Effect of enzymatic treatment on phytochemical compounds and volatile content of mulberry (*Morus nigra*) must by multivariate analysis

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Summary

The objective of this work was to investigate the effect of enzymatic treatment on the phytochemical properties and volatile composition of mulberry must. A correlation was found between browning index, total phenolics, total flavonoids and total anthocyanins as $r = 0.731$, $r = -0.785$ and $r = 0.561$, respectively. Hue correlated ($r = 0.76$) with the browning index. The highest polyphenol concentration (2.768 $g \cdot l^{-1}$) and total volatile concentration (620.86 $\mu g \cdot l^{-1}$) were found after Pectinex UF (Novozymes, Bagsvaerd, Denmark) treatment. The must treated with Klerezyme 150 (DSM, Heerlen, Netherlands) had the highest flavonoid and anthocyanin concentration (5.652 $g \cdot l^{-1}$ and 0.559 $g \cdot l^{-1}$, respectively), but it has the lowest total volatile concentration (305.99 $\mu g \cdot l^{-1}$). Instead, the major aroma-active compounds were esters (36.3%). Multivariate analysis revealed that the aroma profile was mainly affected by the enzymatic treatment, more than the phytochemical compounds and chromatic properties.

Keywords

mulberry; enzyme; polyphenol; aroma compound; colour

Mulberry is a native fruit of Asia and is mainly produced in China. Mulberry fruits are very rich in polysaccharides and phytochemical compounds such as phenolics, flavonoids and anthocyanins [1]. The fresh fruits are not available all year round and they are generally processed into jam, pulp, beverage and wine [2].

Currently, wine consumers are more concerned about the quality of products, they buy and demand products that are rich in natural bioactive compounds [3], have a specific aroma and colour, which are important parameters for wine [4]. Maceration process is one of the key steps during juice and wine processing, and it can affect the final quality of the product. During the process, enzymes rich in polygalacturonase, pectin esterase, cellulase and hemicellulase are commonly used in juice processing and winemaking during the maceration process [5]. These maceration enzymes could improve the yield of juice, phytochemical compounds and colour extracted [6]. Moreover, they could release more volatile aroma compounds such as alcohols, esters and aldehydes according to their glycosidase activity [7]. However, some studies reported a negative effect of some maceration enzymes on phytochemical compounds and the content of volatiles [8, 9]. Maceration enzymes can hydrolyse phytochemical compounds, change their profile and affect the quality of the product [10, 11]. Hence, the determination of quality parameters of the must, which are mostly affected by maceration enzymes, is imperative.

The aim of this study, therefore, was to investigate the effect of enzymatic treatment on phytochemicals (total phenolics, total flavonoids and total anthocyanins) and on volatile compounds of mulberry must. In addition, chromatic properties were also considered.

MATERIALS AND METHODS

Chemicals

The standard of *n*-alkanes (C5–C25) was obtained from Anpel Scientific Instrument (Shang-

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hai, China). 3-Octanol was purchased from Tokyo Chemical Industry (Tokyo, Japan). Gallic acid and rutin were purchased from J&K Scientific (Beijing, China), the purity was 99%. Folin–Ciocalteu phenol reagent was obtained from Shanghai Labaide Biotechnology (Shanghai, China). All other chemicals were of reagent grade and were purchased from Sinopharm Chemical Reagent (Beijing, China).

Plant material

The material used in this study was ripe mulberry (*Morus nigra* var. *Zhen Jiang mulberry* No. 1) harvested in Zhenjiang farms (China). The fruits were washed and stored at –20 °C until the experiments.

Maceration and enzyme treatment

Frozen mulberry (2.5 kg) was thawed for 8 h at 4 °C before mashing for 45 s using a household blender. Ascorbic acid (0.5 g·kg^{-1}) was added immediately after mashing to inhibit oxidative browning. Must was divided into 8 lots (300 g each) and treated separately with different maceration enzymes: Lafase Fruit (LF, Laffort, Bordeaux, France), Lafase HE GC (LHG, Laffort) and Novarom Blanc (NB, Novozymes, Bagsvaerd, Denmark) at the average dosage recommended by the manufacturers in a range of 30–1000 g per kilogram of must; and Pectinex USP-L (PUL, Novozymes), Pectinex UF (PUF, Novozymes), Pectinex Ultra Colour (PUC, Novozymes) and Klerezyme 150 (K150, DSM, Heerlen, the Netherlands) at the average dosage of 0.01–0.03 l per kilogram of must. Control was not added any enzyme. Treated samples were incubated in an orbital shaker (IS-RDD3, Crystal Technology and Industries, Jiangsu, China) at 20 °C for 1 h with continuous shaking (3 Hz). After incubation, musts were centrifuged (Anke GL-20B, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4224 × *g* for 15 min at 4 °C. The supernatant was filtered through a cheesecloth and stored at –20 °C until analysis.

Colour measurement

The colour parameters were measured by a ColourQuest XE (Hunter lab, Reston, Virginia, USA), with illuminant D65 and 10° observer angle in the total transmission mode. The colorimeter was calibrated using a white standard tile ($L^* = 99.1, a^* = -0.17, b^* = -0.07$). The CIELAB parameters chroma (C^*) and the hue angle ($H°$) were calculated from CIELAB a^* and b^* coordinates using the following formulas:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$H° = \arctan(b^*/a^*) \quad (2)$$

where a^* is redness/greenness and b^* is yellowness/blueness

Browning index

Browning index (*BI*) was measured using the method of Meydav et al. [12] and calculated according to Buglione and Lozano [13] as follows:

$$BI = A_{520}/A_{420} \quad (3)$$

where A_{520} is absorbance at 520 nm, and A_{420} is absorbance at 420 nm.

Total phenolics concentration

Total phenolics concentration (*TPC*) was determined by the Folin-Ciocalteu method [14] using gallic acid as a standard. *TPC* was expressed as grams of gallic acid per litre.

Total flavonoids concentration

Total flavonoids concentration (*TFC*) was measured by the aluminium chloride colorimetric assay [15] using rutin as a standard. *TFC* was expressed as grams of rutin per litre.

Total anthocyanins concentration

Total anthocyanins concentration (*TAC*) was determined by the pH differential method and calculated as cyanindin-3-glucoside [16]. *TAC* was expressed as grams per litre.

Volatile extraction

Volatile compounds were extracted with a headspace solid-phase microextraction method as described by Butkhup et al. [2]. The 2 cm long, 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) stable Flex fibre (Supelco, Bellefonte, Pennsylvania, USA) was used for the extraction. A 15 ml vial containing 5 ml of sample, spiked with 50 μl of 3-octanol (800 μg.l^{-1}), was used as the internal standard. NaCl (1.5 g) was added to increase the volatility of flavour compounds. The vials were tightly closed with a silicone septum and equilibrated at 40 °C for 10 min with constant stirring (12.5 Hz). The fibre was then extended through the needle and exposed to the headspace above the sample for 30 min. After extraction, the fibre was inserted into the injection port of the gas chromatograph (250 °C) for 5 min to desorb the analytes.

Gas chromatography-mass spectrometry (GC-MS)

Samples were analysed using an Agilent 6890N- 5973B GC-MS instrument (Agilent, Wilmington, North Carolina, USA), equipped

with a capillary column Agilent J&W DB-WAX 122-7062 (60 m, 0.25 mm, 25 μm). Carrier gas was helium (1 ml·min^{-1}), ion source temperature was set at 230 °C, quadrupole at 150 °C. The oven temperature was held at 50 °C for 10 min. It was raised at 6 °C·min^{-1} to 150 °C, followed by an increase of temperature to 200 °C at a rate of 8 °C·min^{-1}. Then the temperature was held at 200 °C for 7 min. Injector port temperature was 250 °C. Injection mode was splittless. Energy ionization was 70 eV. An electron ionization mass spectrum was acquired over the m/z range from 33 to 350, and the detector transfer line was 250 °C. The total run time was 39.92 min. Inlet pressure was 137895.145 Pa with an average velocity of 19 cm·s^{-1}.

Compounds identification and semi-quantification

Identification of compounds was done by comparing: (i) mass spectra of the samples with databases (NIST 2005 v. 2.0 libraries) using AMDIS 3.2 software (NIST, Gaithersburg, Maryland, USA), and (ii) retention indices of *n*-alkanes to those of the literature [17–33, 9, 34–63].

The retention indices were calculated as described by Bianchi et al. [64] using a mixture of *n*-alkanes (C_5–C_{25}) under the chromatographic conditions described previously. Semi-quantification of the compounds was carried out by the internal standard method.

Statistical analysis

All the analyses were run in triplicates, and the results are presented as means ± standard deviation. Tukey test ($p < 0.05$) was used to compare the means and correlation between chromatic properties, and phytochemical compounds were studied by means of Pearson correlation coefficients using OriginPro version 9.0 (OriginLab, Northampton, Massachusetts, USA). Multivariate analysis was performed with Statistica version 10.0 (StatSoft, Tulsa, Oklahoma, USA). Cluster analysis was used to determine the similarities between mulberry must treated by different enzymes, while principal component analysis (PCA) was used to explain the variances observed in the overall properties of mulberry musts and to understand the relationship between the different parameters.

