

Antioxidant capacities of extracts in relation to toasting oak and acacia wood

PAVEL HÍC – IVO SOURAL – JOSEF BALÍK – JANA KULICHOVÁ – NADĚŽDA VRCHOTOVÁ – JAN TŘÍSKA

Summary

The paper reports on preparation and evaluation of extracts of toasted wood as a potential alternative for wine barrique technology. Various methods of heat treatment were monitored for two types of wood (oak and acacia). Heat treatment led to a weight loss of wood, which increased with increase in temperature of toasting; colour parameters of wood were also changing, with the inner sections of wood showing the same colour change in the value of lightness (L^*) as the surface, which pointed to even toasting throughout the wood volume. The weight of matter extractable by 80 % (v/v) ethanol was each time about 5 % of the total weight of the treated wood. The antioxidant capacity of the wood extracts obtained was dependent of the temperature of toasting. The antioxidant capacity (AC) measurement methods involved ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and total polyphenols (TP) by means of Folin-Ciocalteu reagent. For acacia wood, a steady decrease was recorded, for all the three antioxidant parameters, as the wood toasting temperature increased. All of the measured values (AC by both methods and TP) mutually correlated.

Keywords

oak; acacia; toasting wood; barrique extract; antioxidant capacity; weight loss

Storing and maturing wine in wooden barrels is a traditional method in wine technology. Substances that conventional wooden barrels release into the wine can increase the feeling of fullness and sweetness of the beverage [1]. Of the wines aging in barrels, barrique wines are now preferred, being sought after by a large group of consumers. These wines mature in barrels the inner part of which underwent toasting. As maturing occurs in barrels, various compounds are released from the toasted wood into the wine, causing several qualitative changes. These involve a change in taste related to the increased stability of the wine [2, 3]. There is also a change in colour, which is caused by the extraction of pigmented compounds and flavour-active substances, mainly phenols, which have antioxidant activity [4–7], and vanillin, vanillic acid, syringaldehyde, syringic acid, coniferaldehyde, sinapaldehyde, gallic acid, coumarins and others [8]. Reasons for paying attention to these

compounds often include the antioxidant properties [9, 10].

The process of making barrique barrels mainly employs wood of oak, acacia and chestnut [11]. The wood type has a large influence on the quantity and quality of substances extracted from barrique barrels or wood chips. Important are also other factors such as duration of contact with the wine, the extent of the contact area, temperature, alcohol content and pH [12, 13]. Critical parameters include the degree of wood toasting, which depends on the temperature and the duration of its action. For barrels, the degree of toasting wood can be light, medium or heavy. Various researchers have different opinions about the processes of toasting. According to CANAS et al. [14], traditional way, which is commonly done by coopers, lasts 10 min for light, 20 min for medium and 25 min for heavy toasting. DE SIMÓN et al. [15] used temperature 200 °C for 35 min for creating chips toasted at

Pavel Híc, Ivo Sural, Josef Balík, Jana Kulichová, Department of Post-Harvest Technology of Horticultural Products, Faculty of Horticulture, Mendel University in Brno, Valtická 337, 69144 Lednice, Czech Republic.

Naděžda Vrchotová, Jan Tříška, Laboratory of Metabolomics and Isotope Analyses, Global Change Research Centre, Academy of Sciences of the Czech Republic, v. v. i., Na Sádkách 7a, 37005 České Budějovice, Czech Republic.

Correspondence author:

Pavel Híc, e-mail: pavel.hic@mendelu.cz

medium level. ARAPITSAS et al. [16] enriched wines by adding different-sized chips, which were toasted at 200 °C for 2 h. Other processes of toasting are described by RIBEREAU-GAYON et al. [17] and HALE et al. [18].

The aim of this study was to compare the effects of different temperatures of toasting oak and acacia wood on colour, weight loss, the amount of extracted substances and the antioxidant capacity (AC) of the extracts.

MATERIALS AND METHODS

Oak and acacia wood

The experiment made use of samples containing wood of sessile oak (*Quercus petraea*) and acacia (*Robinia pseudoacacia*). The wood came from the Small Carpathian region in Slovakia. According to the annual rings, the oak wood originated from a 58-year-old oak tree, felled in January 2013, which was splinted and loose-dried in a dry chamber for 4 months. The 41-year-old acacia tree was felled in May 2013 and was used immediately. Heartwood (i.e. the inner portion) of tree trunk at a height of 0.5–2.0 m from the ground was used from both of the species.

The wood was splintered into blocks, each measuring 3 cm × 3 cm × 20 cm. Six blocks with a total mass approximately 1 kg were used for each sample. The monitored variants of heat treatment were as follows: wood with no heat treatment, wood dried for 5 h at 110 °C (to remove moisture content), wood dried in the same man-

ner as above and further toasted for 2 h at 150 °C, 175 °C, 200 °C and 225 °C (Tab. 1). Toasted wood samples are denoted as “O” for oak and “A” for acacia. At all times, heat treatment of wood was carried out in a laboratory drier in the presence of air (Model 100-800; Memmert, Schwabach, Germany) and with the flue gases being removed.

The temperature of drying and subsequent toasting was reached by steadily increasing the original laboratory room temperature over 2 h (constant values of temperature were held for 5 h for drying and 2 h for toasting). Re-cooling to the laboratory temperature was carried out by evenly decreasing the temperature for 1 h.

