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Technical Report

Influence of grapevine flower treatment with gibberellic acid (GA_3) on *o*-aminoacetophenone (AAP) content and sensory properties of white wine

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'Riesling' and 'Sauvignon blanc' grapevine flowers were treated at full bloom with 20ppm aqueous gibberellic acid (GA_3) solution. The resulting wines were analysed on 2-aminoacetophenone (AAP) content by means of GC/MS. Compared to the untreated control the GA_3 treated wines showed higher AAP contents: control 0.72µg/l, GA_3 1.29µg/l ('Riesling'); control 0.83µg/l, GA_3 1.26µg/l ('Sauvignon blanc'). Panellists rather detected the untypical ageing (UTA) off-flavour in the respective control wines. We speculate that the higher polyphenol content of the GA_3 treated wines is partially responsible for the masking effect of the UTA off-flavour and, hitherto, the general better sensory properties of wines made from GA_3 treated berries.
Keywords: gibberellic acid; 2-aminoacetophenone (AAP); untypical ageing flavour (UTA); GC/MS

2-Aminoacetophenone (AAP) is an aroma compound, which is associated with the so-called "untypical ageing off-flavour" (UTA) in *Vitis vinifera* L. white wines and generally occurs after a few months storage (RAPP et al., 1993; DOLLMANN et al., 1996; HOENICKE et al., 2002). The off-flavour is described by various aroma descriptors, such as "acacia blossom, naphthalene, furniture polish, wet wool or fusel alcohol". Depending on wine flavour UTA can be organoleptically recognised at concentrations as low as 0.5µg/l AAP (RAPP et al., 1993).
Gibberellins are an important class of natural growth regulators in plants. They are products of the diterpenoid pathway and their formation is initiated by cyclisation of the common C_{20} precursor geranylgeranyl diphosphate (GGPP) (GRAEBE et al., 1965; HEDDEN and PROEBSTING, 1999). GA_3 has been widely used in table grape production and its effects on grape quality have been intensively studied (GUELFAT-REICH and SAFRAN, 1973; BEN-TAL, 1990; KORKAS et al., 1999). Generally, GA_3 application reduced berry set, increased berry weight, and improved juice quality. It also led to increased petiole length, more rigid pedicels, less firm berry skin, reduced the number of seeds, and enhanced shoot growth.
Recent research has shown, that GA_3 treatment leads to higher polyphenol and anthocyanin content, respectively (TESZLÁK et al., 2005), and also to higher concentrations of indole-3-acetic acid (IAA) (POUR NIKFARDJAM et al., 2005). Research performed on bayberries (*Myrica rubra* Bieb.) showed that GA_3 spraying of bayberry flower buds leads to inactivation of the enzyme indole-3-acetic acid oxidase (LI et al., 2003), which could explain the higher IAA contents recently found in white wines made from GA_3 treated grapes.
IAA is regarded as an important precursor of 2-aminoacetophenone (2-AAP). Higher IAA contents of wines in consequence of GA_3 treatment of grapevine flowers could, thus, lead to higher 2-AAP formation and increase UTA off-flavour potential in wine. The aim of our project was to analyse wines made from GA_3 treated grapes on their 2-AAP formation potential and assess the wines with regard to their sensory profile.

Chemicals

All reagents used were of analytical grade unless otherwise stated. Gibberellic acid (GA_3) and Tween 20® were from Fluka (Buchs, Switzerland). Deionised, nanopure water was from Szkarabeusz (Pécs, Hungary). 2-AAP was purchased from Sigma-Aldrich (Steinheim, Germany) and 2-methyl anthranilate (2-AME) from Fluka (Buchs, Switzerland).

