Diacetyl Formation, Reduction & Removal

Testing Methods
- Small, Medium, Large Craft Breweries.

Daniel I McCulloch - vb
When I started Young Henrys, it was known as the butter factory of Newtown.

Cows now in the pen
VDKs or Vicinal Diketones are a group of flavor components commonly found in beer. VDKs found in beer are most notably 2,3-butanedione (Diacetyl) and 2,3-pentanedione.

So what does VDK DO?

VDKs can have a significant influence on a beer’s flavour profile.

<table>
<thead>
<tr>
<th>VDK</th>
<th>Flavour threshold (mg L⁻¹)</th>
<th>Perceived Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacetyl</td>
<td>0.1</td>
<td>Butterscotch</td>
</tr>
<tr>
<td>Pentanedione</td>
<td>0.9</td>
<td>Honey</td>
</tr>
</tbody>
</table>
What is perceivable?

- 2,3-Butanedione aka Diacetyl makes up about 90% of VDK present during fermentation. It is perceived as Butter, Butterscotch, Yoghurt or Caramel.

- 2,3-Pentanedione makes up about 10% of VDK present during fermentation and is perceived as Honey.

- Note this ratio depends heavily on the yeast strain used.
Genetically most of the population can detect Diacetyl, but a small portion are not able to detect. Think of people that like coriander and those that don’t.
When are VDKs created?

Figure 2. Concentrations of diacetyl and α-acetolactate during a lager beer fermentation. Values are derived from Haukeli and Lie (22).


Source: http://educate.beer/off-flavor-diacetyl/
The strain?

Table 3.6  Diacetyl Formation in Three Yeast Strains

<table>
<thead>
<tr>
<th>Day</th>
<th>Diacetyl level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-206</td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.23</td>
</tr>
<tr>
<td>7</td>
<td>0.18</td>
</tr>
<tr>
<td>9</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Source: google image search “typical ‘country’ photo

Source: Fix Principles of Brewing science.
An example ale strain chromatogram – end of fermentation

- Diacetyl
- Pentanedione
An example lager strain chromatogram – end of fermentation

Diacetyl

Pentanedione

More on this later
Figure 1. The pathways for diacetyl and 2,3-pentandione formation and reduction, as well as valine and isoleucine synthesis, in Saccharomyces spp. yeast. AHA, acetoxyhydrox acid; DHA, dihydroxyacid; BCAA, branched chain amino acid (13–16).
Diak?

Diacetyl - Formation

Oh sweet wort

Outside and independent reaction of the yeast cell

Yeast Cell

Source: Adapted from GBS notes, Can't seem to find the original Author. If anyone knows please let me know. Thanks.
Diacetyl - Are you getting it?

Product placement?

Source: google image search
Diacetyl - Are you getting it?

Free from product placement everyone is happy...

In Newtown???
Imagine... Gene’s eating diacetyl tacos & pooping a-acetolactate.

The chemical reaction is outside Gene’s body which is a chemical (oxidative) reaction favoured by temp (higher = faster) & low pH (<4.3).

Converting the a-acetolactate into new diacetyl tacos
Pentanедione - Formation

Oh sweet wort

Outside and independent reaction of the yeast cell

2, 3 pentanedione → a - hydroxybutyrate
acetyl ethyl carbinol → a - acetohydroxybutyrate
isoelucine

2, 3 pentanediol

a - acetohydroxybutyrate → oxidative decarboxylation

Yeast Cell

Source: Adapted from GBS notes, Can't seem to find the original Author. If anyone knows please let me know. Thanks.
1. Oxidative decarboxylation in fermenting wort

2. Enzymatic decarboxylation of α-acetolactate

3. Alternative route for diacetyl synthesis

* (TPP) Thiamine pyrophosphate

Source: Brewing Microbiology third edition
Fig 5.2 adapted.
SO how do we measure?

