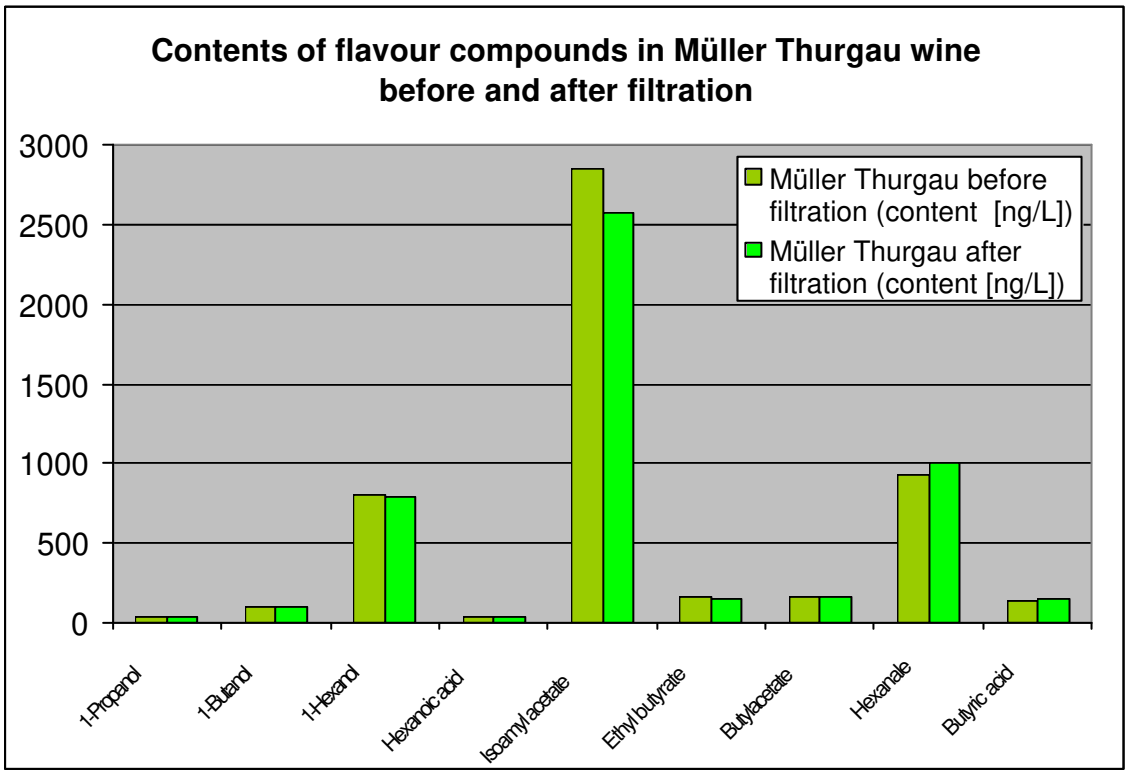

Figure 3: Flavour compounds in Müller Thurgau wine before and after filtration

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 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.

5.2. 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.

Like in the preliminary tests no TBA could be detected in the samples of the main trial after filtration (figure 5).

