

COMPARISON OF DIFFERENT MICROBIOLOGICAL STRATEGIES FOR CURATIVE DIACETYL REDUCTION BY *SACCHAROMYCES CEREVISIAE* IN WHITE WINE

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Diacetyl reduction by *Saccharomyces cerevisiae* is an efficient strategy to reduce the risk of wine spoilage. This study evaluates three different microbiological methods with regard to their effectiveness in curative diacetyl reduction in white wine. In the first variant, the wine lees were stirred after adding 20 mg/l diacetyl directly to a 'Riesling' wine. Although in this variant the highest yeast viability was found, there was no significant decrease in diacetyl concentration during the experimental period. In the second variant 20 mg/l diacetyl were added to a sterile-filtered 'Riesling' wine and *Saccharomyces cerevisiae* was inoculated according to the manufacturer's instructions. Here a slight reduction of the initial diacetyl concentration was observed. In the third variant, a sterile-filtered 'Riesling' wine was blended with a 72 h fermented wine and diacetyl concentration was set on 20 mg/l. Although this variant possessed the lowest yeast viability, there was a nearly complete consumption of the initial diacetyl concentration. To the best of the author's knowledge, this is the first study, which compares different microbial strategies to reduce diacetyl concentration in white wine. Furthermore this work demonstrates the efficiency of easy- to- handle methods for a curative diacetyl reduction during vinification.

Keywords: 2,3-butanedione, spoilage, yeast, degradation; consumption, winemaking vinification

Vergleich verschiedener mikrobiologischer Strategien zur kurativen Diacetylreduktion durch *Saccharomyces cerevisiae* in Weißwein. Die Diacetylreduktion durch *Saccharomyces cerevisiae* ist eine effiziente Strategie, um das Risiko von Weinverderb zu reduzieren. Diese Studie bewertet drei verschiedene mikrobiologische Methoden hinsichtlich ihrer Wirksamkeit bei der kurativen Diacetylreduktion in Weißwein. In der ersten Variante wurde der Weintrub nach Zugabe von 20 mg/l Diacetyl direkt zu einem Riesling-Wein gerührt. Obwohl in dieser Variante die höchste Hefeviability gefunden wurde, gab es während der Versuchsdauer keine signifikante Abnahme der Diacetylkonzentration. In der zweiten Variante wurden 20 mg/l Diacetyl einem sterilfiltrierten Riesling-Wein zugesetzt, und *Saccharomyces cerevisiae* wurde gemäß den Anweisungen des Herstellers inokuliert. Hier wurde eine leichte Reduktion der anfänglichen Diacetylkonzentration beobachtet. In der dritten Variante wurde ein sterilfiltrierter Riesling-Wein mit 72 Stunden fermentiertem Wein gemischt und die Diacetylkonzentration wurde auf 20 mg/l eingestellt. Obwohl diese Variante die geringste Lebendzellzahl der Hefe zeigte, gab es einen nahezu vollständigen Verbrauch der anfänglichen Diacetylkonzentration. Nach bestem Wissen des Autors ist dies die erste Studie, die verschiedene mikrobielle Strategien vergleicht, um die Diacetylkonzentration in Weißwein zu reduzieren. Darüber hinaus demonstriert diese Arbeit die Effizienz einfach zu handhabender Methoden zur kurativen Diacetylreduktion während der Vinifikation.

Schlagwörter: 2,3-Butandion, Verderb, Hefe, Abbau; Verbrauch, Weinherstellung

The vicinal diketone diacetyl (2,3-butanedione) is a volatile compound and is associated with a buttery flavor (BARTOWSKY et al., 2002). The sensory threshold of diacetyl in wine varies between 0.2 and 2.7 mg/l and depends on the wine type and style (MARTINEAU et al., 1995a). In lower concentrations diacetyl can improve the wine stylistic and the wine bouquet (RANKINE et al., 1969). However, diacetyl concentrations above 5 to 7 mg/l induce the typical buttery wine spoilage (DAVIS et al., 1985).

During wine making, most diacetyl is formed by lactic acid bacteria as a consequence of the citric acid and pyruvate metabolism (BARTOWSKY et al., 1997; MINK et al., 2015). But to a much lesser extent wine yeasts of the species *Saccharomyces cerevisiae* (*S. cerevisiae*) can also release small amounts of diacetyl (MINK et al., 2012). In that case, diacetyl derives from intermediates of the branched-chained amino acid synthesis.