RESULTS AND DISCUSSION

Chromatic properties

Lightness (*L**)

Individual enzyme treatments were found to have none significant ($p > 0.05$) effect on L^*, except for PUL, which had the highest value, as shown in Tab. 1. The slight differences between the samples may be due to oxidation rather than the enzymatic treatment as reported by Oszmiański et al. [8, 65]. According to Mihalev et al. [66], a large amount of ascorbic acid is required to inhibit polyphenol oxidase responsible for the oxidation, which was not the case in this study.

Hue angle (*H*°)

The $H°$ angle of the control was significantly different ($p < 0.05$) from enzyme-treated samples apart from PUF and LF, as shown in Tab. 1. Almost all treated samples had $H°$ values significantly higher than the control, except for K150 and PUC. Based on Pearson correlation coefficients analysis (Tab. 2), $H°$ was significantly correlated to b^* ($r = 0.76$). It could be attributed to the enzymatic hydrolysis of the fruit pigment as described by Sun et al. [67].

Chroma (*C**)

The C^* values for treated samples were significantly different from the control. The C^* value of

Tab. 1. Chromatic properties of mulberry musts.

Enzymes	*L**	*a**	*b**	*C**	*H*°	*BI*
Control	2.42 ± 0.01^{e}	16.35 ± 0.05^{a}	3.57 ± 0.03^{ac}	16.74 ± 0.05^{b}	12.33 ± 0.08^{ac}	1.49 ± 0.00^{a}
Lafase fruit	2.52 ± 0.02^{abf}	16.89 ± 0.05^{b}	3.78 ± 0.01^{b}	17.31 ± 0.04^{a}	12.61 ± 0.06^{ab}	1.12 ± 0.01^{d}
Lafase HE GC	2.50 ± 0.04^{acg}	16.56 ± 0.10^{c}	3.93 ± 0.03^{e}	17.02 ± 0.09^{c}	13.34 ± 0.16^{d}	1.31 ± 0.01^{e}
Pectinex USP-L	2.29 ± 0.01^{d}	15.49 ± 0.01^{d}	3.53 ± 0.04^{ad}	15.88 ± 0.01^{d}	12.85 ± 0.15^{b}	1.46 ± 0.00^{b}
Pectinex UF	2.65 ± 0.02^{h}	17.61 ± 0.01^{e}	3.82 ± 0.03^{b}	18.02 ± 0.02^{e}	12.25 ± 0.09^{c}	1.48 ± 0.01^{ab}
Pectinex Ultra Colour	2.54 ± 0.03^{bc}	17.05 ± 0.03^{f}	3.33 ± 0.03^{f}	17.37 ± 0.02^{a}	11.06 ± 0.11^{e}	1.38 ± 0.01^{c}
Novarom Blanc	2.33 ± 0.01^{d}	15.31 ± 0.05^{g}	3.61 ± 0.01^{cd}	15.73± 0.05^{f}	13.25 ± 0.08^{d}	1.37 ± 0.01^{c}
Klerezyme150	2.46 ± 0.02^{efg}	15.87 ± 0.02^{h}	3.17 ± 0.02^{g}	16.19 ± 0.02^{g}	11.28 ± 0.05^{e}	1.12 ± 0.00^{d}

The same letter in the same column indicates that the difference of the means is not significant at the level of 0.05%.
*L** – Lightness, *a** – redness, *b** – yellowness, *C** – chroma, *H*° – hue angle, *BI* – browning index.

Tab. 2. Pearson's correlation coefficients of chromatic properties and phytochemical compounds of mulberry musts.

	*L**	*a**	*b**	*C**	*H°*	*BI*	*TPC*	*TFC*	*TAC*
*L**	1	0.94789[a]	0.27106	0.94244[a]	–0.39312	–0.12244	0.09313	–0.13413	–0.39264
*a**		1	0.36155	0.99812[a]	–0.32898	0.05997	0.15345	–0.39985	–0.53467
*b**			1	0.41785	0.76056[a]	0.19735	0.26994	–0.11554	–0.05295
*C**				1	–0.27087	0.06879	0.1646	–0.39383	–0.52291
H°					1	0.18978	0.19482	0.13045	0.29668
BI						1	0.73099[a]	–0.7852[a]	0.5610
TPC							1	–0.42947	0.01874
TFC								1	0.40148
TAC									1

a – Correlation significant at the level of 0.05%.
*L** – Lightness, *a** – redness, *b** – yellowness, *C** – chroma, *H°* – hue angle, *BI* – browning index, *TPC* – total phenolics concentration, *TFC* – total flavonoids concentration, *TAC* – total anthocyanins concentration.

the control was significantly higher than NB, PUL and K150 (Tab. 1). The C^* for all samples was significantly correlated to a^* and L^* ($r = 0.998$ and $r = 0.942$, respectively), which were also significantly correlated to each other ($r = 0.948$). This implied that the addition of ascorbic acid tended to inhibit the polyphenol oxidase-catalysed oxidation, which affects the colour of the sample [66].

Phytochemical compounds

Total phenolics concentration

TPC of mulberry must was affected by enzymatic treatments and varied depending on the enzyme used (Tab. 3). Activity of all the enzymes caused a significant increase in TPC, while LF and K150 had a negative effect, similar to the of Sandri et al. [11]. This might be due to the specific characteristics of these enzymes regarding hydrolysis of pectin and influence on procyanidins [65], which are major polyphenols of mulberry fruit [68]. As shown in Tab. 2, the relationship between *BI* and *TPC* was significant ($r = 0.731$). Jiang [69] reported that the presence of phenolic compounds strongly stimulated oxidation of the pigment by polyphenol oxidase, leading to the development of brown pigments.

Total flavonoids concentration

The enzymatic treatment of the mash by LF, LHG, NB and K50 caused an increase in *TFC* of mulberry must. No significant effect was observed after PUF, PUL and PUC treatment (Tab. 3). According to Sun et al. [70], enzymes from *Aspergillus niger* have a lower rutinase activity and some of them can degrade rutin to quercetin, but not in a significant manner. *TFC* had a significantly negative correlation with *BI* ($r = –0.785$), which indicated that flavonoids were degraded or little affected by oxidative enzymes, similar to data reported previously [71].

Tab. 3. Concentrations of phytochemical compounds in mulberry musts.

Enzymes	*TPC* [g·l^{-1}]	*TFC* [g·l^{-1}]	*TAC* [g·l^{-1}]
Control	2.318 ± 0.01[g]	3.486 ± 0.04[abd]	0.524 ± 0.00[a]
Lafase fruit	2.098 ± 0.02[h]	4.660 ± 0.06[h]	0.442 ± 0.00[b]
Lafase HE GC	2.618 ± 0.03[abd]	5.024 ± 0.11[g]	0.555 ± 0.00[c]
Pectinex USP-L	2.618 ± 0.02[ace]	3.628 ± 0.12[ace]	0.529 ± 0.00[a]
Pectinex UF	2.768 ± 0.02[i]	3.587 ± 0.09[bcf]	0.497 ± 0.00[d]
Pectinex Ultra Colour	2.661 ± 0.03[bcf]	3.648 ± 0.09[def]	0.440 ± 0.00[b]
Novarom Blanc	2.602 ± 0.02[def]	5.085 ± 0.06[g]	0.515 ± 0.00[e]
Klerezyme150	2.173 ± 0.03[j]	5.652 ± 0.07[i]	0.559 ± 0.00[c]

The same letter in the same column indicates that the difference of the means is not significant at the level of 0.05%.
TPC – total phenolics concentration, *TFC* – total flavonoids concentration, *TAC* – total anthocyanins concentration.

Total anthocyanins concentration

The enzymatic treatment with PUL, LF, PUF and NB had a negative effect on *TAC* (Tab. 3). This could be attributed to the fact that enzymes from *Aspergillus niger* group have β-galactosidase, α-arabinosidase and/or β-glucosidase activities, which can affect selected anthocyanin pigments in fruit juices [72]. No significant effect was observed with PUL, whereas LHG and K50 had a positive effect, causing an increase in *TAC*. A similar observation was made by other researchers [73, 74]. A positive correlation between *TAC* and *BI* (r = 0.561) suggests that anthocyanase activity of enzymes has an effect on anthocyanins [74] by breaking the bond between glucose and anthocyanin to form anthocyanidins [75]. Their degradation products are potential substrates for polyphenol oxidase [76].

Volatile composition

Data on profiles of volatile compounds of mulberry musts are presented in Tab. 4. A total of 67 compounds were identified and quantified in the volatile fraction of untreated and enzymatically treated musts. The quantified volatiles were grouped into seven groups, including 5 acids, 25 alcohols, 2 aldehydes, 26 esters, 5 hydrocarbons, 1 ketone and 3 phenols.

Acids

Isovaleric acid was identified to be the major fatty acid (accounting for 94.4%). Enzymatic treatment tended to increase the release of fatty acids (butanoic acid, hexanoic acid and octanoic acid). Indeed, polygalacturonase enzymes according to their specificity (endo- or exo-polygalacturonases) hydrolyse fruits in different ways and may affect fatty acid composition, which differ in biosynthetic pathways among the fruit tissues. The presence of short-chain organic acids (C_2–C_8) considered as a potential precursor of volatile aroma-active compounds [77] had a significant contribution to the global aroma because of their low perception thresholds and also to harsh aromas [78].

