

Influence of pH and temperature on the conversion of the mousiness causing agent acetyl-tetra-hydro-pyridine (ATHP) to acetyl-pyridine (AP)

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The thermodynamic parameters of the oxidation of the mousy off-flavor causing agent in wine 2-acetyl-tetra-hydro-pyridine (ATHP) to 2-acetyl pyridine (AP) via 2-acetyl-dihydro-pyridine (ADHP) were calculated. The results show that the first oxidation step is rather entropy-driven, while the second oxidation step is more enthalpy-driven. We could show that at higher temperatures, a potentially present off-flavor is oxidized to AP, which shows a tenfold higher sensory threshold and, thus, the off-flavor would be diminished. In wines without off-flavor, the temperature should be constantly kept at wine cellar temperature levels to avoid a possible off-flavor formation by various microbiota or via chemical processes.

Keywords: mousy off-flavor, wine, pH, temperature

Die thermodynamischen Parameter der Oxidation von 2-Acetyl-tetra-hydro-pyridin (ATHP), das im Wein für den Fehlton „Mäuseln“ verantwortlich ist, zu 2-Acetylpyridin (AP) über 2-Acetyl-dihydro-pyridin (ADHP) wurden berechnet. Die Ergebnisse zeigen, dass der erste Oxidationsschritt eher durch den Entropiegewinn getrieben wird, während der zweite Schritt eher durch den Gewinn an Enthalpie möglich wird. Wir konnten zeigen, dass ein bereits vorhandener Fehlton bei höheren Temperaturen zu AP umgewandelt wird. Dieses hat eine zehnfach höhere Geruchsschwelle und der Fehlton würde so verringert. In Weinen, die noch keinen Fehlton aufweisen, sollte die Temperatur bei konstanter Kellertemperatur gehalten werden, um die Bildung des Fehltons durch verschiedene Mikroorganismen und chemische Prozesse zu vermeiden.

Schlagwörter: Mäuseln, Wein, pH-Wert, Temperatur

The wine defect mousiness or mousy off-flavor has grown in intensity during the recent years, presumably through increasing pH values and more microbial spoilage. It is also linked to a significant decrease in free sulfur dioxide and the use of microbiota present in the vineyard rather than using specific commercially available yeasts (MASSINI and VUCHOT, 2015). Several compounds have been associated with this off-flavor, namely 2-acetyl-tetra-hydro-pyridine (ATHP), ethyl-tetra-hydro-pyridine (ETHP), and 2-acetyl-1-pyrroline (APY) (STRAUSS and HERESZTYN, 1984; CRAIG and HERESZTYN, 1984; HERDERICH et al., 1995). The mousy off-flavor has been described as a particularly unpleasant defect, reminiscent of rodent urine, and grilled food, such as popcorn, rice, crackers, and bread crust (STRAUSS and HERESZTYN, 1984; HERDERICH et al., 1995; TUCKNOTT, 1977; BUTTERY et al., 1983, BARTOWSKY, 2009). The off-flavor is only perceived, when the wine comes

into contact with saliva and may persist as long as ten minutes after swallowing or spitting. As a matter of fact, all three compounds are not volatile at wine pH, which is around 3.0 to 4.0. Saliva pH is near 7.0 and favors the imino form, which is much more volatile than the protonated amino form (BARTOWSKY and HENSCHKE, 1995; GRBIN et al., 1996).

Generally, these compounds are formed from the amino acids lysine or ornithine and acetaldehyde by *Brettanomyces* yeast or *Lactobacillus* or *Oenococcus oeni* species (GRBIN and HENSCHKE, 2008; COSTELLO and HENSCHKE, 2002; ROMANO ET AL., 2008). More recently, a pure chemical pathway has also been proposed, where ATHP is formed from the amino acid proline and methylglyoxal (KÜNZLER and POUR NIKFARDJAM, 2013). It has also been shown that ATHP is not stable and oxidizes over time to 2-acetyl pyridine (AP), Figure 1.

