Methoxypyrazines in Sauvignon Blanc Wines, Detection of Addition of Artificial Aroma

Aleš RAJCHL, Helena ČÍŽKOVÁ, Michal VOLDŘICH, Dobromila LUKEŠOVÁ and Zdeňka PANOVSKÁ

Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

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Methoxypyrazines are the principal aroma components responsible for the vegetative and herbaceous green bell-pepper flavour of Sauvignon blanc wines produced in Moravia. The aroma profiles of 8 samples of Moravian Sauvignon wines were analysed; the levels of 3-isobutyl-2-methoxypyrazine varied in the range of 4.7–17.0 ng/l. The commercial Sauvignon aroma preparation Aroma Fantasia S, available in the region, was analysed; the product contained the 3-isobutyl-2-methoxypyrazine, and in negligible concentrations also anethol and ethylbenzoate. The Moravian Sauvignon blanc wine samples spiked with different amounts of aroma preparation were evaluated by hedonic sensory analysis, to estimate the meaningfulness of such illegal improvement. The most preferred concentration ranged from 5 ng/l to 10 ng/l, which are the natural levels of MP in Moravian Sauvignon blanc wines, therefore the addition of aroma at this level, which can be detectable with difficulties, has no reason. The less sophisticated adulteration of wine, such as the production of artificial Sauvignon blanc wine by the addition of MP into less distinctive wines, is easily detectable according to the aroma profile.

Keywords: methoxypyrazines; SPME; wine; Sauvignon blanc; adulteration

Wine is an adulterated food commodity very often. The non-routine, analytical control of wine and wine-based drinks must be addressed to a series of problems very different in nature, e.g. the search for additives, particularly stabilisers, the identification of sugars other than those coming from grapes and the determination of alcohol derived from sugars other than those contained in grape (Bononi & Tateo 1995). The more sophisticated ways of adulteration also include the addition of aromas, e.g. spices, essential oils, herbal extracts, synthetic linalool, or artificial aromas, which are sometimes added to wine to enhance or change the natural aroma of the product. The addition of such compounds is illegal in most cases, except for the group of "aromatised wines" (vermouth, americano).

The addition of coriander essential oil or aroma extracts into muscat wine was easily detectable due to the presence of compounds foreign to muscat wines, namely camphor. Since elder flowers have the aroma composition similar to that of muscat wines, the detection of elder extract addition was not always possible even when added in large amounts (5 g/l), however, the ratio of some terpenes present in both elder flowers and muscat wines

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(trans-pyranlinalool oxide, citronellol, geraniol, nerol) can be a useful indicator in aromatised muscat wine evaluation (Mazza & Ubigli 2001). The separation of volatile compounds on a chiral cyclodextrin stationary phase combined with gas chromatographic/mass spectrometric analysis is a rapid, reliable technique for profiling chiral aroma compounds in "suspicious" samples of wines. Several enantiomeric terpenes, esters, alcohols, norisoprenoids, and lactones were identified in flavoured wine and malt beverages. As an example, linalool isomeristion to α-terpineol follows a specific mechanism leading to a chiral compound and allows the addition of the R(–) isomer or racemic linalool in to musts and wines to be detected, since only the S(+) isomer of linalool is present in grape musts (Moruno 1999).

Sauvignon Blanc, the second most popular white wine variety in the world (after the cv. Chardonay), is distinctive and one of the varietal wines to be recognised easier by its often sharp, aggressive smell. This typical vegetative and herbaceous green bell-pepper or nettle flavour is based on the presence of methoxypyrazines (MPs), which are typical also for the flavours of other Cabernet wines, e.g. the cvs Cabernet Sauvignon, Cabernet franc, but to the aroma of Sauvignon blanc wine they contribute significantly (Sala *et al.* 2002).

