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Atypical aging off-flavour and relation between sensory recognition and 2-aminoacetophenone in Croatian wines

Netipična aroma starenja u hrvatskim vinima i odnos između senzorne prepoznatljivosti i sadržaja 2-amino acetofenona

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ABSTRACT

Atypical aging off-flavour (UTA) is not a common wine fault but it is still an interesting topic, due to the complexity of the occurrence, chemical markers, and sensory descriptors. Although 2-aminoacetophenone (AAP) is the main chemical marker, an increase in AAP concentration does not necessarily result with the higher sensory expression of UTA. The goal of this study was to investigate the presence of UTA and the link between UTA olfactory perception and AAP concentration in Croatian wines. All the samples that were rejected due to the UTA in sensory testing during the certification procedure for the market during 2013 - 2017 were included in research. The relevant data of origin were analyzed to filter out potential factors of UTA appearance. Sensory analyses were performed with the „100 points" and the „Yes/No" methods, and AAP analyses were done by GC-MS. The appearance of UTA may be associated with the regional origin and extremely poor climatic conditions of a particular year. The results of AAP analyses corroborated the presence of UTA in all samples; the concentrations of AAP were 0.3 - 4.4 µg/L. A respective correlation between sensory recognition and the concentration of AAP was specified. This is the first study of sensory detection of UTA and the interconnection with 2-AAP in Croatian wines. The results indicate the need to detect as many volatiles as possible, to explain the difference in olfactory perception of this fault.

Keywords: Atypical aging off-flavour, sensory recognition, 2-AAP, Croatian wines

SAŽETAK

Netipična aroma starenja (UTA, ATA) nije česta mana vina, ali je stalno zanimljiva tema zbog kompleksnosti čimbenika pojave, kemijskih markera i senzornih atributa. Iako je 2-aminoacetofenon (AAP) glavni kemijski marker, s povećanjem njegove koncentracije u vinu ne mora nužno rasti i senzorna izraženost ove mane. Cilj istraživanja bio je analizirati prisutnost i vezu između senzorne prepoznatljivosti UTA-e i sadržaja AAP u hrvatskim vinima. Istraživanje je provedeno u razdoblju 2013.–2017. Obrađeni su podaci senzornih ispitivanja uzoraka koji su odbačeni zbog UTA-e u postupku kontrole kakvoće prije trženja. Provedena je analiza svih relevantnih podataka o podrijetlu i kakvoći uzoraka radi ekstrakcije mogućih čimbenika pojave UTA-e. Senzorne analize provedene su metodama "100 bodova" i "Da/ne", a analiza 2-AAP GC-MS tehnikom. Obzirom na grupiranje uzoraka, pojava UTA-e može biti vezana uz vinogradarsko područje i klimatske uvjete određene godine. Vrijednosti sadržaja AAP potvrdile su prisutnost mane u ispitivanim uzorcima; koncentracije AAP bile su između 0,3 µg/L i 4,4 µg/L. Dobivena je korelacijska veza između senzorne prepoznatljivosti i koncentracije AAP Ovo je prvo istraživanje senzorne dijagnostike UTA-e i povezanosti s 2-AAP u hrvatskim vinima. Rezultati istraživanja upućuju na potrebu analize što većeg broja hlapljivih spojeva radi sigurnijeg objašnjenja različitih mirisnih percepcija uzoraka s vrlo sličnim koncentracijama ključnih spojeva, kao što je AAP.

Ključne riječi: Netipična aroma starenja, senzorna prepoznatljivost, 2-AAP, hrvatska vina

INTRODUCTION

Atypical aging off-flavour ("Untypische Alterungston", UTA) is a relatively new problem in white wines; the first data are related to German research in the late 1980s (Raap et al., 1993; Christoph et al., 1995). This problem is known in wine countries around the world as a fault of white wines and it is characterized by the loss of typical varietal aroma in young wines. Modified unpleasant off-flavour is described as the smell of naphthalene, acacia blossom, old wet wax, and even as a fox ton of hybrid grape (Rapp et al., 1993). The occurencce of UTA is related to stressful conditions in the vineyard; various climatic, pedological, and agrotechnical factors. Due to the complexity of factors that can influence UTA development and sensory expression, the actual presence of this fault is probably higher than the knowledge about it.

The main chemical marker of UTA is 2-aminoacetophenone (AAP) and its pathways are still the subject of research. AAP is formed by the degradation of indole-3-acetic acid (IAA) and L-tryptophan, whose content and conversion to AAP depend on wine composition, in relation to natural factors, the activity of yeasts, the availability of nutrients and free SO_2, and other factors (Christoph et al., 1998; Hühn et al., 1999, Hoenicke et al., 2002; Linsenmeier et al., 2007; Maslov et al., 2011; Horlacher et al., 2016). Although the IAA content can be higher in red than in white wines, UTA is still not detected in red wines and it is probably due to their specific components that can prevent the oxidative degradation of IAA to AAP (Bonerz et al., 2008). UTA symptoms can occur very early, in young wines after fermentation and the sulfiting, as well as after several months.