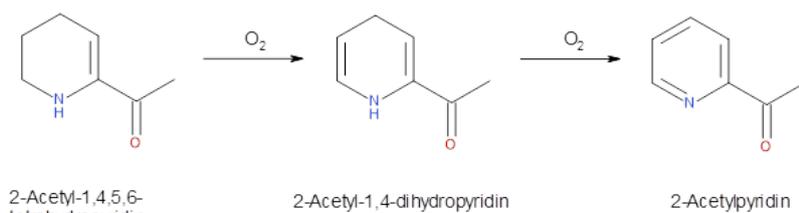


Figure 1. Oxidation route of 2-acetyl-1,4,5,6-tetra-hydro-pyridine

AP shows a tenfold higher detection threshold than ATHP and, thus, a conversion from ATHP to AP will significantly lower the perceived off-flavor (BELITZ et al., 2008). It was also proposed that an intermediate is formed, 2-acetyl-dihydro-pyridine (ADHP), and that its formation is pH dependent as two protons would have to be released into the wine from ATHP (POUR NIKFARDJAM and KUNZ, 2021). This supports also work by other authors (WEERAWATANAKORN et al., 2015; SCHIEBERLE, 1995). Other authors have stated that some wines only express mousiness after oxidation, which would oppose the aforementioned results (GRBIN et al., 1996).

In general, when a wine shows a mousy off-flavor the winemaker is advised to bring the wine to a more reductive stage using ascorbic acid or tannins, to lower the pH value in order to achieve a higher proportion of molecular sulfur dioxide against microbial spoilage, and to filter the wine to get rid of spoiling bacteria or yeast. Furthermore, the winemaker should store the wine at lower temperatures as warmth has been shown to intensify the formation of the mousy off-flavor due to better microbial growth or faster chemical formation of ATHP (MARGALIT, 2012; KÜNZLER and POUR NIKFARDJAM, 2013).

From this perspective, it was interesting to analyze the physico-chemical factors, which would determine the formation of AP from ATHP via the intermediate product ADHP. We took a focus on temperature and pH, as these are the most important factors favoring mousy off-flavor formation according to the above-mentioned research. Furthermore, since pH and temperature are going to further increase in years to come due to climate change, they may ultimately also have a direct effect on the formation of mousy off-flavor. We took a special focus on normal cellar temperature, i.e. 14 degrees Celsius, as well as on an elevated temperature of 30 degrees as might be reached on a warm summer day or during wine storage in a non-conditioned environment.

MATERIALS & METHODS

Thermodynamic parameters of the oxidation, i.e. deprotonation, of the 2-acetyl-1,4,5,6-tetrahydro-pyridine were determined as follows: the total energies of the interacting species were calculated at semi-empirical AM1 level using the geometry optimization performed by Fletcher-Reeves algorithm implemented in the HyperChem 8 code (BearingPoint France SAS, Paris, France). The molecular environment was considered by the TIP3P method, while the simulation box was randomly filled by the water molecules. The change in enthalpy associated with the oxidation steps was considered as the energy change calculated by subtracting the total energies of the reactants from the total energies of the products. Similarly, the entropy changes were calculated by subtracting the entropy terms of the reactants from the entropy terms of the products. Calculation of the entropy term is implemented in the HyperChem code as follows: after calculating the vibrational frequencies limited to the harmonic approximation, the entropy was then determined using the following equation:

$$S_{vib} = R \sum_i \left\{ \frac{h\nu_i/kT}{e^{(h\nu_i/kT)} - 1} - \ln[1 - e^{(-h\nu_i/kT)}] \right\}$$

Here the ν_i is the frequency of vibration and T is the temperature. Temperature was set to 14 and 30 degrees Celsius, respectively.

The equilibrium constants were determined by the algorithm implemented in the HyperChem 8 code either using the Gibbs free energy calculated within the procedure above and also the equilibrium geometry of the molecules optimized in AM1 level. Species distribution was calculated using the HyperQuad code (GANS et al., 1996).

RESULTS AND DISCUSSION

The thermodynamic parameters associated with the oxidation route of 2-acetyl-1,4,5,6-tetrahydro-pyridine were calculated for two temperatures: the usual winery cellar temperature of 14°C and a significantly higher temperature of 30°C, considered as warm shelf temperature in stores or at the consumer side. Table 1 summarizes the results. The results reflect the significantly different thermodynamic background of the **1** → **2** and the **2** → **3** reaction. In both cases the Gibbs free energies are negative

suggesting a spontaneous oxidation of the 2-acetyl-1,4,5,6-tetrahydro-pyridine or 2-acetyl-1,4-dihydro-pyridine molecules. Yet, the **1** → **2** reaction shows a more entropy-driven character, while the **2** → **3** reaction shows an enthalpy-driven character. This means that the endothermic character of the second reaction is overcompensated with the entropy gain in the first dissociation step. In the second dissociation step, a large entropy decrease is observed due to the improvement of the molecular symmetry, i.e. formation of a resonance-stabilized pyridine ring, which is energetically favored.