Weight loss due to toasting

Oak and acacia wood was monitored for weight loss resulting from different temperature of toasting (150 °C, 175 °C, 200 °C, and 225 °C), when the default comparing material involved wood dewatered by drying at 110 °C, until reaching a substantially constant weight (changes below 0.5 g from 1 kg of sample weight). The weight of dried and subsequently also toasted blocks of wood was approx. 1 kg. Both pre-toasting and post-toasting weighing was done using a laboratory balance with accuracy of 0.01 g (Model EW 2200-2NM; Kern & Sohn, Balingen, Germany).

Changes in colour due to toasting

The heat-treated wood blocks were measured for changes in colour along the surface and in the inner portions of the wood using a colorimeter (Lovibond RT850i-X-Rite; The Tintometer, Amesbury, United Kingdom). The wooden blocks were cleaved by an axe to obtain the inner layer. The colorimeter was used to measure the spatial coordinates of the CIELAB colour space, i.e. lightness (L^*) and colour-opponent dimensions (a^* and b^*).

Chemicals and solvents

For extraction, ethanol (absolute, grade for high-performance liquid chromatography; Fisher Scientific, Pittsburgh, Pennsylvania, USA) was used. For antioxidant measurement, the following chemicals were used: 2,2-diphenyl-1-picrylhydrazyl (DPPH) (97%, Sigma-Aldrich, St. Louis, Missouri, USA), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97%, Sigma-Aldrich), 2,4,6-tris(2-pyridyl)-s-triazine (99%, Sigma-Aldrich), hydrochloric acid (p.a.; Penta, Prague, Czech Republic), acetic acid (99.99%, Penta), iron trichloride (97%, Sigma-Aldrich), sodium acetate trihydrate (p.a., Penta). Folin-Ciocalteu reagent (Sigma-Aldrich), gallic acid monohydrate (for

Tab. 1. Treatment of wood.

Wood	Sample	Treatment conditions		WL [%]	PES [%]
		Drying	Toasting		
Oak	O1	–	–	na	4.7
	O2	110 °C, 5 h	–	0.0	5.0
	O3	110 °C, 5 h	150 °C, 2 h	2.8	4.1
	O4	110 °C, 5 h	175 °C, 2 h	4.5	5.5
	O5	110 °C, 5 h	200 °C, 2 h	8.5	6.2
	O6	110 °C, 5 h	225 °C, 2 h	16.6	5.3
Acacia	A1	–	–	na	6.9
	A2	110 °C, 5 h	–	0.0	6.5
	A3	110 °C, 5 h	150 °C, 2 h	3.3	6.3
	A4	110 °C, 5 h	175 °C, 2 h	6.1	5.9
	A5	110 °C, 5 h	200 °C, 2 h	9.4	5.4
	A6	110 °C, 5 h	225 °C, 2 h	16.3	4.1

WL – weight loss, PES – proportion of ethanol-soluble substances in mass of the toasted wood, na – not analysed.

HPLC, 99%, Sigma-Aldrich), sodium carbonate (p.a., Penta).

Ethanol extracts

Having undergone different heat treatment, wood blocks (size 3 cm × 3 cm × 20 cm) were chopped to splinters with a diameter of approximately 3 mm and a length of 20 mm. The splinters were crushed using a laboratory mill (Model MF 10 Basic; IKA, Staufen, Germany), sieve mesh size of 2.00 mm. The resulting pulp was made free of dust particles by sieving through a stainless steel sieve (Preciselekt ISO 3310; Preciselekt, Dolní Loučky, Czech Republic) with a mesh size of 0.500 mm. The obtained fraction measuring from 0.500 mm to 2.00 mm was sampled; the sample that weighed exactly 2.50 g was put into a 50 ml volumetric flask. 80% (v/v) ethanol was added into each flask (up to the mark, it means that volume of ethanol was slightly smaller than 50 ml) and placed into a shaker at harmonic oscillatory movement with amplitude 2.5 cm, frequency 200 Hz (LT1; Kavalier, Prague, Czech Republic) for 5 days; extraction took place during the period. After five days, the samples were allowed to settle for two days and then the clear fractions were removed for analysis. Until analysis, the obtained samples were stored in a dark place at 4 °C.

Antioxidant capacity

Determination by the ferric reducing antioxidant power (FRAP) method was done in a pH 3.6 acetate buffer (23 mmol·l⁻¹ sodium acetate trihydrate in solution of 34 mmol·l⁻¹ acetic acid). The reaction mixture contained 12 mmol·l⁻¹ FeCl₃ solution, 10 mmol·l⁻¹ 2,4,6-tris(2-pyridyl)-s-triazine in 40 mmol·l⁻¹ HCl solution, and buffer in a ratio of 1:1:10. A volume of 2 ml of the reaction mixture was mixed with 25 ml of a sample diluted with deionized water in a disposable plastic cuvette (10 mm lightpath) and the obtained solution was measured after 10 min at a wavelength of 593 nm using a spectrophotometer (Specord 50 Plus; Analytik Jena, Jena, Germany). Blank was prepared in the same way but 25 ml of deionized water was used instead of the diluted sample. The antioxidant capacity (*AC*) was calculated from the calibration curve of Trolox.

