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Indole-3-acetic acid and tryptophan in Istrian Malvasia grapes and wine

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Abstract

The phenomenon of "untypical aging off-flavor" (UTA) is associated with higher concentrations of 2-aminoacetophenone (2-AAP) in *Vitis vinifera* white wines. It can stem from some technological treatments applied in grape and wine production. In order to find out if a wine is liable to "untypical aging off-flavor", it is necessary to determine the concentrations of indole-3-acetic acid (IAA) and L-tryptophan, the main precursors of 2-aminoacetophenone. The formation of 2-AAP is caused by an oxidative degradation of the phytohormone indole-3-acetic acid, triggered by sulfuration after fermentation. Metabolism of tryptophan and indole-3-acetic acid formation during vinification is correlated with yeast strain. 2-AAP formation considered to be greater in wines from grapes which where grown under stress conditions like insufficient water or nitrogen supply in soil. The purpose of this investigation was to determine the influence of soil type and different yeast strains on the levels of L-tryptophan and indole-3-acetic acid in the Malvasia wine from Istria. Grapes were harvested from two different soil type locations in Istria (terra rossa and terra bianca) wine growing region and separately undergo alcoholic fermentation, spontaneous fermentation and by use of 4 commercial yeast strains (Fermol Cryoarome, Fermol Assosiee, Uvaferm CS2, Anchor VIN 13). Young wine samples were taken for analysis immediately after completed alcoholic fermentation. Chemical analyses of compared musts and wines showed differences in L-tryptophan and indole-3-acetic acid concentrations that depend more upon the soil type than yeast strain used in this investigation. More intense use of tryptophan was noticed during fermentation of must from terra bianca soil. Significantly higher concentrations of indole-3-acetic acid were noticed in wines produced by indigenous yeasts.

Key words: Indole-3-acetic acid, IAA, L- tryptophan, Trp, 2-aminoacetophenone, 2-AAP, untypical aging off-flavor, UTA, Malvasia Istria, wine, yeast.

Introduction

Tryptophan (Trp) and its metabolites, especially the phytohormone indole-3-acetic acid (IAA) are considered to be potential precursors of 2-aminoacetophenone (2-AAP), an aroma compound that causes an "untypical aging off-flavor"(UTA) in wines [1, 2]. The bouquet has been described as odor taint such as acacia blossom or floor polish. In some years, about 20% of the rejected white wines in the vintage wine certification of different wine growing regions in Germany were connected with UTA, which consequently caused high losses of economic value [3]. It is assumed that UTA is the result of a stress reaction in grape. As a consequence, the IAA concentrations in must have been considered as stress indicator with increasing stress leading to higher IAA concentrations [4]. At higher pH, the indole ring is relatively stable, but the phenomenon of an increase in the IAA level, which is probably due to an oxidative conversion of some other abundant indolic components, such as the precursor Trp itself, has been reported [5]. 2-APP can be formed by an oxidative degradation of IAA, which is triggered by a sulfuration, a measure indispensable for white wine making [1]. It could be demonstrated that pyrrole ring cleavage of IAA by superoxide radicals, which can be generated by aerobic oxidation of sulfite during storage of sulfurized wines, leads in several steps to N-formyl-2-aminoacetophenone (FAP) which is further decomposed to 2-AAP [6,7]. The response of Trp in grapes to stress conditions is yet unknown. Trp levels may be reduced by enhanced UV-B radiation [8]. Lower amounts of Trp, IAA and other Trp metabolites were analyzed in musts and wines of a dry vintage as well as in musts and wines from an early grape vintage [9]. Trp concentrations of must essentially depended on weather; hot years resulted in low values, while in cool years values were very high [10]. During alcoholic fermentation the level of free Trp decreased, the consumption by the yeast was between 60% and 100% [8]. This utilization was dependent on the amount of Trp in the must [9]. If the Trp content of the must was low, nearly 100% was utilized. At Trp concentrations above a threshold of about 15 mg L^{-1} in the must, its utilization was between 65 and 100%, at an average of 85%. Utilization of Trp by the yeast was also reported by other studies [11, 12]. Certain amino acids such as leucine, isoleucine, phenylalanine and tryptophan are preferentially incorporated by yeast [13]. Tryptophan was totally consumed in all fermentations, independently of the concentration of amino acids added to the must [14]. Consequently, it may be concluded that the specific permease of the yeast for the assimilation of aromatic amino acids (Tat1p) could present a greater affinity for tryptophan than for

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tyrosine or phenylalanine. The aim of this study was to determine the influence of soil type and different yeast strains on the levels of L-tryptophan and indole-3-acetic acid in Istrian Malvasia grapes and wines.