Grapes

Grapes were grown during the 2004 vintage in the vineyards of the FVM Research Institute for Viticulture and Oenology on the south-facing slopes of the Mecsek Hills in Pécs/Hungary (latitude: 46°07' N, longitude: 18°17' E, 180 to 200m above sea level). The soil is Ramann-type brown duff on red sandstone. The vines were not irrigated. The five-year-old 'Riesling' and 'Sauvignon blanc' grapevines were grown according to the Lenz-Moser system. In each case 40 grapevines were selected from each cultivar, of which 20 were kept untreated and 20 were treated with GA_3 as follows.

GA_3 treatment

Grapevine flowers were sprayed at full bloom to the drip point with an aqueous 20ppm GA_3 solution (containing 0.2 % Tween 20®). The control group remained untreated.

Winemaking technology

The grapes were harvested by hand at physiological ripeness, destemmed and crushed by means of a Cantinetta C.D.A. TR (Nuova Zambelli, Camisano Vicentino, Saonara, Italy) destemmer/crusher. After crushing the grapes received 20mg/kgSO_2. After destemming and crushing the mashes were immediately pressed by means of a hydraulic press and the resulting must divided in three 5l plastic containers resulting in three replicates for each cultivar and treatment. They were then inoculated with a selected yeast starter culture (Lalvin® EC 1118, Lallemand, Rexdale, Canada) and fermented for one week. After fermentation was complete, the wines were racked off the lees, received 50 mg/l SO_2, and then were stored at 14 °C in the dark till analysis.

FTIR analysis

Because of the small volume left for analysis, standard wine parameters, such as alcohol, extract etc., were measured by means of FTIR spectrometry according to PATZ et al. (1999).

GC/MS analysis

2-AAP analysis was performed according to HÜHN (2004) and SPONHOLZ et al. (2001) with some modifications. Briefly, sample extraction was performed by placing 10 ml of thermally treated (72 h at 50 °C) wine in a 10 ml vial, adding a stir bar (Twister®, Gerstel, Mühlheim/Ruhr, Germany) and 100µl of internal standard solution (2-AME). The stir bar consists of a 10 mm magnetic stirring rod incorporated in a glass jacket and coated with a 1mm layer of polydimethylsiloxane (PDMS; equivalent to 110 ml PDMS). After stirring for 5h the stir bar was removed, rinsed with water, dried with a lint free tissue and placed into a glass thermal desorption tube (Gerstel). The thermal desorption tube was then introduced into a thermal desorption unit (TDS 2 and CAS 4, Gerstel), where the stir bar was thermally desorbed (temperature programme TDU: 30°C; 720°C/min; 250°C (10min) to release the extracted compounds into the cryogenically pre-cooled PTV (temperature programme of the CAS:-150°C, 12.0°C/s, 250°C (10min)) for subsequent GC/MS analysis. GC/MS analysis was performed using a GC (GC 6890, Agilent Technologies, Little Falls, USA) equipped with a ZB-WAX column (30.0 m x 250 mm x 0.25 mm; Phenomenex, Aschaffenburg, Germany) and a mass selective detector (MSD 5973, Agilent Technologies). GC/MS conditions were as follows: temperature programme GC 130 °C, 4°C/min, 250°C (10min); carrier gas helium with a constant flow of 1.3 ml/min; splitless time 1 min; transfer line temperature 250°C; MS source temperature 230 °C; MS Quad temperature 150°C; MSD SIM-Parameter: m/z92/119/120/135/151. Operating the MSD in selected ion monitoring mode (SIM) increased the sensitivity of the method up to a quantitation limit of 50ng/l and detection limit of 10ng/l. Analysis was performed in duplicate.

Sensory analysis

A trained panel evaluated the wines. Before the actual wine tasting, a test was performed to analyse the sensitivity of the panel on 2-AAP. All members of the panel were able to detect artificially added 2-AAP in an UTA-free 'Riesling' wine in a concentration range between 0.5 and 20.0µg/l. More than 70 % were capable to correctly arrange wines according to UTA-intensity with 2-AAP added artificially in concentrations between 0.5 and 20.0µg/l.