As most diacetyl is formed by lactic acid bacteria, it is assumed that wines which have undergone malolactic fermentation (MLF) are frequently affected by buttery off-flavors (MARTINEAU et al., 1995b). *Oenococcus oeni* (*O. oeni*) is the preferred species for MLF. The wine industry offers numerous *O. oeni* strains with different diacetyl formation tendencies (MINK et al., 2012). To prevent buttery flavors, it is suggested to use *O. oeni* strains with a low diacetyl formation potential. However, there are much more factors than the bacteria strain, which influence the diacetyl concentration in wine (BARTOWSKY and HENSCHKE, 2005; MINK et al., 2015).

One factor, that is still underestimated, is the diacetyl degradation potential by *S. cerevisiae*. It has been shown, that active *S. cerevisiae* strains are able to reduce diacetyl concentrations up to 50 mg/l (Mink et al., 2014). This is much more than normally expected during the wine making procedure.

Diacetyl reduction by *S. cerevisiae* contains two enzymatically driven steps. First of all diacetyl is converted into acetoin by the butanedione-dehydrogenase (WAINWRIGHT, 1973). After that acetoin is reduced to 2,3-butanediol (GUYMON and CROWELL, 1965). Acetoin, as well as 2,3-butanediol are molecules with high sensory thresholds and do not impart the typical buttery flavor of diacetyl (BERTRAND et al., 1984).

In order to reduce the buttery off-flavor in wine, one frequently used oenological procedure is stirring the wine lees and to age the wine on the lees. However, to the best of our knowledge there is no scientific evidence that diacetyl concentration can be reduced by these methods.

In order to improve the understanding of diacetyl reduction strategies the present study evaluates three different oenological methods regarding their effectiveness in the reduction of diacetyl in white wine.

MATERIAL AND METHODS

WINE MATRIX, MICROORGANISMS AND EXPERIMENTAL FERMENTATION

Experiments were performed in 3 l-Erlenmeyer flasks covered with sterile bungs, equipped with ethanol (70 %vol.) filled airlock bubblers. Wine matrix composition was determined by FTIR Spectroscopy (FOSS WineScan FT 120, Foss, Hillerod, Denmark) with the following results:

glucose 0.5 mg/l, fructose 1.7 mg/l, L-malic acid 2.6 mg/l, ethanol 94.2 mg/l, tartaric acid 4.3 mg/l and pH-value 3.14.

Characterisation of the diacetyl reduction efficiency by *S. cerevisiae* Lalvin CY 3079 (Lallemand, Montreal, Canada) was performed with three different oenological techniques:

1. Stirring the wine lees.
2. Blending with 1/12 of a 72 h fermented wine.
3. Yeast inoculation according to manufacturer's instructions.

All variants were fermented at room temperature and in triplicate replication.

For the first variant pasteurized 'Riesling' must was fermented with *S. cerevisiae*. The yeast inoculation was performed with a yeast dosage of 25 g/hl according to the manufacturer's instructions (Lallemand, Montreal, Ca-

nada). In order to generate the lees the wine was stored for three weeks after alcoholic fermentation. Diacetyl concentration (20 mg/l) was adjusted by adding diacetyl (Sigma-Aldrich, Darmstadt, Germany) directly to the wine. After that the wine was stirred to dissolve the lees. For the second variant 2.75 l sterile-filtered 'Riesling' wine was blended with 0.25 l of a 72 h unfiltered fermented 'Riesling' wine. The sterile-filtered 'Riesling' wine as well as the unfiltered fermented 'Riesling' wine, which were used in the present study, derived from the same 'Riesling' must.

Subsequently diacetyl was added to a final concentration of 20 mg/l.

For the third variant 3 l sterile-filtered 'Riesling' wine was inoculated with *S. cerevisiae*. The inoculation procedure was performed with a yeast dosage of 25 g/hl according to the manufacturer's instructions.

