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REGULAR ARTICLE

INFLUENCE OF DIFFERENT OAK CHIPS ON AROMA COMPOUNDS IN WINE

Jana Návojská[1], Walter Brandes[2], Stefan Nauer[2], Reinhard Eder[2], Helena Frančáková[1]*

Address: [1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food
Sciences, Department of Storing and Processing Plant Products,
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2] Department of Chemistry, Lehr- und Forschungszentrum für Wein- und Obstbau,
Wienerstrasse 74, A-3400 Klosterneuburg, Austria

*Corresponding author: jana.navojska@uniag.sk

ABSTRACT

Oak wood has considerable influence on amount of aroma compounds in wine. During the time, when wine is in contact with oak wood, compounds typical for wood diffuse into wine and enrich its aromatic complexity.

Concentration of diffused aroma compounds in wine depends on several factors as contact time, storage conditions, wine properties, geographical origin and toasting degree of oak wood. Because of very small quantities of volatile aroma compounds in wine, it is necessary to use sensitive method as gas chromatography with appropriate sample preparation, to concentrate and analyse the relevant volatiles. In our work, four different wines and eight chips samples with different geographic origin and degree of toasting were used. With GC-MS following substances were determined in wines after 30 days of maceration with oak chips: furfural, 5-methylfurfural, guaiacol, 4-ethylguaiacol, eugenol, isoeugenol, *cis*- and *trans*-whiskey lactones, 4-ethylphenol.

Our results show, that the degree of toasting influences the concentration of furfural, guaiacol and *cis*- and *trans*- whiskeylactone. Higher toasting leads to an increase of furfural and guaiacol and a decrease of *cis*- and *trans*-whiskeylactone in wine. The highest increase of volatile aroma compounds was determined in wine samples with French oak chips with intense toasting. We did not find any specific, definite differences among chips with different geographic origin.

Keywords: oak wood, chips, aroma compounds, wine aroma, gas chromatography.

INTRODUCTION

Usage of wood belongs to one of the traditional winemaking practices, because of its positive effect on organoleptic and chemical quality of wine. Oak wood is the most frequently used wood for improvement of wine aroma. The role of wood in wine aging is to transfer volatile aroma compounds and astringency-related phenolics to wine, to improve the intensity and complexity of wine flavour and aroma. Additionally, wine aging in wood barrels leads to gentle oxidation of certain compounds resulting in reduction of astringency and bitterness, colour stabilization, and the disappearance of excessive vegetative-herbaceous aromas (**Scheiblhofer *et al.*, 2005; Garde-Cerdán *et al.*, 2006; Bozalongo *et al.*, 2007; Macedo *et al.*, 2008; Rayne *et al.*, 2011**).

Wine aging in wood barrels has also some disadvantages as are long duration and relative high price of barrels, also requirement of much space in winery and limited lifetime of barrels (**Rodríguez-Rodríguez *et al.*, 2011**). Furthermore the difficult cleaning of barrels enhances hygienic problems of wine as infection with *Brettanomyces* yeasts.

These facts lead to application of alternative methods for improving the wine quality. Usage of oak chips combined with micro-oxygenation system presents a valid alternative for production of wine with woody character. Oak chips are small pieces of oak wood which can be obtained from wood scrap wastes produced during barrel manufacturing, and are prepared using traditional methods in cooperage and subjecting them to boiling in water and toasting (**Bozalongo *et al.*, 2007**). Chips can be added to wine fermented in stainless steel tanks, to obtain wines like those fermented in barrels, but with better manipulation conditions (better temperature control, also able for larger amounts, better control of hygiene) (**Pérez-Coello *et al.*, 2000; Rodríguez-Rodríguez *et al.*, 2011**).

The amount of volatile aroma compounds extracted from oak wood into wine depends on several conditions, mainly on the quantity of compounds that are potentially extractable, contact time between wine and wood, storage temperature, chemical properties of wine, wood properties such as oak species, geographic origin, seasoning of wood, degree of toasting (**Del Alamo *et al.*, 2000; Garde-Cerdán *et al.*, 2004; Garde-Cerdán *et al.*, 2006; Fernández de Simón *et al.*, 2006; Nonier *et al.*, 2006; Garde-Cerdán *et al.*, 2010**). These factors affect the concentration of oak lactones, volatile phenols and phenolic aldehydes. (**Del**

Alamo *et al*., 2004). For example, American oak contributes more cis- and trans- isomers of whiskylactone than French oak (**Chatonnet and Dubordieu, 1998**).