Alcohols

As indicated in Tab. 4, quantitatively, the most abundant compounds for all samples were aliphatic alcohols, which accounted for 47.5% of the total volatile component. Among the alcohols, ethanol was predominant (82.3%). Ten aliphatic alcohols were present in all samples (etanol, 1-propanol, 2-butanol, 2,3-butanediol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, benzyl alcohol, phenylethyl alcohol and terpene-4-ol). Maceration enzymes, in addition to their main activities, possess other enzyme "side activities", including glycosidases, which differ largely as a function of their pectinase activities. These enzymes can hydrolyse the aglycone moieties of glycosides and have an impact on the release of aliphatic alcohols according to the saccharides moiety of the substrates [79]. This may explain the variation of seventeen other alcohols identified. As reported by Cabaroglu et al. [80], the use of exogenous glycosidase can thereby accelerate the formation of odour-active volatiles. In most cases, the amounts of herbaceous alcohols are affected by the enzymatic treatment [81].

Aldehydes

Aldehydes are common in the flavour of fruits and are believed to play an important role in many fruits. For this reason, it was surprising to observe only two aldehydes (acetaldehyde and benzaldehyde), and these represented only 2.1% of the total volatile compounds. This result is in agreement with those reported by Samappito and Butkhup [82]. It was noted that LF and LHG had a negative effect on acetaldehyde, probably due to cellulosic activities of these enzymes, which can reduce aldehydes to their corresponding alcohols [83].

Esters

The major aroma-active compounds identified in this study were esters, which represented 36.3% of all the volatile compounds. This result agrees with Kalua and Boss [84], who reported that esters were the major volatiles characteristic for berries. Ethyl acetate, ethyl lactate, ethyl 2-hydroxyhexanoate and diethyl succinate were the major esters found in high concentration. Several aliphatic esters (ethyl 2-methylbutanoate, ethyl pentanoate, ethyl decanoate, ethyl tetradecanoate and others), which are important flavour contributors [85, 86], were released by enzymatic treatment and they provided more floral, cherry, stone-fruit and dryplum aroma [86]. However, due to esterase activities, some enzymes could induce the cleavage of esters [83]. This may explain the disparity of the eleven esters (methyl acetate, ethyl propanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl pentanoate, isoamyl lactate, ethyl 9-decenoate, diethyl pentanedioate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate).

Other groups of aroma-active compounds

As mentioned previously, maceration enzymes can hydrolyse pectin in different sites, which affects the hydrocarbon composition (dodecane, tridecane, tetradecane, pentadecane and nonadecane) and produce significant volatile compounds

Tab. 4. Volatile compounds in mulberry musts.

Compounds	*RI*	*RIL*	Control [$\mu g \cdot l^{-1}$]	LF [$\mu g \cdot l^{-1}$]	LHG [$\mu g \cdot l^{-1}$]	PUL [$\mu g \cdot l^{-1}$]	PUF [$\mu g \cdot l^{-1}$]	PUC [$\mu g \cdot l^{-1}$]	NB [$\mu g \cdot l^{-1}$]	K150 [$\mu g \cdot l^{-1}$]
Acids										
Acetic acid	1452	1306–1480	2.12 ± 0.04	2.03 ± 0.05	2.44 ± 0.08	2.24 ± 0.07	2.44 ± 0.05	2.37 ± 0.04	2.06 ± 0.02	2.44 ± 0.02
Butanoic acid	1634	1443–1650	ND	ND	ND	ND	0.59 ± 0.00	0.53 ± 0.00	ND	ND
Isovaleric acid	1645	1642–1674	74.99 ± 0.36	47.02 ± 0.38	48.23 ± 0.45	63.89 ± 0.89	77.50 ± 0.72	59.65 ± 0.15	47.60 ± 0.16	34.29 ± 0.20
Hexanoic acid	1850	1625–1872	ND	ND	2.69 ± 0.06	1.14 ± 0.00	ND	2.81 ± 0.06	ND	0.54 ± 0.04
Octanoic Acid	2060	1809–2098	ND	ND	ND	ND	0.35 ± 0.00	ND	ND	ND
Subtotal			77.11	49.05	53.36	67.27	80.88	65.36	49.66	37.27
Alcohols										
Ethanol	944	819–1010	235.61 ± 0.27	160.26 ± 0.81	152.96 ± 0.52	221.64 ± 0.86	282.94 ± 0.86	213.21 ± 0.68	188.56 ± 0.94	124.22 ± 0.02
2-Butanol	1034	988–1041	11.75 ± 0.89	9.50 ± 0.08	9.08 ± 0.01	8.09 ± 0.03	9.39 ± 0.08	8.45 ± 0.09	9.98 ± 0.08	5.05 ± 0.04
1-Propanol	1053	1035–1065	3.98 ± 0.02	2.90 ± 0.08	2.69 ± 0.04	2.91 ± 0.09	3.59 ± 0.04	2.82 ± 0.09	3.22 ± 0.03	1.68 ± 0.01
3-Methyl-2-butanol	1094	996–1580	ND	ND	ND	ND	ND	0.21 ± 0.00	0.15 ± 0.00	ND
2-Methyl-1-propanol	1103	920–1125	0.96 ± 0.06	0.88 ± 0.08	0.85 ± 0.01	0.73 ± 0.05	0.69 ± 0.07	0.84 ± 0.08	0.82 ± 0.02	0.56 ± 0.04
2-Methyl-1-butanol	1214	1072–1249	2.30 ± 0.08	1.81 ± 0.01	1.79 ± 0.02	1.92 ± 0.05	2.22 ± 0.01	1.90 ± 0.09	1.95 ± 0.04	1.27 ± 0.04
3-Methyl-1-butanol	1217	1187–1255	11.09 ± 0.02	9.47 ± 0.21	9.65 ± 0.09	10.00 ± 0.57	10.53 ± 0.21	10.80 ± 0.37	10.24 ± 0.29	6.68 ± 0.72
3-Methyl-3-buten-1-ol	1260	1121–1339	ND	ND	ND	0.31 ± 0.00	0.27 ± 0.02	0.27 ± 0.00	ND	ND
4-Methyl-1-Pentanol	1327	1301–1347	ND	ND	ND	ND	0.35 ± 0.00	ND	ND	ND
3-Methyl-1-pentanol	1333	1313–1345	ND	ND	ND	ND	ND	ND	0.12 ± 0.00	ND
2-Heptanol	1340	1212–1338	ND	ND	0.46 ± 0.01	ND	ND	ND	0.45 ± 0.02	ND
1-Hexanol	1362	1237–1392	ND	ND	ND	ND	0.28 ± 0.01	ND	ND	ND
3-Ethyl-4-methyl-pentanol	1510	1507–1531	ND	ND	1.68 ± 0.00	1.03 ± 0.01	ND	1.17 ± 0.07	ND	ND
2-Nonanol	1527	1369–1654	2.06 ± 0.04	0.70 ± 0.01	ND	0.91 ± 0.03	0.35 ± 0.00	ND	0.40 ± 0.00	ND
1-Octanol	1547	1064–1605	1.60 ± 0.01	1.12 ± 0.03	1.11 ± 0.00	ND	ND	0.71 ± 0.05	0.92 ± 0.06	0.37 ± 0.02
2,3-Butanediol	1551	1345–1620	3.52 ± 0.02	2.15 ± 0.01	1.52 ± 0.03	3.51 ± 0.07	5.16 ± 0.04	2.87 ± 0.08	6.44 ± 0.04	2.78 ± 0.02
1,3-Butanediol	1583	1576–1619	23.78 ± 0.11	0.81 ± 0.00	ND	0.95 ± 0.08	20.64 ± 0.2	ND	ND	ND
Terpene-4-ol	1605	1571–1606	1.18 ± 0.05	1.14 ± 0.01	0.84 ± 0.03	0.96 ± 0.06	0.70 ± 0.02	1.09 ± 0.09	0.63 ± 0.01	0.55 ± 0.08
2-Decanol	1657	1657	ND	ND	ND	ND	ND	0.23 ± 0.00	0.28 ± 0.01	ND
2-Undecanol	1720	1710–1723	1.26 ± 0.04	ND	ND	0.83 ± 0.06	ND	ND	0.75 ± 0.02	0.91 ± 0.07
2-Butyl-1-octanol	1864	1848–1863	ND	ND	ND	ND	ND	ND	ND	0.24 ± 0.00
Benzyl alcohol	1882	1830–1896	0.73 ± 0.01	0.52 ± 0.08	0.57 ± 0.01	0.40 ± 0.05	0.41 ± 0.07	0.54 ± 0.07	0.60 ± 0.02	0.38 ± 0.02
2-Tetradecanol	1926	1923–1958	0.83 ± 0.05	ND	ND	0.22 ± 0.00	0.36 ± 0.01	ND	ND	0.08 ± 0.07
Phenylethyl alcohol	1932	1840–1941	5.07 ± 0.03	4.44 ± 0.01	5.21 ± 0.08	3.48 ± 0.05	3.22 ± 0.06	5.22 ± 0.02	3.18 ± 0.02	2.31 ± 0.08
2-Pentadecanol	2094	2095–2128	0.47 ± 0.00	0.31 ± 0.00	ND	0.64 ± 0.02	0.49 ± 0.01	ND	0.34 ± 0.00	0.95 ± 0.07
Subtotal			306.19	196.01	188.41	258.53	341.59	250.33	229.03	148.03

