Enzymatic release of flavour compounds from Heritage apple varieties

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Abstract

Heritage apple varieties such as *Ilzer Rose*, which is domestic to the southern area of Austria, are often used as a raw material for the production of apple wines due to their pleasant flavour properties. In this study, we investigated if the concentrations of terpenes that had been identified in the skin of this variety might be increased upon modification of the fermentation strategy by the addition of pectolytic enzyme with pronounced β-glucosidase activity. Furthermore, the impact of prolonged juice maceration as well as mash fermentation were investigated regarding a potential release of terpenes from glycosides. These strategies led to a significant increase of the investigated compounds in apple wine; however, the resulting concentrations were still below their odour threshold values in the matrix apple wine. Nevertheless, the modified fermentation management resulted in interesting products that will be in the centre of future investigations.

Keywords: apple wine, heritage apple varieties, terpenes, glycosides, odour threshold

Introduction

With the recent trend to increase the consumption of local and regional foods, heritage apple varieties have regained popularity in Austria. Several hundreds of apple varieties are domestic to meadow orchards; some of them have also been cultivated in plantations during the last decade. Many of these heritage varieties are characterised by excellent sensory properties and are, thus, of high interest especially for the production of apple wine and cider. In a recent study, we showed that a large number of interesting terpenes is present in the skin of the heritage apple variety *Ilzer Rose*, an apple variety that is known for its floral, rose-like flavour [1]. We assume that these terpenes contribute to the floral properties of *Ilzer Rose* wine.

Oenology has been tackling glycosilated aroma compounds for years to enhance the flavour profile of certain wines. Approximately 90 % of monoterpenes were found in their glycosylated form in Muscat wines [2]. Glycosides are built by an aglycone linked to at least one sugar molecule as glycone. Hydrolysis of the glycosidic bonds then leads to the release of the aglycone and the glycone. If the released aglycone is an aroma active compound, the flavour profile of the resulting wine can be affected. Some members of the class of monoterpenes are important representatives for aroma compounds with floral and 'sweet' characteristics. Especially, in grape varieties of aromatic wines such as Gewürztraminer, Muskateller or Riesling these monoterpenes are essential. However, these compounds mainly occur as glycosides in grapes meaning that they are odourless and most likely transported to the vacuoles, where they are stored as potential flavour precursors [3]. However, the odour-active terpenes may be released from these precursors by the activity of certain glucosidases, which are added during the vinification of aromatic white wines.

In this study, we transported this concept to the production of apple wines. We investigated if the concentrations of terpenes and sesquiterpenes in apples wines from *Ilzer Rose* apples were increased upon enzymatic treatment during apple wine production, whereas we followed two different strategies, (i) maceration of the juice, and (ii) mash fermentation over several days. The assumed release of terpenes from their glycosides during the fermentation similar to their release from specific grape varieties could boost the flavour of *Ilzer Rose* wines and make them even more attractive to consumers.

Experimental

Material

A total of 157 kg *Ilzer Rose* apples was harvested from an apple orchard at their commercial ripeness in October 2018. After the harvest, the apples were stored for further two weeks under cooled conditions to promote postharvest ripening and flavour development. Juice preparation and the subsequent apple wine (AW) production was carried out in special micro fermentation devices. Figure 1 gives a summary about the processing steps for the five apples wines. AW1 was produced as a reference product without maceration; the mash was pressed

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immediately after milling of the apples; pectinase (Enzym MS flüssig, Preziso, 5 ‰) was added to the juice for AW1. A pectolytic enzyme with β-glucosidase activity (Trenolin® Bouquet PLUS, Erbslöh, Germany) was added to the mashes of the apple wines AW2-5. For the production of AW2 and AW4, the juice was let to macerate for 8 hours prior to fermentation. For the production of AW4 and AW5, the fining agent Gerbinol® CF (Erbslöh, Germany; 2 ‰) was added to the mash with the purpose to bind unbalanced tannins and for clarification. Mash fermentation for AW3 and AW5 was performed for 10 days with subsequent fermentation after pressing of the mash. *Saccharomyces cervisiae* var. *bayanus* (LALVIN® EC-1118; Lallemand Inc.; Canada; 250 mg/L) was added to all approaches to initiate the fermentation. Fermentation was controlled for AW1, AW2 and AW4 (FC, temperature control (15.5 °C); addition of thiamine and diammonium phosphate (Vitamon Liquid; Erbslöh; Germany) as nutrients during the fermentation; addition of bentonite 2.5 g/L); for AW3 and AW5 neither temperature was not controlled nor nutrient was added (NFC).