For the DPPH method, 1.9 ml of DPPH radical solution in methanol (0.1 mmol·l⁻¹) was mixed with 0.1 ml of a sample diluted with deionized water in a disposable plastic cuvette (10 mm lightpath). Absorbance at 515 nm was measured after 30 min using the spectrophotometer Specord 50 Plus. Blank was prepared in the same way but 0.1 ml of deionized water was used instead of 0.1 ml of the

diluted sample. *AC* was calculated from the calibration curve of Trolox.

AC measured by both methods was expressed as millimoles of Trolox per kilogram of wood mass (WM).

Total polyphenols

Total polyphenols (*TP*) were determined using the Folin-Ciocalteu reagent. In this method, 0.040 ml of extract was put into a 50 ml volumetric flask with approximately 20 ml of deionized water and mixed with 1 ml of the Folin-Ciocalteu reagent. The flask was shaken and then, after 3 min, 5 ml of 20% Na₂CO₃ was added to the mixture, shaken thoroughly again and the flask was filled with water. After 30 min of standing, the colour of mixture was measured in a cuvette (10 mm lightpath) at a wavelength of 700 nm using the Specord 50 Plus spectrophotometer (as opposed to the blank sample). *TP* were calculated from the calibration curve of gallic acid. Content of *TP* was expressed as grams of gallic acid per kilogram WM.

Mass fraction of ethanol-soluble substances

A volume of 10 ml of each extract was dried for 4 h at approximately 50 °C to remove the solvent (80% v/v ethanol). Subsequently, the samples were dried 24 h in a freeze-dryer and then weighed using an analytical balance (Model SBP32; Scaltec Instrument, Göttingen, Germany) with the accuracy of 0.0001 g. The weight values were converted to the proportion of ethanol-soluble substances (*PES*), which was expressed in percentage of weighed wood prior to extraction.

Statistical analysis

All ethanol extracts were measured three times. Averages, standard deviations and $p \leq 0.01$ or $p \leq 0.05$ (significant vs non-significant differences) were calculated. The data were analysed by two-way ANOVA, applying Tukey's multiple range test for making comparisons with Statistica Cz 12 (StatSoft, Tulsa, Oklahoma, USA) and MS Excel 2010 (Microsoft, Redmond, Washington, USA) software.

RESULTS AND DISCUSSION

Oak and acacia wood

Weight loss due to toasting

The results showed that increasing temperature of toasting caused the weight of the toasted wood to reduce. Toasting at a temperature of 225 °C decreased the weight of wood by more than 16% for both oak and acacia as opposed to the

sample that was only dried at 110 °C. This means that 16.3% or 16.6% of weight was changed to gases and aerosols, which were discharged via the exhaust. The resulting weight losses are indicated in Tab. 1. SHCHUPAKIVSKYY et al. [19] measured the change of oak wood density depending on different temperatures during processing. The temperature 200 °C decreased the wood density by approximately 7.8% and temperature 220 °C decreased the wood density by approximately 12.7%. This decrease corresponded with weight losses determined in this paper.

Changes in colour parameters due to toasting

The measured data implied that an increase the temperature of toasting caused detectable changes in each parameter of wood colour range. The greatest dependence was shown for lightness (L^*), which decreased as the temperature of toasting increased, i.e. toasting was causing the wood turning darker.

The colour (L^*) of the surface of oak wood changed from 62.14 for raw wood to 31.32 for the wood toasted at 225 °C. The decrease in L^* was dependent on the toasting temperature (Fig. 1A). In their study, BARCÍK et al. [20] measured colour changes of thermo-treated wood at 210 °C and found the value $L^* = 64.46$ for raw wood to decrease to $L^* = 42.9$. The reported changes of colour parameters correlate with values in this paper.

A similar tendency was seen in acacia wood, where there was an even stronger decrease in the surface lightness from $L^* = 67.42$ for raw acacia to $L^* = 27.13$ for the acacia wood toasted at 225 °C. The change in lightness was uniform through-

out the volume of the treated wood, with mostly no statistically significant differences detected between the surface and internal portions, except for toasting temperatures of 110 °C, 150 °C and 175 °C where inner sections of wood were found to be statistically darker than the surface portions (Fig. 1B). There is colloid water in the inner parts of wood, which can be the reason of the darker hue of the inner parts of toasted wood. The water, which is not evaporated under lower temperature of toasting, can cause the thermal hydrolysis of hemicellulose and thus darker hue of the inner part of wood. SEHLSTEDT [21] also observed the influence of present water on the increased colour change of wood treated with heat, due to hydrolysis of hemicellulose.

However, the fact described above involved a slight variation of lightness. Within various modifications of thermal treatment, the deviations were much larger. Importantly, the two-hour period of toasting of each variant ensured that an even change occurred throughout the wooden mass, not just on the surface.

Colour parameter a^* (changes in the redness) showed no correlation with temperature (Fig. 2), but there was some variation, having an average value 7.16 and ranging from the maximum of $a^* = 10.92$ to the minimum of $a^* = 4.00$. In this case, statistically significant differences were determined between the heat treatment variants, however, with no trend of dependency on the temperature of toasting.