Different authors have found a positive correlation between AAP content and atypical aroma (Christoph et al., 1995; Schneider, 2013). However, others have obtained a very low connection between AAP and UTA (Linsenmeier et al., 2009). The recognition threshold varies from 0.5 to 1.5 μg/L AAP, and it depends primarily on wine complexity (Christoph et al., 1995; Fisher and Sponholz, 2000; Linsenmeier et al., 2009). Intensive fresh and fruity aroma can mask UTA smell even in wines with AAP concentrations of 1.5 μg/L (Linsenmeier et al., 2007). In the case of light and meager wines, the problem with off-flavour can be evident even if the AAP concentration is less than 0.5 μg/L (Christoph et al., 1995).

The occurrence of this defect in Europe is associated with cooler climates of Germany, while some authors recognized UTA in wines from different climates in different world regions, with AAP content below the recognition threshold (Cheng et al. 2004; Henick-Kling et al. 2008). In this case, wines described as UTA correlated with TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), one of the compounds responsible for the so-called petrol smell, which can also be associated with UTA. Due to insufficient knowledge, differences in the expression of defects at different stages of production, and differences in terminology, etc., confusion about UTA is possible and still happens. An example of confusion is the associating of some reductive wine compounds with an atypical aroma, which can be tested by a copper test (Rauhut et al., 2003). Therefore, it is of particular importance to define the exact descriptors to avoid confusion and substitution of atypical aging off-flavour with other defects.

The sensory attributes of UTA can be classified into two groups (Fisher et al., 2000; Schneider, 2014). The aromas in the first group are described as scents of naphthalene beads, naphthalene, soap scents, laundry detergents, furniture polishes, shoe pastes, old wax, jasmine scent, acacia blossom, lemon blossom, and dry laundry. The intensity of aroma increase with free sulfur dioxide content. The smells of wet towels, wet wool, dirty dishes, dishwashers, and dried urine are classified in the second group. These odours imply a transition to the reductive stage in sensory profiling and can make it difficult to identify the fault. In both cases, the fruit, flower or mineral variety character disappears, and the UTA symptoms dominate (Schneider, 2014). Wine loses colour over time, becomes lighter, its taste thin and empty, with a typical metallic bitterness. This aroma complexity, along with the diversity of taster's sensory knowledge, further complicates the simple determination of UTA descriptors (Fischer and Sponholz, 2000). "Model wine" with the addition of AAP will be described by aromas from the first group, such as the smell of naphthalene beads or acacia blossoms, while the aromas of the second group are difficult to simulate in wine. According to some authors (Christoph et al., 1995; Gessner et al., 1999), descriptors from the second group are related to other IAA metabolites, such as indole and skatole, although their concentrations may be below the recognition threshold. Particular attention should be given on skatole because of its dominant faeces odour (Schneider, 2014).

Thanks to ecological conditions and technological standards in grape and wine production, the risk of UTA appearance is very low in Croatia (Mihaljević Žulj et al., 2015b, Maslov et al., 2011). Some authors confirmed this assumption by monitoring of precursors and synthesis of AAP concerning the grape ripeness, soil type, and yeasts strain, in the case of white varieties Malvazija istarska and Riesling (Jakobović et. al., 2014; Maslov et al., 2011).

The certification of wines in Croatia implied a mandatory procedure, which includes chemical and sensory analysis before the market for all wines. The purpose of sensory testing is to verify compliance with the Protected designation of origin (PDO) specifications and quality concerning wine defects. This system of continuous quality monitoring before placing the wine on the market is a factor of increasing the wine quality and protecting both producers and consumers. Based on the results of the analyses it is possible to apply appropriate oenological practices to ensure the wine quality before bottling without affecting the declared parameters. Therefore, it is essential to ensure the reliability of the analytical results. In case of wine quality control in Croatia, both types of methods, chemical and sensory are acreditied. It means permanent internal and external control of results. In case of sensory testing the assessors participate in periodical interlaboratory analysis (DRRR) and they analyse internal control samples before and during the commission work. Control samples are therefore not included in this research.

This research aimed to increase the knowledge about UTA in Croatian white wines, through the analysis of the frequency of this off-flavour, and the connection between UTA sensory recognition and AAP content. All the samples that were sensory rejected because of UTA in the official control protocol for market were analysed in this study. A detailed analysis of the commissions in relation to the results of sensory evaluation and assessors was performed.

