This first note in a series on Beverage Flavor notes features the BIG Issue of Diacetyl: 2,3-Butanedione.

Basic Chemical Properties

Diacetyl (a vicinal diketone) is described as a yellowish-green liquid having a strong odor (at full strength) that resembles quinone (others report chlorine). It occurs naturally in bay oil and butter and is used as a flavorant to convey buttery properties in popcorn and margarines etc. \( \text{CH}_3\text{COCOCH}_3 \) aka: Biacetyl (esp. in wine literature), 2,3-Butanediol, Butan-2,3-dione, Butane-2,3-dione or Diacetyl (most commonly known as). Called the Big D in brewing circles and once associated with “Sarcina sickness” in the brewing industry.

Physical Properties

Molecular formula: \( \text{C}_4\text{H}_6\text{O}_2 \)
Molecular weight: 86.0892 g/mol.
Boiling point: 88 °C. [190.4 °F]
Flash point: 6 °C/Melting temp: -3 °C
Vapor pressure: 7.6 kPa (25 °C) or 56.8 mm Hg at 25 °C.

Index of refraction: 1.3951 @ 20 °C. Density: 0.990 g/cm³ (15 °C/15 °C). [van der Waals space filling model shown]
Diacetyl has plagued brewers for centuries and the compound is known as "the big D" in brewing circles. Its production by contaminating microorganisms was once difficult to curtail in certain breweries and forced them to close due to the "Sarcina" sickness.

Described as having typical levels of from 8-600 ppb. in beer - it was once considered a desired flavor note in some English ales. Though a major fault if detected at all in lagers.

The amount of diacetyl produced depends upon fermentation conditions (wort nitrogen levels - free amino nitrogen, pH and temperature.) The amount of the precursor (α-acetolactate) can be limited by operating at lower fermentation temperature and by ensuring good yeast growth and by adequate aeration.

Brewers will force precursor over to diacetyl by performing a diacetyl rest (see column on page 3). This is done by increasing slightly the temperature of the fermented beer before cooling. With sufficient healthy yeast left in suspension diacetyl is then reabsorbed and reduced to the less flavorful compounds shown in the front page figure. The topic is described further in Part II/corresponding section on the next page.

Diacetyl in Wine - Part I

As with brewing diacetyl is produced during fermentation. However, while it is formed by yeasts within the valine biosynthetic pathway most of the diacetyl in wine is associated with lactic acid bacteria (LAB). Wines rarely contain excessive concentrations of diacetyl at the end of primary fermentation.

Diacetyl is associated with a nutty and toasty flavor when present above its flavor threshold in a wine matrix of between 0.2-2.8 mg/L. At higher concentrations it imparts a buttery or lactic off-odor. At 1-4 ppm., depending on style and type of wine, it contributes a desirable buttery or butterscotch flavor. At high concentration (exceeding 5-7 ppm.) regarded by many as undesirable.

The diacetyl concentration in US Chardonnays is reported as between 0.005-1.7 ppm. Australian whites recorded as from 0.1-2.3 ppm. and red wines at 0-7.5 ppm!

Many wines undergo a secondary or malolactic fermentation. Lactobacilli in wine involved in malolactic fermentation not only metabolize malate to lactate but may also metabolize citrate into several compounds including lactate and diacetyl. The amount of diacetyl is influenced by the amount of citrate, the oxidation-reduction potential of the wine and the oxygen concentration present. The topic is described further in Part II/corresponding section on the next page.

Diacetyl in the Spirits - Part I

A small amount of diacetyl in distilled products can be pleasant (whiskies and rums), though large amounts are often considered as a serious flavor defect. The precursors of diacetyl (formed during fermentation) can be rapidly converted to diacetyl during the early stages of distillation when heat is applied. With its low flavor threshold (1 ppm.) and similar volatility to ethanol it is not easily or totally removed once formed. Distillers sometimes, like brewers, will allow for a diacetyl rest whereby the yeast are allowed to remove excess diacetyl from the wort or distillers wash. The final concentration of Diacetyl in a beverage is determined by the balance between the rate of formation and the rate of degradation. Fermentation oxygen influences spirit diacetyl content and aeration at still filling or aerating the wash for continuous distillation can also impact its concentration.

Levels in spirits: Scotch whiskies (0.09-0.32 ppm.), Brandy (0.07-0.31 ppm.), Rum (0.03-4.4 ppm. = 4400 ppb. in Buttery rums), and Vodka (0.01-0.10 ppm.)

The topic is described further in Part II/corresponding section on the next page.
DIACETYL: Production and Reduction:
Diacetyl is produced by citrate-metabolizing lactic acid bacteria and in yeast fermentations. Diacetyl is also a product of nitrogen metabolism. As a carbonyl compound it is formed from α–acetolactate which is involved in amino acid production. It is then decarboxylated and reduced to acetoine and 2,3-butanediol. Essentially the key intermediate product of mainstream metabolism (pyruvate) is converted to α–acetolactate which feeds valine and other metabolite formation. Any excess α–acetolactate is secreted from the yeast cell where it can then be spontaneously decarboxylated into diacetyl. Yeast cells reabsorb the diacetyl (under suitable conditions) whereby it can be metabolized into the aforementioned acetoin and butanedione (see main figure on the front page). Diacetyl can also be produced from bacterial metabolism.

While bacterial contaminants including Lactobacillus species and Pediococcus species can give rise in beer and distillers mash to substantial amounts of diacetyl and lactic acid (“buttery sour-milk” notes) desirable bacterial strains give rise to malolactic fermentations in winemaking which also result in diacetyl production. [See box below.]