Materials and Methods

Fermentation: Istrian Malvasia grapes from two vine growing producers (Pilato, Rossi) having vines planted in 1994, Guyot trained, grafted on 420A rootstock, on different soil type in Istria wine growing region (terra rosa, terra bianca), harvested in 2007 year, was used to produce the wines by traditional vinification for white wine. For the winemaking 300 kg of grapes was de-stemmed, crushed and separated in three 100 L stainless-steel tanks for fermentation. All treatments were carried out in triplicate. After completion of alcoholic fermentation, the wines were racked and in all samples chemical analysis were performed.

Yeast: Alcoholic fermentation was carried out without yeast inoculation (spontaneous fermentation) and by use of 4 commercial yeast strains (Fermol Cryoarome, Fermol Assosiee, Uvaferm CS2, Anchor VIN 13). For the inoculums all of the commercial strain yeast cultures were preincubated in sterilized grape must for 48 h at 25°C and inoculated at a final level of $5x10^6$ cells m L^{-1}.

Chemicals: Indole-3-acetic acid, indole-3-propionic acid and potassium dihydrogen phosphate were purchased from Fluka (Germany). Tryptophan was obtained from Sigma (Steinheim, Germany). Methanol (HPLC grade) was purchased from Mallinckrodt Baker (Deventer, Holland). All other chemicals (p.a. purity) were obtained from Kemika (Zagreb, Croatia). AccuBOND II ENV PS DVB cartridges (1000 mg/6 mL) were from Agilent Technologies (USA). Vacuum manifold was obtained from Agilent Technologies, USA.

Chemical analysis: Basic chemical analyses of must and wine were done using methods proposed by O.I.V. [15]. Free α-amino nitrogen (FAN) was calculated by formol (10% solution of formaldehyde in water) titration [16]. Must turbidity level was determined by nephelometer analyzer, model 2100P (Hach, USA), expressed in NTU units.

Determination of IAA and Trp: AccuBOND II ENV PS DVB cartridges (1000 mg/ 6 mL) were conditioned by 10 mL of methanol, followed by 10 mL of buffer solution (0.025 M phosphate buffer).Then 10 mL of sample was loaded. The column was washed twice with 10 mL of buffer solution. The elution was carried out with 2 x 5 mL buffer solution in 50% (v/v) methanol.

HPLC analysis: Analyses were performed using Agilent 1100 equipped with autosampler and fluorescence detector. Separation was achieved on Luna C_{18} (endcapped) column (5 µm packing, 250 x 4.6 mm i.d.), (Phenomenex, USA), protected with guard column from same material (Phenomenex, USA). The elution conditions were as follows: 0.5 mL min^{-1} was flow rate. The injection volume was 50 µL. The column temperature was kept at 15°C. Excitation was at 225 nm and emission was at 365 nm. The mobile phase consisted of solvent A, 0.025 M phosphate buffer and solvent B, methanol. The following gradient was used: from 25% to 100% B in 30 min, 5 min from 100% B to 25% B followed by re-equilibration for 5 min.

Statistical analysis: One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) comparison test of SAS (SAS Institute, Cary, NC, USA) were used to interpret differences in means, if any, at the 95% and 99% confidence level.

Results and Discussion

Determination of IAA and Trp: Recovery percentages of spiked wines with 26.7 µg L^{-1} of IAA, 131.7 µg L^{-1} and 38.8 µg L^{-1} of IPA using cartridges were determined. Three extractions were carried out. Each extract was analyzed in duplicate by HPLC. The recoveries are presented in Table 1. Optimizing procedure, at first, elution was done by 35% methanol in phosphate buffer then with 40%, 50% and 75% methanol in phosphate buffer solution. Best recoveries were obtained with 50% methanol in phosphate buffer.

HPLC-FLD method was optimized and validated. The column was stable under the chosen conditions. A flow rate of 0.5 mL min^{-1} was optimal for separation of Trp, IAA and indole-3-propionic acid (IPA) as internal standard [17]. The method was linear for all three compounds. Correlation coefficient for IAA was 0.9990, for Trp was 0.9995 and for IPA was 0.9991, respectively (Table 2). Limits of detection (LOD) was calculated from the signal to noise ratio (S/N), 3:1. Limits of quantification (LQD) were obtained from 10:1 S/N. The chromatograms of standard solution and sample are shown in Fig.1.

Sample results: Table 3 shows basic chemical composition of musts used in this investigation. As it can be seen there were no marked differences between samples from two types of soil. Must sugar concentration and total acidity pointed out that at the time of vintage grape was ripe, while the NTU values and FAN concentrations were relatively low. A range between 100 NTU and 250 NTU units is generally recommended as an optimum turbidity[18].