MATERIALS AND METHODS

Samples and sensory analysis

All the samples that were rejected due to the UTA in sensory testing during the certification procedure for the market during 2013 - 2017 were included in research. Technical requirements for sensory analyses were under ISO/DIS 8589 standards, in the laboratory accredited to HRN EN ISO/IEC 17025. Wines with PDO were tested by a method „100 points". The final result is a median of individual results. Wines without PDO were tested using the descriptive „Yes/No" method. The negative result had to be explained in the case of both methods (Official Gazette). The assessors indicated one or more quality descriptors from the list in software created for this purpose. The negative result is acceptable when at least 60% of assessors describe the same reason for sample rejection. Each commission (7 members) was allowed to analyze a maximum of 50 samples per day. 42 assessors participated in analyses; 18 female and 24 male. They were professionals in wine sensory testing: with the academic level of education in viticulture and/or enology and with at least 5 years of work experience in the wine sector. They participate periodically in the aptitude tests and they were participated in the "UTA training program" in 2012, at the Faculty of Agriculture in Zagreb. Control samples, wines with and without UTA were included in both, sensory and chemical analyses.

Chemical Analysis

The basic parameters of wine quality were determined according to the official methods of the International organisation OIV. 2-aminoacetophenone (AAP) was analyzed according to Mihaljević et al. (2015a). For DI-SPME analysis, 15 mL of wine and 3 g of NaCl spiked with 1 µg/L of 2-nitroacetophenone as internal standard were placed into 15 mL vials and tightly capped with polytetrafluoroethylene-silicon septum. After equilibration at 30 °C for 5 min with stirring at 800 rpm, the SPME fiber was exposed to the sample for 45 min, removed, and inserted into GC-injection port. Determination of AAP was performed by GC HP6890 coupled to a mass spectrometer 5973N (Agilent, Palo Alto, USA). The carrier gas employed was helium at 1 mL/min constant flow. The oven temperature program was 40 °C for 2 min, raised to 250 °C a to a rate of 6 grad/min, and maintained for a further 10 min. The separation was achieved on a fused silica capillary column (ZB-5ms Phenomenex, USA), length 30 m with 0.25 mm internal diameter, and 0.25 µm film thickness. Injector temperature was 230 °C. Desorption was made in splitless mode using 0.75 mm i.d.liner. The mass spectrometer was operated in the electron impact mode with the following conditions: the transfer line was 280 °C, the voltage was 70 eV and the solvent delay was 6 min. The source temperature of 230 °C and quadrupole temperature of 150 °C were selected. Compounds were measured in the SIM-mode with different time windows. The following m/z ratios were monitored: for 2-AAP m/z 120 (quantifier ion) and m/z 135 (qualifier ion), and for 2-nitroacetophenone as internal standard, m/z 150 (quantifier ion) and m/z 165 (qualifier ion).

Data analyses

The data were analysed in Microsoft Excel, 2017 (Microsoft Office, Redmond, WA, USA), and the results of the sensory evaluation were analysed in PanelCheck software (www.panelcheck.com). In addition to actual points, the results were transferred to normalized values too. Each score consistent with the final score was considered a "0", and deviations were normalized in the range ±1- ± 3, according to the standardized score ranges for the individual quality group and Traditional Terms. The ranges of points were as follows: 1-59, 60-71, 72-81, 82-100. In the case of the „Yes/No" method, the normalized values were 0 (correct result) and 1 (incorrect result).

RESULTS AND DISCUSSION

Considering the results of sensory quality control in white wine certification, it can be assumed that UTA is not a problem in Croatian wines. In the period 2013-2017, only 0.001% of all white white samples, and 0.8% of all samples with faults were rejected because of UTA.

The data of origin and quality of samples that are rejected because of UTA are given in Table 1. The important information, such as variety, vintage, climatological zone, and PDO of samples were presented to assessors before sensory testing of each sample. All results of basic chemical parameters were within the legal limits and indicated correct basic quality. Eighty percent of samples had a volatile acidity of 0.4 g/L and less, and an average total SO_2 content of 153 mg/L. However, the concentrations of free sulfur dioxide in samples were lower than recommended doses for white wines, especially for the years with earlier harvests and poor grape quality. Only a few samples were sulphited as recommended. It was found in previous research that free SO_2 in concentrations above 25 mg/L prevents UTA (Gafner, 2002). The samples from the vintage 2014 and from the zone B were characterized with the high total acidity, with lower pH, and lower alcohol content in comparison to other vintages from this study.

