Vinifera master thesis abstract

Thesis title: Effects of must turbidity on fermentative aroma development in Sauvignon blanc

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Abstract (max 1500 words)

Wine became New Zealand's 6th biggest export good by 2015 with an annual sale of 1.42 billion $. 66% of grapes grown in the country are Sauvignon blanc and the most rewarded growing region is Marlborough, where a unique style developed, which distinguished itself not only from famous archetypes around the world but also from other winegrowing regions inside New Zealand. The aroma of Marlborough Sauvignon blanc is mainly driven by the complex interaction of the two aroma compounds Methoxypyrazines and thiols. While Methoxypyrazines are responsible for greenish, grassy and vegetal flavor compounds, thiols deliver the impression of tropical nuances like grapefruit, passion fruit, boxtree and guava. This masterthesis with the title "Effects of must turbidity on fermentative aroma development in Sauvignon blanc" aimed to give an overview over the state of research on Sauvignon blanc aroma with a focus on the three most important thiols 3MH,3MHA and 4MMP. This work tried to answer the question if thiol expression may be affected due to different additions of grape solids, which are in a commercial winemaking process separated after the pressing of grapes to ensure a clean fermentation bouquet and the absence of off flavors. The lees, obtained after the pressing of grapes consist of earth, skin, stem fragments, cellular debris from grapepulp and insoluble residues from vineyard treatment products During bibliographic review, grape solids were suspected to affect thiol metabolism in three different ways. First grape solids consist mainly of cell wall debris which is known to house a big pool of precursors for 3MH. Further, solids are known to act as crystallization points for CO_2 and therefore help to release CO_2 from a ferment which prevents from its enzyme denaturizing effect on the yeast cells. Finally, turbidity delivers unsaturated fatty acids (UFAs) which are not only a limiting factor for cell growth but have been suggested to affect yeasts enzyme activity. Especially alcohol acetyltransferase, the enzyme responsible for the transfusion of 3MH to 3MHA was suspected to increase its activity

Further this work tried to improve the small scale winemaking protocol of Plant and Food research (PFR) in Blenheim, New Zealand, where this work has been conducted. As fermentation trials are carried out through all the year at PFR, lots of juices are generally cold stored after cross flow filtration and addition of high amounts of SO2. Later usage of this juices for fermentation purposes has encountered multiple difficulties which were related to the clarity of juices. This work aimed to find solutions to be able to ferment in conditions close to commercial setups throughout all the year.