The decrease in colour parameter b^* (changes in yellowness) had a similar trend (Fig. 3). Values of b^* decreased with the increase in toasting temperature, while the inner portions of wood showed

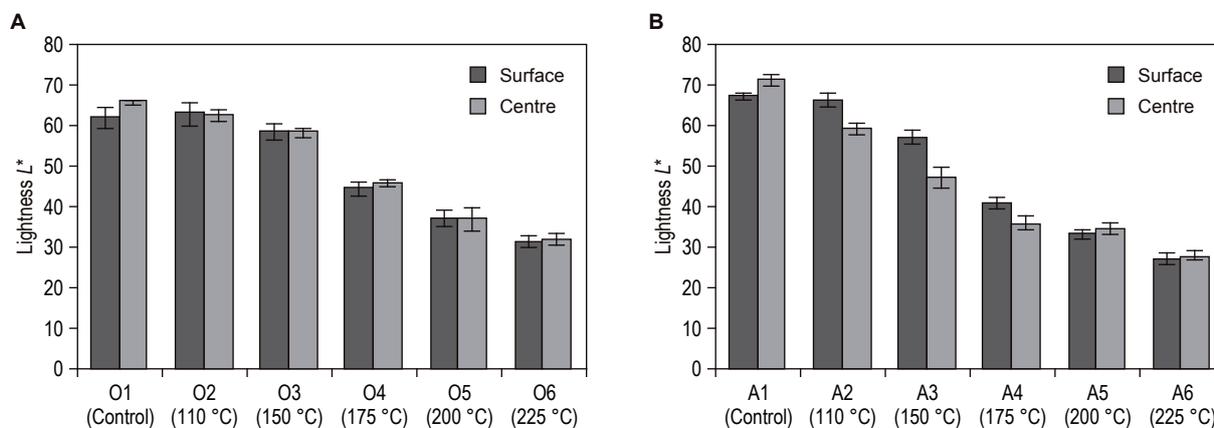


Fig. 1. Dependence of lightness of wood on toasting temperature.

A – oak wood, B – acacia wood.

O1–O6, A1–A6 – designation of samples, description given in Tab. 1.

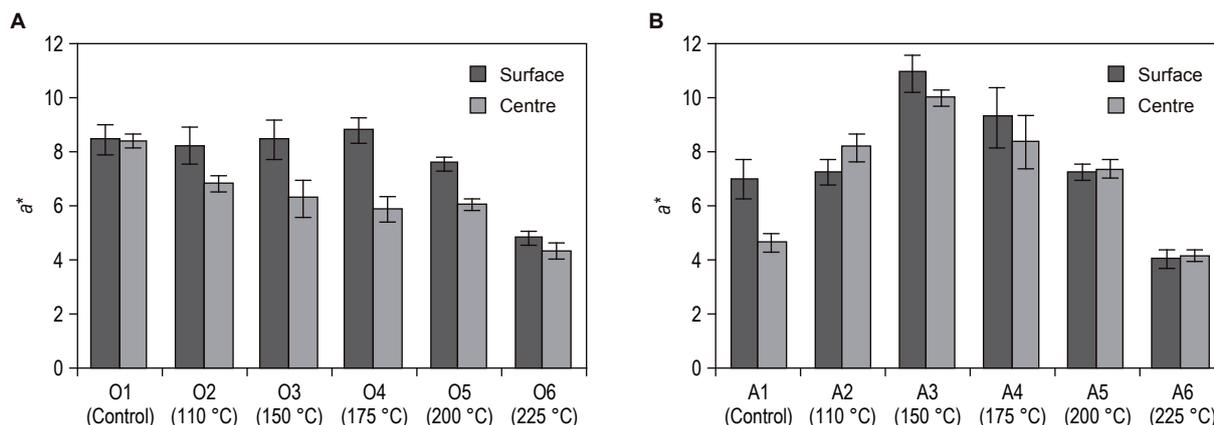


Fig. 2. Dependence of colour parameter a^* of wood on toasting temperature.

A – oak wood, B – acacia wood.

O1–O6, A1–A6 – designation of samples, description given in Tab. 1.

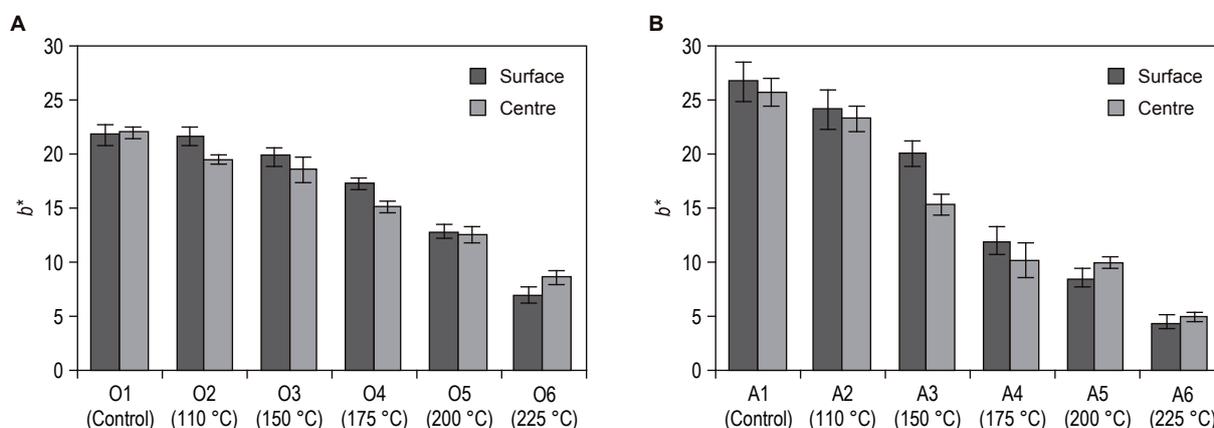


Fig. 3. Dependence of colour parameter b^* of wood on toasting temperature.