To enforce UTA formation one half of the wines was stored at 50 °C for 72 h. The other half remained untreated. The wines were presented in randomised order in Duo-tests and the panellist was asked to score the intensity of the different attributes on a 10 cm unstructured scale. A volume of 50ml wine was provided in tulip-shaped ISO tasting glasses at room temperature in the sensory laboratory of the FVM Research Institute for Viticulture and Oenology in Pécs (Hungary).

Results and Discussion

General chemical composition

Chemical composition of the wines analysed by FTIR is shown in Table 1 and 2. Due to higher degrees Oechsle in the must (Teszlák et al., 2005) the wines from GA_3 treated berries show higher alcohol contents and less density. Titratable acidity is also decreased but only in case of 'Sauvignon blanc' the difference was significant. Thus, pH-value was also only significantly increased in the latter cultivar.

2-AAP content

GC/MS analysis showed that 2-AAP content is significantly increased in wines made from GA_3 treated grapevines (Table 3). We found a high correlation between contents of IAA and 2-AAP in 'Riesling' (R^2 = 0.8068; n = 12). 'Sauvignon blanc' showed a much weaker correlation (R^2 = 0.2489; n = 12). These results are, thus, in agreement with Simat et al. (2004), who found a significant correlation between unbound IAA and 2-AAP concentration after heat treatment of wines of the grape cultivar 'Müller-Thurgau', indicating that about 30 to 50 % of the 2-AAP values might be traced back to the precursor IAA.

One explanation for the difference between cultivars might be the fact that depending on cultivar 2-AAP is being formed from different precursors as already discussed by Dollmann et al. (1996), Hoenicke et al. (2002), and Simat et al. (2004). A different explanation might involve the antioxidative influence of polyphenols in wine. Recent research has shown that antioxidants are able to inhibit the formation of 2-AAP from IAA (Simat et al., 2004). The generally lower polyphenol contents in 'Sauvignon blanc' could have led to less antioxidative protection in the wines and, thus, permitted more AAP formation during storage and heat

Table 1:
Chemical composition of 'Riesling' wines made from gibberellic acid treated (GA_3) and untreated grapevines (n = 3)

Parameter	Unit	Riesling GA_3	Riesling Control
Density	20/20	0.9901 ± 0.0000	0.9909 ± 0.0002*
Alcohol	%vol	13.57 ± 0.03	13.29 ± 0.08 *
Residual sugar	g/l	0.21 ± 0.10	0.71 ± 0.13**
Glucose	g/l	0.0 ± 0.0	0.0 ± 0.0
Fructose	g/l	0.07 ± 0.12	0.40 ± 0.12*
Titrateable acidity	g/l	7.04 ± 0.09	7.14 ± 0.07
pH		3.23 ± 0.02	3.20 ± 0.01
Tartaric acid	g/l	2.64 ± 0.05	2.38 ± 0.04*
Lactic acid	g/l	0.12 ± 0.04	0.08 ± 0.05
Malic acid	g/l	2.80 ± 0.11	2.73 ± 0.04
Citric acid	g/l	0.37 ± 0.03	0.35 ± 0.02
Volatile acidity	g/l	0.41 ± 0.01	0.42 ± 0.01
Glycerol	g/l	5.66 ± 0.23	6.39 ± 0.06*
Polyphenols (Folin)	mg/l	285 ± 20	265 ± 16

* $p < 0.05$, ** $p < 0.01$

Table 2:
Chemical composition of 'Sauvignon blanc' wines made from gibberellic acid treated (GA_3) and untreated grapevines (n = 3)

