The Microbial Population Dynamics in Wine


Fig. 1. The change in yeast community composition, temperature, and ethanol concentration during a traditional wine ferment. Shown is the change in population size (colony forming units, cfu) of the non-Saccharomyces yeasts (thin black dashed lines) and S. cerevisiae (thin black solid lines) in four separate barrels over 20 days of fermentation. Also shown is the average change in temperature (heavy red line) and ethanol levels estimated from the change in specific gravity (heavy blue line) for these four barrels over days 6–16 of the fermentation.
Pre-fermentation yeast can help you keep/bring out the full potential of the grapes.

Grape juice is chemically quite complex and the sensory potential is large (Terpenoids, Nor-isoprenoids, Thiols, Methoxypyrazines, Acids, Alcohols).

Is the use of one Saccharomyces yeast bringing out this potential?

Pre-fermentation yeast will help bring out more of the potential.

Bring back the complexity and full potential of the wine.
When screening for pre-fermentation yeast it is not the alcoholic fermentation activity which is our focus

- Instead we are looking for activities, which can increase the quality of the wine (Flavor, structure, bioprotection etc.)
- And securing that no off-flavors are produced by these non-saccharomyces yeast
- We then ensure compatibility with our other yeast & malolactic cultures

- We work with institutes from all over the world to find new yeast
  - Sometimes, we also isolate them internally from grape juice and wines

- According to OIV regulations all yeast and bacteria sold for winemaking have to be isolated from grapes, juice or wine
  - This we always comply to
 Viniflora® FROOTZEN™

- *Pichia kluyveri*
- The amazing “Fruit expression”

- Generates Esters, Thiols and flavor precursors
- Reduces oxydation impact
Background of FrootZen™

- FrootZen™ was found in New Zealand, during a research project at Auckland University on New Zealand Sauvignon blanc
  - They were investigating why New Zealand Sauvignon blanc was special
  - What was giving it these intense tropical flavours that it is known for

- They found *Pichia* to be the source of the increased thiol liberation and ester production
- Then started a characterization of all the found Pichia strains and FrootZen was chosen as the best for production of thiols
  - We then purchased the right to the strain

- We have since found out that FrootZen™ can do much more than bring out the tropical passion fruit flavour in Sauvignon blanc
  - FrootZen’s™ high hydrolysis activity of thiols increases fruitiness in all varieties - also reds
  - Furthermore, it has a high production of Esters

- NB! FrootZen is NOT a strong fermenter
  - Do not wait on a decrease in density (sugars)
Sauvignon blanc, 2010, New Zealand

Volatile thiols (ng/l)

- Control
- Pk Co-inoc
- Pk Seq

3MH
3MHA

CHR HANSEN
Improving food & health
Viniflora® FrootZen™ the first wine yeast for direct inoculation

- FrootZen™ is a frozen yeast (-50°C) for direct inoculation
  - Take the FrootZen out of the freezer
  - Place in lukewarm water for a couple of minutes, to loosen the yeast from the plastic
  - Open the pack with a knife or scissor (be careful!)
  - Throw the yeast directly in the tank!

- Inoculation timings
  - In the harvest trucks -> to protect the grapes and extract as many thiols and thiol precursors as possible
  - In the tank
    - Before or after cold settling (whites)
    - Before, being more for bioprotection
  - During the tank filling (reds)

- Optimal temperature 15-25°C for aromas
- Tolerance 10-30°C

- Recommendation is to inoculate FrootZen as early as possible
Sensory profile improvement with FrootZen™
white wine example (Sauvignon blanc)

Flavors frequency mentioned by external trained/non professional jury
Loire Valley wine (Sancerre - Sauvignon blanc)
Data: In senso veritas, Feb. 2013
Sensory profile improvement with FrootZen™ red wine example (Pinot Noir)

Descriptive sensory evaluation: Red bars: S. cerevisiae + FrootZen®, Blue bars: S. cerevisiae only

Quantification of 3-mercato ethanol, a flavoring thiol
# Viniflora® FrootZen™ application notes

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<th>Strengths</th>
<th>Application</th>
<th>Why</th>
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<tr>
<td>Boosted fruity aroma in all varieties</td>
<td>White, rosé, red wine (Pinot noir)</td>
<td>Flavour impact</td>
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<td></td>
<td>Increase quality of high yield, low aromatic grapes</td>
<td>Better colour in rosé (more pink)</td>
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<td>(white grapes in warm areas)</td>
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<td></td>
<td>Blending tool for high and base white wine</td>
<td>Blackcurrant aroma in pinot noir</td>
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<td>White wines with skin contact</td>
<td>Oxygen scavenging</td>
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<td>Rosé and especially No SO₂ rosé</td>
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<td></td>
<td>Pinot noir – increased blackcurrant and overall fruitiness</td>
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Lachancea thermotolerans (ex Kluyveromyces thermotolerans)

- Produces lactic acid
- Improves acid balance
- Increases red berry aroma
Concerto™ was selected due to its flavour production as well as lactic acid production.