Tab. 4. *continued*

Compounds	*RI*	*RIL*	Control [$\mu g \cdot l^{-1}$]	LF [$\mu g \cdot l^{-1}$]	LHG [$\mu g \cdot l^{-1}$]	PUL [$\mu g \cdot l^{-1}$]	PUF [$\mu g \cdot l^{-1}$]	PUC [$\mu g \cdot l^{-1}$]	NB [$\mu g \cdot l^{-1}$]	K150 [$\mu g \cdot l^{-1}$]
Aldehydes										
Acetaldehyde	716	669–905	0.28 ± 0.03	ND	ND	0.19 ± 0.07	0.17 ± 0.03	0.22 ± 0.00	0.18 ± 0.00	0.13 ± 0.01
Benzaldehyde	1556	1354–1562	13.12 ± 0.27	11.02 ± 0.18	12.72 ± 0.02	10.00 ± 0.09	8.64 ± 0.07	13.20 ± 0.84	7.85 ± 0.50	6.25 ± 0.08
Subtotal			13.4	11.02	12.72	10.19	8.81	13.42	8.03	6.38
Esters										
Methyl acetate	813	647–902	0.28 ± 0.00	0.31 ± 0.03	ND	0.27 ± 0.03	0.24 ± 0.04	0.30 ± 0.04	0.21 ± 0.02	0.13 ± 0.04
Ethyl acetate	822	824–977	56.38 ± 0.86	52.88 ± 0.46	46.89 ± 0.19	47.21 ± 0.98	46.47 ± 0.15	51.42 ± 0.89	39.59 ± 0.91	27.96 ± 0.56
Ethyl propanoate	960	915–1021	0.49 ± 0.04	0.34 ± 0.03	ND	0.22 ± 0.00	0.28 ± 0.00	0.31 ± 0.01	0.24 ± 0.05	ND
Propyl acetate	976	935–1030	1.56 ± 0.03	1.42 ± 0.01	1.21 ± 0.01	1.08 ± 0.01	1.08 ± 0.02	1.11 ± 0.09	1.06 ± 0.05	0.63 ± 0.04
Ethyl butanoate	1040	1009–1086	2.04 ± 0.02	1.95 ± 0.06	1.49 ± 0.01	1.96 ± 0.06	2.15 ± 0.02	1.95 ± 0.01	1.59 ± 0.02	1.15 ± 0.08
Ethyl 2-methylbutanoate	1056	1035–1107	ND	ND	ND	0.20 ± 0.01	0.40 ± 0.01	0.22 ± 0.00	0.46 ± 0.02	0.21 ± 0.02
Ethyl 3-methylbutanoate	1071	1051–1096	0.25 ± 0.00	ND	ND	ND	ND	ND	0.27 ± 0.01	0.24 ± 0.00
Isopentyl acetate	1123	1106–1187	1.45 ± 0.06	1.53 ± 0.03	1.26 ± 0.04	1.15 ± 0.07	1.23 ± 0.05	1.11 ± 0.06	1.32 ± 0.01	0.60 ± 0.09
Ethyl pentanoate	1136	1118–1170	ND	ND	ND	0.26 ± 0.01	0.25 ± 0.01	0.23 ± 0.00	0.19 ± 0.02	0.03 ± 0.00
Ethyl hexanoate	1236	1185–1270	4.05 ± 0.08	3.50 ± 0.02	3.49 ± 0.04	4.05 ± 0.04	4.40 ± 0.04	3.62 ± 0.03	3.78 ± 0.05	2.90 ± 0.01
Ethyl lactate	1324	1325–1372	31.80 ± 0.14	23.71 ± 0.9	24.84 ± 0.16	29.75 ± 0.07	34.94 ± 0.17	29.37 ± 0.59	23.26 ± 0.08	16.88 ± 0.09
Ethyl octanoate	1438	1405–1497	0.91 ± 0.01	ND	0.61 ± 0.05	ND	0.55 ± 0.00	0.47 ± 0.03	ND	ND
Ethyl 2-hydroxyhexanoate	1536	1531–1544	25.97 ± 0.01	20.99 ± 0.03	22.38 ± 0.98	21.80 ± 0.02	23.76 ± 0.02	25.07 ± 0.04	19.50 ± 0.06	14.24 ± 0.02
Isoamyl lactate	1574	1558–1570	1.54 ± 0.01	1.26 ± 0.01	1.39 ± 0.02	1.51 ± 0.00	ND	1.60 ± 0.01	1.35 ± 0.03	0.67 ± 0.09
Ethyl decanoate	1622	1395–1680	ND	2.54 ± 0.01	ND	ND	2.42 ± 0.01	ND	ND	0.23 ± 0.00
Diethyl succinate	1684	1664–1702	80.17 ± 5.58	73.47 ± 2.86	85.35 ± 2.37	53.42 ± 2.58	47.77 ± 1.22	75.78 ± 3.77	48.07 ± 0.91	35.02 ± 0.78
Ethyl 9-decenoate	1698	1690–1712	0.48 ± 0.01	0.40 ± 0.02	ND	0.52 ± 0.05	0.23 ± 0.00	0.40 ± 0.02	0.36 ± 0.06	ND
Benzyl acetate	1727	1726–1743	0.67 ± 0.01	0.55 ± 0.01	0.50 ± 0.08	0.42 ± 0.01	0.35 ± 0.08	0.53 ± 0.09	0.37 ± 0.05	0.29 ± 0.02
Methyl salicylate	1745	1556–1787	2.00 ± 0.01	1.64 ± 0.08	1.88 ± 0.04	1.22 ± 0.09	1.14 ± 0.04	1.53 ± 0.07	1.24 ± 0.11	0.91 ± 0.10
Diethyl pentanedioate	1765	1768–1780	0.54 ± 0.07	0.34 ± 0.02	0.33 ± 0.05	0.33 ± 0.00	0.23 ± 0.01	0.41 ± 0.03	0.21 ± 0.03	ND
Ethyl phenylethanoate	1790	1755–1803	2.17 ± 0.03	1.73 ± 0.06	1.89 ± 0.03	1.37 ± 0.07	1.52 ± 0.02	2.27 ± 0.01	1.26 ± 0.03	1.01 ± 0.08
2-Phenylethyl acetate	1812	1777–1871	5.83 ± 0.02	5.48 ± 0.05	6.22 ± 0.04	3.64 ± 0.01	3.29 ± 0.08	5.80 ± 0.03	3.30 ± 0.08	2.39 ± 0.02
Ethyl dodecanoate	1844	1489–1882	3.73 ± 0.09	2.33 ± 0.00	2.69 ± 0.02	1.89 ± 0.01	ND	ND	1.34 ± 0.04	1.78 ± 0.01
Ethyl 3-phenylpropanoate	1886	1862–1908	3.50 ± 0.02	3.67 ± 0.02	3.94 ± 0.01	2.52 ± 0.06	2.35 ± 0.04	4.44 ± 0.06	2.27 ± 0.04	1.86 ± 0.06
Ethyl tetradecanoate	2056	2015–2065	1.42 ± 0.05	0.96 ± 0.02	1.21 ± 0.02	0.44 ± 0.05	0.62 ± 0.01	0.92 ± 0.06	0.58 ± 0.02	0.63 ± 0.04
Ethyl hexadecanoate	2250	2224–2302	1.13 ± 0.04	0.73 ± 0.01	0.95 ± 0.03	0.53 ± 0.04	0.42 ± 0.01	0.46 ± 0.00	0.96 ± 0.00	0.96 ± 0.05
Subtotal			228.36	201.73	208.52	175.76	176.09	209.32	152.78	110.72
Hydrocarbons										
Dodecane	1201	1200–1203	0.56 ± 0.01	ND	ND	0.33 ± 0.04	0.38 ± 0.08	0.21 ± 0.00	0.30 ± 0.00	0.15 ± 0.01

Tab. 4. *continued*

Compounds	*RI*	*RIL*	Control [μg·l^{-1}]	LF [μg·l^{-1}]	LHG [μg·l^{-1}]	PUL [μg·l^{-1}]	PUF [μg·l^{-1}]	PUC [μg·l^{-1}]	NB [μg·l^{-1}]	K150 [μg·l^{-1}]
Tridecane	1303	1262–1349	0.53 ± 0.07	ND	ND	0.42 ± 0.03	0.37 ± 0.01	0.35 ± 0.08	0.32 ± 0.00	0.04 ± 0.00
Tetradecane	1398	1392–1400	1.10 ± 0.02	1.18 ± 0.00	1.61 ± 0.01	ND	ND	1.80 ± 0.02	ND	0.05 ± 0.00
Pentadecane	1500	1494–1500	0.33 ± 0.02	0.24 ± 0.09	0.20 ± 0.00	0.49 ± 0.08	0.49 ± 0.07	0.59 ± 0.05	0.14 ± 0.00	0.66 ± 0.01
Nonadecane	1901	1899–1900	ND	ND	0.78 ± 0.06	ND	ND	ND	0.42 ± 0.08	ND
Subtotal			2.52	1.42	2.59	1.24	1.24	2.95	1.18	0.9
Ketone										
3-Hydroxy-2-butanone	1287	1269–1320	7.81 ± 0.04	ND	1.91 ± 0.09	6.36 ± 0.03	7.69 ± 0.06	5.18 ± 0.07	ND	ND
Subtotal			7.81	ND	1.91	6.36	7.69	5.18	ND	ND
Phenols										
2-methoxyphenol	1873	1840–1883	ND	ND	ND	ND	0.16 ± 0.00	ND	ND	ND
4-Methyl-2-methoxyphenol	1963	1191–1965	0.57 ± 0.09	0.50 ± 0.03	0.56 ± 0.07	ND	0.29 ± 0.01	0.44 ± 0.02	0.32 ± 0.08	ND
2,4-Di-*tert*-butylphenol	2315	2277–2330	10.31 ± 0.04	4.60 ± 0.02	6.12 ± 0.02	4.04 ± 0.06	4.11 ± 0.06	5.55 ± 0.09	5.12 ± 0.02	2.69 ± 0.01
Subtotal			10.88	5.1	6.68	4.04	4.56	5.99	5.44	2.69
Total			**646.27**	**464.33**	**474.19**	**523.39**	**620.86**	**552.55**	**446.12**	**305.99**