Parameter	Unit	Sauvignon bl. GA_3	Sauvignon bl. Control
Density	20/20	0.9877 ± 0.0002	0.9903 ± 0.0008*
Alcohol	%vol	15.40 ± 0.08	13.55 ± 0.36*
Residual sugar	g/l	0.88 ± 0.11	0.47 ± 0.22*
Glucose	g/l	0.0 ± 0.0	0.0 ± 0.0
Fructose	g g/l	0.93 ± 0.10	0.45 ± 0.06**
Titrateable acidity	g/l	5.30 ± 0.02	6.73 ± 0.44*
pH		3.44 ± 0.01	3.27 ± 0.07*
Tartaric acid	g/l	1.92 ± 0.05	2.26 ± 0.15*
Lactic acid	g/l	0.18 ± 0.02	0.24 ± 0.10
Malic acid	g/l	2.44 ± 0.03	2.75 ± 0.02**
Citric acid	g/l	0.48 ± 0.03	0.34 ± 0.04*
Volatile acidity	g/l	0.43 ± 0.01	0.41 ± 0.03
Glycerol	g/l	7.17 ± 0.14	6.90 ± 0.28
Polyphenols (Folin)	mg/l	249 ± 17	182 ± 9 ***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3:
Indole-3-acetic acid (IAA) and *o*-aminoacetophenone (*2-AAP*) content of wines made from gibberellic acid treated (GA_3) and untreated grapevines (n=6)

Variety	Treat-ment	IAA [µg/l]	*o*-AAP [µg/l]	Correlation coefficient R^2 (IAA-AAP)
Riesling	Control	34.8 ± 15.6	0.72 ± 0.26	0.8068
	GA_3	86.6 ± 36.1*	1.29 ± 0.43 *	
Sauvignon blanc	Control	37.7 ± 25.3	0.83 ± 0.53	0.2489
	GA_3	54.2 ± 12.2	1.26 ± 0.28 *	

* $p < 0.05$

Table 4:
Mean molar conversion rate (n=3) of indole-3-acetic acid (IAA) to *o*-aminoacetophenone (*2-AAP*)

Variety	Treatment	IAA [µmol/l]	*o*-AAP [µmol/l]	Conversion rate [µmol%]
Riesling	Control	198.8	5.3	2.0
	GA_3	494.9	9.5	3.1
Sauvignon blanc	Control	215.4	6.1	3.2
	GA_3	309.7	9.3	4.9

treatment, respectively (Table 1, 2 and 3). Given the fact that IAA content in GA_3 treated 'Riesling' had been much higher than in 'Sauvignon blanc' and presuming that all 2-AAP had been formed from IAA, the calculated molar conversion rate from IAA to 2-AAP had been lower in 'Riesling' than in 'Sauvignon blanc' (Table 4), which might be again referred to the higher polyphenol content in 'Riesling'.

Sensory properties

The wines stored at 50 °C did not show any significant difference with regard to their sensory profile (results not shown). Panellists struggled to detect the UTA off-flavour in the wines, which showed other off-flavours and thermally formed flavours as well, which might have superimposed the UTA off-flavour. Due to the extensive formation of "caramel"-like flavours formed during heat treatment no descriptive sensory analysis like in the thermally untreated wines could be performed. Interestingly, in the thermally untreated wines panellist rather detected the UTA off-flavour in the control wines (Fig. 1 and 2). We speculate that the higher polyphenol contents measured in the GA_3 treated wines partially masked the UTA off-fla-