Table 1. The main quality parameters and description of the samples with UTA

Sample	Varieties, DOP[A]	Zone[B]	Vintage	Alcohol[C] (% vol)	Reducing sugars (g/L)	Total acidity[D] (g/L)	Volatile acidity[E] (g/L)	SO_2 total (mg/L)	SO_2 free (mg/L)
1	Blend[F]	B	2011.	12.0	3.8	6.6	0.4	152	14
2	Blend	B	2011.	11.9	19.8	7.3	0.3	169	32
3	Blend	B	2012.	12.7	1.8	4.3	0.5	196	5
4	Blend, PDO[G]	B	2012.	11.2	1.9	5.7	0.2	194	20
5	Blend	B	2013.	12.1	3.5	7.9	0.3	159	20
6	Chardonnay, PDO	B	2013.	12.1	10.8	6.7	0.3	183	26
7	Maraština, PDO	C II	2013.	12.8	2.0	6.0	0.3	87	12
8	Graševina, Moslavac	B	2014.	11.0	1.7	9.3	0.4	169	52
9	Graševina, PDO	B	2014.	13.0	1.9	7.2	0.5	109	16
10	Graševina, Riesling	C I	2014.	13.0	2.8	9.5	0.3	112	15
11	Graševina, PDO	B	2014.	10.6	1.2	7.1	0.4	117	40
12	Graševina	B	2014.	11.0	2.8	7.5	0.3	97	17
13	Riesling, PDO	C I	2014.	12.4	11.4	8.5	0.4	141	17
14	Riesling, PDO	B	2014.	12.1	8.7	7.6	0.5	181	10
15	Blend, PDO	B	2014.	10.1	1.8	8.3	0.3	83	12
16	Graševina, Riesling	B	2014.	12.2	2.1	6.5	0.3	149	14
17	Blend	C I	2014.	11.8	2.6	6.8	0.4	178	21
18	Blend	C I	2014.	11.8	3.9	5.8	0.5	178	19
19	Blend	B	2014.	11.6	3.6	6.4	0.4	152	11
20	Blend	C II	2014.	12.6	4.8	5.5	0.6	108	12

[A]: DOP: Denomination of protection, [B]: Climatological zone, according to Commission Regulation (EC) No 1308/2013, [C]: Actual alcoholic strength, [D]: g/L, as tartaric acid, [E]: g/L, as acetic acid, [F]: Blend of white varieties, [G]: PDO: Protected designation of origin.

Most of the samples are from the harvest of 2014 and the "Sjeverozapadna Hrvatska" subregion in Croatia (Table 1). This area belongs to climatological zone B, according to the Winkler index and the classification of wine-growing climate zones, adopted for the regulation of oenological practices in the EU. Taking into account climatic conditions conserning zone B, we can discuss the presence of UTA in relation to the stress variables caused by weather conditions. The weather-related vine stress conditions included a short duration of sunshine that was below the minimum 1500 hours required during the vine vegetation period, and very wet and extremely wet conditions prevailed in August and September (DHMZ).

Assessment of climate anomalies in Croatia for 2014 shows significant anomalies in temperatures and precipitation compared to perennial averages. Figure 1 quantifies precipitation anomalies in 2014 for climatological stations across the country with respect to the referent 30-year period. It shows that about 70% of the country was extremely wet (upper 2 percentile), 25% of the country was very wet (91-98 percentiles) while 5% of Croatia (the South Adriatic islands) was wet. Extreme climatic conditions in 2014 for the Varaždin station (climatological zone B) are presented in Figure 2, with deviations defined with respect to the 1981-2010 period. Previous authors have found a strong influence of

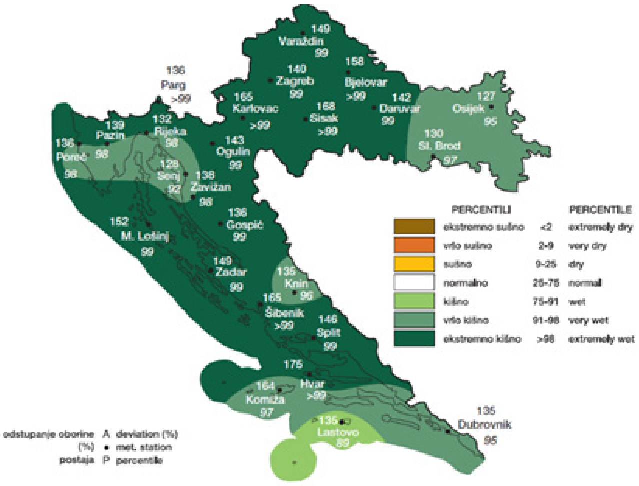


Figure 1. Annual precipitation amounts, in percentages of multiannual mean, period 1961-1990, Croatia, the year 2014 (DHMZ)