Brewers and Diacetyl - Part II (A bit about control)

Diacetyl formation and reduction is a complex topic with many more details than could be discussed here. Three key references are listed at the end of this review (Anderson, et al. 2000; Krogerus, et al. 2015 and Krogerus & Gibson, 2013) which provide much more in-depth discussions of all the subtleties of the topic and laboratory personnel and those wishing to go very deep should read the book on Diacetyl by Inoue.

A bit more on control of diacetyl:
Yeast metabolism is complex - here is a quick synopsis for control in brewing. Wort low in amino acids or too rich in oxygen content or lipids, or if the temperature and agitation of the wort are too high, can lead to a situation whereby the consumption by yeast of certain nutrient amino acids occurs. Valine synthesis occurs and the α–acetolactate (see notes elsewhere in this review) is produced. For ales (fermented at 20-25°C) the chemical conversion of the α–acetolactate to diacetyl is rapid and the diacetyl quickly utilized by the yeast. In bottom fermentation, at lower temperatures, the conversion of α–acetolactate to diacetyl is slow and elimination of diacetyl by yeast can take several days. Conditions for diacetyl reduction and recommended reading on the topic can be discussed with the team at BDAS, LLC.

Diacetyl in Wine - Part II - On Malolactic fermentation and control!

More on the malolactic fermentation and origin of diacetyl.

The lactic acid and other bacteria involved (including Oenococcus oeni) utilize a citric acid metabolic pathway to diacetyl. Citric acid is metabolized to pyruvate (see box at bottom of page) which is then enzymatically converted to an activated form of acetaldehyde (acetaldehyde-TTP). Then is driven to α–acetolactate and to diacetyl. The diacetyl can then be reduced to acetoine and on to 2,3-butanediol.

The formation of diacetyl and malolactic fermentation is governed by the strain(s) of bacteria used, inoculation rate, pH, citrate concentration, temperature of the wine, extent of contact with wine lees, sulfur dioxide (SO2) concentration and the degree of aeration during winemaking.

The diacetyl concentration rises as the citric acid is catabolized but then often decreases due to diacetyl reduction to 2,3-butanediol.

Diacetyl may be rapidly bound by SO2 in wine and only the unbound form is considered from a sensory detection viewpoint. Free and bound diacetyl is then governed by pH and SO2 concentration and acetaldehyde and pyruvate levels which can also bind to SO2.

Diacetyl in the Spirits - Part II - A bit of control please!

Very little is available in the literature about diacetyl and its role and control in distilled spirits production. See the section part I for some detail or contact us for more insights here. Most useful information has been generated with respect to Scotch whisky and a review of works leading to the now famous Scotch whisky wheel describing whisky flavor would be a good start for the interested reader. In Bourbon production lactic acid bacteria play a role (sour mash) and careful control of contaminating organisms should be entertained.

As for brewing fermentation O2 influences spirit diacetyl content. The amount of diacetyl depends upon FAN (nitrogen) level, pH and temperature. Limiting the amount of precursor (α–acetolactate) by lower temp. fermentations, good yeast growth and adequate aeration is to be noted (but reduced in final wort/mash). α–acetolactate can be converted to diacetyl during the distillation. Late reduction of pH helps spontaneous decarboxylation of α–acetolactate – proceeds rapidly at pH 3.5. Conversion of α–acetolactate to diacetyl can be minimized by use of inert gases to protect wash from oxygen. Also noted: treatment with charcoal (eg., for white spirits such as vodka can) remove undesirable compounds such as diacetyl.

More on yeast, fermentation and diacetyl production and reduction.
Fermentation involves the conversion of fermentable sugars (maltose and glucose) into a key metabolite - pyruvate. This then feeds pathways for the production of ethanol and carbon dioxide. Yeast uptake amino acids and other nutrients but can synthesize their own amino acids including valine. Pyruvate feeds into enzymic reaction pathways to produce α–acetolactate which, via enzymic reactions, leads to the essential amino acid valine. Excess formation of α–acetolactate leads to non-enzymatic formation of diacetyl outside the yeast cell. Later uptake of the diacetyl then leads to enzymic formation of acetoin with a flavor threshold much higher than for diacetyl (approx. 17 ppm.) and ultimately to 2,3-butanediol (see figure on frontpage). Other vicinal diketones are involved (e.g., 2,3-pentanedione) but are not as flavor active and as big of an issue as diacetyl (2,3-butanediol) and are not discussed here. See biochemistry and microbiology texts on metabolism or the references cited here for more on this.
Analytical determination of diacetyl.

Diacetyl (2,3 butanedione) has been measured in many different ways over the years. In simple terms the brewer can do a simple odor filter test in beer to make decisions as to when the diacetyl rest is complete (details available on the BDAS, LLC website: www.alcbevtesting.com).

Winemakers and brewers have and continue to measure total VDK’s (Diacetyl and its less flavorful cohort: 2,3-pentanedione) spectrophotometrically following a rapid steam distillation of the beer or wine. A leading expert on this method in the US is Scott Bruslind at Analysis Labs in Lebanon, OR.

However, headspace gas chromatography (see figure) with electron capture detection is the best method today for determination (and discrimination) of diacetyl and related vicinal diketones. Testing can look at current (actual total or “as is” values) and potential levels (following a precursor chase) - total potential levels. Note: any precursor present can be converted during beverage storage into diacetyl and is noted by an increasing butteriness to the beer.

2,3-Butanedione

DIACETYL CH$_3$COCOCH$_3$


Major recent reviews -with extensive references contain within- worth looking at include:


Further details on diacetyl, other flavors involved in alcoholic beverages and the testing of beer, wine and spirits may be obtained from the author and team at Brewing and Distilling Analytical Services, LLC in Lexington, KY. www.alcbevtesting.com 859-278-2533.