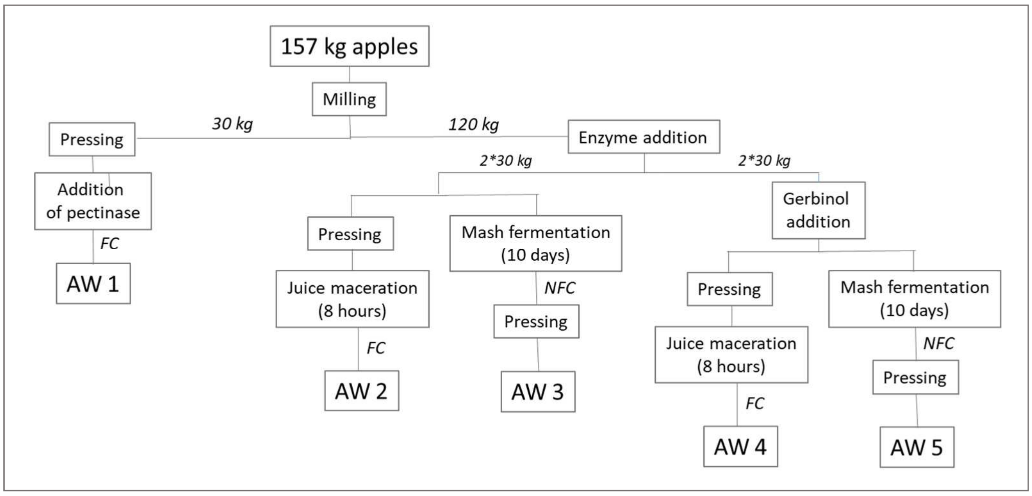

Figure 1: *Survey about the fermentation strategies, AW apple wine, FC fermentation control, NFC no fermentation control.*

Gas chromatography-mass spectrometry (GC-MS) of the volatile compounds

An aliquot of 50 µL of apple wine was transferred into 20 mL headspace vials with the addition of 50 mg NaCl. 2-Octanol was added as an internal standard (10 ng absolute). The extraction/enrichment of the volatiles was performed by headspace solid-phase-microextraction (HS-SPME; 2cm stable flex fibre, 50/30 µm DVB/Car/PDMS fibre) for 20 minutes at 40°C. Samples were stirred thoroughly during the enrichment process. The GC-MS analysis was conducted on a non-polar column (Rxi-5ms 30 m × 0.25 mm × 1 µm) and as well a as polar column (ZB-Wax 20 m × 0.18 mm × 0.18 µm), GC-MS chromatograms were recorded on Shimadzu GC-2010 Plus, MS QP 2020 (Shimadzu Europa GmbH; EI 70 eV, scan range 35-350 amu). The samples were analysed in fourfold in randomized order. The identification of the volatiles was based on probability-based matching of their mass spectra with those from MS libraries, authentic reference compounds and linear temperature-programmed retention indices. Retention indices were calculated by measuring the homologous series of *n*-alkanes (C6-C26) using the same GC-MS conditions as for the samples. Data deconvolution and integration was done with the PARADISe software, based on the PARAFAC2 model [4]. Semi-quantification was done via the internal standard (2-octanol) assuming a response factor of 1 for all compounds.

Threshold Determination

The determination of odour thresholds (detection as well as recognition thresholds) of selected compounds with floral, rose-like odour (i.e. linalool, rose oxide, 2-phenylethanol) was performed according to the ASTM method E679-04 [5]. Threshold values were calculated as the 'best estimate thresholds' (BET) from the geometric means of individual thresholds. The thresholds were determined in water and in a neutral apple wine that was produced from apples poor in aroma. Sensory experiments were performed with a well-trained expert panel (n=12) under standardized conditions in the sensory laboratory.

Statistical evaluation of the results

Statistical analysis was performed using one-way analysis of variance (ANOVA) to identify statistically significant differences between the samples. For statistical analysis, the MS excel add-in XLSTAT was used (Addinsoft 2020, Long Island, NY, USA).

Results and discussion

Floral and rose-like odour is part of the pleasant flavour of apple wines making the product attractive to consumers. Apples from the heritage apple variety *Ilzer Rose* which is domestic to the southern regions of Austria express a pronounced floral, rose-like aroma. In this work, we investigated if this specific floral and rose-like aroma might be intensified by technological measures. A large variety of terpenes was identified recently in the skin of *Ilzer Rose* apples [1]. In accordance to oenological practices, we applied different fermentation regimes to *Ilzer Rose* apple juice by adding enzymes with pronounced β-glucosidase activity. Maceration of the juices as well as mash fermentation – two common oenological practices leading to increased exposure time of the flash or the apple skin, respectively, to the enzymes – were investigated with respect to the concentration of terpenes, as we hypothesized an enzymatic release of terpenes from their glycosides.

Table 1 shows the concentrations of the selected compounds of interest in the different apple wines. For the terpenes under investigation that show floral and rose-like sensory properties (i.e., linalool, linalool oxide, α-farnesene) as well as for 2-phenylethanol we observed significant, however, low increases in concentrations when comparing the reference apple wine (AW1) to the apple wines AW2-AW5 where a pectolytic enzyme with β-glucosidase activity had been added and different fermentation regimes had been applied. For all compounds, the maceration of the juice or mash fermentation led to an increase in concentration of these volatiles, however, no clear trend is visible depending on the fermentation strategy. In comparison to the apple juice as starting material, we noticed a decrease in concentration by a factor 2 to 10 (data not shown) for the terpenes. 2-Phenylethanol, which also shows expressed floral sensory notes, is significantly higher in overall concentrations in the apple wines compared to terpenes; again, no clear trend can be observed in dependence of the fermentation strategy. However, 2-phenylethanol – which is most likely formed as a metabolite of the amino acid phenylalanine by *Saccharomyces* via the Ehrlich pathway – shows a 300-fold increase in concentration upon the fermentation process.