The Type - Free & Total:

• Free Diacetyl is the concentration of Diacetyl in beer.
• Total Diacetyl is the concentration after heat treatment of α-acetolactate to Diacetyl.

The Methods:

• Organoleptic Method (the sniff test).
• Distillation then Colourimetric.
• Gas Chromatography.
The Budget Method

Organoleptic Method (the sniff test):

• Heat a filtered sample of your beer. This forces the reaction:
  a-acetolactate → Diacetyl
  heat + low pH + oxygen = conversion
• Ensure the lid is sealed during heating to trap the volatiles
• Cool to room temperature
• Smell for Diacetyl – Butter, etc.
The Budget Method

Organoleptic Process:

1. On a stable specific gravity of two days - take a sample. Note: raising the temperature towards the end of ferment will assist in absorption aka Diacetyl rest.

2. Take a second sample as a positive control with a beer that is on the second to third day of fermentation. E.g. 1.028 SG

<table>
<thead>
<tr>
<th>Date</th>
<th>Temp</th>
<th>Gravity</th>
<th>Plato</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/05/2018</td>
<td>15.5</td>
<td>1.0406</td>
<td>10.14</td>
</tr>
<tr>
<td>16/05/2018</td>
<td>16.2</td>
<td>1.0385</td>
<td>9.63</td>
</tr>
<tr>
<td>17/05/2018</td>
<td>16.1</td>
<td>1.028</td>
<td>7.16</td>
</tr>
<tr>
<td>18/05/2018</td>
<td>16</td>
<td>1.016</td>
<td>4.08</td>
</tr>
<tr>
<td>19/05/2018</td>
<td>17.8</td>
<td>1.010</td>
<td>2.56</td>
</tr>
<tr>
<td>20/05/2018</td>
<td>15.1</td>
<td>1.010</td>
<td>2.56</td>
</tr>
<tr>
<td>21/05/2018</td>
<td>15</td>
<td>1.010</td>
<td>2.56</td>
</tr>
</tbody>
</table>
Organoleptic Process:

3. Filter through a Coffee Filter ($0.12c) to remove yeast or a Centrifuge ($1000-2700) to remove yeast.

Why remove the yeast cell??

When you heat the cell, you lyse the cell releasing α-acetolactate → Diacetyl during heating

Cooking other flavours e.g. hops/yeast confusing the Panel.
Organoleptic Process:

4. Place samples in a reagent container ($10.90) or a resealable bottle ($2).
Organoleptic Process:

5. Place both samples in Water bath (Water bath costs $400 - $700) or Soup Cooker (Soup cooker for $99 [Setting 8]) for 20 minutes (Kitchen timer $8) at 65°C. Microwave if the sample is sealed, but can give mixed results, requires tweaking based on microwave type and is risky. Some people just use a bucket of hot water from the Brewhouse hot liquor tank.
The Budget Method

Organoleptic Process:

6. Assemble your VDK panel usually three people. Rate, high, medium, low or pass. Some hop flavours & caramel malt flavours will confuse the panel, this is where the positive control sample comes into play.

<table>
<thead>
<tr>
<th>High</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Pass</td>
<td><strong>Two Consecutive days we chill our beer for processing.</strong></td>
</tr>
</tbody>
</table>
The Spectrophotometer method

Colourimetric. Distillation of 100ml beer sample to 50ml mix with chemicals that reacts with alpha-napthol giving a colour result @ 530 nm.