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Two fermentation trials were carried out. The first one was aiming to clarify the effect of four yeast doses, 150, 250, 350 and 450 mg/l in combination with two different doses of Superfood yeast nutrient consisting of DAP (Diammonium Phosphate), yeast cell hulls and yeast extract.
Samples were analyzed in the PFR laboratories (excluding thiol analysis), using the following methods.°Brix, pH, total acidity, tartaric acid, malic acid, lactic acid, citric acid, ammonia, alpha amino acid N, glucose, fructose, potassium, ethanol, anthocyanins, Folin C Index, OD280 and OD520. The infrared (IR) spectra of wine samples were first obtained and analyzed by a Foss Winescan FT2. Afterwards further analysis was carried out. Alcohol was determined using an Anton Parr Wine Alcolyzer. Glucose and fructose and ammonia were quantified by enzymatic assay. All spectrophotometric assays were run on a Molecular Devices Spectramax 384 Plus. Tartaric, malic, ascorbic, citric and succinic acids were quantified on a Shimadzu Prominence HPLC. Titrable acidity and pH were determined on a Mettler Toledo T70 autotitrator system
Analysis of the volatile thiols 3-MH, 3-MHA, and 4-MMP, was undertaken by Hill Laboratories in Hamilton, New Zealand. In summary wine volatile thiols were analyzed by HS-SPME (Head Space Solid-Phase Micro-Extraction) with GC-MS/MS (Gas Chromatography-Two Dimensional Mass Spectrometry).
Fermentation time was affected by both, Superfood additions and yeast dose and caused differences up to four days. Biggest effects were seen during the lag phase of fermentation. Once the stationary phase of fermentation was reached, behavior was similar within the ferments. Although it was hard to point out clear trends, as behavior changed, within different nutrition levels, multiple changes in the organic acid profiles of the derived wines were detected during organic acid analysis. Effects were seen on malic acid, tartaric acid, succinic acid and acetic acid, changing pH up to 0.06. Higher inoculation rates yielded up to 0.46% lower alcohol, most likely due to the effect of maloethanolic fermentation. This result was very satisfying as low alcohol wines get more attention nowadays. As other countries, New Zealand searches for options to lower the impact of climatic change. Further, it was seen, that higher yeast inoculations are able to preserve ascorbic acid in a ferment. The highest inoculation dose of 450 mg yeast/l yielded in significant lower amounts of 4MMP and 3MHA. These effect did not change the overall sum of thiols. This result was very unexpected and although more research has to be carried out to reveal the reason for these results it must be concluded that increasing inoculation doses have a negative effect on thiols when passing a threshold.
The second trial aimed to clarify the effect of different turbidity levels during fermentation of juices derived from three different vineyards within the Marlborough wine region. Therefore, eight levels of turbidity were adjusted, 50, 100, 150, 200, 250, 500 and 950 NTU, aswell as one fermenter set at 150 NTU with the help of Turbicell, an inert cellulose product which delivers the physical advantages of must turbidity without delivering chemical compounds, affecting the yeast cell. During the turbidity trial it became obvious that the different origins of the vineyards and their different initial YAN values (Yeast assimilable nitrogen) had a very strong effect which in some cases exceeded the actual treatment effects. Although all YAN values were adjusted to an equal level, following the PFR winemaking protocol, the initial vineyard derived nitrogen status caused different fermentation speeds. Therefore, for statistical analysis, fermentation time was used as a covariate. Within one dataset, turbidity levels caused changes of up to 5 days in fermentation speed. Slight changes of pH were monitored during this trial. But other than in the inoculation trial, no analysis of organic acids was carried out and no clear conclusions of this effect could have been drawn.
The most impressive finding during the turbidity trial was the increase of 3MH expression, monitored at wines derived from ferments of 500 and 950 NTU. 3MH levels were increased to 1.76 respectively up to 2.72-fold. This effect was suggested to be caused by the formation of thiol precursors due to the interaction of H_2S with green leave volatiles. The occurrence of 3MH is known to distinguish the Marlborough region from other Sauvignon blanc growing regions. Therefore, an increase of this compound is crucial to further contrasting the Marlborough region. Although no analysis on sulphur compounds was carried out during this trial, it is expectable that also strong off flavors like carbon disulphide, dimethylsulphide and dimethyldisulphide have been formed during fermentation with high turbidity, due to a high yeast cell mass accomplied by a depletion of nutrients, which would have decreased overall wine quality. A recently published patent has revealed a practical implementation of the effect of H_2S on thiol expression including sparging of gaseous H_2S into a ferment, starting 2 days before fermentation and lasting half way through fermentation. This method was able to deliver

high amounts of 3MH without the creation of sulphurous off flavors. The production of 4MMP and 3MHA, which relay mainly on the activity of the enzymes beta lyase and alcohol acetyltransferase were not affected by turbidity. It is known that beta lyase only transfers a small part of its precursors into 4MMP. This research states that also acetyltransferase is not affected by the media composition and only transfers a certain percentage of precursors. Therefore, initial assumptions had to be rejected. Further research may focus on the oxidation of unsaturated fatty acids like linoleic and linolenic acid to increase green leave volatiles which may in reaction with gaseous H_2S further increase the presence of 3MH.

Finally, this work was able to point out yeast nutrition and juice clarity as weak points within the Plant and Food Research small scale winemaking protocol. Suggestions to improve this winemaking protocol included the introduction of frozen grape solids derived from juices pressed during harvest and further a change towards another nutrient mixture which would ideally not only increase YAN by the addition of ammonia but by the addition of amino acids ad fatty acids. The addition of Turbicell alone was not able to mimic the effects of rape solid derived turbidity but Turbicell might be added besides a new nutrition mixture.

Keywords (5): Sauvignon blanc, thiol, turbidity, inoculation dose, nitrogen