A – oak wood, B – acacia wood.

O1–O6, A1–A6 – designation of samples, description given in Tab. 1.

a greater decrease in b^* than its surface layers, although the reduction was not statistically significant.

Ethanol extracts

Antioxidant capacity

The oak wood was not found to show any trend in changes of AC due to temperature, as measured by the FRAP and DPPH methods (Fig. 4A). However, statistically significant differences were found between certain options of treatment (Tab. 2). The highest value of AC found by both methods occurred for the treatment at 175 °C: 267 mmol·kg⁻¹ WM by FRAP method and 236 mmol·kg⁻¹ WM by DPPH method. The lowest value was found for the treatment at 150 °C: 167 mmol·kg⁻¹ WM by FRAP method and 144 mmol·kg⁻¹ WM by DPPH method. CANAS et al.

[14] compared antioxidant capacities of alcohols after maceration in oak wood barrels obtained at different toasting temperatures, as percentage of discoloration of DPPH solution. Unfortunately, values were not expressed using any standards, thus their results cannot be directly compared with ours. However, results of those authors are similar to ours in the fact that they did not find any upward or downward trend of AC depending on the temperature of toasting.

A statistically significant influence ($p \leq 0.01$) of toasting temperature on values of AC of acacia wood was observed. Increasing the temperature of wood treatment resulted in an even decrease of AC (Fig. 4B). The greatest determined AC (435.1 mmol·kg⁻¹ WM by DPPH method) was determined for a sample without heat treatment. Gradually, the values decreased to 123.6 mmol·kg⁻¹ WM by DPPH method, which was

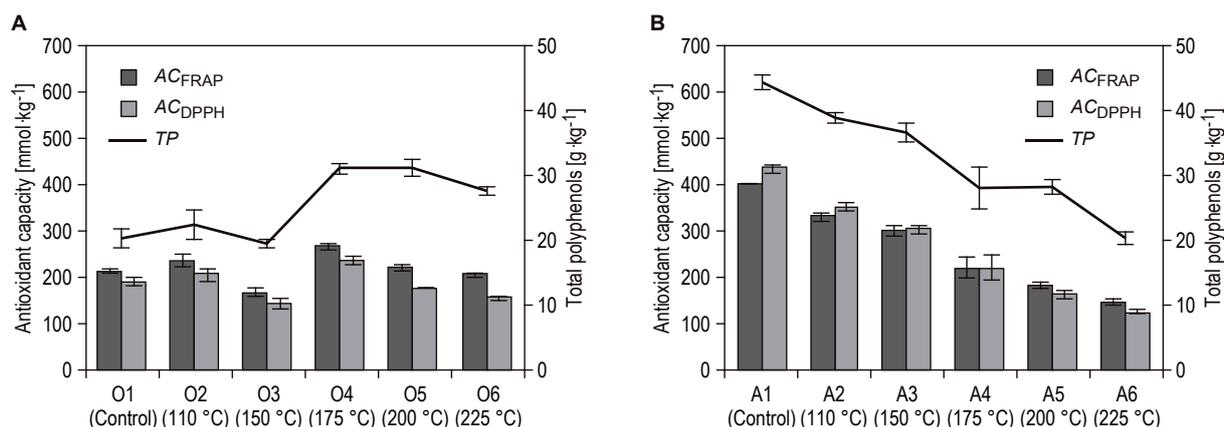


Fig. 4. Dependence of antioxidant capacity and total polyphenols of wood extracts on toasting temperature.

A – oak wood, B – acacia wood.

O1–O6, A1–A6 – designation of samples, description given in Tab. 1.

AC_{FRAP} – antioxidant capacity measured by ferric reducing antioxidant power method, AC_{DPPH} – antioxidant capacity measured by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. Antioxidant capacity is expressed in millimoles of Trolox per kilogram of wood mass.

TP – total polyphenols expressed as grams of gallic acid per kilogram of wood mass.

Tab. 2. Statistical evaluation of the influence of toasting temperature on antioxidant capacity and total polyphenols of the oak extract, and lightness of oak wooden blocks.

		O1	O2 (110 °C)	O3 (150 °C)	O4 (175 °C)	O5 (200 °C)	O6 (225 °C)	Values							
		Lightness L*													
		S	C	S	C	S	C	S	C	S	C	S	C	S	C
O1	AC _{FRAP}														
	AC _{DPPH}		ns	*	**	**	**	**	**	**	**	**	**	62.1	65.8
	TP														
O2 (110 °C)	AC _{FRAP}	*													
	AC _{DPPH}	ns		**	**	**	**	**	**	**	**	**	62.9	62.8	
	TP	**													
O3 (150 °C)	AC _{FRAP}	**	**												
	AC _{DPPH}	**	**		**	**	**	**	**	**	**	**	58.6	58.3	
	TP	ns	**												
O4 (175 °C)	AC _{FRAP}	**	**	**											
	AC _{DPPH}	**	**	**	**	**	**	**	**	**	**	**	44.5	45.7	
	TP	**	**	**											
O5 (200 °C)	AC _{FRAP}	ns	ns	**	**										
	AC _{DPPH}	ns	**	**	**	**	**	**	**	**	**	**	37.3	37	
	TP	**	**	**	**	ns									
O6 (225 °C)	AC _{FRAP}	ns	**	**	**	**	**	ns							
	AC _{DPPH}	**	**	ns	**	**	*						31.3	31.9	
	TP	**	**	**	**	**	**	**							
Values	AC _{FRAP} [mmol·kg ⁻¹]	213.1	236.1	167.3	266.6	220.3	206.2								
	AC _{DPPH} [mmol·kg ⁻¹]	190.8	205.9	143.5	235.6	175.8	155.6								
	TP [g·kg ⁻¹]	20.3	22.3	19.5	31	31.2	27.7								

Tukey's honest significance test for different toasting temperatures of oak wood (values are means calculated from three measurements): ** – $p \leq 0.01$, * – $p \leq 0.05$, ns – not significant.