This is a measure of total VDK it does not separate Diacetyl from Pentanedione.
### The Spectrophotometer method

**ASBC**: Beer 25. Diacetyl

**EBC**: 9.24.1 Vicinal Diketones in Beer: Spectrophotometric Method

<table>
<thead>
<tr>
<th>Method</th>
<th>Time</th>
<th>Cost</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBC</td>
<td>30 minutes distilling, 20-30 minutes in the dark, minutes to read on Spectro</td>
<td>$0.08 per test</td>
<td>Longer distillation time due to no anti-foam also reaction time is longer, note to blank with distillate</td>
</tr>
<tr>
<td>ASBC</td>
<td>10-20 minutes distilling Spectro 6-7 minutes</td>
<td>$0.11 per test 150,000 tests to get 15L of chemical to dispose @ $275</td>
<td>Can use anti-foam</td>
</tr>
</tbody>
</table>
The Spectrophotometer method

The kit – Spectrophotometer $2500 - $5000
or second hand, risky like eating a raw onion....
The chemicals

Understand the chemicals you are working with from a safety and disposal point of view. You will require a fume cupboard & collection for disposal of the used chemicals.
Fume cupboard will cost about $15,000
Or second hand approx. $3000
Tell him he's dreaming!

$50,000!!

$391.50
Distillation

unknown

known....

Known..

Ensure tap water is on

100ml beer plus 3 drops anti-foam

Rack to ensure 50ml capture air tight

Distill to 50ml

Set timer to 12 minutes
Was hoping for a blue colour reaction but got pink
These chemicals age/Oxidise/light sensitive – monitor light sensitive chemicals for age
So the standard curves

<table>
<thead>
<tr>
<th>Diacetyl Concentration (mg/L)</th>
<th>(VDK) ppb (calc)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>1500</td>
<td>0.307</td>
</tr>
<tr>
<td>0.75</td>
<td>750</td>
<td>0.151</td>
</tr>
<tr>
<td>0.5</td>
<td>500</td>
<td>0.107</td>
</tr>
<tr>
<td>0.25</td>
<td>250</td>
<td>0.051</td>
</tr>
<tr>
<td>0.225</td>
<td>225</td>
<td>0.046</td>
</tr>
<tr>
<td>0.2</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>0.175</td>
<td>175</td>
<td>0.038</td>
</tr>
<tr>
<td>0.15</td>
<td>150</td>
<td>0.034</td>
</tr>
<tr>
<td>0.125</td>
<td>125</td>
<td>0.028</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>0.022</td>
</tr>
<tr>
<td>0.075</td>
<td>75</td>
<td>0.018</td>
</tr>
<tr>
<td>0.05</td>
<td>50</td>
<td>0.014</td>
</tr>
<tr>
<td>0.025</td>
<td>25</td>
<td>0.011</td>
</tr>
</tbody>
</table>

0.038 outlier excluded

Graph showing absorbance against concentration with a linear regression line.
Why I’m not worried about the standard curves?

• We have used this method for about 10 months to chill our beers.
• We no longer create the standard curve or use it.
• For both our lager and ale strain we ran this method based on absorbance along with the organoleptic method until we switched over. We found that we could chill our Ales <0.015 and our lagers <0.020 Absorbance (ABS).
• We sent our lager off for GC analysis and found that our lager yeast was making very little 2-3,(BD) Diacetyl and very high 2-3, Pentanedione and this was the reason why we were not smelling Diacetyl but getting a higher number compared to our Ale yeast.
Why I’m not worried about the standard curves?

• You can make the standard curve up each time to test the concentration of your standard solution. This can help to identify errors however most errors occur in the distillation stage.
• Distillation needs to be exactly **50 mls**. Result value is higher.
• If the chemical oxidises or your distillate is incorrect the value is always higher. Luckily you don’t chill your beer.
• Isopropyl alcohol or alcohol in your sample ports not rinsed will lower your result value.
• The standard curve takes up a great deal of time, and is often inconsistent. At the YH lab we have two staff, maybe at the second site we will have time.
• There would be more value in the data set if you were able to separate the two VDK compounds, which this method cannot.
The ASBC method has saved us days of production. Where a panel of tasters would take a few days to reach a decision, we can now chill most of our beers in 1-2 days after terminal gravity, which was 3 to 4 days with the old method.

**Think about your gains:**

- **Tank turn around** - knowing your beers are ready for chilling and tanks are ready to be re-filled days earlier.
- **Sleep Factor** - we can chill beer with certainty.
- **Brewer hours** - no taking brewers off the floor for a panel/chat.
- **Precision** - we now have a quantifiable number in shorter time.