Table 1: Concentrations of terpenes in apple wine produced with different fermentation approaches; concentrations are given in average concentrations µg/L (n=4); letters indicate significant differences between compound concentrations in the different wines (p<0.05).

	linalool [µg/L]	**linalool oxide* [µg/L]**	**α-farnesene [µg/L]**	**2-phenyl-ethanol [µg/L]**
AW1	3.61 ± 14.5% *d*	5.91 ± 2.3% *c*	1.98 ±20.3% *c*	629 ± 8.9% *ab*
AW2	5.45 ± 2.8% *a*	9.04 ± 6.1% *a*	7.29 ±7.3% *ab*	727 ± 8.8% *a*
AW3	4.75 ± 3.2% *bc*	9.18 ± 5.9% *a*	9.15 ±22.7% *a*	618 ± 3.2% *b*
AW4	5.06 ± 3.0% *ab*	7.37 ± 0.7% *b*	6.32 ± 8.1% *b*	612 ± 7.4% *b*
AW5	4.42 ± 4.6% *c*	8.06 ± 2.2% *b*	8.06 ± 14.7 % *ab*	558 ± 4.3% *b*

* furanoid form; enantiomer not identified

It is well known that odour thresholds of odour-active compounds are highly matrix dependent. In the literature, most values for odour thresholds can be found for the matrices water and oil. In the context of our investigation, we wanted to receive detailed information on the how an apple wine matrix with an average ethanol content of 5% would impact the perception of selected aroma compounds with pronounced floral and rose-like aroma. As a matrix, we chose an apple wine that only showed weak, and especially not rose-like flavour; the investigated volatiles could also not be detected in this apple wine. As it was not possible to receive an α-farnesene-free apple wine, we did not determine the odour thresholds for this compound. Table 2 gives the results from the odour threshold experiments. The results demonstrate that the matrix shows significant impact on the odour thresholds of linalool. Even though the recognition and detection thresholds are close to one another, significantly higher concentrations are required for the sensory detection in the apple wine matrix compared to water. These results are in good accordance with previously reported odour thresholds for linalool in orange juice as matrix [6]. Interestingly, the odour thresholds for rose oxide as well as 2-phenylethanol are affected by the matrix apple wine, but to a far lesser extent than for linalool.

Table 2: Odour thresholds for selected compounds of interest given in water and apple wine in terms of the group BET in µg/L (n=12); group BET was calculated as geometric means of individual BET values.

	Detection threshold § [µg/L]		Recognition threshold $ [µg/L]	
	water	apple wine	water	apple wine
Linalool	4.9	90	5.1	330
Rose oxide %	0.02	0.3	0.1	1.0
2-phenylethanol	0.01	3.60	0.5	4.8

§ The value for the detection threshold is the level at which the differing sample is selected correctly without being able to describe the sensory properties of the compound; $ The value for the recognition threshold is the level at which the differing sample is selected correctly and the sensory properties could be described. % Rose oxide was incorporated to the study due its odour properties and the structure similarities to linalool oxide.

While setting the odour thresholds in relation to the observed concentrations of the compounds of interest, it is obvious that the investigated terpenes do not play an important role for the rose-like flavour of *Ilzer Rose* apple wine, independently of the way the fermentation is carried out. Obviously, in contrast to aroma-rich grape varieties, terpenes are present mainly in the apple skin, but mostly in their free form and not as glycosides. On the other hand, 2-phenylethanol must be considered an important contributor to the floral aroma of *Ilzer Rose* wines. The formation of 2-phenylethanol via the Ehrlich pathway is highly dependent on the yeast strain. Thus, the selection of a different from the applied *Saccharomyces* strain might be an option to further increase its concentration and to influence the floral aroma of the resulting apple wines.

Conclusion

The results of this study confirmed our initial hypothesis assuming that the treatment of *Ilzer Rose* juice with enzymes showing pronounced β-glucosidase activity would increase the concentration of terpenes due to their release from the corresponding glycosides. However, unfortunately observed concentration increases were too small for a significant impact on the flavour of the apple wines. We assume that the rose-like flavour is more likely attributed to the presence of 2-phenylethanol than to the investigated terpenes. However, the resulting apple wines showed completely different and nonetheless interesting flavour properties in comparison to the reference product. As a consequence, the detailed investigation of the flavour compounds in these apple wines will be subject of future investigations. Refashioning the technology by integrating oenological measures into the production of fruit wines and enhancing flavour release/formation from heritage apple varieties such as *Ilzer Rose* may help develop new flavour attributes in addition to the well-known apple wine properties.

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