Natural seasoning has an effect on the aromatic profile of wood. Oak wood dried and seasoned outside undergoes the action of microorganisms, rain, sun, and air, what eliminates a portion of the ellagitannins through hydrolysis, leaching, and oxidation. Opposite to artificial seasoning, natural seasoning has predominantly a positive effect on volatile composition and sensorial characteristics of oak wood (**Matricardi and Waterhouse, 1999; Vivas *et al*., 2004; Cadahía *et al*., 2007; Fernández de Simon *et al*., 2010**).

Toasting of wood causes, that large polymers lignin or cellulose present in wood are decomposed due to a high temperature forming compounds as aldehydes, phenols, furfural derivatives, lactones, and other (**Nonier *et al*., 2006**).

Volatile oak compounds which influence wine aroma are mainly furfurals (smell like dried fruits), guaiacol (burn overtones), whisky lactone (woody and coconut notes), eugenol (spices, cloves and smoke character), 4-ethylphehol, 4-ethylguaiacol, vanillin (vanilla character) and syringaldehyde (related to vanilla character), with a low sensory thresholds (**Díaz-Plaza *et al*., 2002; Fernández de Simon *et al*., 2006; Bozalongo *et al*., 2007; Scheiblhofer *et al*., 2005**).

All mentioned volatile compounds are present in wine in small quantities ($\mu g.dm^{-3}$ or less); therefore their determination is commonly performed by sensitive analytical techniques such as gas chromatography (**Carillo and Tena, 2006; Brandes *et al*., 2002**). Before chromatographic analysis, sample preparation is needed, what is imposed by several factors as low concentrations of desired compounds, great variation of other chemicals (alcohols, acids, esters, etc.) or presence of chemicals as complex compounds (**Castro *et al*., 2008**).

The objective of our study was to find out, if there are any significant differences in analyzed volatile aroma compounds after using oak chips with different origin and toasting degree by specified conditions (amount of chips, contact time, temperature).

MATERIAL AND METHODS

Wine samples

For our experiment, we used four different wines, two pure varieties: Cabernet Sauvignon (CS), Blaufränkisch (BF) and two cuvees: red (RC) and white cuvee (WC).

Table 1 Initial basic parameters of wine samples

Wine samples	density (kg.dm^{-3})	alcohol (vol. %)	reducing substances (g.dm^{-3})	titr. acidity (g.dm^{-3})	pH
CS	0.9937	13.2	1.1	4.9	3.7
BF	0.9945	12.3	2.1	5.4	3.6
RC	0.9940	12.9	2.1	5.0	3.7
WC	0.9915	12.7	1.4	6.2	3.4

CS – Cabernet Sauvignon, BF – Blaufränkisch, RC – Red cuvee, WC – White cuvee

Oak chips differed in origin and degree of toasting. We used chips from American oak with medium and high degree of toasting (OakyVin AM and AH; Erbslöh, Geisenheim, Germany), German oak, not toasted, medium toasted and premium toasted (Best Oak type 2 NTF, 8 MT and 8 PTH; Keller Mannheim, Germany), French oak medium and medium-plus toasted (OakArome M and M+; Begerow, Germany) and high toasted (OakyVin FH; Erbslöh, Geisenheim, Germany).

Nine 1-litre glass bottles of each wine, closed with crown cork, were prepared: one litre as a blank and eight bottles with 1 g.dm^{-3} oak chips – according to the supplier's recommendation. Chips were kept in bottles for 30 days, in dark conditions by the temperature 4 °C. Samples were shaken twice a week to facilitate the extraction of oak compounds.

Samples preparing

Before the chromatographic analyse performance, the samples were prepared by water steam-distillation, liquid-liquid extraction and evaporation according to modified method of **Brandes, Wendelin and Eder (2002).**

To 50 ml of wine sample, 0.5 ml internal standard, 50 µl concentrated H_2SO_4 and a bit of silicon against foaming were added and distilled using water steam distillation system up to the amount of 70-75 ml of distillate. Distillate was additionally acidified with 50 µl concentrated H_2SO_4 and extracted twice with 10 ml dichloromethane in separatory funnel. Total extract was dried with a half of spoon of Na_2SO_4. 200 µl of toluol was added to dried extract and subsequently, evaporated at 135 mbar in 40 °C water bath on rotary evaporator, till the amount of residue reached approximately 200 µl. Residue was then injected directly into GC-MS system.