DETERMINATION OF THE INITIAL YEAST CELL VIABILITY

Determination of the initial yeast viability was performed immediately prior to diacetyl addition by determination of the colony forming units on YPM-Agar (Sigma-Aldrich, Darmstadt, Germany). Viable cells were enumerated after the plates were incubated at 20 °C for five days.

DETERMINATION OF DIACETYL

Diacetyl was analyzed after derivatisation with 1,2-diaminobenzene (Sigma-Aldrich, Darmstadt, Germany) by gas chromatography-mass spectrometry (GC-MS) as described in the compendium of international methods of analysis of wines and musts (OIV-MA-AS315-21). The GC instrument used was an Agilent 6890N Gas chromatograph (G1530A, Agilent Technologies, Santa Clara, USA), equipped with a split/splitless injector connected to a Agilent 5975 C VL MSD (G3170A, Agilent Technologies, Santa Clara, USA). Samples (1.5 µl) were injected in split mode (20:1) using an MPS 2 Multi Purpose Autosampler (Gerstel, Mülheim, Germany), with an injector temperature of 250 °C. A 30 m × 0.25 mm i.d. fused silica capillary, coated with 0.25

µm of a polyethylene glycol stationary phase (ZB-Wax, Phenomenex, Aschaffenburg, Germany) was used as a separation column. Helium was used as carrier gas with a constant flow of 1.2 ml/min. The oven temperature was initially held at 60 °C for 2 min and then raised to 240 °C with a rate of 8 °C/min, finally held for 5 min. The MS transfer line and ion source temperature were held at 240 °C, the quad temperature at 150 °C. Electronic ionisation was realized at 70 eV. Detection (EI+) in the SIM mode (m/z 117.0, 158.1, 171.0) and quantification was done on ion traces of fragment ions. Quantifier and qualifier ions were m/z 117.0 and 158.1 for diacetyl, respectively m/z 158.1 and 171.0 for 2,3-hexanedione (Sigma-Aldrich, Darmstadt, Germany), the latter used as internal standard. Instrument control and data acquisition were performed with Agilent Chem Station (Version E 02), quantification with Agilent Mass Hunter Workstation Software, Quantitative Analysis 5.0.

RESULTS

INITIAL YEAST VIABILITY

All three variants showed significant differences in the initial viability (Fig. 1). The highest viability ($8,5 \times 10^7$ CFU/ml) was found in the variant, in which the wine lees were stirred. In the variant in which dry selected wine yeasts were added according to the manufacturer's instructions, the initial viability was $9,4 \times 10^5$ CFU/ml. The lowest initial viability (5×10^4 CFU/ml) was found in the variant which was blended with the 72 h fermented wine.

DIACETYL CONCENTRATION

Diacetyl concentration was determined by GC-MS after derivatisation according to the OIV Method OIV-MA-AS315-21. In all variants native diacetyl concentration was <0,5 mg/l (results not illustrated here). After the addition of 20 mg/l diacetyl, diacetyl concentration was found significantly higher than the theoretically expected concentration (Fig. 2).