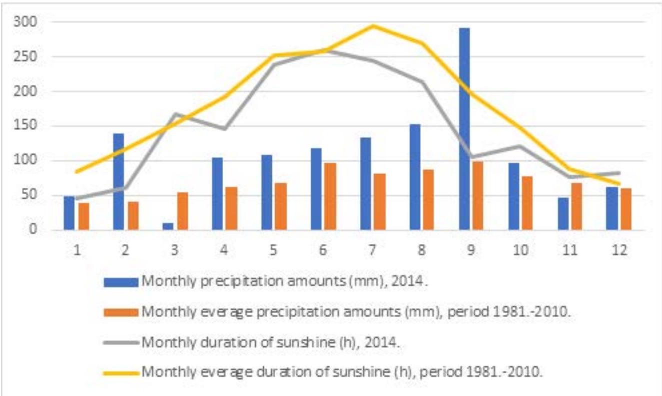
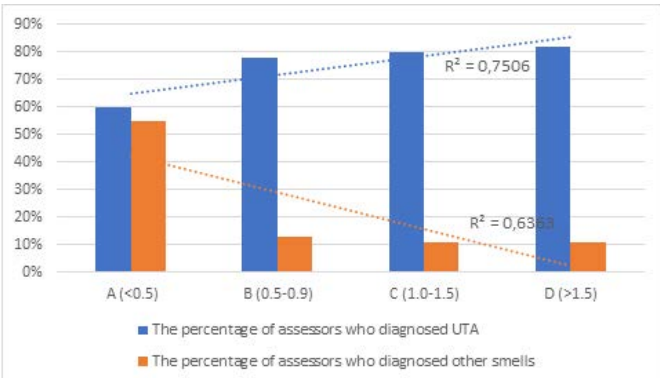


Figure 2. Monthly duration of sunshine (h) and monthly precipitation amounts (mm), the year 2014 compared to the period 1981-2010, station Varaždin (zone B)

the vintage on UTA (Linsenmeier et al., 2007, 2009). Apart from weather conditions and other factors such as yield (Linsenmeier et.al., 2007, 2007a), earlier harvest (Kohler et al., 1995; Schwab et al., 1999), permanent green cover, N supply, and plant disease have been found to preclude the formation of UTA (Schneider, 2013).

In the case of 2014 and climatic conditions in Croatia, in particular climatological zone B, the cumulative influence could be the trigger for the synthesis of AAP and other compounds related to UTA. Continuous rain delayed the physiological path of maturation, sugar accumulation, and provoked symptoms of infection with Botrytis. The harvest 2014 was characterized by high yields, and technologically immature grapes, with poor aromatic maturity. Both of these factors have been found to play an important role in UTA occurrence (Schwab et al., 1999). Stress factors that can cause AAP synthesis are more pronounced in climatic conditions with extremes, either humid or arid (Rapp et al, 1993). Muller (1999) reported that the occurrence of UTA in Europe and the connection with AAP is associated to cooler climates. Although the vine is a very adaptable plant species, severe climatic stress can alter grape quality potential. Stressful climatic conditions with consequences to wine quality can be long-term, but also short-term as was the case in this observed five-year period with one extreme year.

The percentage of the assessors who described UTA and corresponding AAP concentrations are presented in Table 2. The concentrations of AAP in samples with off-flavour ranged from 0.3 to 4.4 µg/L. The samples are classified A, B, C and D (Figure 4), based on the previous research of detection thresholds 0.5-1.5 µg/L, and correlation between AAP concentrations and sensory recognition of UTA (Christoph et al., 1995; Fan et al., 2007; Linsenmeier et al., 2007; Rauhut et al., 2007). Each sample is accompanied by the percentage of assessors who diagnosed the UTA. The samples with less than 0.5 µg/L AAP are in serie A and UTA was diagnosed by 60% of all assessors who participated in analyses. However, it has to be taken into account that other negative odours were associated with these samples, and 55% of assessors described the smell of mold (Figure 3).



A, B, C and D: Classification of the samples in relation to AAP concentration (µg/L) and literature detection thresholds of UTA: : A:≤0,5 (4 samples,); B:AAP 0,5-0,9 (10 samples,); C:AAP 1,0-1,5 (3 samples), and D:AAP≥1,6 (3 samples). Total number of assessors: 17 assessors in serie A, 38 assessors in serie B, 13 assessors in serie C, and 9 assessors in serie D

Figure 3. The relation and correlation between AAP concentration (µg/L) and level of sensory diagnosis of UTA (%), and between AAP concentration(µg/L) and sensory diagnosis of other smells (%)

Table 2. The concentrations of AAP (µg/L) and percentage of assessors who diagnosed UTA, and classification of samples in relation to these two variables