Standards for calibration were manipulated by the same way as the samples.

GC-MS analyses

Oak volatile substances and other reagents used in analyses were: 2-nonanol (97 %), furfural (99 %), 5-methylfurfural (99 %), whiskeylactone (98 %), 4-ethylguaiacol (98 %), o-kresol (99 %), m- and p- kresol (98 %), 4-ethylphenol (98 %), eugenol (99 %), dichloromethane (99.6 %), toluol (99.7 %), water-free Na_2SO_4 obtained by Sigma Aldrich, Austria; guaiacol obtained by Extrasynthese, Genay, France; ethanol absolute p.a. by Riedel de Haën, Austria and sulphur acid (98 %) obtained by Fluka, Austria.

Stock solutions were prepared in pure ethanol, with concentration 1 $g.dm^{-3}$. Standards for calibration were prepared in 10 % ethanol. Internal standard 2-nonanol was prepared in pure ethanol with final concentration 13.6 $mg.dm^{-3}$.

Volatile substances were analysed using modified method according to **Brandes, Wendelin and Eder (2002)**, with following conditions:

GC - instrument: GC System 7890 A, Agilent Technologies (Vienna)
Carrier gas: Helium
Column: DB-5 60 m x 250 µm x 0.25 µm
Flow: 1.267 ml/min constant
Injection volume: 1 µl splitless
Injector-temperature: 250 °C
Transfer line temperature: 240 °C
Oven program: 70 °C for 2,5 min, then 20 °C/min to 110 °C for 0 min, then 2 °C/min to 226 °C for 0 min, than 50 °C/min to 255 °C for 15 min.
Run Time: 78.08 min

MS - instrument: Mass-selective detector 5975 C, Agilent Technologies, Vienna
Coupling: direct interface
Mode: Single Ion Monitoring
Mass fragments: IS 2-nonanol (45, 55, 69), furfural (39, 95,96), 5-methylfurfural (53,109,110), guaiacol (81, 109, 124), cis-whiskeylactone (69, 71, 99), trans-whiskeylactone (69, 71, 99), 4-ehtylguaiacol (122, 137, 152), o+p+m-kresol (79, 107, 108), eugenol (77, 103, 149, 164), 4-ethylphenol (77, 107, 122, 164), cis-isoeugenol (131, 149, 164), trans-isoeugenol (131, 149, 164).

RESULTS

All results of our experiment are presented in the following **Figures 1-10**. Wine samples are labeled as CS for Cabernet Sauvignon, BF for Blaufränkisch, RC as red cuvee, WC as white cuvee. Chips samples are marked with geographical origin (at the first place) A / G / F – as American / German / French oak wood. Degree of toasting is marked at the second place with N / M / M+ / H / P – as not toasted / medium / medium plus / high / premium toasted. Zero samples, without any influence of wood, are labeled as 0.

Furfural compounds

In our experiment, we found the greatest differences in amount of furfural compounds. Initial concentration of furfural ranged between 15.9 μg.dm^{-3} in white cuvee and 95.8 μg.dm^{-3} in red cuvee. Concentration of 5-methylfurfural was determined between 0.7 μg.dm^{-3} (in CS) and 3.9 μg.dm^{-3} in RC. Compared to zero samples, all wines treated with oak chips reached increase of furfural and 5-methylfurfural amount. The highest increase of furfural was detected in wine samples treated with French oak chips with high degree of toasting (**Fig 1**), particularly in red wine varieties (540.3 μg.dm^{-3} in RC). The highest increase of 5-methyl furfural showed medium toasted American chips (between 6.1 μg.dm^{-3} in WC and 53.8 μg.dm^{-3} in RC) and high toasted French oak chips (between 11.6 μg.dm^{-3} in WC and 50.7 μg.dm^{-3} in RC) what showes **Figure 2**. The smallest increas or eventually decrease of furfural compounds was evident in wine samples with not-toasted German oak.