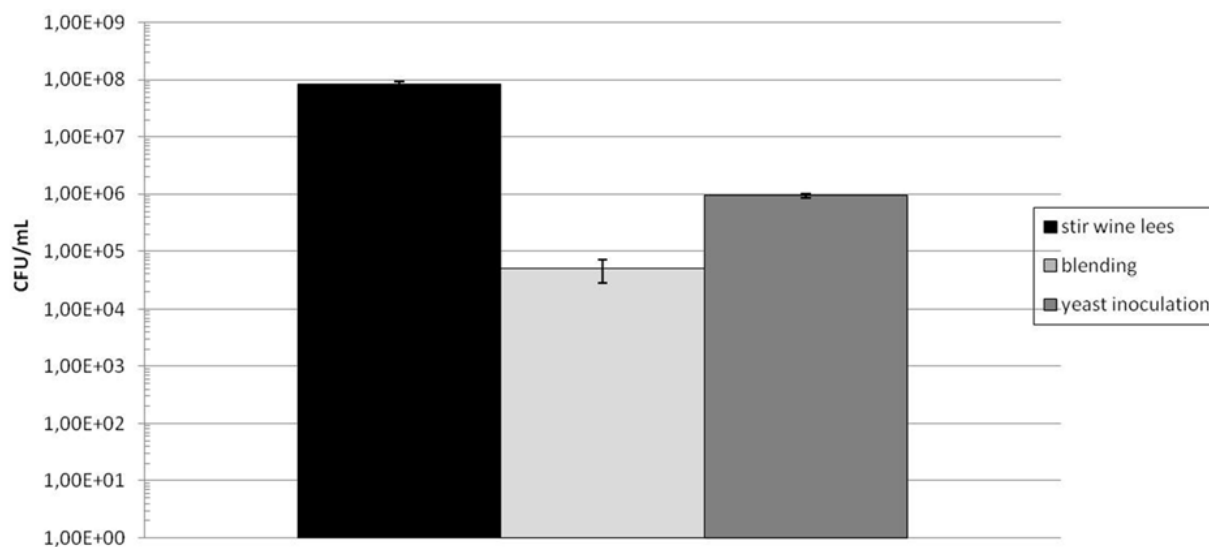


Fig. 1: Initial viability of *S. cerevisiae*. Each bar presents the average results of triple repeat experiments.

Within the experimental period (14 days) only the variant which was blended with a 72 h fermented wine showed a nearly complete diacetyl reduction (Fig. 2, Fig. 3). In contrast, there was no significant decrease in diacetyl concentration after the lees were stirred. In the variant in which *S. cerevisiae* was added according to the manufacturer's instructions, the initial diacetyl concentration decreased from 31 mg/l to 17 mg/l.

DISCUSSION

A small amount of diacetyl is associated with a buttery, nutty and toasty aroma and this may improve wine stylistic (RANKINE et al., 1969). However, higher diacetyl concentrations impart buttery off-flavors (DAVIS et al., 1985; RANKINE et al., 1969). Therefore, an optimal dia-

cetyl management is necessary to reduce the risk of wine spoilage.

Although there is enough evidence, that *S. cerevisiae* is able to reduce diacetyl concentration much more than normally expected during vinification (MINK et al., 2014), the potential of the diacetyl degradation behaviour by *S. cerevisiae* is still underestimated.

To the best of our knowledge, the vast majority of studies focus on the diacetyl formation by malic acid bacteria and barely on the diacetyl reduction by *S. cerevisiae*. Therefore, the present study should help to improve the understanding of the effectiveness in diacetyl reduction by *S. cerevisiae* during the vinification procedure.

After the addition of 20 mg/l diacetyl to the wine, the diacetyl concentration was found significantly higher than theoretically expected. Here, the most obvious explanation is an insufficient homogenisation of the added diacetyl.