The ester production by Concerto™ depends as with other yeast on the wine conditions e.g. the temperature.

- **Saccharomyces**
- **Concerto + Saccharomyces**
- **Prelude + Saccharomyces**
- *Lachancea termotolerans*'s acid production with different dosage of Saccharomyces

- **Source:** Comitini, F., et al., Selected non-Saccharomyces wine yeasts in controlled multistarter f..., Food Microbiology (2011)
  - Pasteurized grape must at pH 3.2 under laboratory conditions

- This knowledge can also be used for inoculation timing decision with Concerto
  - If you give it more time to dominate, you will have more effect
  - We recommend 1-3 days before inoculating a Saccharomyces
Valpolicella Negrar

- Tank 1  Jazz  20 g/hL
- Tank 2  Concerto + Jazz  25 g/hL Concerto + 20 g/hL Jazz (48 hours after Concerto)
- Tank 3  HD Concerto  75 g/hL Concerto

- 100L tanks
- Alcoholic fermentation proceeded similar in the 3 tanks (potentially with help from the spontaneous flora)
Valpolicella Negrar

- Tank 1 Jazz 20 g/hL
- Tank 2 Concerto + Jazz 25 g/hL Concerto + 20 g/hL Jazz (48 hours after Concerto)
- Tank 3 HD Concerto 75 g/hL Concerto

- Lactic acid was produced by Concerto the first 3 days
  - Concerto produces lactic acid, in it’s growth phase
  - Fits well with literature saying that *Kluyveromyces thermotolerans* can produce lactic acid from sugar, when oxygen is present

The production of lactic acid by Concerto is not dosage dependent -> Normal dosage 25g/hL is enough
Ethyl lactate synthesis
Ethyl lactate production

![Ethyl lactate production graph]

- Ethyl lactate production for different strains:
  - S. cerevisiae
  - Prelude
  - Concerto
  - Spontaneous

![Strawberries image]
Viniflora® Concerto™

- To keep/increase acidity in warm climate wines
- Enhance red berry aromas in red and rose

- New data is showing that Concerto™ has good bioprotection potential in must and wine (It is more than just flavor)
  - Data shown in bio-protection session
## Viniflora® Concerto™ application notes

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<tr>
<td><strong>Concerto™</strong> Freshness, fruitiness and acidity</td>
<td>Red, white, sparkling and Porte Sparkling base wine, with and with-out sequential inoculation of a saccharomyces yeast Warm climate reds – to give some acidity and elegance Thermo vinification red wine – less cooked sensory profile White wine blending tool</td>
<td>Freshness, fruitiness and acidity Bio-protection</td>
</tr>
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</table>
PRELUDE™
Torulaspora delbrueckii

- Improves mouthfeel
- Dark fruit & Candies flavor
  - Low VA

The perfect choice to get ‘wild ferment’ benefits without the risks.
Viniflora® Prelude™

- Prelude™ was selected for polysaccharide production and resistance to osmotic stress.

- What is a polysaccharide?
  - They are polymeric carbohydrates -> So, chains of sugar e.g. glucose chains
  - They give texture and viscosity

- Therefore, Prelude™ increases the mouth feel of a wine (Used in red, white and sweet wines)
Simultaneous inoculation, with different dosage of Saccharomyces

- The dominance of Prelude™ has a clear effect on the Polysaccharide concentration in the final wine

• Same effect can be achieved by sequential inoculation
• Prelude 1-3 days later Saccharomyces
• The more time by it self to dominate the more polysaccharides

Source: Comitini, F., et al., Selected non-Saccharomyces wine yeasts in controlled multistarter f…, Food Microbiology (2011)
- Pasteurized grape must at pH 3.2 under laboratory conditions
Prelude™ increases fruit flavor and could mask green flavor often faced in merlot

The values in the spider web are given as odor activity value (OAV) - concentration/threshold

**Tasting notes**
- Tank Prelude: Dark fruit e.g. plums
- Tank S.c.: Dark fruit and spicy
- Tank Spontaneous: Harsh and alcoholic

Merlot is often subject to green flavor issues.

Prelude was also associated to dry fruit, tropical fruit or marmalade Merlot, South Africa, 2015 and 2016. The green perception was significantly reduced.

Hein et al. (2008) have shown the green flavor could be masked by the fruity flavor in red wine.
Viniflora® Prelude™ Effect on volatile acidity
High sugar content wine from Semillon

Wine made from high sugar Semillon must, Bordeaux, France 2009
Marina Belly’s laboratory, ISVV Bordeaux
### Viniflora® Prelude™ application notes

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| Prelude™ Versatile yeast, successful in white and red | Red and whites  
- Cold soak both red and white  
- "normal" sequential inoculation | Mouthfeel  
Non fruity complexity |
| Mouthfeel | Australia and NZ mainly use Prelude in white wines  
In the rest of the world mainly red varieties as Pinot noir, Cabernet franc, merlot etc. | More flavor  
Bio-protection |
Pre-fermentation yeast from Chr. Hansen’s impact on the sensory profile
All with Merit 2 days later