RI – retention index, relative to C5-C25 n-alkanes, *RIL* – Retention index from the literature [17-33, 9, 34-63]. Maceration enzymes: LF – Lafase fruit, LHG – Lafase HE GC, PUL – Pectinex USP-L, PUF – Pectinex UF, PUC – Pectinex Ultra Colour, NB – Novarom Blanc, K150 – Klerezyme150.

[87]. Enzymatic treatment was found to have a negative impact on ketones (3-hydroxy-2-butanone) and volatile phenols (4-methyl-2-methoxyphenol, 2-methoxyphenol and 2,4-Di-tert-butylphenol).

Multivariate Analysis

Cluster analysis

In order to evaluate the effect of enzymatic treatment on the release of volatile compounds, cluster analysis was carried out to find data grouping among different treatments. Ward's method and Euclidean distances were selected as the measure of similarity. Groups of compounds were used as classifying variables (Fig. 1).

The dendrogram allowed the identification of various groups of enzymatic treatments. The first great division in groups A and B separated musts according to their total volatile concentration. Group A consisted of musts treated with CON and PUF, which had in common a high concentration of volatile aroma. It also contained musts with high concentrations of ethanol, 2-methyl-1-butanol, ethyl lactate, and high concentrations of alcohols. Group B was constituted by musts that had a low concentration of acids. This group was split into two subgroups: subgroup C (K150), which was characterized by the presence of 2-butyl-1-octanol, and lowest concentrations of acids, esters and aldehydes. Subgroup D was also divided into two subgroups: E (LF and LHG), characterized by the absence of acetaldehyde, dodecane, tridecane and 2-methoxyphenol, and subgroup F, which had a moderate concentration of alcohols and acids. Group H (NB) was characterized by 3-methyl-1-pentanol, and high concentrations of 2,3-butanediol and ethyl 2-methylbutanoate. Group F could be differentiated from group G (PUL and PUC) on the basis of the presence of 3-hydroxy-2-butanone. It is noteworthy that similar subgroups E and G were constituted by musts tretaed with enzymes supplied by the same company. This means that these enzymes produced musts that had a similar basic volatile composition.

Principal component analysis

To highlight the effect of the enzymatic treatment, PCA was performed on all parameters (Fig. 2A). The portion of 88.7% of the total variance could be explained by the

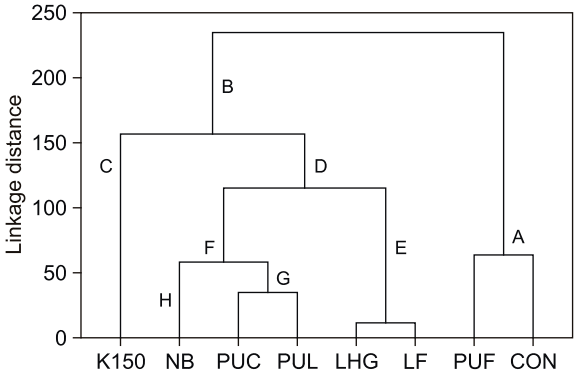

Fig. 1. Dendrogram of cluster analysis of groups of volatile compounds.

Dendrogram represents Euclidean distances as results of Ward's method application.
CON – control. Maceration enzymes: LF – Lafase fruit, LHG – Lafase HE GC, PUL – Pectinex USP-L, PUF – Pectinex UF, PUC – Pectinex Ultra Colour, NB – Novarom Blanc, K150 – Klerezyme150.

first four principal components (PC). Individual components were responsible for 44.0%, 19.2%, 14.3% and 11.2%, respectively. Loading values > 0.60 of the chromatic properties, phytochemical compounds and volatile compounds are marked throughout Tab. 5.

As presented in Tab. 5, the evaluated parameters indicated that PC1 strongly correlated with the volatile composition, *TFC* and *BI*. PC2 (*L**, *a**, *c**) and PC4 (*b** and *H*°) were mainly linked with colour properties. The correlation of phytochemical compounds (*TPC* and *TAC*) was shared between PC1 and PC2. Nevertheless, aldehydes were not negligible in PC3.

Mulberry musts were divided into four groups, according to the correlation between enzyme treatments (scores) and their properties (loadings) as shown in Fig. 2B. The first group was on the positive side of PC1 and PC2, and on the negative side of PC4. It included control and PUC, which were characterized as having a high concentration of volatile compounds (esters and aldehydes), and middle values of colour properties *L** and *C**.

Mulberry musts situated on the negative side of PC1 and PC4, and on the positive side of PC2 formed the second group, containing PUL and K150. These were described by middle values of their colour properties *a** and *b**, and lower concentrations of volatile phenols.

The third group was characterized by the lowest concentration of volatile compounds, high concentration of phytochemical compounds (*TAC* and *TFC*), lower *BI* and colour properties (*b** and *H*°). It was situated on the negative side of PC1 and on the positive side of PC2, represented by K150. This third group was represented by NB, LF and LHG, which could be split into two subgroups based on their correlation between PC4 (NB) and PC1 (LF and LHG). It is noteworthy that the musts treated with LF and LHG, which were ob-

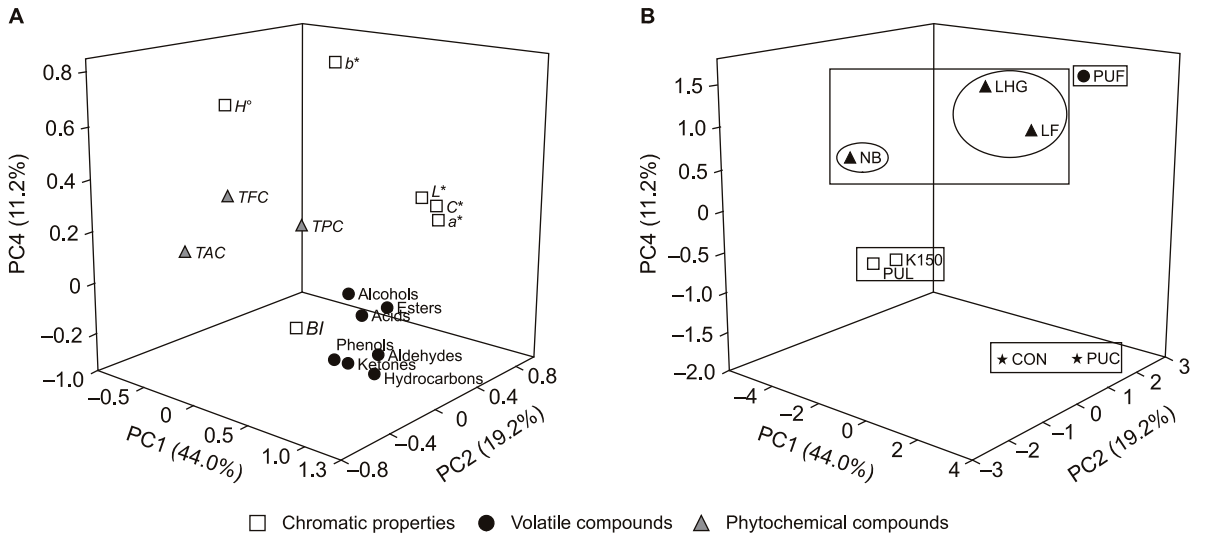

Fig. 2. Principal component analysis of mulberry musts.

A – Loadings plot, B – Scores scatter plot.
*L** – Lightness, *a** – redness, *b** – yellowness, *C** – chroma, *H*° – hue angle, *BI* – browning index, *TPC* – total phenolics concentration, *TFC* – total flavonoids concentration, *TAC* – total anthocyanins concentration, CON – control.
Maceration enzymes: LF – Lafase fruit, LHG – Lafase HE GC, PUL – Pectinex USP-L, PUF – Pectinex UF, PUC – Pectinex Ultra Colour, NB – Novarom Blanc, K150 – Klerezyme150.

Tab. 5. Loadings of the first four principal components.