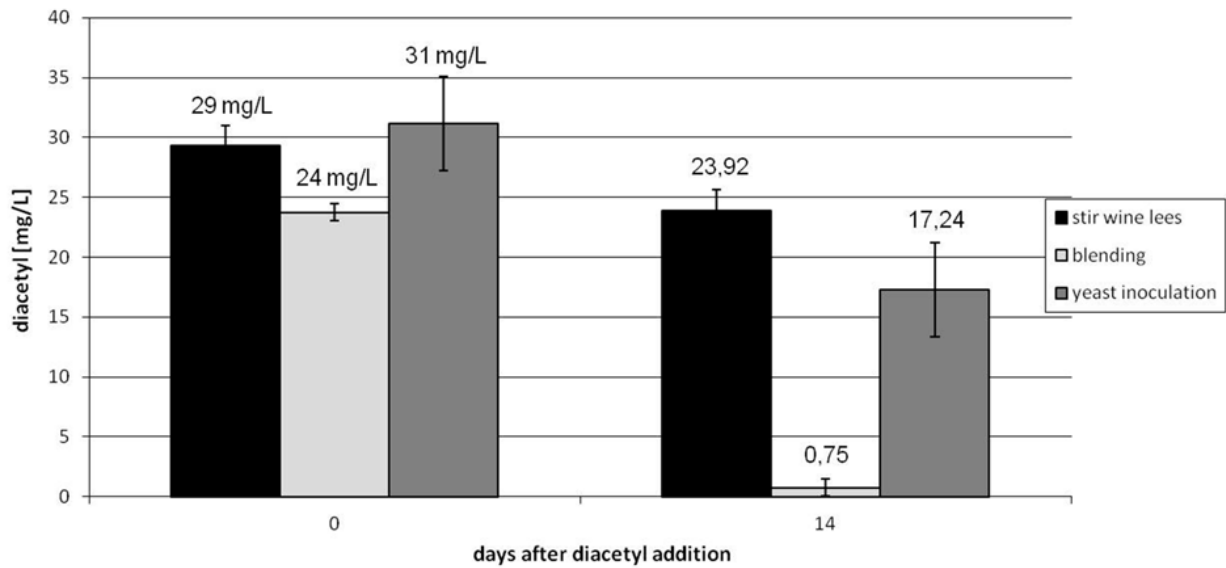


Fig. 2: Initial diacetyl concentration (0) and diacetyl concentration after 14 days (14). Each bar presents the average results of triple repeat experiments.

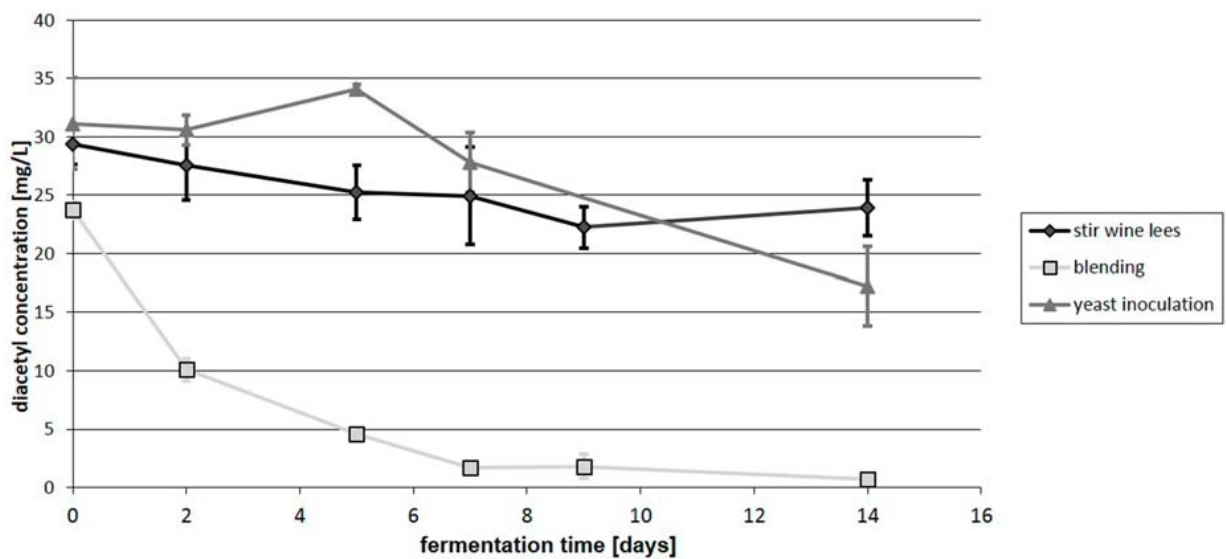


Fig. 3: Dynamics of diacetyl concentration during the experimental period. Each point presents the average results of triple repeat experiments.

The presence of lees, especially when stirred, is frequently recommended as an effective method for diacetyl reduction (BARTOWSKY and HENSCHKE, 2004; RANKINE et al., 1969). However, the scientific evidence of this statement has never been provided.

The results of this study show no significant diacetyl reduction after the lees were stirred. It is assumed that based on the reduced metabolic activity in the lees the genes which were involved in the diacetyl reduction metabolism, especially the *bdh1* gene, are much lower expressed than during alcoholic fermentation. Therefore, most of the diacetyl will not be significantly reduced and remains in the wine. This is further supported by the missing correlation between the yeast viability and diacetyl reduction activity during the experimental period. Therefore, it can be assumed, that the diacetyl reduction

potential of *S. cerevisiae* in wine is primarily influenced by the yeast activity.

Further experiments are necessary to determine the amount and activity of *S. cerevisiae* for an optimal diacetyl reduction. Furthermore, gene expression studies of the *bdh1* gene should be performed, to verify the relation between the *bdh1* gene and the diacetyl degradation capacity of *S. cerevisiae*.

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