The most important MPs in wines are 3-isobutyl-2-methoxypyrazine (IBMP), 3-sec-butyl-2-methoxypyrazine (SBMP), and 3-isopropyl-2-methoxypyrazine (IPMP). IBMP, SBMP and IPMP have extraordinarily low sensory thresholds in wine, which are generally reported at low ng/l levels. In contrast, the concentrations of grape-derived MPs in grapes and wines can be well above their sensory thresholds. IBMP concentrations have been recorded at 307 ng/l and 56.3 ng/l in grapes and wines, respectively. IPMP amounts have been up to 48.7 ng/l in grapes and up to 4.5 ng/l in wines. The highest reported concentration of SBMP in grapes and wines is 11.2 ng/l. The grape maturity at the harvest and sunlight exposure is the main factor affecting the levels of MPs in wines. Even though IBMP is generally more abundant than IPMP and SBMP in grapes and wine, high IPMP concentrations are also considered undesirable in most wines and have been described as having a 'pea-asparagus' type aroma (Kotseridis *et al.* 2008).

The artificial addition of MPs into wine was also described in literature (Morfia 2007). The most "famous" case was in 2004 in South Africa. The MPs was added into Sauvignon Blanc wine in order to increase the intensity of the typical flavour. The extensive testing of Sauvignon Blanc wines produced in 2004 were induced by several complaints about the wine with atypical flavour. The tests proved the use of illegal flavorants (MPs) by two big winemakers. The proof of MPs addition was also based on the comparison of volatiles concentration (monoterpenes, C13-norisoprenoids and 3-isobutyl-2-methoxypyrazine (IBMP)) in grapes, must and wine. The scandal arose when the levels of IBMP found in bottled wine did not correspond with those in the grape juice (Morfia 2007).

Another artificial increase of MPs concentration in wine was caused by insects. When *Harmonia axyridis* (multicoloured Asian lady beetles) are inadvertently incorporated in with the grapes at harvest, elevated MP concentrations are found; concurrent with this incorporation is the off-flavour known as ladybug taint (Pickering *et al.* 2006).

Recently, the matter of wine adulteration by the addition of aroma flavourings has been increasingly discussed among the viticultural authorities in the region. The flavour of wine can be modified by available aroma preparations of unknown composition, which are often originally intended for the fortification of beverages other than wine. On the market, aroma concentrates are available with Sauvignon, Chardonnay, Muscatel, and Muller Thurgau flavours. The fortification of wines with these concentrates would result in increased or absolutely modified wine variety aromas. The consequence of such an adulteration would involve not only the deception on the consumer but also negative impacts on the regional high quality wine producers and on the general Czech wine industry reputation.

The aim of the study present was to analyse the aroma profiles of Moravian Sauvignon wines and to evaluate the possibilities to detect the adulteration of those wines by the addition of artificial flavourings.

MATERIAL AND METHODS

Samples. Eight samples of Moravian Sauvignon blanc wines were analysed.

Commercial Sauvignon aroma preparation Aroma Fantasia S (Zan Aromi s.r.o. Brno, Czech Republic, recommended dilution 1:1000) was purchased from the market.

Artificially fortified model wines were prepared by diluting the aroma preparation in five samples of methoxypyrazines-free wines Green Veltliner (in the range from 1:1000 to 1:1 000 000).

Reagents and solutions. The standard reference compound of SBMP: 2-methoxy-3-(1-methylpropyl)-pyrazine (purity > 90%), IBMP: 2-isobutyl-3-methoxypyrazine (purity > 99%), and internal standard 3-octanol (purity > 95.0%) were purchased from Sigma-Aldrich Chemie GmbH. (USA). The standard solutions were prepared in distilled water, ethanol (12% v/v) and tartaric acid solution (1 g/l).

HS-SPME procedure (Vas *et al.* 1998; Hartmann *et al.* 2002; Wampfler & Howell 2004). 100 µm polydimethylsiloxane fibre (Supelco Inc., Belefonte, USA) was inserted into the headspace of 22 ml vial filled with 10 ml of sample and 3 g of NaCl, which was agitated at 900 rpm. The optimised extraction conditions were: 30 min at 25°C

Gas chromatography condition. The sample analyses were performed on an Agilent Technologies (GC 6890N), equipped with a mass detector (MS 5973) and DB-5MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The split (1:10) GC inlet was maintained at 250°C and desorption time of 2 min was used. The carrier gas (He) flow was 1.2 ml/minute.