O1–O6 – designation of samples, description given in Tab. 1.

AC_{FRAP} – antioxidant capacity obtained by ferric reducing antioxidant power method, AC_{DPPH} – antioxidant capacity obtained by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. Values of antioxidant capacities are expressed in millimoles of Trolox per kilogram of wood mass.

TP – total polyphenols. Values of total polyphenols are expressed as grams of gallic acid per kilogram of wood mass.

S – lightness of wood on surface of blocks, C – lightness of wood in centre of blocks.

by as much as 71.6 % off the original level. A similar downward trend was observed in *AC* when applying the FRAP method, where there was almost always a highly significant difference ($p \leq 0.1$) between the thermal variations. Only a significant difference ($p \leq 0.05$) was observed between the samples treated at 175 °C and 200 °C. A statistically insignificant reduction was found between the sample treated at 110 °C and that treated at 150 °C (Tab. 3). The decreasing trend of *AC* and *TP* content in dependence on increasing temperature of processing is shown in Fig. 4B.

The average value of *AC* in oak measured by DPPH method was 181 mmol·kg⁻¹ WM, the fluctuation (variance) accounting for 14.7 % of this value. This was less than the fluctuation of values for acacia, where the variance was up to 52.4 % of the average value *AC* (267 mmol·kg⁻¹ WM). *AC* values for thermally untreated acacia were approximately twice that of oak for the same antioxidant methods

(FRAP, DPPH). For temperatures above 175 °C, both of the determined values of *AC* were higher for oak than for acacia.

The results of *AC* in both of the methods (FRAP vs DPPH) correlated very well, with the correlation coefficient of 0.9790. This indicated that extracts with higher *AC* (for the DPPH method) had not only a high ability to inactivate free radicals but also greater reduction properties (FRAP).

Total polyphenols

The determined values of *TP* for oak were statistically significantly influenced by the temperature of toasting. Yet, there was no statistically significant change at toasting temperatures below 150 °C. At 175 °C, there was an increase in the value of *TP*, which did not change statistically significantly even at 200 °C. Increase in toasting temperature to 225 °C caused a statistically sig-

Tab. 3. Statistical evaluation of the influence of toasting temperature on antioxidant capacity and total polyphenols of the acacia extract, and lightness of acacia wooden blocks.

		A1	A2 (110 °C)	A3 (150 °C)	A4 (175 °C)	A5 (200 °C)	A6 (225 °C)	Values							
		Lightness L*													
		S	C	S	C	S	C	S	C	S	C	S	C	S	C
A1	AC _{FRAP} AC _{DPPH} TP			ns	**	**	**	**	**	**	**	**	**	67.4	71.3
A2 (110 °C)	AC _{FRAP} AC _{DPPH} TP	**			**	**	**	**	**	**	**	**	**	66.5	59.4
A3 (150 °C)	AC _{FRAP} AC _{DPPH} TP	**	ns				**	**	**	**	**	**	**	57.3	47.3
A4 (175 °C)	AC _{FRAP} AC _{DPPH} TP	**	**	**	**	**		**	ns	**	**	**	**	41.1	36.1
A5 (200 °C)	AC _{FRAP} AC _{DPPH} TP	**	**	**	**	*	**	ns		**	**	**	**	33.3	34.8
A6 (225 °C)	AC _{FRAP} AC _{DPPH} TP	**	**	**	**	**	**	**	*	**	**	**	**	27.1	27.8
Values	AC _{FRAP} [mmol·kg ⁻¹] AC _{DPPH} [mmol·kg ⁻¹] TP [g·kg ⁻¹]	399.7	329.6	299.2	219.8	181.3	144.9								

Tukey's honest significance test for different toasting temperatures of oak wood (values are means calculated from three measurements): ** – $p \leq 0.01$, * – $p \leq 0.05$, ns – not significant.

A1–A6 – designation of samples, description given in Tab. 1.

AC_{FRAP} – antioxidant capacity obtained by ferric reducing antioxidant power method, AC_{DPPH} – antioxidant capacity obtained by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. Values of antioxidant capacities are expressed in millimoles of Trolox per kilogram of wood mass.

TP – total polyphenols. Values of total polyphenols are expressed as grams of gallic acid per kilogram of wood mass.

S – lightness of wood on surface of blocks, C – lightness of wood in centre of blocks.

nificant decrease in *TP* compared to 200 °C, which was, however, still higher than 150 °C (Fig. 4A). For acacia wood, the levels of *TP* were statistically significantly influenced by the temperature of toasting, except for a few adjacent values the overall trend of which was similar to that seen for antioxidants, i.e. *TP* decreased with an increase in temperature of toasting (Fig. 4B).