	PC1	PC2	PC3	PC4
Acids	0.912419 +	–0.256705	–0.284785	0.008550
Alcohols	0.824599 +	–0.286629	–0.396258	0.080340
Aldehydes	0.727791 +	0.073030	0.604881 +	–0.234348
Esters	0.815425 +	0.062506	0.555337	–0.033467
Hydrocarbons	0.635933 +	0.137662	0.540212	–0.333694
Ketones	0.831774 +	–0.300100	–0.349205	–0.177014
Phenols	0.621373 +	–0.216931	0.573139	–0.206512
Lightness *L**	0.407931	0.802994 +	–0.242134	0.242583
Redness *a**	0.640599 +	0.717610 +	–0.159770	0.191949
Yellowness *b**	0.394382	0.001334	0.336437	0.848685 +
Chroma *C**	0.648192 +	0.700989 +	–0.131741	0.242926
Hue angle *H*°	–0.021930	–0.514356	0.442900	0.718388 +
Browning index	0.730449 +	–0.622467 +	–0.236070	0.005641
Total phenolics concentration	0.484311	–0.354421	–0.337740	0.298570
Total flavonoids concentration	–0.878774 +	0.200547	0.240537	0.206368
Total anthocyanins concentration	–0.394092	–0.539066	0.012051	0.137387

(+) – loading values > 0.60.

tained from the same manufacturer, had similar colour properties (L^*, a^* and C^*) and volatile composition (alcohols and esters).

The last group, formed by PUF, was situated on the positive side of PC1, PC2 and PC4, being characterized by the highest concentration of volatile compounds (alcohols and acids), highest colour property values (L^*, a^* and C^*) and the highest concentration of *TFC*, but had the highest *BI* values.

CONCLUSION

Enzymatic treatment of mulberry must had significant effects on chromatic properties, phytochemical compounds and volatile aroma compounds in the mulberry must. The commercially available enzymes, which are widely used in the maceration process, had an effect on the concentrations of volatile compounds, phytochemical compounds and chromatic properties. Indeed, some enzymes released more phytochemical compounds and/or more volatile compounds, whereas others could give better colour parameters. Therefore, the quality of the final product depends on the choice of the enzyme.

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REFERENCES

1. Lee, J. S. – Synytsya, A. – Kim, H. B. – Choi, D. J. – Lee, S. – Lee, J. – Kim, W. J. – Jang, S. – Park, Y. I.: Purification, characterization and immunomodulating activity of a pectic polysaccharide isolated from Korean mulberry fruit Oddi (*Morus alba* L.). International Immunopharmacology, *17*, 2013, pp. 858–866. DOI: 10.1016/j.intimp.2013.09.019.
2. Butkhup, L. – Jeenphakdee, M. – Jorjong, S. – Samappito, S. – Samappito, W. – Chowtivannakul, S.: HS-SPME-GC-MS analysis of volatile aromatic compounds in alcohol related beverages made with mulberry fruits. Food Science and Biotechnology, *20*, 2011, pp. 1021–1032. DOI: 10.1007/s10068-011-0140-4.
3. González-Molina, E. – Domínguez-Perles, R. – Moreno, D. – García-Viguera, C.: Natural bioactive compounds of *Citrus limon* for food and health. Journal of Pharmaceutical and Biomedical Analysis, *51*, 2010, pp. 327–345. DOI: 10.1016/j.jpba.2009.07.027.
4. Lattey, K. – Bramley, B. – Francis, I.: Consumer acceptability, sensory properties and expert quality judgements of Australian Cabernet Sauvignon and Shiraz wines. Australian Journal of Grape and Wine Research, *16*, 2010, pp. 189–202. DOI: 10.1111/j.1755-0238.2009.00069.x.
5. Nguyen, V. – Le, T. – Le, V.: Application of combined ultrasound and cellulase preparation to guava (*Psidium guajava*) mash treatment in juice processing: optimization of biocatalytic conditions by response surface methodology. International Food Research Journal, *20*, 2013, pp. 377–381. <http://www.ifrj.upm.edu.my/20%20(01)%202013/52%20IFRJ%20

20%20(01)%202013%20Le%20Vietnam%20(107).pdf>
6. Nattaporn, W. – Pranee, A.: Effect of pectinase on volatile and functional bioactive compounds in the flesh and placenta of 'Sunlady' cantaloupe. International Food Research Journal, *18*, 2011, pp. 819–827. <http://ifrj.upm.edu.my/18%20(02)%202011/(49)%20IFRJ-2010-004.pdf>
7. Armada, L. – Fernández, E. – Falqué, E.: Influence of several enzymatic treatments on aromatic composition of white wines. LWT – Food Science and Technology, *43*, 2010, pp. 1517–1525. DOI: 10.1016/j.lwt.2010.06.009.
8. Oszmiański, J. – Wojdyło, A. – Kolniak, J.: Effect of pectinase treatment on extraction of antioxidant phenols from pomace, for the production of puree-enriched cloudy apple juices. Food Chemistry, *127*, 2011, pp. 623–631. DOI: 10.1016/j.foodchem.2011.01.056.
9. Riu-Aumatell, M. – López-Tamames, E. – Buxaderas, S.: Assessment of the volatile composition of juices of apricot, peach, and pear according to two pectolytic treatments. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 7837–7843. DOI: 10.1021/jf051397z.
10. Laaksonen, O. – Sandell, M. – Nordlund, E. – Heiniö, R.-L. – Malinen, H.-L. – Jaakkola, M. – Kallio, H.: The effect of enzymatic treatment on blackcurrant (*Ribes nigrum*) juice flavour and its stability. Food Chemistry, *130*, 2012, pp. 31–41. DOI: 10.1016/j.foodchem.2011.06.048.
11. Sandri, I. G. – Lorenzoni, C. M. T. – Fontana, R. C. – da Silveira, M. M.: Use of pectinases produced by a new strain of *Aspergillus niger* for the enzymatic treatment of apple and blueberry juice. LWT – Food Science and Technology, *51*, 2013, pp. 469–475. DOI: 10.1016/j.lwt.2012.10.015.
12. Meydav, S. – Saguy, I. – Kopelman, I. J.: Browning determination in citrus products. Journal of Agricultural and Food Chemistry, *25*, 1977, pp. 602–604. DOI: 10.1021/jf60211a030.
13. Buglione, M. – Lozano, J.: Nonenzymatic browning and chemical changes during grape juice storage. Journal of Food Science, *67*, 2002, pp. 1538–1543. DOI: 10.1111/j.1365-2621.2002.tb10318.x.
14. Singleton, V. L. – Orthofer, R. – Lamuela-Raventós, R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Lester P. (Ed.): Methods in enzymology. Volume *299*. San Diego: Academic Press, 1999, pp. 152–178. ISBN 0076-6879. DOI: 10.1016/S0076-6879(99)99017-1.
15. Zhishen, J. – Mengcheng, T. – Jianming, W.: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, *64*, 1999, pp. 555–559. DOI: 10.1016/S0308-8146(98)00102-2.
16. Lee, J. – Durst, R. W. – Wrolstad, R. E.: Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. Journal of AOAC International, *88*, 2005, pp. 1269–1278. <http://www.usmarc.usda.gov/SP2UserFiles/ad_hoc/53581000FoodChemistry/PDF/LEEJAOAC.pdf>
17. Gyawali, R. – Seo, H.-Y. – Lee, H.-J. – Song, H.-P. – Kim, D.-H. – Byun, M.-W. – Kim, K.-S.: Effect of γ-irradiation on volatile compounds of dried Welsh onion (*Allium fistulosum* L.). Radiation Physics and Chemistry, *75*, 2006, pp. 322–328. DOI: 10.1016/j.radphyschem.2005.07.001.
18. Garruti, D. S. – Franco, M. R. B. – da Silva, M. A. A. P. – Janzantti, N. S. – Alves, G. L.: Assessment of aroma impact compounds in a cashew apple-based alcoholic beverage by GC-MS and GC-olfactometry. LWT – Food Science and Technology, *39*, 2006, pp. 373–378. DOI: 10.1016/j.lwt.2005.02.006.
19. Goodner, K. L.: Practical retention index models of OV-101, DB-1, DB-5, and DB-Wax for flavor and fragrance compounds. LWT – Food Science and Technology, *41*, 2008, pp. 951–958. DOI: 10.1016/j.lwt.2007.07.007.
20. Tripathi, J. – Chatterjee, S. – Gamre, S. – Chattopadhyay, S. – Variyar, P. S. – Sharma, A.: Analysis of free and bound aroma compounds of pomegranate (*Punica granatum* L.). LWT – Food Science and Technology, *59*, 2014, pp. 461–466. DOI: 10.1016/j.lwt.2014.05.055.
21. Mallouchos, A. – Paul, L. – Argyro, B. – Koutinas, A. – Komaitis, M.: Ambient and low temperature winemaking by immobilized cells on brewer's spent grains: Effect on volatile composition. Food Chemistry, *104*, 2007, pp. 918–927. DOI: 10.1016/j.foodchem.2006.12.047.
22. Radovic, B. S. – Careri, M. – Mangia, A. – Musci, M. – Gerboles, M. – Anklam, E.: Contribution of dynamic headspace GC–MS analysis of aroma compounds to authenticity testing of honey. Food Chemistry, *72*, 2001, pp. 511–520. DOI: 10.1016/S0308-8146(00)00263-6.
23. Du, X. – Finn, C. E. – Qian, M. C.: Volatile composition and odour-activity value of thornless 'Black Diamond' and 'Marion' blackberries. Food Chemistry, *119*, 2010, pp. 1127–1134. DOI: 10.1016/j.foodchem.2009.08.024.
24. Pasini, F. – Gardini, S. – Marcazzan, G. L. – Caboni, M. F.: Buckwheat honeys: Screening of composition and properties. Food Chemistry, *141*, 2013, pp. 2802–2811. DOI: 10.1016/j.foodchem.2013.05.102.
25. García-Carpintero, E. G. – Sánchez-Palomo, E. – González-Viñas, M. A.: Aroma characterization of red wines from *cv*. Bobal grape variety grown in La Mancha region. Food Research International, *44*, 2011, pp. 61–70. DOI: 10.1016/j.foodres.2010.11.013.
26. Lee, P.-R. – Chong, I. S.-M. – Yu, B. – Curran, P. – Liu, S.-Q.: Effects of sequentially inoculated *Williopsis saturnus* and *Saccharomyces cerevisiae* on volatile profiles of papaya wine. Food Research International, *45*, 2012, pp. 177–183. DOI: 10.1016/j.foodres.2011.10.011
27. Tao, Y. – Li, H. – Wang, H. – Zhang, L.: Volatile compounds of young Cabernet Sauvignon red wine from Changli County (China). Journal of Food