For the evaluation of the characteristic aromagrams of Moravian Sauvignon blanc wines, the following temperature programme was used: 50°C (held 5 min), ramped at 5°C/min to 150°C, ramped at 10°C/min to 220°C (held 2 min).

For the determination of MPs, the following temperature programme was used: 50°C (held 5 min), ramped at 5°C/min to 110°C, ramped at 30°C/min to 220°C (held 2 min), the SIM mode (*m/z* 165, 151, 138, 124) was used for the quantification.

Sensory analysis. The test room was equipped according to the requirements of the international standard (ISO 8589 – Sensory analysis – General guidance for the design of test rooms). The samples were divided into two groups, the concentrations were in group A 1, 5, 10, and 50 ng/l of IBMP, in group B 5, 10, 15, and 20 ng/l of IBMP. The samples were evaluated by the same assessors. The assessors evaluated the pleasantness of green odour and that of green taste using the rank test. The samples from each group tested were served at a session, each time 25 ml of the sample in a 50 ml coded beaker. The samples for odour evaluation were placed in 50 ml coded flasks with stoppers. The temperature of all serving samples was 20°C. The samples were neutralised with water, cheese, and bread. The sample serving was in agreement with the respective international standard (ISO 6658 – Sensory analysis – Methodology – General guidance). The rank tests were performed using the international standard (ISO 8587 – Sensory analysis – Ranking) and were evaluated after Friedman at the probability level of $P = 0.95$. The data were calculated using the software STATVYD Version 2.0 beta.

The sensory evaluation was performed by a total of 23 panelists (19 female and 4 male) from the Faculty of Food and Biochemical Technology at the Institute of Chemical Technology in Prague (Ph.D. students, the staff of the department and students of the fourth year MSc study). The assessors were selected, trained, and monitored according to the respective standard (ISO 8586 – Sensory analysis – General guidance for selection, training and monitoring of assessors – selected assessors).

RESULTS AND DISCUSSION

SPME/GC/MS method optimisation

To evaluate the aroma profiles of Moravian sauvignon blanc wines, the SPME/GC/MS procedure was proposed and validated. The SPME/GC/MS method for the analyses of aroma profiles of wine has often been used for the analyses of aroma profiles of wine. The applications and conditions used are summarised in the literature (Sala *et al.* 2008).

Two types of fiber coating were compared (nonpolar polydimethylsiloxane, polar polyacrylate and divinylbenzene/carbowax/polydimethylsiloxane) (Figure 1) and also various conditions of the sorption, desorption, and sample preparation (sorption temperature 25°C, 35°C, 50°C) during various time durations (10, 30, 50 min), desorption temperature (220°C, 250°C) and time (2, 5, and 10 min), sample saturation with salt, and stirring (Hartmann *et al.* 2002; Wampfler & Howell 2004). The comparison of different conditions of extraction is given in Figures 2 and 3.

The optimised method described in Material and methods chapter was used. Although the described SPME procedure is not ideal for the quantification of MPs, especially of the minor MPs, the detection limit for IBMP was 1.2 ng/l, the recovery was 92%, range of linearity of 4–100 ng/l, and repeatability

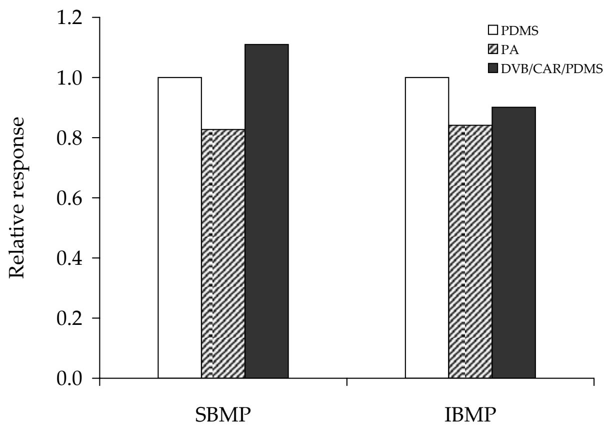


Figure 1. Performance of selected SPME fiber types on the relative response of SBMP and IBMP

was 7.3% (expressed as RSD). For the determination of IBMP is this sufficient.