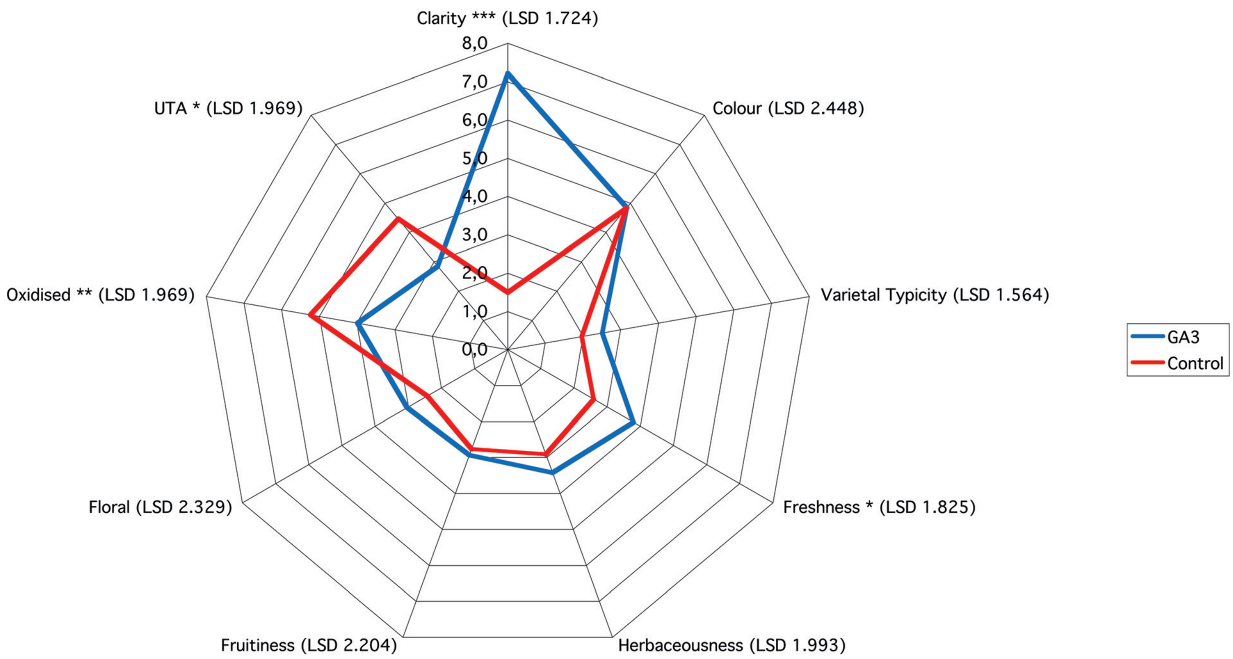

Figure 1: 'Riesling' aroma radar graph of wines stored at 15°C made from GA_3 treated and untreated berries (LSD: linear standard deviation; n = 3)