Composition and Analysis, *21*, 2008, pp. 689–694. DOI: 10.1016/j.jfca.2008.05.007.
28. Howard, K. L. – Mike, J. H. – Riesen, R.: Validation of a solid-phase microextraction method for headspace analysis of wine aroma components. American Journal of Enology and Viticulture, *56*, 2005, pp. 37–45. <http://www.ajevonline.org/content/56/1/37.full.pdf+html>
29. Lee, J. – Sugawara, E. – Yokoi, S. – Takahata, Y.: Genotypic variation of volatile compounds from flowers of gentians. Breeding Science, *60*, 2010, pp. 9–17. DOI: 10.1270/jsbbs.60.9.
30. Jirovetz, L. – Buchbauer, G. – Abraham, G. T. – Shafi, M. P.: Chemical composition and olfactoric characterization of *Acmella radicans* (Jacq.) R. K. Jansen var. *radicans* from southern India. Flavour and Fragrance Journal, *21*, 2006, pp. 88–91. DOI: 10.1002/ffj.1524.
31. Fan, W. – Qian, M. C.: Identification of aroma compounds in Chinese 'Yanghe Daqu' liquor by normal phase chromatography fractionation followed by gas chromatography / olfactometry. Flavour and Fragrance Journal, *21*, 2006, pp. 333–342. DOI: 10.1002/ffj.1621.
32. Li, H. – Tao, Y.-S. – Wang, H. – Zhang, L.: Impact odorants of Chardonnay dry white wine from Changli County (China). European Food Research and Technology, *227*, 2008, pp. 287–292. DOI: 10.1007/s00217-007-0722-9.
33. Lee, P.-R. – Boo, C. – Liu, S.-Q.: Fermentation of coconut water by probiotic strains *Lactobacillus acidophilus* L10 and *Lactobacillus casei* L26. Annals of Microbiology, *63*, 2013, pp. 1441–1450. DOI: 10.1007/s13213-013-0607-z.
34. Gamero, A. – Hernández-Orte, P. – Querol, A. – Ferreira, V.: Effect of aromatic precursor addition to wine fermentations carried out with different *Saccharomyces* species and their hybrids. International Journal of Food Microbiology, *147*, 2011, pp. 33–44. DOI: 10.1016/j.ijfoodmicro.2011.02.035.
35. Sun, S. Y. – Jiang, W. G. – Zhao, Y. P.: Evaluation of different *Saccharomyces cerevisiae* strains on the profile of volatile compounds and polyphenols in cherry wines. Food Chemistry, *127*, 2011, pp. 547–555. DOI: 10.1016/j.foodchem.2011.01.039.
36. Mo, X. – Fan, W. – Xu, Y.: Changes in volatile compounds of Chinese rice wine wheat Qu during fermentation and storage. Journal of the Institute of Brewing, *115*, 2009, pp. 300–307. DOI: 10.1002/j.2050-0416.2009.tb00385.x.
37. Lee, P.-R. – Chong, I. S.-M. – Yu, B. – Curran, P. – Liu, S.-Q.: Effect of precursors on volatile compounds in papaya wine fermented by mixed yeasts. Food Technology and Biotechnology, *51*, 2013, pp. 92–100. <http://hrcak.srce.hr/file/146878>
38. Kocsis, N. – Márkus, F. – Mednyánszky, Z. – Amtmann, M. – Korány, K.: Recognition experiments of the vintage year 1997 hot and red paprika (*Capsicum annuum*) varieties grown in Kalocsa. Acta Alimentaria, *32*, 2003, pp. 63–75. DOI: 10.1556/AAlim.32.2003.1.8.
39. Giri, A. – Okamoto, A. – Okazaki, E. – Ohshima, T.: Headspace volatiles along with other instrumental and sensory analyses as indices of maturation of horse mackerel *Miso*. Journal of Food Science, *75*, 2010, pp. S406–S417. DOI: 10.1111/j.1750-3841.2010.01780.x.
40. Marlatt, C. – Ho, C. T. – Chien, M.: Studies of aroma constituents bound as glycosides in tomato. Journal of Agricultural and Food Chemistry, *40*, 1992, pp. 249–252. DOI: 10.1021/jf00014a016.
41. Shiratsuchi, H. – Shimoda, M. – Imayoshi, K. – Noda, K. – Osajima, Y.: Volatile flavor compounds in spray-dried skim milk powder. Journal of Agricultural and Food Chemistry, *42*, 1994, pp. 984–988. DOI: 10.1021/jf00040a028.
42. Pino, J. A. – Marbot, R. – Vázquez, C.: Characterization of volatiles in strawberry guava (*Psidium cattleianum* Sabine) fruit. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 5883–5887. DOI: 10.1021/jf010414r.
43. Fu, S.-G. – Yoon, Y. – Bazemore, R.: Aroma-active components in fermented bamboo shoots. Journal of Agricultural and Food Chemistry, *50*, 2001, pp. 549–554. DOI: 10.1021/jf010883t.
44. Moreira, R. F. A. – Trugo, L. C. – Pietroluongo, M. – De Maria, C. A. B.: Flavor composition of cashew (*Anacardium occidentale*) and marmeleiro (*Croton* species) honeys. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 7616–7621. DOI: 10.1021/jf020464b.
45. Lee, S.-J. – Noble, A. C.: Characterization of odor-active compounds in Californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 8036–8044. DOI: 10.1021/jf034747v.
46. Ledauphin, J. – Saint-Clair, J.-F. – Lablanquie, O. – Guichard, H. – Founier, N. – Guichard, E. – Barillier, D.: Identification of trace volatile compounds in freshly distilled Calvados and Cognac using preparative separations coupled with gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 5124–5134. DOI: 10.1021/jf040052y.
47. Choi, H.-S.: Aroma evaluation of an aquatic herb, Changpo (*Acorus calamus* Var. *angustatus* Bess), by AEDA and SPME. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 8099–8104. DOI: 10.1021/jf040239p.
48. Chida, M. – Sone, Y. – Tamura, H.: Aroma characteristics of stored tobacco cut leaves analyzed by a high vacuum distillation and canister system. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 7918–7924. DOI: 10.1021/jf049223p.
49. Ferrari, G. – Lablanquie, O. – Cantagrel, R. – Ledauphin, J. – Payot, T. – Fournier, N. – Guichard, E.: Determination of key odorant compounds in freshly distilled Cognac using GC-O, GC-MS, and sensory evaluation. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 5670–5676. DOI: 10.1021/jf049512d.
50. Culleré, L. – Escudero, A. – Cacho, J. – Ferreira, V.: Gas chromatography-olfactometry and chemical quantitative study of the aroma of six