Table 1. Thermodynamic parameters associated with the oxidation steps of 2-acetyl-1,4,5,6-tetrahydro-pyridine

reaction step	ΔG (14 °C)	ΔG (30 °C)	ΔH	ΔS
	kJ/mol	kJ/mol	kJ/mol	J/K·mol
1 → 2	-18,53	-23,04	62,50	282,15
2 → 3	-32,16	-29,89	-72,91	-141,92

Table 2. Dissociation constants (log K values) related to the oxidation steps of 2-acetyl-1,4,5,6-tetrahydro-pyridine at two characteristic temperatures

temperature	equilibria	
	1 ↔ 2	2 ↔ 3
14 °C	3,37	5,85
30 °C	3,97	5,15

Table 3. Distribution [%] of different derivatives of 2-Acetyl-tetrahydro-pyridine at various pH levels selected within the common pH range of wines

pH	14° Celsius			30° Celsius		
	2-Acetyl-tetrahydro-pyridine	2-Acetyl-dihydro-pyridine	2-Acetyl-pyridine	2-Acetyl-tetrahydro-pyridine	2-Acetyl-dihydro-pyridine	2-Acetyl-pyridine
3,0	69,53	30,29	0,04	89,75	10,07	0,07
3,2	59,04	40,68	0,09	85,43	14,29	0,16
3,4	47,79	51,83	0,19	77,55	21,89	0,41
3,6	36,96	62,48	0,35	69,13	29,84	0,85
3,8	27,39	71,76	0,62	57,90	40,07	1,83
4,0	17,67	80,90	1,23	46,44	49,81	3,53

Table 2 summarizes the dissociation constants (log K values) related to the oxidation steps of 2-acetyl-1,4,5,6-tetrahydro-pyridine at two characteristic temperatures.

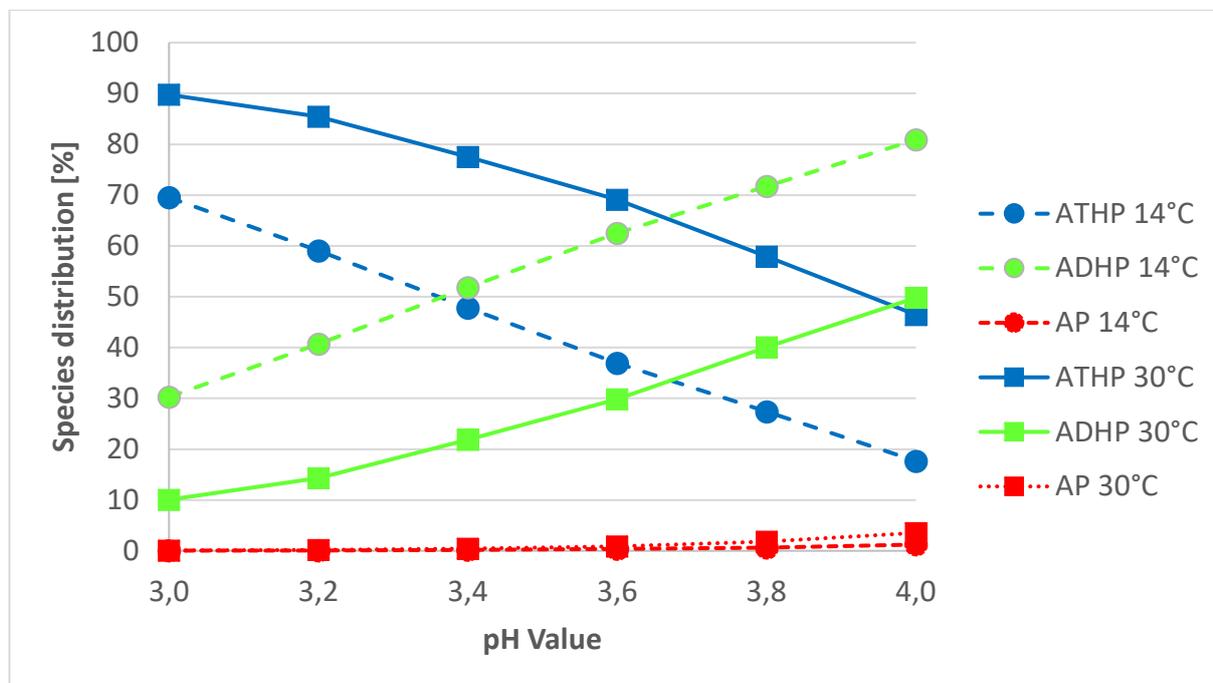


Figure 2. Distribution of different derivatives of 2-acetyl-tetrahydropyridine as function of the pH