Aroma profiles of Moravian Sauvignon blanc wines

Eight samples of Moravian Sauvignon blanc white wine were analysed.

The analytical method allowed a correct identification and quantification of over 40 compounds in the volatile fraction of the wine samples. The results are shown in Table 1. The majority consist of higher alcohols, ethyl esters, fatty acids, carbonyl compounds, and acetates from higher alcohols. For the compounds identification, the mass spectrum was used (matching with standard NIST library spectrum was higher then 95%). The presence of the selected compounds was verified by standard addition. The composition of volatiles of the Moravian Sauvignon blanc wine samples corresponds with the literature data (Vas *et al.* 1998; Aznar & Arroyo 2007; Hernanz *et al.* 2008; Swiegers *et al.* 2009). The aroma composition of the samples is strongly affected by the light and temperature conditions, season, and origin (Kozina *et al.* 2008), the profiles contain similar compounds in similar ratios, but the levels of MPs are high as it is expected due to the colder climate conditions.

The volatiles profile analysis can serve as a tool for the wine varietal authentication, especially as the first screening methods to sort out the suspected samples for subsequent more sophisticated analyses or to confirm the sensorial off-grade samples. Such an application of aroma profiles is limited by the procedure used (concentration of isolated compounds, separation, etc.), and mainly by the database of profiles which is available. Unfortunately, there are not very many published data dealing with the aroma profiles of individual wine cultivars and the effects of origin, season, agro technical conditions, etc. on the profiles either. However, the TIC mode is not sufficiently sensitive for the determination of some volatile components such as minor methoxypyrazines (2-methoxy-3-isobutylpyrazine) and sulphur compounds (4-mercapto-4-methylpentan-2-one), which are considered among to belong the most important Sauvignon blanc impact aroma components and are present in ng/l levels (just at or below the quantification limit of the method). But the SIM method proposed for the determination of methoxypyrazines was successfully applied to both authentic wine and spiked wine samples, the major methoxypyrazine IBMP having been detected and quantified. The

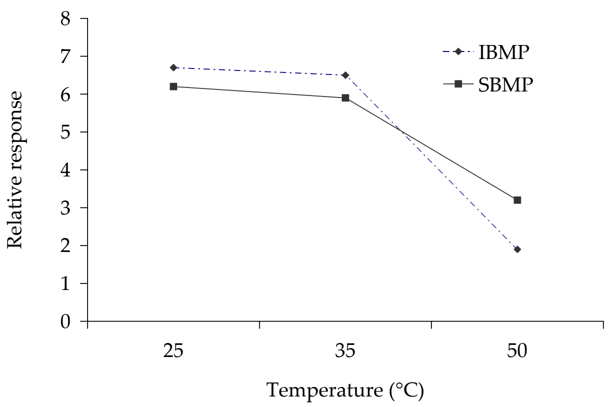


Figure 2. Effect of sample temperature on the relative response of IBMP and SBMP extracted on PDMS SPME fiber