Over clarification can decrease the fruity aroma of dry white wines, it is followed by difficult fermentation conditions, excessively slow fermentations with increased volatile acidity production. In our case, fermentation duration was relatively short, from 17 to 20 days while the volatile acidity ranged between 0.49 and 0.72 mg L^{-1} (Table 4). Minimum requirement of free amino nitrogen (FAN) reported is 70 to 140 mg L^{-1} for complete fermentation of must with initial sugar concentration between 183 and 258 g L^{-1} [19], and 140 mg L^{-1} of FAN for satisfactory fermentation [20]. Our results showed that even with relatively low FAN concentrations all yeast strains managed to complete fermentation of Malvasia must. As shown in Table 4 there were no marked differences in basic chemical composition of wines produced with tested yeast strains. Results of wine chemical analysis showed significant difference only in volatile acidity concentrations, pointing out indigenous yeasts as the highest volatile acidity producers.

The IAA concentration in both Malvasia musts was similar ranging from 3.2 to 4.3 µg L^{-1}, what is in accordance whit published data [9]. During the vintage period of 1996-1999 only traces of free IAA (<3 µg L^{-1}) was found in the examined grape musts. In the wines, the free IAA varied between 3 and 90 µg L^{-19}.Marked changes of IAA, compared to must concentrations were noticed in all Malvasia wines. IAA concentration significantly increased in wine produced with grapes from terra bianca by indigenous yeasts, while in other wines concentrations were similar ranging from 25.35 to 32.89 µg L^{-1} (Table 5) indicating either a neosynthesis

Table 1. Trp, IAA and IPA recoveries from Malvasia wine (n=3).

Cartridge	Tryptophan	Indole-3-acetic acid	Indole-3-propionic acid
AccuBOND II ENV PS-DVB	84.5±4.9	99.3±6.9	81.9±3.3

Table 2. Calibration range for determination of Trp, IAA and IPA and limits of detection and quantification.

Compound	Range (μg L^{-1})	Correlation coefficient	LOD (μg L^{-1})	LOQ (μg L^{-1})
Tryptophan	50-1600	0.9995	2.1	6.9
Indole-3-acetic acid	5-110	0.9990	1.8	5.9
Indole-3-propionic acid	20-300	0.9991	6.3	21

Table 3. Chemical composition of Malvasia must from two different soil type (terra rossa, terra bianca).

Compounds	Pilato Terra rossa	Rossi Terra bianca
Sugar °Oe	89	92
pH	3.25	3.30
Total acidity (g L^{-1})*	6.9	6.4
FAN (mg L^{-1})	85	70
NTU	89	76
Indole -3-acetic acid (μg L^{-1})	4.3	3.2
Tryptophan (μg L^{-1})	3305	2611

* As tartaric acid

Table 4. Basic chemical analysis of Malvasia wines from two different soil type (terra rossa, terra bianca).

Compounds	Pilato Terra rossa						Rossi Terra bianca					
	Indigenous yeasts	Fermol assosiee	Fermol cryoarome	Anchor VIN13	Uvaferm CS2	LSD	Indigenous yeasts	Fermol assosiee	Fermol cryoarome	Anchor VIN13	Uvaferm CS2	LSD
Alcohol (vol. %)	12.2	12.2	12.1	12.1	12.2	n.s.	12.2	12.7	12.9	12.9	12.9	n.s.
Red. sugar (g L^{-1})	< 1	< 1	< 1	< 1	< 1	n.s.	1.3	1.2	1.5	< 1	1.2	n.s.
Total acidity (g L^{-1})*	5.7	5.8	5.7	5.9	6.0	n.s.	5.2	5.0	5.2	5.4	5.0	n.s.
Volatile acidity (g L^{-1})**	0.68	0.40	0.35	0.41	0.40	5%=0.15	0.70	0.50	0.58	0.51	0.48	5%=0.20
pH	3.1	3.0	3.1	3.1	3.2	n.s.	3.3	3.3	3.4	3.2	3.3	n.s.

* As tartaric acid; ** As acetic acid.

Table 5. IAA and tryptophan concentrations in Malvasia wines from two soil type (terra rossa, terra bianca).