The distribution of the different derivatives of 2-acetyl-pyridine as function of the pH and the percentages of the species existing in the pH values selected from the common pH range of wines are presented in Figure 2 and Table 3, respectively. The opposite temperature dependence of the Gibbs free energy is observed at usual wine cellar temperature and at elevated temperature. Hence, the equilibrium constants associated with the two oxidation steps result in a quite different species distribution.

The unexpected opposite temperature dependence of the Gibbs free energies associated with the $1 \rightarrow 2$ and $2 \rightarrow 3$ reactions reflect a quite different reordering of the molecules surrounded with the water solvent. The mapped isosurface of the compounds **1**, **2** and **3** suggest that two processes compete in association with the entropy change (Figure 3).

First process: During the $1 \rightarrow 2$ reaction the slightly positively charged surface of the molecule weakens due to solute–solvent interactions. Therefore, water molecules will leave the hydration shell resulting in entropy gain. Second process: During the $2 \rightarrow 3$ reaction the water molecules in the hydration shell are stabilized due to the increased Coulomb forces between the nitrogen or oxygen atoms and the solvent water molecules. This has a number of consequences: the order increases, the molecular skeleton is stabilized by the repulsive interaction between the nitrogen and oxygen atoms, and hence, the entropy term decreases. This thermodynamic background results in the enhanced formation of the compound **3** at any given pH and elevated temperature, while the formation of compound **2** is slightly inhibited at elevated temperature.

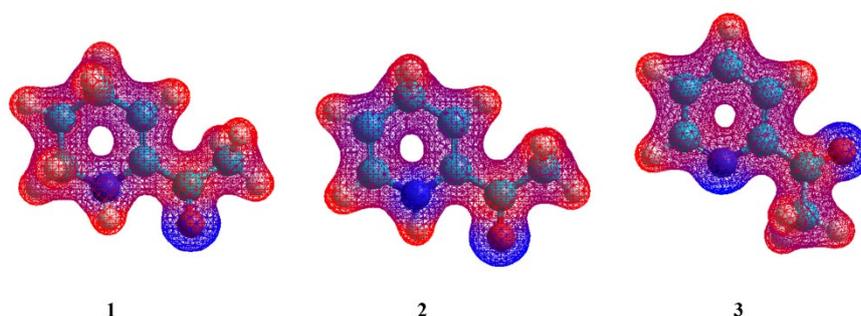


Figure 3. Mapped isosurface of charge densities of compounds **1**, **2** and **3** plotted at total charge density contour value of 0.05

In summary, the results show that at higher temperatures a reaction towards ADHP is not favored. This means that the mousy off-flavor will rather persist when temperature is at higher levels. Also lower temperatures seem to hinder the reaction from ATHP to AP. This is somewhat surprising, since winemakers are generally advised to lower pH and temperature, when a wine shows a distinct mousiness (MARGALIT, 2012). A lower pH would ensure a better effect of the molecular sulfur dioxide against microbial spoilage. Lower temperature would also hinder a faster growth of microbes and slow down thermally induced formation of ATHP. From this perspective lower temperatures can be beneficial, keeping bacterial growth at a minimum and also slowing down chemically induced formation of ATHP from its precursors proline and methylglyoxal. This is especially important, when a wine has not yet developed a mousy off-flavor. Yet, if mousiness is already present, the wine should be rather brought to warmer temperatures to convert any existing ATHP to AP, which has an about tenfold higher sensory threshold and so, the wine would lose mousy off-flavor intensity. This would mean that before treating any mousy wine, first the wine should be microbiologically stabilized, i.e. filtrated and adequate amounts of free sulfuric acid given, and then afterwards brought up to higher temperatures to convert the existing ATHP to AP.

These are completely new insights in a possible treatment of mousy off-flavor tainted wines. Further research has to look at these processes in detail not only in model solutions, but also in real wine situations to assure the practicability of the presented theoretical results for the winemakers.

ACKNOWLEDGMENTS

The research in Hungary was funded by NKFIH within the framework of the University of Pécs project TKP2021-EGA-17.

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