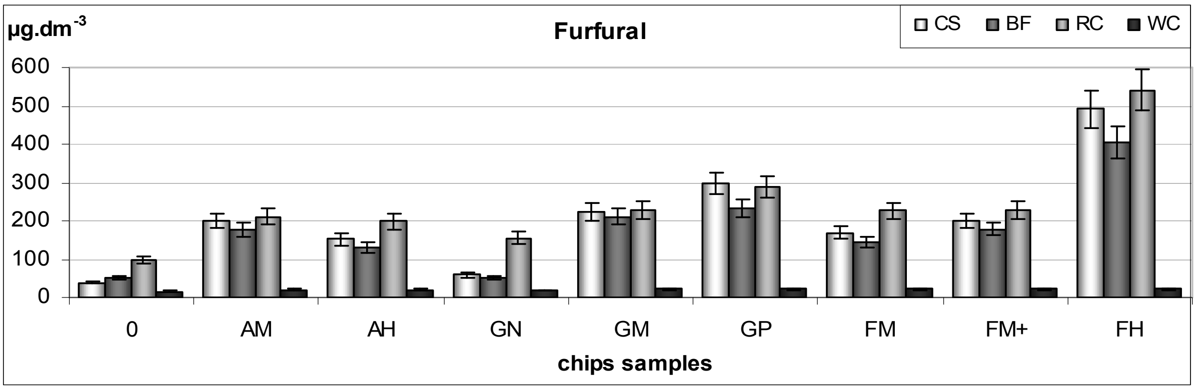

Figure 1 Variances of furfural content in wine with different chips samples.

(0 – zero sample without chips, AM – chips from American wood with medium degree of toasting, AH – American wood with heavy toasting degree, GN – German wood without toasting, GM – German wood, medium toasted, GP – German wood, premium (heavy) toasted, FM – French wood, medium toasted, FM+ - French

wood, more than medium toasted, FH – French wood, heavy toasted; CS – Cabernet Sauvignon, BF – Blaufränkisch, RC – Red cuvee, WC – White cuvee)

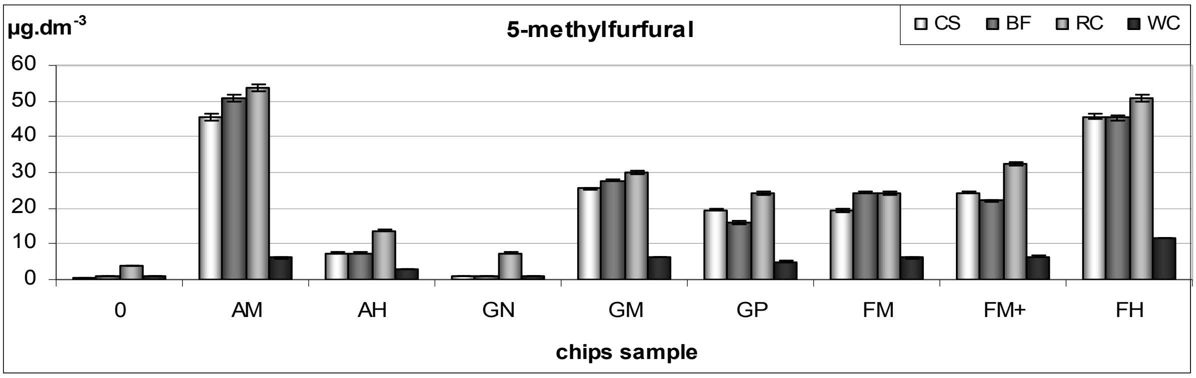

Figure 2 Variances of 5-methylfurfural content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

Lactones

Concentration of *cis*-whiskeylactone was too small in wine CS (0 – 0.17 μg.dm^{-3}) and BF (0.4 – 0.7 μg.dm^{-3}) to claim, that there are differences among samples. On the other hand, it came to evident increase of this compound in both of cuvee wines (**Fig 3**). **Figure 4** showes, that concentration of *trans*-whiskeylactone rose in all wine samples treated with oak chips, in comparison with zero samples. The highest accumulation of *trans*-whiskeylactone was determined in all wine samples with medium toasted American oak chips – AM (between 25,9 μg.dm^{-3} in WC and 106,7 μg.dm^{-3} in RC). The lowest increase of whiskeylactones was measured in samples with high / premium toasted chips.