The investment of equipment has a significant pay back over time.
HACH kits, still need to distill, chemicals come pre-mixed, dose and read with a Hach Spectro.
Other solutions in the this space

CDR beer lab, still requires the distillation step, with a smaller volume, chemicals come pre-mixed, add distillate to reagent place in reader & result is displayed.
REACH OUT to crafty people?

Get your Science friends or patrons interested in your beer to help out with a Bevie even if your first data set is wrong!

Hooch – YH regular and Science mate.
REACH OUT to crafty people?

Watched the ASBC talk online and got amazing assistance with Dan @ Avery Brewing that gave the talk and just asked a heap of probably annoying questions which got the cost down and here we are.

The moral: no matter how small a brewery you are, nor the continent you reside in, the beer community will help you even when we have breweries in space! So be human and ask a mate.

Massive thanks to Dan!
Gas Chromatography. The sample is heated in a sealed vial with oxygen present to convert all precursors. The headspace is injected into the gas chromatograph in which Diacetyl and Pentanedione are separated and detected by electron capture. This method provides an accurate separation of the compounds.
Gas Chromatography (GC)

$80,000+ require a skilled operator. Training and safety commissioning about $20,000 and usually a GC will have a life of 7 years. Nitrogen safety requirements. Cheaper ones available at $50,000.

**EBC:** 9.24.2 - Vicinal Diketones in Beer: Gas Chromatographic Method

**ASBC:** Methods of Analysis - Beer-25: Diacetyl

The glass vials that you put the sample in are reusable but you need new caps each time at approx. $1 a cap (they come in a pack of 1000). Then, it’s just the power and nitrogen usage.
Gas Chromatography (GC)

So, Diacetyl = high. These results (~0.2 ppm) would generally indicate Diacetyl is above the taste threshold (>0.1 ppm)

Pentanediione = low. Hex is the control.

Run time is one hour & each subsequent sample is 10 minutes additional there after.

<table>
<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>Time [Min]</th>
<th>Height [µV]</th>
<th>Area [µV.Min]</th>
<th>Area % [%]</th>
<th>Quantity [mg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,3-BD (DIACETYL)</td>
<td>3.71</td>
<td>26759.3</td>
<td>1279.1</td>
<td>22.731</td>
<td>0.195</td>
</tr>
<tr>
<td>3</td>
<td>2,3-PENTANEDIIONE</td>
<td>5.05</td>
<td>2997.6</td>
<td>191.4</td>
<td>3.401</td>
<td>0.008</td>
</tr>
<tr>
<td>4</td>
<td>2,3-HEXANEDIIONE</td>
<td>6.89</td>
<td>31902.3</td>
<td>2962.6</td>
<td>52.647</td>
<td>0.000</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>77527.6</td>
<td>5627.2</td>
<td>100.000</td>
<td>0.202</td>
<td></td>
</tr>
</tbody>
</table>

Hexanediione peak area must be between 2200 and 3000
Worts low in FAN mean yeasts have to make more amino acids like Valine (which is needed for diacetyl reduction). Research shows higher levels of Diacetyl in worts low in FAN (Quain 2006). These can typically be assimilated from the wort.
Oh sweet wort

Outside and independent reaction of the yeast cell

Yeast Cell

Source: Adapted from GBS notes, Can't seem to find the original Author. If anyone knows please let me know. Thanks.
The wort-substrate – yeast food?

If you are not getting enough from your grist or using high adjuncts then there are nutrients out there to add.

Methods to measure FAN:

**ASBC**: Beer-31, Wort-12


Also CDR beer lab has a FAN kit with their device.
Bacterial?

Pediococci are traditionally associated with "sarcinae sickness" characterised by acid formation and the "buttery" aroma of diacetyl. But, Lactobacilli are also a cause.