The determined values of *TP* correlated with the total *AC* determined by both of the methods (FRAP, DPPH). Correlation coefficients were 0.8801 for DPPH method and 0.9011 for FRAP method. This suggests that most of the antioxidants in the wood is of a phenolic character. ŠERUGA et al. [22] assumed that phenolic compounds are the main source of antioxidants in wine. Studies of ORTEGA-HERAS et al. [23] and BARRIO-GALÁN et al. [24] confirmed that phenolic compounds are released from wood barrels into wine. GORTZI et al. [25] reached similar conclusions. The latter authors supposed that antioxidant effects are caused by the phenolic components extracted from wood chips. In contrast, GÓMEZ et al. [26] did not observe significant influence of added oak chips into wine on the amount of *TP*. Possible explanation of this fact is that although the extract from oak chips has a high content of polyphenols, the amount of polyphenols released from oak chips into wine is very low.

Mass fraction of ethanol-soluble substances

The determined values demonstrate that the proportion of ethanol-soluble substances (*PES*) extractable from wood did not change significantly with temperature. Their range was 4.1 % to 6.9 %, while the average value of the two types of wood was 5.5 % for a single-stage extraction process (Tab. 1). It is worth comparing *AC* of the product obtained by drying of the sourced extract. It appeared that its *AC* was comparable with pure Trolox (103.5 % ± 19.2 %). It was therefore a strong antioxidant, which can be assumed to act strongly in wine.

CONCLUSIONS

The determined changes in the colour of the toasted wood showed a uniform toasting throughout the section of the wood. In the process of toasting, the loss of weight of the dry wood under treatment was related to the temperature. The largest decrease was determined at the highest temperature (225 °C) and corresponded to about 16 % of the original weight of both types of wood. For toasted wood, the aver-

age weight of substances extracted by 80% (v/v) ethanol was 51 g·kg⁻¹ ± 7 g·kg⁻¹ for oak wood and 59 g·kg⁻¹ ± 10 g·kg⁻¹ for acacia wood. *AC* of ethanol extracts had, when re-calculated to dry mass, very high levels, which were comparable with *AC* of pure Trolox (103.5 % ± 19.2 %). The results obtained by FRAP and DPPH methods showed that one kilogram of toasted wood could provide *AC* equivalent to about 50 g of pure Trolox. The ethanol extracts obtained can thus be used as a good source of antioxidants.

Acknowledgements

Financial support for this research was provided by project IGA-ZF/2015-AP008 of Mendel University, Brno, Czech Republic. The authors would like to thank Petra Pavelková for sample preparation.

REFERENCES

- Vidal, S. – Francis, L. – Williams, P. – Kwiatkowski, M. – Gawel, R. – Cheynier, V. – Waters, E. J.: The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry*, 85, 2004, pp. 519–525. DOI: 10.1016/S0308-8146(03)00084-0.
- Atanasova, V. – Fulcrand, H. – Cheynier, V. – Moutounet, M.: Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta*, 458, 2002, pp. 15–27. DOI: 10.1016/S0003-2670(01)01617-8.
- Fulcrand, H. – Benabdejalil, C. – Rigaud, J. – Cheynier, V. – Moutounet, M.: A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry*, 47, 1998, pp. 1401–1407. DOI: 10.1016/S0031-9422(97)00772-3.
- Sartini, E. – Arfelli, G. – Fabiani, A. – Piva, A.: Influence of chips, lees and micro-oxygenation during aging on the phenolic composition of a red Sangiovese wine. *Food Chemistry*, 104, 2007, pp. 1599–1604. DOI: 10.1016/j.foodchem.2007.03.010.
- McCord, J.: Application of toasted oak and micro-oxygenation to ageing of Cabernet Sauvignon wines. *Australian and New Zealand Grapegrower Winemaker*, 474, 2003, pp. 43–53. ISSN: 0727-3606.
- Kennison, K. R. – Gibberd, M. R. – Pollnitz, A.P. – Wilkinson, K. L.: Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. *Journal of Agricultural and Food Chemistry*, 56, 2008, pp. 7379–7383. DOI: 10.1021/jf800927e.
- Spillman, P. J. – Iland, P. G. – Sefton, M. A.: Accumulation of volatile oak compounds in a model wine stored in American and Limousin oak barrels. *Australian Journal of Grape and Wine Research*, 4, 1998, pp. 67–73. DOI: 10.1111/j.1755-0238.1998.tb00136.x.
- Monedero, L. – Olalla, M. – Villalón, M. – López-García, H. – López, M.: Standardisation of the chro-