Sample	AAP (µg/L)	UTA detection[A]	Serie[B]
1	0.3 ± 0.02	60 %	A
2	2.1 ± 0.04	100%	D
3	0.5 ± 0.01	60 %	B
4	1.0 ± 0.02	60%	C
5	1.1 ± 0.03	100%	C
6	0.5 ± 0.01	80 %	B
7	0.3 ± 0.02	60 %	A
8	0.6 ± 0.02	80%	B
9	0.4 ± 0.03	60 %	A
10	0.6 ± 0.01	80 %	B
11	0.5 ± 0.01	80 %	B
12	0.5 ± 0.01	100 %	B
13	4.4 ± 0.04	80%	D
14	2.6 ± 0.04	80%	D
15	0.4 ± 0.01	60 %	A
16	0.6 ± 0.01	80 %	B
17	0.8 ± 0.03	60%	B
18	0.9 ± 0.01	80%	B
19	1.3 ± 0.01	80%	C
20	0.6 ± 0.01	60%	B

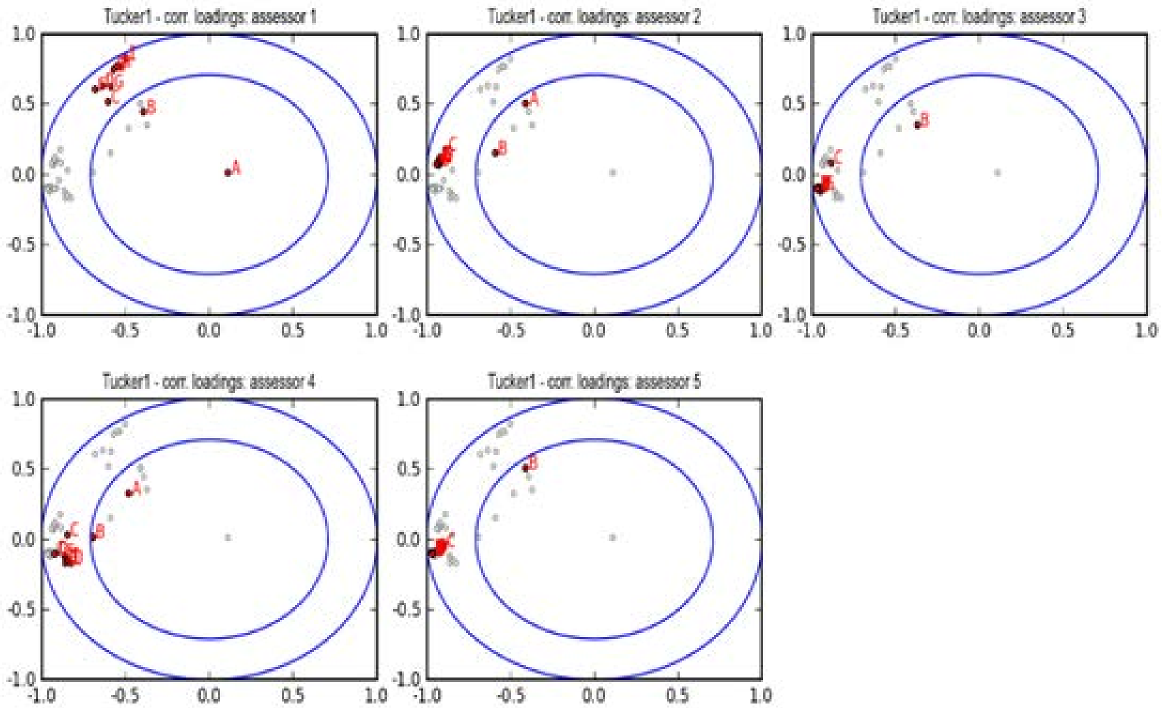
[A]: The percentage of assessors who diagnosed UTA. The number of assessors per serie was: 17/serie A, 38/serie B, 13/serie C, and 9/serie D. [B]: Classification of the samples in relation to AAP concentration (µg/L) and literature detection thresholds of UTA: A::≤0,5; B:AAP 0,5-0,9; C:AAP 1,0-1,5, D:AAP≥1,6

To discuss this results, it should be necessary to know the concentrations of mold chemical markers and more details about the aroma composition. It was probably a synergistic effect of different volatiles here when the final off-flavour is caused by several different compounds and the impression of UTA was probably enhanced with some other volatiles. The samples in serie B have the concentration of AAP between 0.5 and 0.9 µg/L, and they were rejected by 78% of the assessors. With the increase in the concentration of AAP, the recognition of UTA also increased, but there was no significant difference in the UTA recognizability between the series B, C, and D. 80% of assessors in serie C (AAP concentration between 1.0 and 1.5 µg/L) and 82% of assessors in group D (AAP concentration above 1.6 µg/L) detected UTA. The moldy and mercaptan smells were also detected in some samples that belong to series B, C, and D (Table 2).