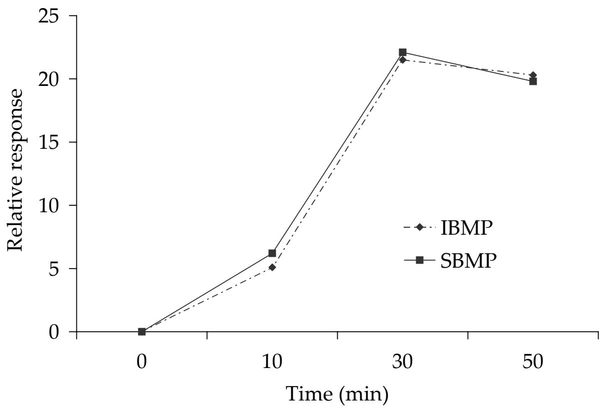
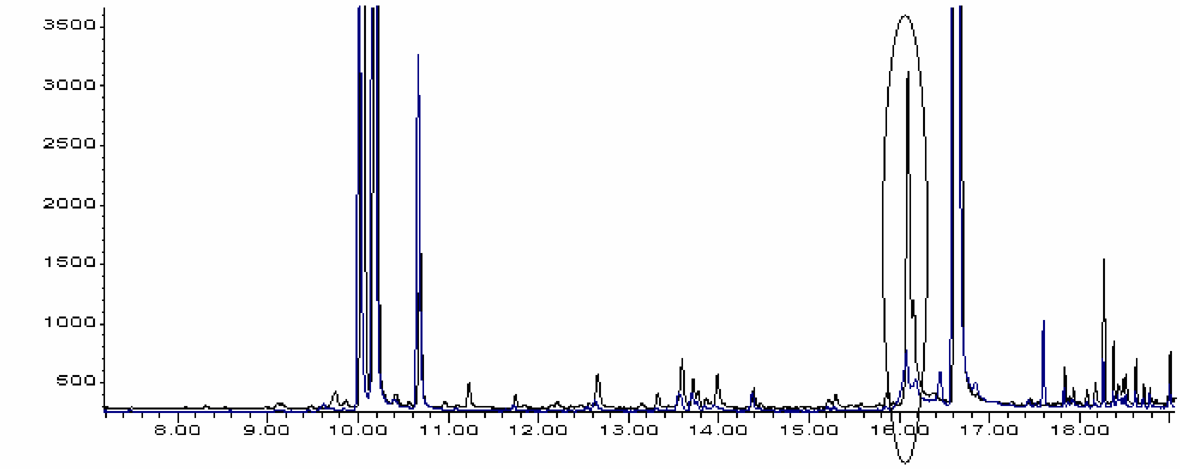


Figure 3. Effect of extraction time on the relative response of IBMP and SBMP extracted on PDMS SPME fiber

Table 1. Volatile compounds compositions of 8 samples of Moravian Sauvignon blanc wine

		RT (min)	Area		
			min	max	average
Ethyl propanoate	1	2.79	4 050	8 850	5 610
2,4,5-Trimethyl-1,3-dioxolane	2	3.01	0	67 410	13 470
1,1-Diethoxyethane	3	3.07	0	118 260	23 640
3-Methylbutan-1-ol	4	3.11	360 990	572 430	482 610
2-Methylbutan-1-ol	5	3.17	112 710	184 950	161 520
Ethyl 2-methylpropanoate	6	3.56	10 080	16 800	12 930
2-Methylpropyl acetate	7	3.84	2 910	4 440	3 480
2,3-Butanediol	8	3.96	5 100	15 120	7 980
Ethyl butanoate	9	4.40	17 130	32 790	25 080
2-Hydroxyethyl propanoate	10	4.68	7 020	26 730	16 230
Methyl 2-ethyl butanoate	11	5.58	4 620	8 550	6 060
Methyl 3-methyl pentanoate	12	5.67	6 900	12 870	9 690
1-Hexanol	13	6.06	32 940	50 520	40 770
1-Butanol-3-methylacetate (isoamylacetate)	14	6.29	34 020	218 520	93 870
2-Methylbutylacetate	15	6.36	6 840	21 660	9 930
2,2,6-Trimethyl-6-vinyltetrahydropyrane	16	8.99	3 450	10 020	5 580
1-(1-ethoxyethoxy) pentane	17	9.11	0	27 390	5 490
Hexanoic acid	18	9.29	0	4 560	2 100
Ethyl hexanoate	19	9.89	317 340	765 240	547 650
Hexyl acetate	20	10.31	4 470	55 260	21 990
Limonene	21	10.70	1 320	2 430	1 890
Ethyl 2-hexenoate	22	11.23	3 690	6 540	4 590
2-Hydroxyethyl 4-methylpentanoate	23	11.59	1 710	3 900	2 850
3,7-Dimethyl-1,6-octadiene (linalool)	24	12.88	8 460	22 140	14 550
3,7-Dimethyl-1,5,7-octatrien-3-ol	25	13.02	1 770	3 300	3 630
2-Phenylethanol	26	13.26	81 750	168 570	124 710
Methyl octanoate	27	13.64	0	8 910	3 750
3,6-Dihydro-4-methyl-2-(2-methyl-1-propyl)-2H-pyrane	28	14.50	3 690	22 740	9 270
Octanoic acid	29	15.11	0	33 180	7 710
Diethyl succinate	30	15.31	54 990	125 220	100 230
α-Terpineol	31	15.54	4650	11 460	7 770
Ethyl octanoate	32	15.79	1 751 610	4061460	2 763 420
Ethyl benzoate	33	17.09	2 550	4 290	3 300
Isopentyl hexanoate	34	17.24	3 450	5 970	5 250
2-Ethylphenyl acetate	35	17.41	10 860	30 570	18 510
Geranylethylether	36	18.28	2 640	12 300	6 900
Ethyl nonanoate	37	18.52	3 360	4 860	4 110
2-Butyloctanoic acid	38	19.88	2 550	3 630	3 030
1,2-Dihydronaftalene	39	20.01	9 990	95 820	29 430
Decanoic acid	40	20.34	0	5 160	1 710
Ethyl decanoate	41	21.14	923 280	1 827 090	1 144 500
3-Methylbutyl octanoate	42	22.40	6 840	11 610	9 570
BHT	43	24.05	4 770	27 990	14 160
Ethyl dodecanoate	44	26.00	5 280	83 790	35 880
1-Methylethyl dodecanoate	45	26.71	2 820	38 700	3 030