- matic characteristics of *sobretablas* wine macerates obtained by an accelerated ageing technique using heating and oak shavings. *Food Chemistry*, *69*, 2000, pp. 47–54. DOI: 10.1016/S0308-8146(99)00235-6.
9. Hollman, P. C. H.: Evidence for health benefits of plant phenolics: local or systemic effects? *Journal of the Science of Food and Agriculture*, *81*, 2001, pp. 842–849. DOI: 10.1002/jsfa.900.
 10. Shrikhande, A. J.: Wine by-products with health benefits. *Food Research International*, *33*, 2000, pp. 469–474. DOI: 10.1016/S0963-9969(00)00071-5.
 11. De Rosso, M. – Panighel, A. – Dalla Vedova, A. – Stella, L. – Flamini, R.: Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels. *Journal of Agricultural and Food Chemistry*, *57*, 2009, pp. 1915–1920. DOI: 10.1021/jf803161r.
 12. Cadahía, E. – Fernández-de-Simón, B. – Jalocho, J.: Volatile compounds in Spanish, French, and American oak woods after natural seasoning and toasting. *Journal of Agricultural and Food Chemistry*, *51*, 2003, pp. 5923–5932. DOI: 10.1021/jf0302456.
 13. Doussot, F. – De Jéso, B. – Quideau, S. – Pardon, P.: Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographical location and single tree-effects. *Journal of Agricultural and Food Chemistry*, *50*, 2002, pp. 5955–5961. DOI: 10.1021/jf020494e.
 14. Canas, S. – Casanova, V. – Belchior, P.: Antioxidant activity and phenolic content of Portuguese wine aged brandies. *Journal of Food Composition and Analysis*, *21*, 2008, pp. 626–633. DOI: 10.1016/j.jfca.2008.07.001.
 15. De Simón, F. – Sanz, M. – Cadahía, E. – Martínez, J. – Esteruelas, E. – Muñoz, A. M.: Polyphenolic compounds as chemical markers of wine aging in contact with cherry, chestnut, false acacia, ash and oak wood. *Food Chemistry*, *143*, 2014, pp. 66–76. DOI: 10.1016/j.foodchem.2013.07.096.
 16. Arapitsas, P. – Antonopoulos, A. – Stefanou, E. – Dourtoglou, V. G.: Artificial aging of wines using oak chips. *Food Chemistry*, *86*, 2004, pp. 563–570. DOI: 10.1016/j.foodchem.2003.10.003.
 17. Ribereau-Gayon, P. – Glories, Y. – Maujean, A. – Dubourdieu, D.: *Traité d'oenologie II – Chimie du vin. Stabilisation et traitements (Treaty of enology II – Chemistry of wine. Stabilization and treatments.)* Paris : DUNOD, 2012. ISBN: 978-2100582365. In French.
 18. Hale, D. – McCafferty, K. – Larmie, E. – Newton, J. – Swan, J.: The influence of oak seasoning and toasting parameters on the composition and quality of wine. *American Journal of Enology and Viticulture*, *50*, 1999, pp. 495–502. ISSN: 0002-9254.
 19. Shchupakivskyy, R. – Clauder, L. – Linke, N. – Pfriem, A.: Application of high-frequency densitometry to detect changes in early- and latewood density of oak (*Quercus robur* L.) due to thermal modification. *European Journal of Wood and Wood Products*, *72*, 2014, pp. 5–10. DOI: 10.1007/s00107-013-0744-x.
 20. Barčík, Š. – Gašparík, M. – Razumov, Y. E.: Effect of temperature on the color changes of wood during thermal modification. *Cellulose Chemistry and Technology*, *49*, 2015, pp. 789–798. ISSN 2457-9459 (Online), ISSN: 0576-9787 (Print). <[http://www.cellulosechemtechnol.ro/pdf/CCT9-10\(2015\)/p.789-798.pdf](http://www.cellulosechemtechnol.ro/pdf/CCT9-10(2015)/p.789-798.pdf)>
 21. Sehlstedt-Persson, M.: Colour responses to heat treatment of extractives and sap from pine and spruce. In: Ispas, M. - Campean, M. - Cismaru, I. - Marinescu, I. - Budau, G. (Eds): *Proceedings of the 8th International IUFRO Wood Drying Conference - Brasov, Romania; 24-29 August 2003 "Improvement and innovation in wood drying - A major issue for a renewable material". Brasov : "Transilvania" University of Brasov, 2003, pp 459–464. ISBN: 973-635-198-X. <<https://www.diva-portal.org/smash/get/diva2:1009196/FULLTEXT01.pdf>>*
 22. Šeruga, M. – Novak, I. – Jakobek, L.: Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry: HPLC and spectrophotometric methods. *Food Chemistry*, *124*, 2011, pp. 1208–1216. DOI: 10.1016/j.foodchem.2010.07.047.
 23. Ortega-Heras, M. – González-Huerta, C. – Herrera, P. – González-Sanjosé, M. L.: Changes in wine volatile compounds of varietal wines during ageing in wood barrels. *Analytica Chimica Acta*, *513*, 2004, pp. 341–350. DOI: 10.1016/j.aca.2003.10.051.
 24. Barrio-Galán, R. D. – Pérez-Magarino, S. – Ortega-Heras, M.: Techniques for improving or replacing ageing on lees of oak aged red wines: The effects on polysaccharides and the phenolic composition. *Food Chemistry*, *127*, 2011, pp. 528–540. DOI: 10.1016/j.foodchem.2011.01.035.
 25. Gortzi, O. – Metaxa, X. – Mantanis, G. – Lalas, S.: Effect of artificial ageing using different wood chips on the antioxidant activity, resveratrol and catechin concentration, sensory properties and colour of two Greek red wines. *Food Chemistry*, *141*, 2013, pp. 2887–2895. DOI: 10.1016/j.foodchem.2013.05.051.
 26. Gómez, G. – Sánchez-Palomo, E. – Hermosín-Gutiérrez, I. – González Viñas, M. A.: Effect of oak chip addition at different winemaking stages on phenolic composition of Moravia Agría red wines. *South African Journal of Enology and Viticulture*, *36*, 2015, pp. 21–31. ISSN: 0253-939X.

Received 4 August 2016; 1st revised 6 February 2017; accepted 8 March 2017; published online 4 May 2017.