The relation between the AAP concentrations and the recognizability of UTA was confirmed through the high correlation coefficient R^2=0,75 between the percentage of assessors who diagnosed UTA and the coresponding concentrations of AAP (Figure 3).

The expression of UTA in this study can also be observed through the frequency of other sensory attributes. The occurrence of other atributes in samples with higher concentrations of AAP can not be neglected, but only 12% of all assessors found some other smells that can be easy checked with chemical analyses. As the concentration of AAP in the samples increased, the occurrence of other attributes decreased, and a correlation coefficient R^2 0,64 was calculated between the percentage of assessors who described other atributes and the coresponding concentrations of AAP (Figure 3).

According to previous studies, there is a large range of AAP recognition thresholds, from 0.5 µg/L (Christoph et al., 2005; Fan et al., 2007) to 2 µg/L (Hoenicke et al., 2002). The detection of UTA depends on the complexity of wine and a higher concentration of AAP does not necessarily respond with the recognition level of the off-flavour (Fan et al., 2007; Linsenmeier et al., 2007, 2007a). The final aroma of a wine depends on many factors and it is unique to each wine (Styger and Bauer, 2011). In this study, the originl wines from production were tested and the off-flavour could be the result of a disturbance of balance of the various compounds constituting the wines aroma. The complexity of the matrix, especially the composition of the volatiles can affect the perceived aroma and flavour (Martineau et al., 1995; Escudero et al., 2004; Chassange et al., 2008; Villamor and Ross, 2013; McKay et al., 2018). The same compound, in the same concentration, is usually expressed differently in different wines (Villamor



Legend: Letters from A to J: parameters of "100 points" method (A: brillance,visual, B: colour, C: nose correctness, D: nose positive intensity, E: nose quality F: taste correctness, G: taste positive intensity, H: taste persistence, I: taste quality, J: harmony,overall perception)

Figure 4. Tucker1 plot for the commission that tested samples 13 and 14

et al., 2013). Le Berre et al.. (2007) have presented how a reduction in alcohol can affect the perceived wine aroma, especially by reinforcing perceptual interaction between woody and fruity wine odorants. Some authors found that blending effects have a severe influence on the final odor character of a mixture (Brodin et al., 2007; Le Berre et al., 2008). Perry and Hayes (2016) also obtained significant differences in the recognition of AAP and methyl anthranilate, depending on the matrix. In this study, the originl wines from production were tested and the off-flavour could be the result of a disturbance of balance of the various compounds constituting the wines aroma.

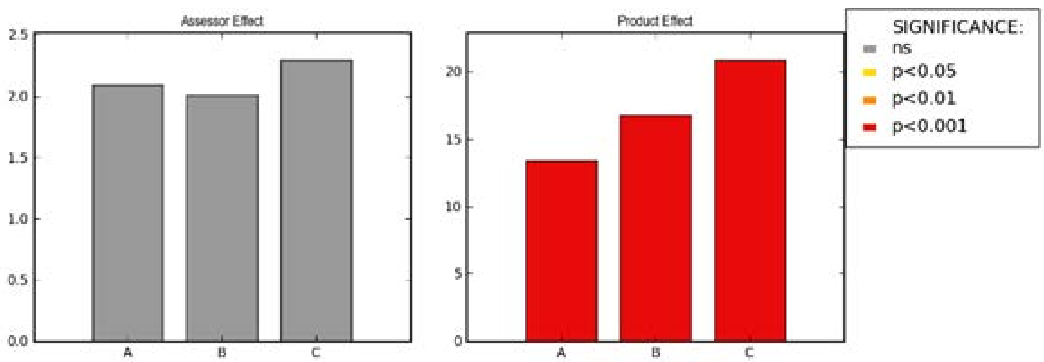
It was important to single out and discuss two samples with the highest AAP concentration in this study; both samples were Riesling wines and in addition to the off-flavour, the assessors find a mercaptan smell. These results correspond with previous studies where the UTA recognition threshold in Riesling was significantly higher in comparison to other varieties (Christoph et al., 1999; Schneider, 2013). In previous research (Cheng et al., 2004; Henick-Kling et al., 2008; Linsenmeier et al., 2009) authors have also found it difficult to determine exact detection limits and the relation between AAP concentration and UTA recognition. Although AAP is a major marker for UTA, the complexity of aroma in wines with UTA is affected by other compounds too, such as indole and scatole (3-methyindol) metabolites (Christoph et al., 1995; Gessner et al., 1999; Rauhut et al., 2003; Linsenmeier et al., 2007).