Compounds	Pilato Terra rossa						Rossi Terra bianca					
	Indigenous yeasts	Fermol assosiee	Fermol cryoarome	Anchor VIN13	Uvaferm CS2	LSD	Indigenous yeasts	Fermol assosiee	Fermol cryoarome	Anchor VIN13	Uvaferm CS2	LSD
Indole -3-acetic acid (μg L^{-1})	40.2	32.9	31.8	30.2	30.5	n.s.	67.1	28.9	30.2	26.1	25.4	5%=5.8
Tryptophan (μg L^{-1})	1044.6	1007.8	828.2	1131.5	880.5	5%=97.56	621.6	693.6	265.1	729.3	726.2	5%=35.83

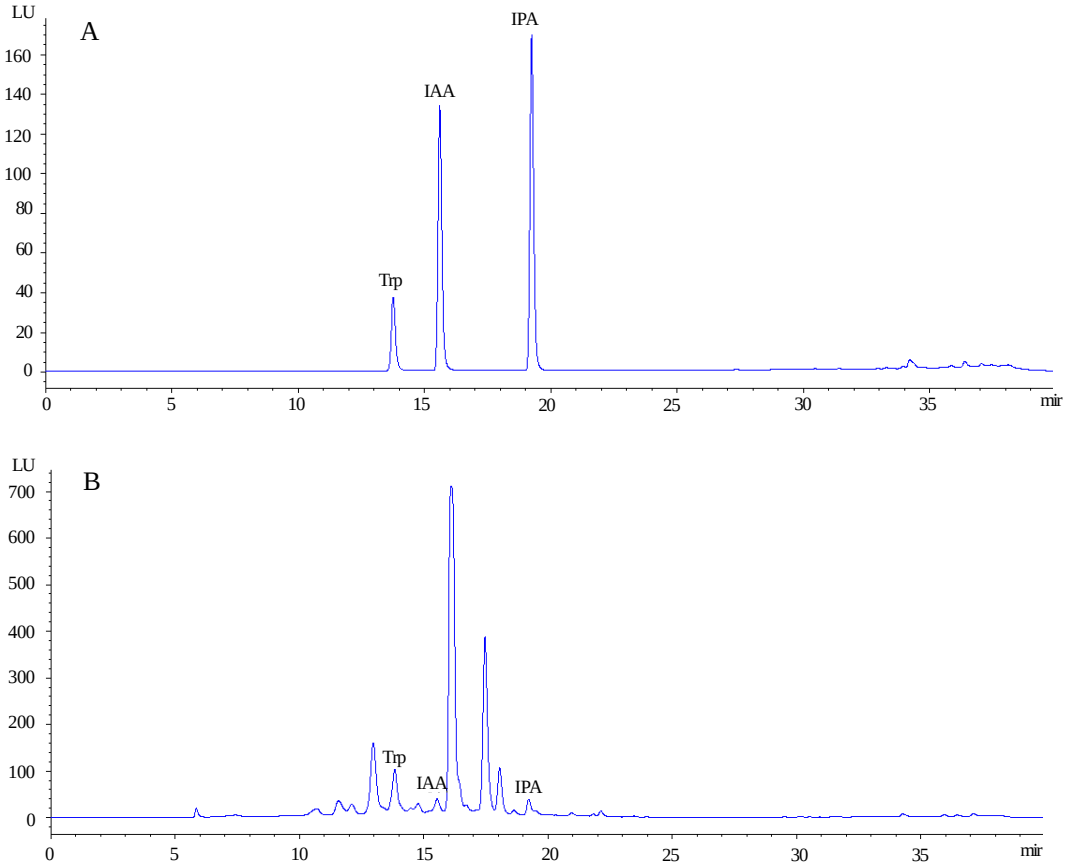

Figure 1. Chromatograms obtained from standard mixture (A) and wine sample (B).

by the yeast or hydrolysis of conjugated IAA during fermentation. From the presented results it can be seen that all commercial yeast strains tested had equal influence on the concentrations of IAA. At the contrary significant changes were noted in the tryptophan concentrations (Table 5). During alcoholic fermentation the level of Trp decreased, the utilization by yeast ranged from 65-75% in wines produced from grapes grown in terra rosa and 72-89% in wines produced from grapes grown in terra bianca. Fermol cryoarome yeast consumed significantly higher quantity of Trp compared to other yeasts used. Trp utilization depends on the amount of Trp in the must, at lower content nearly 100% is utilized while at a content of about 15 mg L^{-1}, its utilization was between 65% and 100%, at an average of 85% [9]. Our results are in agreement with this previously published data.

Conclusions

Results of this study indicate the possible connection between tryptophan and its metabolite indole-3-acetic acid as the results pointed out significant increase in indole-3-acetic acid concentrations and decrease of Trp concentrations during alcoholic fermentation. Commercial yeasts used in this investigation varied in tryptophan consumption during alcoholic fermentation but more pronounced differences in Trp utilization was between soil types examined. More intense use of tryptophan was noticed during fermentation of must from terra bianca. Significantly higher concentrations of IAA were noticed in wines produced by indigenous yeasts. These preliminary results show the complexity of the interactions involved and the need for further research, including more detailed chemical analysis.

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