Figurte 4. Chromatogram (SIM) of original Sauvignon blanc wine and the same sample spiked with 0.1 mg/l of aroma preparation Aroma Fantasia S (100 ng/l of IBMP added)

SIM mode chromatogram of a sample of Sauvignon blanc wine is given in Figure 4.

IBMP contents in the analysed authentic Moravian Sauvignon blanc wine samples varied from 5 ng/l to 17 ng/l (Table 2). These results are in agreement match with those found in the literature (Lacey *et al*. 1991). Although SBMP and IPMP were identified in some of the samples, their levels were always below the quantification limits. As expected, IBMP and other methoxypyrazines were not detected in Green Veltliner wine samples.

Addition of commercial Sauvignon aroma preparation Aroma Fantasia into the authentic sauvignon blanc wine

The commercial Sauvignon aroma preparation Aroma Fantasia S, which is available in the region, contains 3-isobutyl-2-methoxypyrazine (IBMP), and also anethol and ethylbenzoate in negligible concentrations. The addition of the preparation according to the producer's recommendation (1:1000) usually leads to an unacceptable flavour and a very intensive rise of herbaceous green bell-pepper or nettle flavours (see sensory section below), therefore the aroma was added on a lower level of approx. 1:100 000 more acceptable for the sensory properties. An example of a chromatogram of original Sauvignon blanc wine and the same sample spiked with 0.1 mg/l of aroma preparation is shown in Figure 3. The detection of artificial aroma preparation in wine depends on the amount added; the adulteration is detectable when the concentration of IBMP is peculiar. The concentrations of other compounds in the preparation are very low to be detected in adulterated wine.