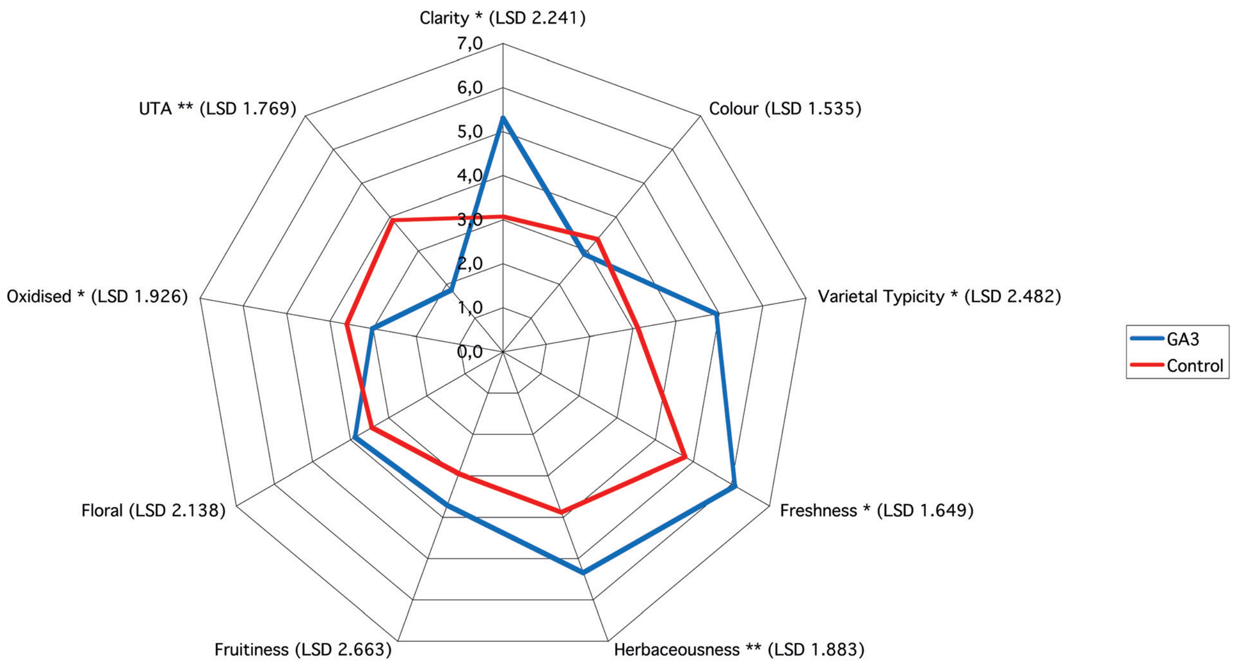

Figure 2: 'Sauvignon blanc' aroma radar graph of wines stored at 15°C made from GA_3 treated and untreated berries (LSD: linear standard deviation; n = 3)

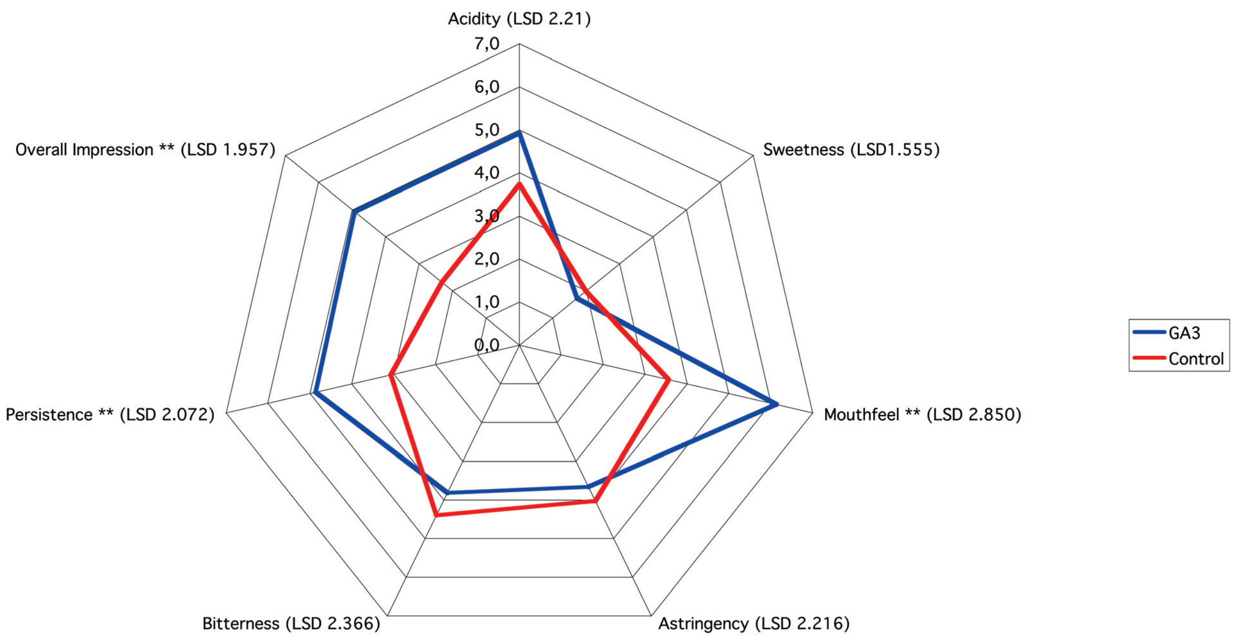

Figure 3: 'Riesling' taste radar graph of wines stored at 15°C made from GA_3 treated and untreated berries (LSD: linear standard deviation; n = 3)