**Lactobacillus brevis**: 1 ml sample inoculated into HLP and incubated for 2 to 7 days at 28-30 °C. Most *Lactobacillus* can usually be described as relatively large, white, inverted tear drop shaped colonies.

**Pediococcus damnosus**: 1 ml sample inoculated into HLP and incubated for 2 to 7 days at 28-30 °C. Most *Pediococcus* can be described as small, white, spherical / comet-like / sesame seeds / tear drop colonies.
Draft Beer Dispense?

It’s very easy to blame the Pub but, are other beers at the pub tasting like Diacetyl? If they aren't then sometimes you have to admit it’s your beer....

Resources to assist your publication:


Source: Draft line cleaning not required under Texas law By Jace Larson - Investigative Reporter
**Prevention: Enzyme?**

**ABV ALPHA ACETOLACTATE DECARBOXYLASE**

Alpha Acetolactate Decarboxylase is a decarboxylase enzyme that prevents the formation of diacetyl by the decarboxylation of alpha-acetolactate to acetoin.

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**Fig. 22.** The removal of alpha-acetolactate during fermentation.

Source: novozymes Enzymes_at_work.pdf
Has your Yeast settled out?

Not from here
technical term: Racking outlet
untechnical term: Donkey dick

Rouse CO2 from the base
technical term: Draining outlet
untechnical term: Pooper

Rousing has its drawbacks but sometimes it is necessary.
Sluggish yeast?

Add a small amount of wort and fresh yeast to kick off fermentation again.
Oh no you chilled it! early?

- Split the first beer into two tanks and brew a fresh beer on top and hope secondary fermentation warms to 18C otherwise you will never get your beer warm again.

- 50% of a tank at 0 degrees mixed with 50% 20 degree wort will still only reach 10 degrees. Tanks need to be split between more than 2 tanks or wort has to be sent in warm which also has its drawbacks.
Yeast Counting? Viability?

Causes of Diacetyl Issues:

- Not enough yeast
- Too much yeast
- Yeast in poor health
- Yeast mutation
- Oxygen not enough or too much
Racking, cone to cone, yeast storage?

Store your yeast at 4°C and no more than a few days.

Don’t take the late flocculated slow lazy yeast

You want the creamy white mixed population of yeast

Not the dead, dark, sluggish lazy yeast

Store your yeast at 4°C Celsius and no more than a few days.
Secondary Fermentation

- Adding of Fruit
- Saccharomyces cerevisiae var. diastaticus (intended or infection?)
- Addition of Dry Hop (Hop Creep) [Add enzyme?]
- Bottle Conditioning

**This strain has been genetically typed in our lab using polymerase chain reaction (PCR). Through this genetic testing, we have determined WLP565 Belgian Saison I Yeast to contain the STA1 gene (glucoamylase), a potential indicator of Saccharomyces cerevisiae var. diastaticus. Brewers yeast are natural hybrids, which make it possible for certain strains to display elements of the STA1 gene. These strains have the ability to utilize some dextrins (unfermentable sugars), resulting in higher levels of attenuation than what is considered typical.
Respiratory deficient yeasts?

When faced with no other root cause for high Diacetyl, you can plate to see if your yeasts are respiratory deficient.

Recipe?

Book: Yeast – White & Zainasheff
p229-231
Diacetyl post packaging?

Pickup in packaging occurs due to unconverted a-acetolactate converting in the presence of O2 to form Diacetyl.

**Lesson:** ensure that all your precursors are converted during primary fermentation and allow enough time for Diacetyl re-absorbance.

This is even greater when packaged beer is pasteurised.
• I consider myself a non-academic life educated scientist
• There are going to be limitations in this talk that we both learn from, hit me up!
• Hope you’ve had as much fun as I have in this journey taming the farm
• Any feedback or assistance

Contact: vb@younghenrys.com
Insta: vonbrews

Let’s just make better beer
hayyyyyyy???
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