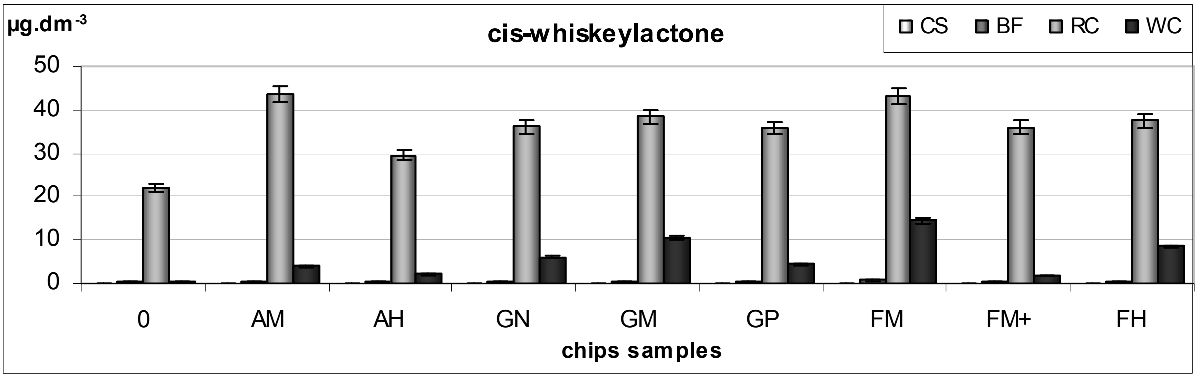

Figure 3 Variances of cis-whiskeylactone content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

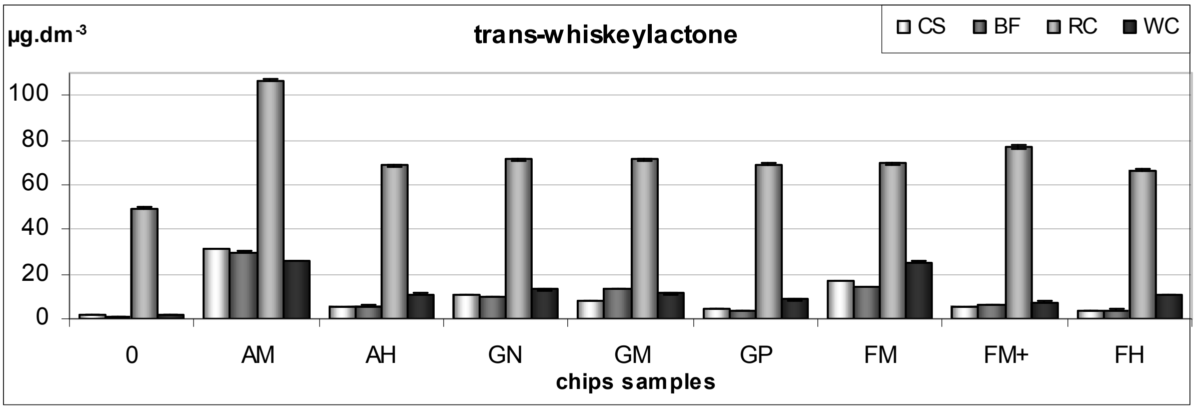

Figure 4 Variances of trans-whiskeylactone content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

Eugenol

All samples of oak chips showed an increase of eugenol in wine except for CS wine, where a small decrease in samples with not-toasted and premium toasted German chips was observed. Eugenol content mostly increased using American medium toasted oak chips – AM (**Fig 5**).

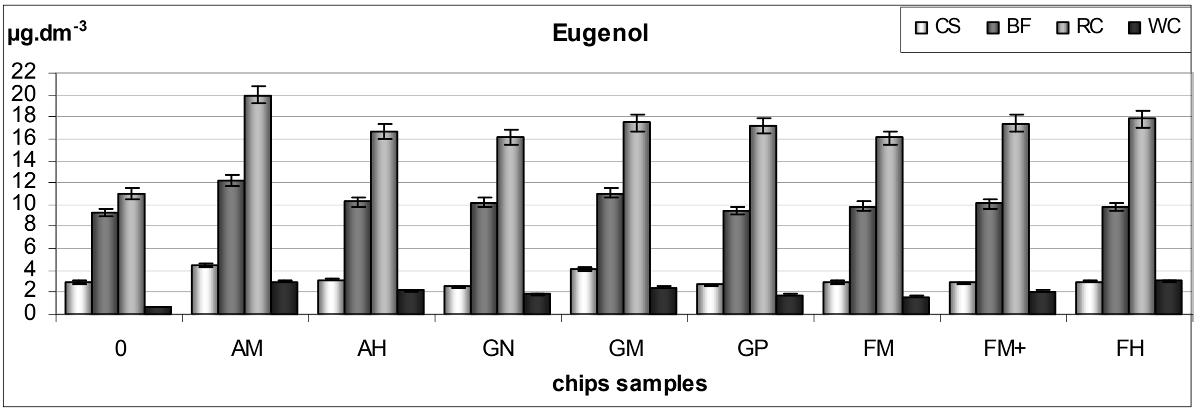

Figure 5 Variances of eugenol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