Sensory effect of IBMP addition

The sensory effect of low levels of artificial aroma addition was investigated using the rank sensorial tests. According to the literature, the concentrations of IBMP and IPMP correlate positively with the perceived green flavours, and inversely with the perceived ripe and fruity flavours. The nuances perceived in the model studies varied from dusty, grassy, to green pepper. It was confirmed that IBMP and methoxypyrazines are important impact components and that their contributions, either positive

Table 2. Concentrations of 3-isobutyl-2-methoxypyrazine (IBMP) in ng/l, in of 8 samples of Moravian Sauvignon blanc wine

Sample	Concentration of 3-isobutyl-2-methoxypyrazine (ng/l)
S 1	14.8
S 2	10.8
S 3	4.7
S 4	17.0
S 5	5.2
S 6	15.5
S 7	8.4
S 8	13.2

Table 3. Rank totals in group A and B

Added artificial aroma (corresponding to ng IBMP /l)	Group A				Group B			
	1	5	10	50	5	10	15	20
Rank sums of pleasantness of odour	41	32	43	54	35	41	56	58
Rank sums of pleasantness of taste	47	29	40	54	36	44	52	58

or negative, varied according to the medium type and composition, as well as to the concentration levels of the components (Allen *et al.* 1991; Marais & Swart 1999; Parr *et al.* 2007).

The preliminary sensory evaluation was done with IBMP spiked samples of Moravian Sauvignon blanc wines, but the hedonic evaluation of the samples with increasing contents of MPs did not lead to consistent results. The assessors were not able to recognise the samples with different concentrations of IBMP because their sensitivity decreased after the first sample spiked with the amount of 1 ng/l of IBMP, probably due to the presence of natural MPs. Therefore, the model samples for the determination of optimal concentration of MPs in wine were prepared using wine cultivars of less typical flavour. Two sets of samples with four variants each were prepared by the addition of artificial aroma to methoxypyrazines-free variety of wine Green Veltliner. Group A contained samples fortified with the aroma preparation corresponding to IBMP concentrations from 1 ng/l to 50 ng/l. Ranking of these samples give the rank sums which are shown in Table 3. The differences are statistically significant when the calculated Friedman value is greater than the critical value (for four samples the critical value is $\chi^2 = 7.81$). The calculated Friedman value (by software STATVYD Version 2.0 beta) for the pleasantness of odour was 8.65, and for the pleasantness of taste 12.04. The significant differences of the pleasantness of odour at the probability level of $P = 0.95$ were between the concentrations 1 ng/l and 50 ng/l, and between 5 ng/l and 50 ng/l of the spiked IBMP. Significant differences of the pleasantness of taste at the probability level of $P = 0.95$ were between the concentrations 1 ng/l and 5 ng/l, and between 5 ng/l and 50 ng/l of the spiked IBMP. The sample with the lowest rank sum was the most pleasant sample for the assessors.

The most pleasant concentration for the added artificial aroma was 5 ng/l for both taste and odour too. The highest concentration, 50 ng/l, was evaluated as unpleasant, therefore group B of model samples was prepared with the concentrations of IBMP in the range from 5 ng/l to 20 ng/l. The rank sums and significant differences for group B are shown in Table 3. The calculated Friedman value for pleasantness of odour was 12.03, and for pleasantness of taste 8.68. The significant differences of pleasantness of odour and taste at the probability level of $P = 0.95$ were the between concentrations 5 ng/l and 15 ng/l, and between 10 ng/l and 20 ng/l of spiked IBMP.

The results of the sensory evaluation of the group B model samples confirmed that the most pleasant odour and taste occur with the concentration of IBMP in the range from 5 ng/l to 10 ng/l, higher concentrations were unacceptable, or evaluated as worse.

The findings show, that the spiking of methoxypyrazines-free wines with the concentrations of IBMP from 5 ng/l to 10 ng/l was the most acceptable for the majority of assessors. We concluded in the above paragraph that a small addition of Aroma fantasia S preparation into the authentic Moravian Sauvignon wine could be detected with difficulties only, but such an addition seems to be groundless, because the concentrations of MPs in these wines are actually within the optimal range (5–17 ng/l) and any addition will worsen the flavour. On the other hand, when the aroma preparation is added into wines of less intensive flavour, or "artificial" Sauvignon or other wines (like Greener Veltliner), this addition is easily detectable according to the aroma profile only.

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Corresponding author:

Ing. Aleš Rajchl, Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie, Technická 5, 160 28 Praha 6, Česká republika
tel.: +420 220 443 024, fax: + 420 233 337 337, e-mail: ales-rajchl@vscht.cz