premium quality Spanish aged red wines. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 1653–1660. DOI: 10.1021/jf0350820.
51. Shimoda, M. – Peralta, R. R. – Osajima, Y.: Headspace gas analysis of fish sauce. Journal of Agricultural and Food Chemistry, *44*, 1996, pp. 3601–3605. DOI: 10.1021/jf960345u.
52. Wada, K. – Shibamoto, T.: Isolation and identification of volatile compounds from a wine using solid phase extraction, gas chromatography, and gas chromatography/mass spectrometry. Journal of Agricultural and Food Chemistry, *45*, 1997, pp. 4362–4366. DOI: 10.1021/jf970157j.
53. Shimoda, M. – Wu, Y. – Osajima, Y.: Aroma compounds from aqueous solution of Haze (*Rhus succedanea*) honey determined by adsorptive column chromatography. Journal of Agricultural and Food Chemistry, *44*, 1996, pp. 3913–3918. DOI: 10.1021/jf9601168.
54. Franco, M. R. B. – Shibamoto, T.: Volatile composition of some Brazilian fruits: Umbu-caja (*Spondias citherea*), Camu-camu (*Myrciaria dubia*), Araça-boi (*Eugenia stipitata*), and Cupuaçu (*Theobroma grandiflorum*). Journal of Agricultural and Food Chemistry, *48*, 2000, pp. 1263–1265. DOI: 10.1021/jf9900074.
55. Biernacka, P. – Wardencki, W.: Volatile composition of raw spirits of different botanical origin. Journal of the Institute of Brewing, *118*, 2012, pp. 393–400. DOI: 10.1002/jib.55.
56. Prat, L. – Espinoza, M. I. – Agosin, E. – Silva, H.: Identification of volatile compounds associated with the aroma of white strawberries (*Fragaria chiloensis*). Journal of the Science of Food and Agriculture, *94*, 2014, pp. 752–759. DOI: 10.1002/jsfa.6412.
57. Fan, W. – Xu, Y. – Jiang, W. – Li, J.: Identification and quantification of impact aroma compounds in 4 nonfloral *Vitis vinifera* varieties grapes. Journal of Food Science, *75*, 2010, pp. S81–S88. DOI: 10.1111/j.1750-3841.2009.01436.x.
58. Luo, T. – Fan, W. – Xu, Y.: Characterization of volatile and semi-volatile compounds in Chinese rice wines by headspace solid phase microextraction followed by gas chromatography-mass spectrometry. Journal of the Institute of Brewing, *114*, 2008, pp. 172–179. DOI: 10.1002/j.2050-0416.2008.tb00323.x.
59. Bianchi, F. – Careri, M. – Mangia, A. – Musci, M.: Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: Database creation and evaluation of precision and robustness. Journal of Separation Science, *30*, 2007, pp. 563–572. DOI: 10.1002/jssc.200600393.
60. Selli, S. – Cabaroglu, T. – Canbas, A. – Erten, H. – Nurgel, C. – Lepoutre, J. P. – Gunata, Z.: Volatile composition of red wine from cv. Kalecik Karasi grown in central Anatolia. Food Chemistry, *85*, 2004, pp. 207–213. DOI: http://dx.doi.org/10.1016/j.foodchem.2003.06.008.
61. Gonzalez-Rios, O. – Suarez-Quiroz, M. L. – Boulanger, R. – Barel, M. – Guyot, B. – Guiraud, J.-P. – Schorr-Galindo, S.: Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. Journal of Food Composition and Analysis, *20*, 2007, pp. 289–296. DOI: 10.1016/j.jfca.2006.07.009.
62. Matos, L. – Moreira, R. – Trugo, L. – De Maria, C.: Componenti aromatici dei mieli brasiliani di *Croton* sp. e *Vernonia* sp (Aroma compounds in morrão de candeia (*Croton* sp.) and assa-peixe (*Vernonia* sp.) honeys.) Italian Journal of Food Science, *14*, 2002, pp. 267–278.
63. Kivçak, B. – Akay, S. – Demirci, B. – Başer, K.: Chemical composition of essential oils from leaves and twigs of *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus* from Turkey. Pharmaceutical Biology, *42*, 2004, pp. 360–366. DOI: 10.1080/13880200490519677.
64. Bianchi, F. – Careri, M. – Mangia, A. – Musci, M.: Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: Database creation and evaluation of precision and robustness. Journal of Separation Science, *30*, 2007, pp. 563–572. DOI: 10.1002/jssc.200600393.
65. Oszmiański, J. – Wojdyło, A. – Kolniak, J.: Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity, and turbidity of cloudy apple juice. Journal of Agricultural and Food Chemistry, *57*, 2009, pp. 7078–7085. DOI: 10.1021/jf900806u.
66. Mihalev, K. – Schieber, A. – Mollov, P. – Carle, R.: Effect of mash maceration on the polyphenolic content and visual quality attributes of cloudy apple juice. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 7306–7310. DOI: 10.1021/jf049480u.
67. Sun, T. – Powers, J. R. – Tang, J.: Effect of enzymatic macerate treatment on rutin content, antioxidant activity, yield, and physical properties of asparagus juice. Journal of Food Science, *72*, 2007, pp. S267–S271. DOI: 10.1111/j.1750-3841.2007.00345.x.
68. Butkhup, L. – Samappito, W. – Samappito, S.: Phenolic composition and antioxidant activity of white mulberry (*Morus alba* L.) fruits. International Journal of Food Science & Technology, *48*, 2013, pp. 934–940. DOI: 10.1111/ijfs.12044.
69. Jiang, Y.: Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. Journal of the Science of Food and Agriculture, *80*, 2000, pp. 305–310. DOI: 10.1002/1097-0010(200002)80:3<305::AID-JSFA518>3.0.CO;2-H.
70. Sun, T. – Tang, J. – Powers, J. R.: Effect of pectolytic enzyme preparations on the phenolic composition and antioxidant activity of asparagus juice. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 42–48. DOI: 10.1021/jf0491299.
71. Lea, A. G. H.: Flavor, color, and stability in fruit products: The effect of polyphenols. In: Hemingway, R. W. – Laks, P. E. (Eds.): Plant polyphenols. Volume *59*. New York : Springer, 1992, pp. 827–847. ISBN 978-1-4613-6540-2. DOI: 10.1007/978-1-4615-3476-1_49.

72. Landbo, A.-K. – Meyer, A. S.: Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 3169–3177. DOI: 10.1021/jf001443p.
73. Romero-Cascales, I. – Ros-García, J. – López-Roca, J. – Gómez-Plaza, E.: The effect of a commercial pectolytic enzyme on grape skin cell wall degradation and colour evolution during the maceration process. Food Chemistry, *130*, 2012, pp. 626–631. DOI: 10.1016/j.foodchem.2011.07.091.
74. Wang, W. D. – Xu, S. Y. – Jin, M. K.: Effects of different maceration enzymes on yield, clarity and anthocyanin and other polyphenol contents in blackberry juice. International Journal of Food Science & Technology, *44*, 2009, pp. 2342–2349. DOI: 10.1111/j.1365-2621.2007.01637.x.
75. Manzanares, P. – Rojas, V. – Genovés, S. – Vallés, S.: A preliminary search for anthocyanin-β-D-glucosidase activity in non-*Saccharomyces* wine yeasts. International Journal of Food Science & Technology, *35*, 2000, pp. 95–103. DOI: 10.1046/j.1365-2621.2000.00364.x.
76. Zhang, Z. – Pang, X. – Ji, Z. – Jiang, Y.: Role of anthocyanin degradation in litchi pericarp browning. Food Chemistry, *75*, 2001, pp. 217–221. DOI: 10.1016/S0308-8146(01)00202-3.
77. Defilippi, B. G. – Dandekar, A. M. – Kader, A. A.: Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 3133–3141. DOI: 10.1021/jf047892x.
78. Rodriguez, S. B. – Amberg, E. – Thornton, R. – McLellan, M.: Malolactic fermentation in Chardonnay: growth and sensory effects of commercial strains of *Leuconostoc oenos*. Journal of Applied Microbiology, *68*, 1990, pp. 139–144. DOI: 10.1111/j.1365-2672.1990.tb02558.x.
79. Günata, Z. – Dugelay, I. – Sapis, J. – Baumes, R. – Bayonove, C.: Role of enzymes in the use of the flavour potential from grape glycosides in winemaking. Progress in Flavour Precursor Studies, *3*, 1993, pp. 219–234.
80. Cabaroglu, T. – Selli, S. – Canbas, A. – Lepoutre, J.-P. – Günata, Z.: Wine flavor enhancement through the use of exogenous fungal glycosidases. Enzyme and microbial technology, *33*, 2003, pp. 581–587. DOI: 10.1016/S0141-0229(03)00179-0.
81. Lao, C. – López-Tamames, E. – Lamuela-Raventós, R. M. – Buxaderas, S. – Torre-Boronat, M.: Pectic enzyme treatment effects on quality of white grape musts and wines. Journal of food Science, *62*, 1997, pp. 1142–1149. DOI: 10.1111/j.1365-2621.1997.tb12231.x.
82. Samappito, S. – Butkhup, L.: Effect of skin contact treatments on the aroma profile and chemical components of mulberry (*Morus alba* Linn.) wines. African Journal of Food Science, *4*, 2010, pp. 52–61. <http://www.academicjournals.org/app/webroot/article/article1380706019_Samappito%20and%20Butkhup.pdf>
83. Schreier, P. – Drawert, F. – Steiger, G. – Mick, W.: Effect of enzyme treatment of apple pulp with a commercial pectinase and cellulase on the volatiles of the juice. Journal of Food Science, *43*, 1978, pp. 1797–1800. DOI: 10.1111/j.1365-2621.1978.tb07417.x.
84. Kalua, C. M. – Boss, P. K.: Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). Journal of Agricultural and Food Chemistry, *57*, 2009, pp. 3818–3830. DOI: 10.1021/jf803471n.
85. Marti, M. P. – Mestres, M. – Sala, C. – Busto, O. – Guasch, J.: Solid-phase microextraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 7861–7865. DOI: 10.1021/jf0345604.
86. Fang, Y. – Qian, M. C.: Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. Journal of Agricultural and Food Chemistry, *54*, 2006, pp. 8567–8573. DOI: 10.1021/jf061396m.
87. Jackson, R. S.: Sensory perception and wine assessment. In: Jackson R. S. (Ed.): Wine science. San Diego : Academic Press, 2008, pp. 641–685. ISBN 978-0-12-373646-8. DOI: 10.1016/B978-012373646-8.50014-7.

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