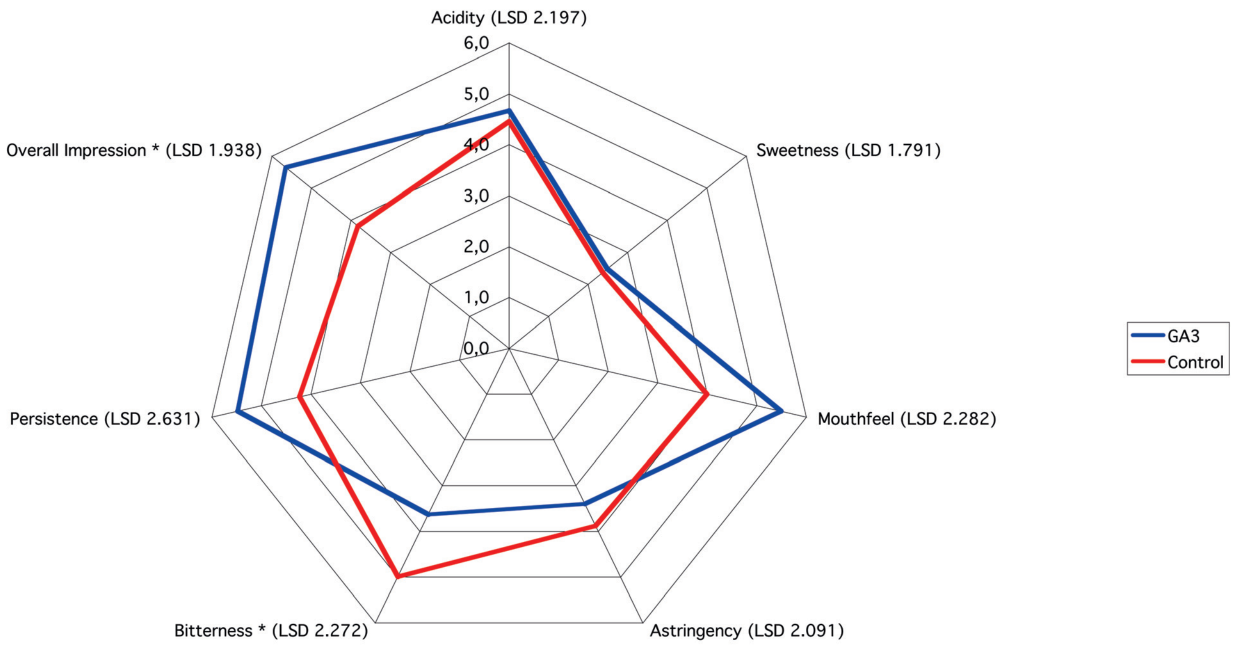

Figure 4: 'Sauvignon blanc' taste radar graph of wines stored at 15°C made from GA_3 treated and untreated berries (LSD: linear standard deviation; n = 3)

vour and, thus, could not be detected during sensory analysis. But this could also mean that other odorous compounds than 2-AAP might contribute to UTA as well. These compounds have yet to be identified by sensory and analytical investigations in the future.

Generally, the GA_3 treated wines were regarded as being fresher, less oxidised, having more varietal character, and showing less UTA and more clarity. The higher polyphenol content could partially also be responsible for the better sensory properties of the GA_3 treated wines. Polyphenols would not only act as natural antioxidants and, therefore, protect aroma compounds from oxidative degeneration during storage but would also contribute to mouthfeel and persistence. In 'Riesling' GA_3 treatment led to significantly ($p < 0.01$) better mouthfeel and persistence in the mouth (Fig. 3), in 'Sauvignon blanc' to significantly ($p < 0.05$) less bitterness and better overall impression (Fig. 4), which certainly can - to a certain extent - be referred to the higher polyphenol contents in GA_3 treated 'Riesling' and 'Sauvignon blanc', respectively. This would emphasize the importance of polyphenols for wine quality not only as masking substances for off-flavours and as mouthfeel providers, but also as antioxidants to protect wine flavour and inhibit off-flavour formation, such as UTA from IAA and other yet to be identified precursors.

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