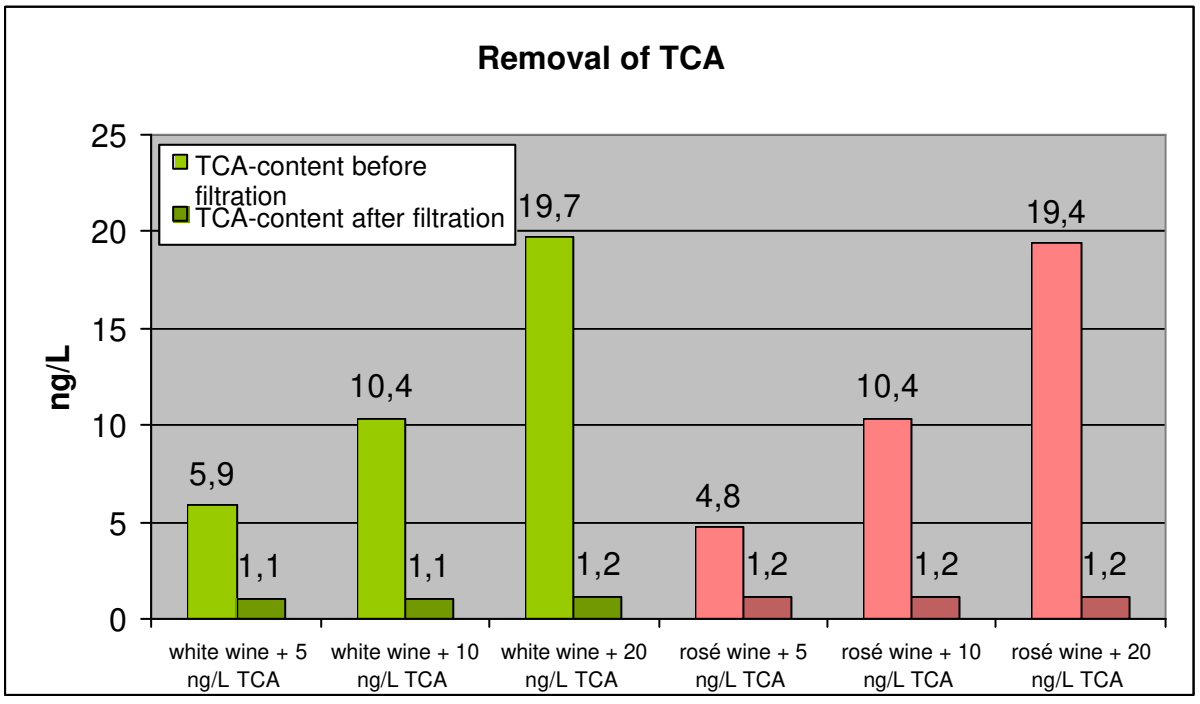

Figure 4: Removal of TCA

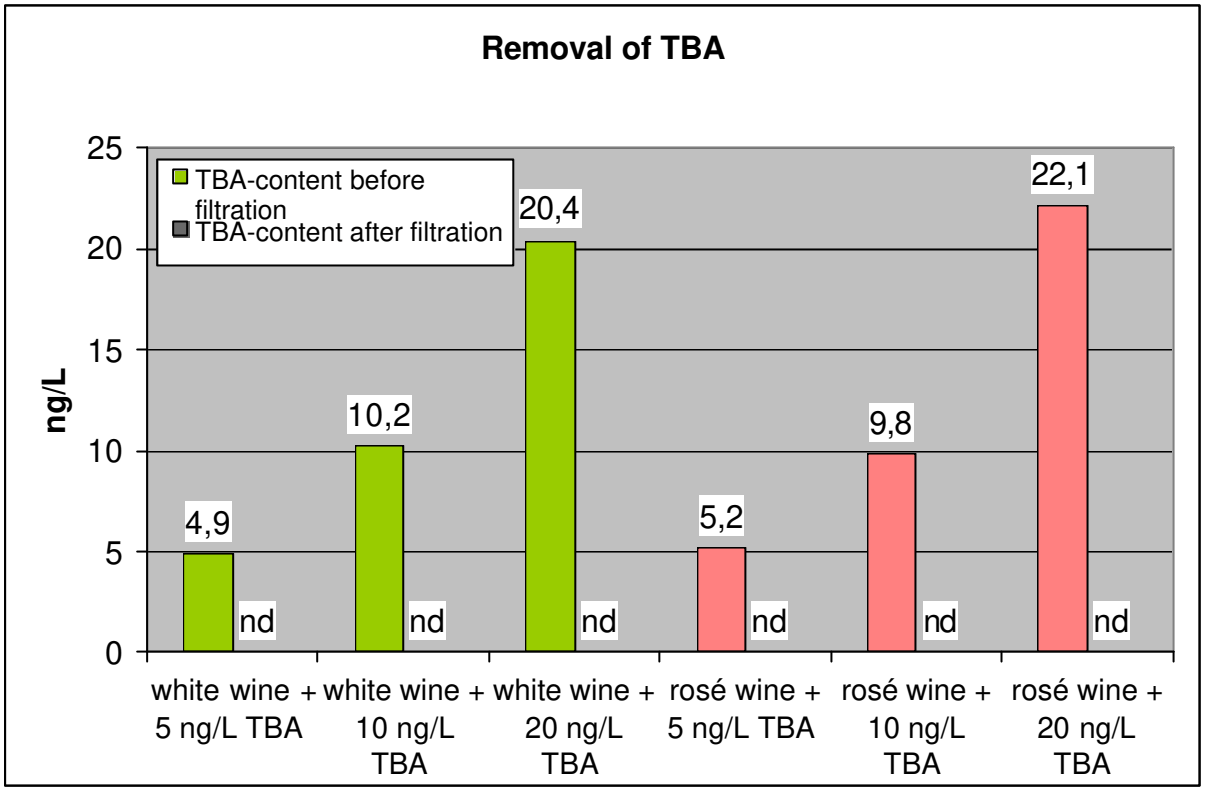

Figure 5: Removal of TBA (nd – not detected)

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

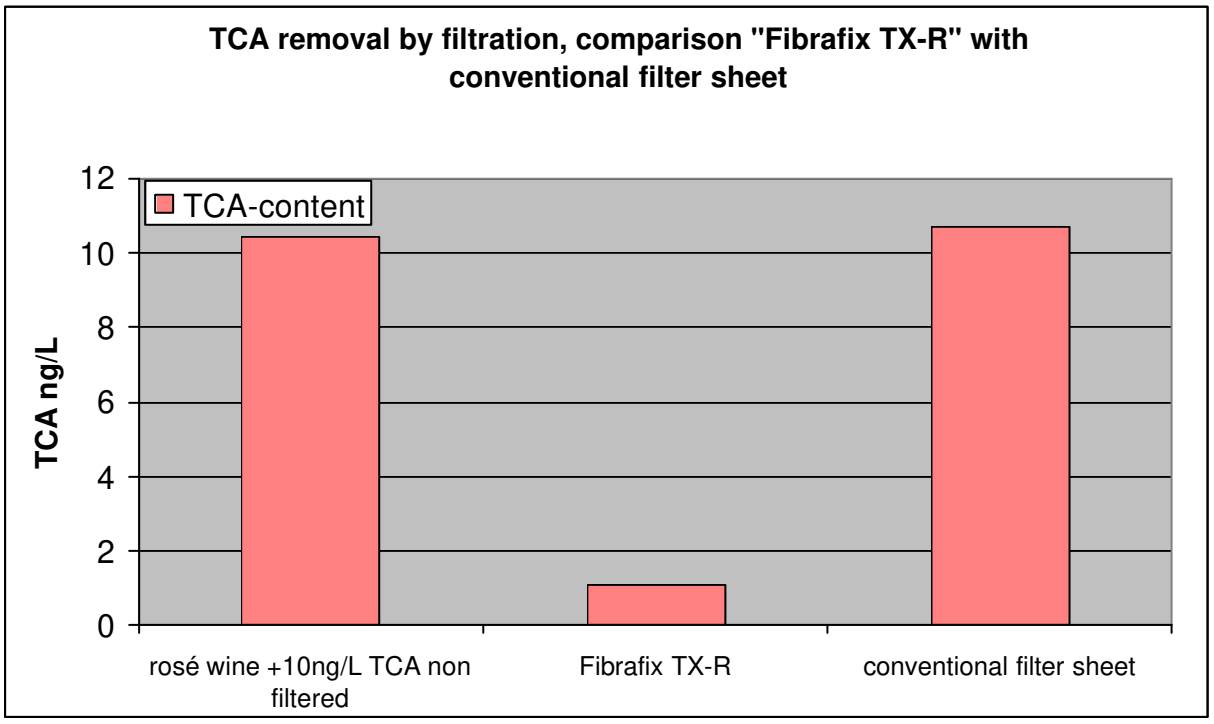

Figure 6: Comparison of TCA removal

5.3. Limit of TCA absorbance

Based on technical data provided by "FILTROX / St. Gallen" 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 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 (see figure 7) showed that up to 120 litres of filtration volume no TCA could be detected in the wine samples.