The interaction of wine volatiles on olfactory perception can be a much more complex aspect of wine aroma, especially in case of off-flavours. These interactions can have synergistic effects and masking effects. A good example is the synergistic effect of ethyl acetate and acetic acid on the impression of volatile acidity in wine (Zoecklein, 2012). It is diffcult to find any information in the literature regarding UTA and the masking effect with other volatiles. Indeed, interaction between compounds, volatile or non-volatile, with a individual negative impact on wine quality have been studied a little in general (McKay and Buica, 2020). Possible confusion in associating some reductive wine compounds with an UTA was presented in a previous study (Rauhut et al., 2003). Volatile sulfur compounds, such as hydrogen sulfide, can mask UTA (Ganß et al., 2014). UTA and reductive smell can also occur simultaneously (Sponholz and Huhn, 2001). In such cases, it is possible to use copper to remove the volatile sulfur compounds that will facilitate the recognition of UTA.

Many subjective factors can affect the sensory skills, such as age, experience, gender, cultural background, level of education, or exercise intensity (Parr et al., 2002; Tempere et al., 2012; Tempere et al., 2011; Kaeppler and Mueller, 2013; Honoré-Chedozeau et al., 2017; Franco-Luesma et al., 2019). Sensitivity can be physiologically caused; under the same conditions, with equal previous experience and education, one assessor may be more sensitive to a specific compound, and less sensitive or anosmic to the others (Kaeppler and Mueller, 2013). Therefore, an specific statistical analysis of the sensory results was performed. In this paper, the statistical results of the commission that tested the samples with the highest concentrations of AAP are presented. This commission was intriquing because one assessor did not diagnose UTA.

The Tucker1 plot (Figure 4) presents the distribution of the parameters of the "100 points" method concerning the assessor. Very good agreement among the assessors was presented and no significant differences among assessors were found. The key parameters; aroma correctness, taste correctness, and overall perception were tested with 2-way ANOVA and the quality of commission was confirmed (Figure 5). A significant difference among samples can be explained by the diversity of samples, in relation to vintage, varieties, technologies, and other quality factors. This commission found 12 samples with different faults; two of them with UTA.

The kind of matrix can affects the sensibility of the assessor. Sensory testing of real wine, wine spiked with particular compound/s or model wine solution with the same compound/s, will probably result in a lack of consensus in the detection of specific odor or odor detection threshold (Grosch, 2001; Perry and Hayes, 2016). The model wines used in the workshop that preceded this research were spiked with AAP only. Other compounds that can influence the specific aromatic profile of UTA have not been used. It is known that wine complexity complicates the possibility of unambiguously recognition the sensory descriptors of UTA (Fischer and Sponholz, 2000). Model wine spiked with AAP will be described by aromas from the first group, such as the smell of naphthalene beads or acacia blossom, while the aromas of the second group are difficult to simulate in wine. According to some authors (Christoph et al., 1995; Gessner et al., 1999), descriptors from the second group are related to other IAA metabolites, such as indole or scatole, although it's concentrations may be below the recognition threshold. Regardless of experience and



Legend: A: Aroma correctness, B: Taste correctness, C: Harmony, the overall perception

Figure 5. 2-way ANOVA of the sensory parameters in case of the commission that tested the samples 13 and 14

other references related to wine experts, some individuals can be sensitive to another compound which is not the main sensory descriptor but can cause confusion and different perception (Tempere et al., 2011). Furthermore, certain similar odours are difficult to differentiate from one another if they belong to the same semantic group (Escudero et al., 2007). This group includes similar specific odours with the same general descriptor as reductive or herbal. This study underlines the complexity of UTA sensory character and that the sensory effect is not caused by one or two compounds acting by themselves, but is rather the result of the interaction of compounds. It is therefore recommended that future studies of UTA include more detailed analyses of volatiles.

CONCLUSION

This study was the first research of the presence of off-flavour UTA and the levels of 2-aminoacetophenone (AAP) in Croatian wines. Based on five-year monitoring it can be concluded that UTA is a minor problem in white wine quality and can be explained by stress climatic conditions in this particular case. The concentrations of AAP confirmed the presence of UTA in all respective samples. The sensory recognition of off-flavour was not proportional to the content of AAP although it is the main chemical marker. Some other descriptors, particularly mercaptan and mold smell were also detected. Most of the professional wine assessors accurately linked the off-flavour to the corresponding UTA descriptors. Although assessors are equally educated and tested, they can present different sensitivity to specific volatiles, which points to the need for permanent training. For a better understanding of the complexity of wine and the relationship between aromatic composition and sensory recognition of UTA, it is necessary to conduct as detailed analysis of volatiles as possible. A further challenge is to improve our understanding about the influence of other volatiles known to contribute to this problem and about factors affecting a particular assessor's perception of UTA.

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