Concentration of both isoeugenol isomers reached too small levels to show evident differences among chips samples in wines CS and BF (**Fig 6**). But we can notice significant differences in isoeugenol quantities among chips samples in both cuvee wines. *Trans*-isoeugenol reached higher levels in cuvee wine samples with medium and higher toasted oak chips, mainly with American and French origin. According to these results (**Fig 7**), we can suppose, that amount of trans-isoeugenol is nearly related with toasting proces of oak wood.

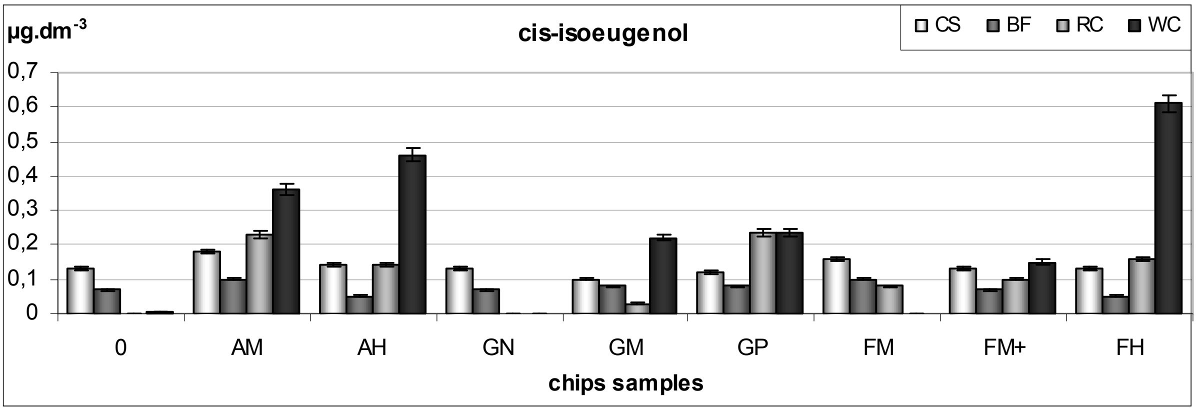

Figure 6 Variances of *cis*-isoeugenol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

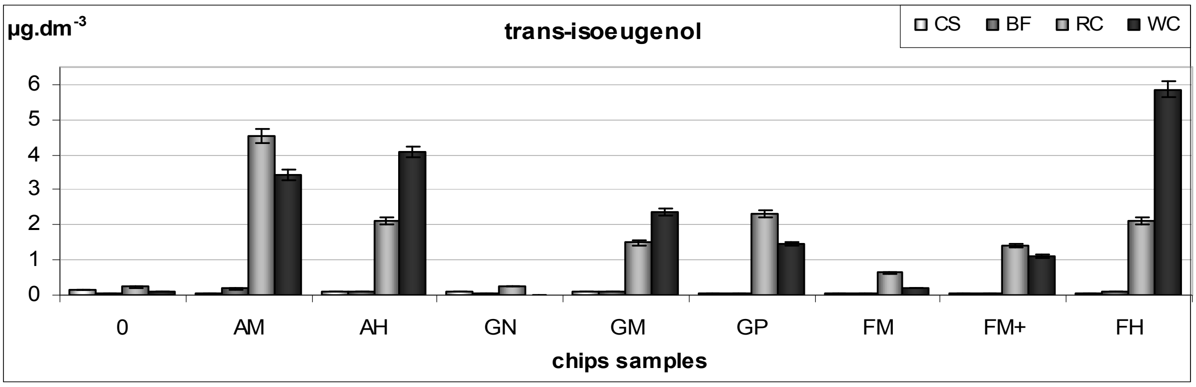

Figure 7 Variances of *trans*-isoeugenol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

Guaiacol

Guaiacol concentrations were mostly influenced by toasting degree of oak chips. It is shown in **Figure 8** and **Figure 9**, higher content of guaiacol and 4-ethylguaiacol was reached in wine samples treated with chips with high degree of toasting, regardless of the geographic origin of oak wood. According to our results, not-toasted chips had not any influence on guaiacol and 4-ethylguaiacol level in our wine samples.