The Red: Cabernet Sauvignon

Winemaking:
AF temp: 25°C
Maceration: 6 days
MLF: Yes

Analysis:
RS: 3.0 g/l
Alc: 13.8%
pH 3.6
TA = 4.8g/l
(as tartaric)

The Rosé: Bobal

Winemaking:
AF temp: 16°C
Maceration: 4 hours
MLF: No

Analysis:
RS: 3.5 g/l
Alc: 14.0%
pH 3.5
TA = 4.8g/l
(as tartaric)
Simultaneous vs. Sequential
- We recommend sequential to get the most out of the pre-fermentation yeast

Viniflora® Pre-fermentation yeast → 1-3 days → Viniflora® Merit

NB! Important to manage the alcoholic fermentation with a Saccharomyces
- As the Pre-fermentation yeast are not specifically selected for their fermentation capacity

Inoculation timing

Alcoholic fermentation

Population
Saccharomyces spp.
Non-Saccharomyces

Malolactic fermentation

Oenococcus oeni

Time
Rehydration of the Saccharomyces and Non-Saccharomyces yeast

- **Viniflora® Merit** (*Saccharomyces cerevisiae*) – Dosage 20g/hL (500g for 650Gal)
  - **Rehydrate** in un-chlorinated water at **86-100°F** for 10 min (1 pack in 1.5Gal)
  - **Activate** add un-sulfured must (5Gal) and leave for approx. 20min
  - **Acclimatize** Add to the and pump over (or other mixing), to ensure a good suspension of the yeast in the must/juice

- **Viniflora® Concerto** (*Lachancea termotolerans*) and **Prelude** (*Torulaspora debrueckii*) – Dosage 25g/hL (500g for 530 Gal)
  - **Rehydrate** in un-chlorinated water at **68-77°F** for 10 min (1 pack in 1.5Gal)
  - **Activate** add un-sulfured must (0.5Gal) and leave for approx. 20min
  - **Acclimatize** Add to the and pump over (or other mixing), to ensure a good suspension of the yeast in the must/juice

- **Viniflora® Prelude™** (*Torulaspora debrueckii*) – Dosage 25g/hL (500g for 530 Gal)
  - **Rehydrate** in un-chlorinated water at **68-77°F** for 10 min (1 pack in 1.5Gal)
  - **Activate** add un-sulfured must (0.5Gal) and leave for approx. 20min
  - **Acclimatize** Add to the and pump over (or other mixing), to ensure a good suspension of the yeast in the must/juice

- Be aware of the slight differences
  - Proper re-hydration will give the customer a better product
Melody™
20% Torulaspora delbrueckii
20% Lachancea thermotolerans (ex Klyuyveromyces thermotolerans)
60% Saccharomyces cerevisiae

- The blend for convenience
  - Mouth feel
  - Complexity
Viniflora® Melody™

- A blend for complexity and convenience
  - 20% Torulaspora delbrueckii (Prelude™)
  - 20% Lachancea (Kluyveromyces) thermotolerans (Concerto™)
  - 60% Saccharomyces cerevisiae (Merit™)

- It gives you the best of all 3 yeast
  - Torulaspora delbrueckii (Prelude™) for mouthfeel and secondary flavors
  - Lachancea (Kluyveromyces) thermotolerans (Concerto™) for acidity, structure and esters (red berries)
  - Saccharomyces cerevisiae (Merit™) For alcoholic fermentation

- Melody use to be sold mainly for Chardonnay
  - However, over the recent years if has become increasingly popular in many red varieties e.g. Shiraz, Grenache and Mourvedre

“Barossa grapes have so much fruit intensity, due to the warm climate. Melody builds the structure to match the fruit – Happy Days”

Jimi Lienert Barossa winemaker

Jimi ads “The slow start is great for the color development in the Grenache and I also get a nice tight structure. Furthermore, it makes it smell of strawberry ice cream”

Jimi Lienert use to favor spontaneous alcoholic fermentation, but now he uses Melody as it gives the structure and aromas he wants and no problems with stuck AF
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<tr>
<th>Culture</th>
<th>Form</th>
<th>Composition</th>
<th>Function</th>
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<tr>
<td>Prelude™</td>
<td>ADY</td>
<td>Torulaspora delbrueckii</td>
<td>Increases mouthfeel</td>
</tr>
<tr>
<td>Concerto™</td>
<td>ADY</td>
<td>Lachancea thermotolerans</td>
<td>Improves acid balance</td>
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<tr>
<td>Frootzen™</td>
<td>Frozen</td>
<td>Pichia kluveri</td>
<td>Amazing fruit expression</td>
</tr>
<tr>
<td>Merit</td>
<td>ADY</td>
<td>Sacharomyces cerevisiae</td>
<td>Strong fermenter</td>
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<tr>
<td>Melody</td>
<td>ADY</td>
<td>S.Cerevisiae (60%)</td>
<td>Mimic traditional flora with a process in control</td>
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<td></td>
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<td>T.Delbriecii (20%)</td>
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<tr>
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<td>L.Thermotolerans (20%)</td>
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Thank you for your attention