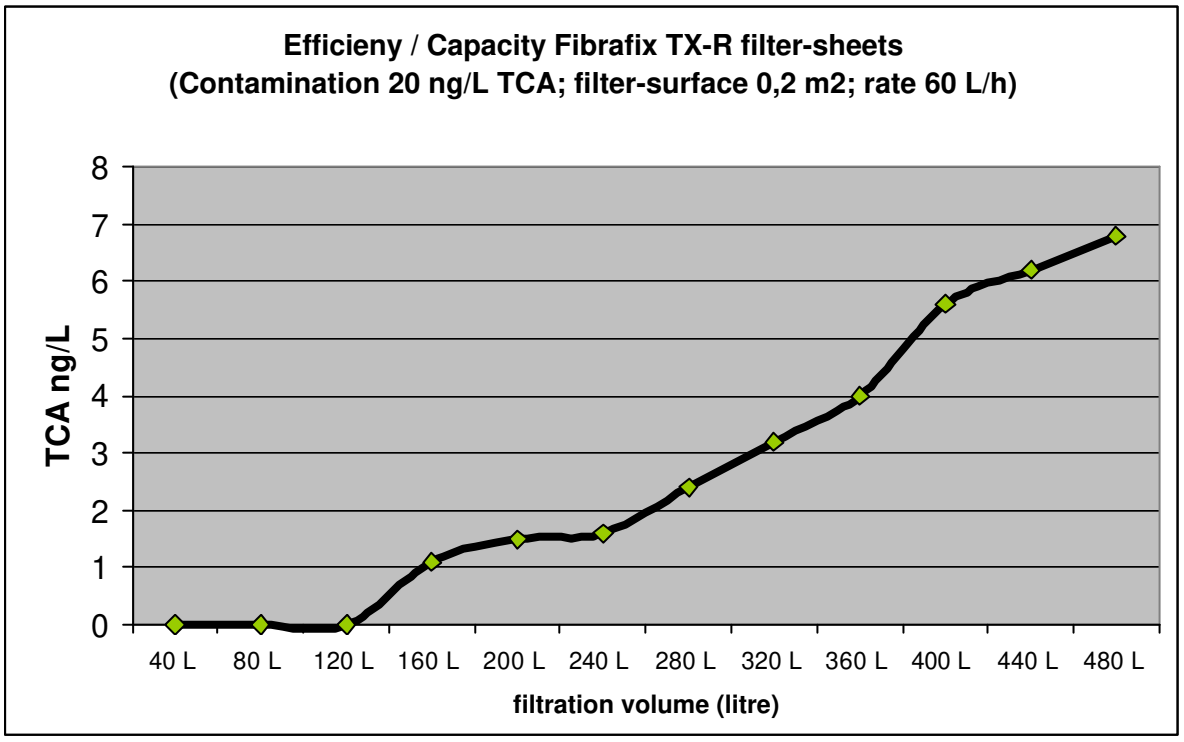

Figure 7: Efficiency/Capacity Fibrafix TX-R filter sheets

The samples taken between 160 and 240 litres filtration volume contained constantly around 1.5 ng/L. Afterwards an increase of the TCA concentration of about 0.8 ng/L each sampling (40 litre 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 1.6 ng/L whereby the TCA concentration rised 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 sensorically described as being "musty-mouldy".

5.4. Sensory analysis

All sensory tests confirmed a TCA-reducing effect of the 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 differ 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 revealed, that the wines could be differentiated by the panelists, but no preference could be ascertained (data not shown).