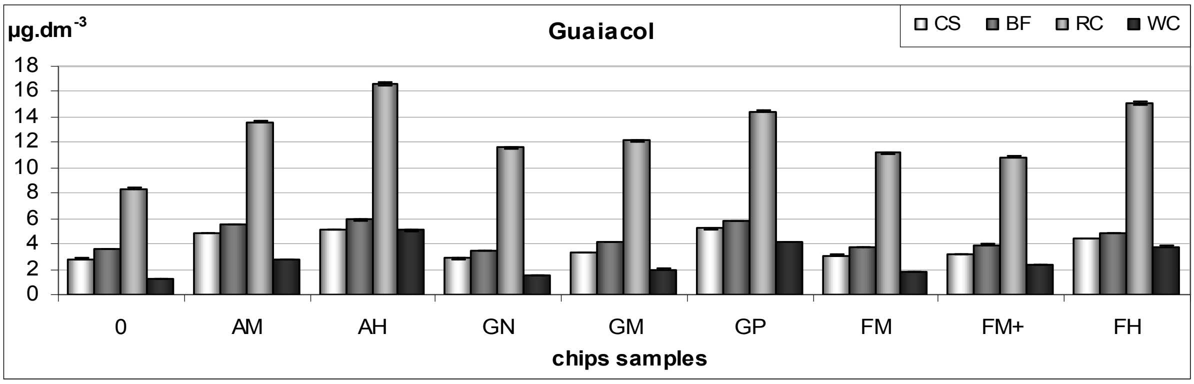

Figure 8 Variances of guaiacol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

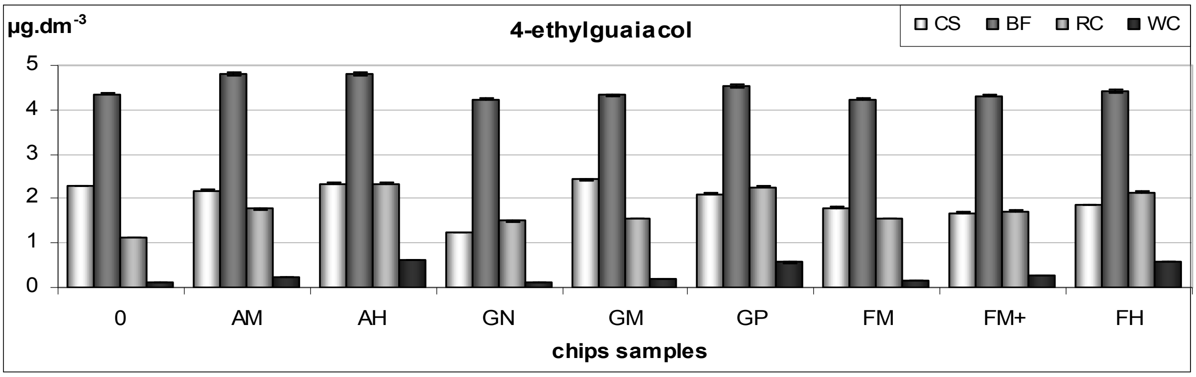

Figure 9 Variances of 4-ethylguaiacol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

Ethylphenol

All four zero wines contained small quantities of 4-ethylphenol (CS: 6.0 µg.dm^{-3}; BF: 12.7 µg.dm^{-3}; RC: 2.5 µg.dm^{-3}; WC: 0.3 µg.dm^{-3}). As shown in **Figure 10**, addition of different oak chips did not significantly change the amount of 4-ethylphenol content.

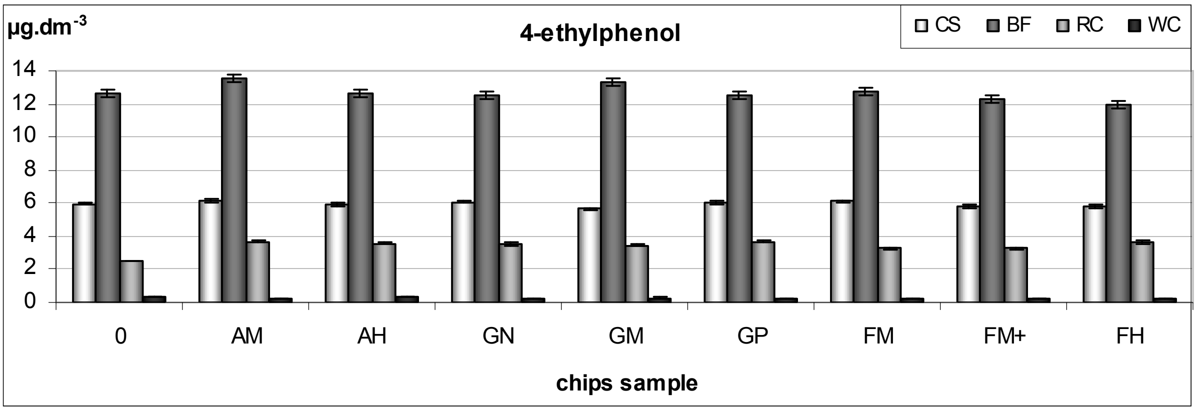

Figure 10 Variances of 4-ethylphenol content in wine with different chips samples.

(Explanation of shortcuts, see Fig 1)

DISCUSSION

Bozalongo *et al.* (2007) found out in their studies, that toasting of oak wood increases amounts of compounds deriving from termal degradation of lignin (vanillin, eugenol, guaiacol and its derivatives) and the pyrolysis of cellulose and hemicellulose (furfural and 5-methylfurfural) and decreases the concentration of whiskey lactone.

In our experiment we reached similar results. We found the highest amounts of eugenol, isoeugenol, guaiacol, 4-ethylguaiacol and furfural in wine samples with high toasted oak chips.

Díaz-Plaza *et al.* (2002) have shown that the furfural, eugenol, *cis*- and *trans*-whisky lactone contents can identify the type of wood (American and French) used to make the barrel in which the wines were aged. The levels of the *cis*- isomer form in the wines aged in American oak were much higher than in those aged in French oak. Also concentration of 4-ethylphenol was presented in low level before aging and its quantity increased significantly during aging in oak wood, mostly in French oak.

Our study does not show the same results and conclusions. We can not exactly determine, what origin was the oak, we used. Also, in our case, 4-ethylphenol did not significantly increase the concentration. Differences were only slight, with the highest increase in wine with medium toasted American oak chips. These results could be due to fact, that we did not use oak barrels but only oak chips for short period.

According to **Díaz-Plaza *et al.* (2002),** furfural is one of the most abundant components after being submitted to aging due to its extraction from the wood of the barrels. This particular compound is created by toasting of oak wood during the manufacturing stage.

Furfural was also the most represented compound in our wine samples treated with oak chips. The highest concentration was measured in wine samples with French oak chips with high degree of toasting.

Frangipane *et al.* (2007) were dealing with differences between using oak barrels and oak chips in their research. They claimed, that wines treated with chips, can show different results as wines, where oak barrels were used for wine aging. It can be due to several variations in production of oak chips without any standardization.

According to our results, we can support this thesis, using comparison of our results with autors, who studied wine behaviour in oak barrels.

CONCLUSION

Under given conditions (amount of chips, storage temperature, contact time and wine properties), our results show, that the greatest differences were reached among chips samples with different toasting degree. Concentrations of furfural, guaiacol and both whiskeylactones were related with the degree of toasting, where furfural and guaiacol amounts increased with toasting intensity. On the other hand, concentrations of *cis*- and *trans*- whiskey lactone were lower in variants with heavy toasted chips.

We also noticed that wine sample alone had a notable influence on diffused volatile compounds. Differences between red and white wine samples were recognized. For example: concentration of *cis*- and *trans*- isoeugenol increased the most in white cuvee wine with almost all samples of toasted oak chips.

Geographic origin played not so significant role in our experiment, as we expected. We did not find any definite signs for its determination, as it was found by other authors.

In a view of overal assessment, we can submit, that the higher increase of desirable volatile aroma compounds was reached by French oak chips with high degree of toasting. On the other hand, usage of not-toasted German oak chips showed only low increase of analysed volatile compounds. It would be interesting to compare obtained chromatographic results with results of sensory analysis and thereby find out, how does chemical composition reflect into organoleptic properties.

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