Table 2: Results of sensorial tests

Results of tests (Alpha risks)				
	Without	**Answers**	**Answers**	**Signif.**
	Answer	**Taken**	**Right**	**(Risk)**
TX-R filtered wine (no TCA)/ TX-R filtered wine 20 ng/L TCA before filtration	**0**	**15**	**7**	**0,203**
TX-R filtered wine (no TCA)/ EK filtered wine (no TCA)	**0**	**15**	**9**	**0,0308**
TX-R filtered wine (no TCA)/TX-R filtered wine (2,4 ng/L TCA)	**0**	**15**	**13**	**<0.0001**

5.5. 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 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.

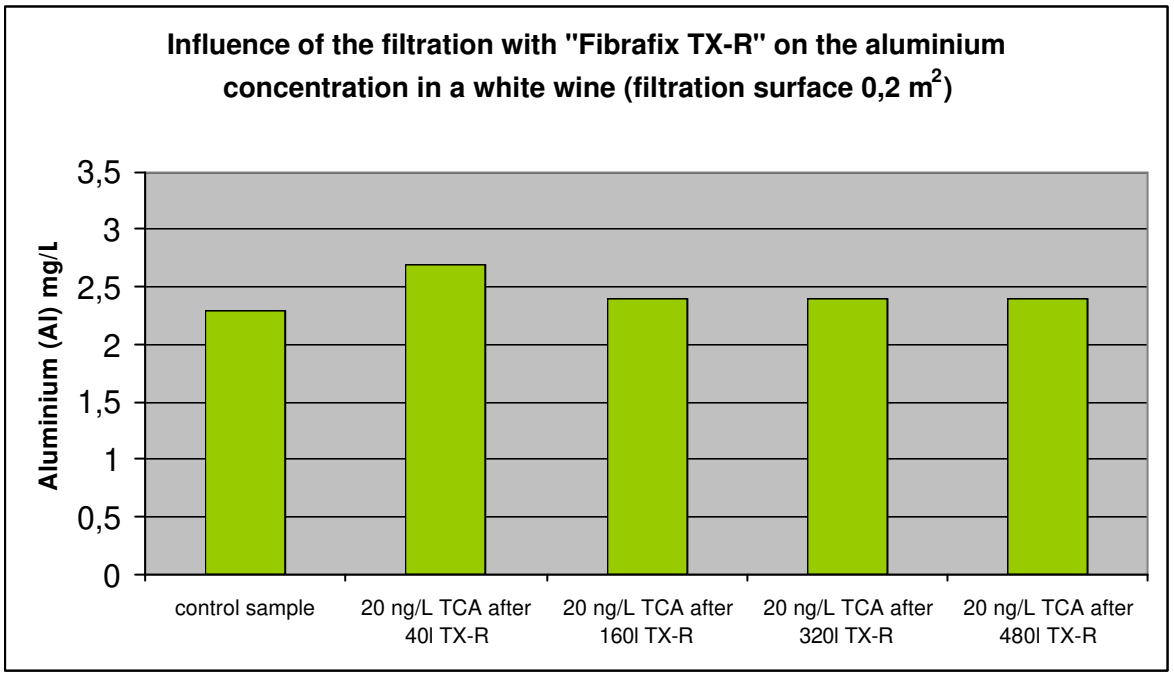

Figure 8: Migration of aluminium ions

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.

6. Summary

In four test series different wines were contaminated with variable amounts of TCA and TBA and then filtered with “Fibrafix TXR” filter sheets.
The purpose of the tests was to determine if there is an effect of the filtration on aroma compounds, the TCA and TBA content and the aluminium content in the wines.
Additionally the effect of a conventional filter sheet as well as the limit of the TCA absorbance of the Fibrafix TXR filter sheets were checked.

The gained data of the sensory and analytical tests revealed, that a successful reduction of TCA and TBA below the detection threshold value could be achieved, while the wine flavour was not affected.
Furthermore the tests regarding the absorbance limit corroborated the data obtained by Filtrox in internal test series.
In addition 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, no effect could be detected after filtration with “Fibrafix TX-R” filter sheets.

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