Application Note
No. 781/2021

Determination of vicinal diketones (VDK) in beer

Distillation unit EasyDist:
Steam distillation and spectrophotometric analysis in comparison with gas chromatography

1. Introduction

Diacetyl (2,3-butanedione) and 2,3-pentanedione are vicinal diketones (VDK) which are formed during beer fermentation. VDK can have a significant effect on beer flavor, presence above their flavor threshold (approx. 0.1 mg/L for diacetyl and 1.0 mg/L for 2,3-pentanedione) can lead to butter/butterscotch like flavors that are undesired in most beers. The concentraiton of VDK depends on numerous process conditions, such as wort composition, fermentation regime, yeast strain, but it can also be influenced by microbial contamination [1].
The control of VDK levels during fermentation is an important aspect of quality control in a brewery. VDK can be determined by a variety of analytical methods. Colorimetric assays, (e.g., complex formation with o-phenylenediamine) and gas chromatography (GC) are most common and recommended methods by the European Brewery Convention (EBC). GC anaylsis according to EBC Analytica 9.24.2 [2] enables the separation and individual quantification of diacetyl and 2,3-pentanedione. The colorimetric assay according to EBC Analytica 9.24.1 [3] reports the VDK concentration as sum of both volatiles and allows no differentiation of diacetyl and 2,3-pentanedione. As the concentration of diacetyl is commonly much greater than the concetration of 2,3-pentanedione and it is also more flavor active, the colorimetric assay is widely used in the brewing industry.
An accurate and reliable method for the determination of VDK in beer is hereby introduced in co-operation with Versuchs- und Lehranstalt für Brauerei Berlin (VLB) e.V. VDK standards and the beer samples are distilled using the distillation unit EasyDist with a glass splash protector. A UV-Vis spectrophotometer is used to determine the VDK content (resp. the complex with o-phenylenediamine is analyzed) after the distillation. The results are further compared with results obtained from GC anaylsis.

2. Equipment

- EasyDist with glass splash protector (11K36512040)
- Sample tubes 300 mL (037377)
- Spectrophotometer (Shimadzu UV-1800 UV-VIS-Spectrophotometer)
- 25 mL, 50 mL, 200 mL and 1000 mL volumetric flasks with stopper (Brand, ISO 9001-14001 certified)
- Thermostated water bath capable of maintaining 20 ± 0.1 °C (WNB29 with CDP 15, Memmert)
- Thermometer for the thermostat, tolerance 18-24°C ± 0.02 °C
- Recirculating Chiller F-314 (11F31401K)
- Constricted distillate outlet tube Ø 5 mm (043096)
- 20 mL Headspace-Screwcap Vial (VWR, article number: 548-0248)
- Magnetic screwcap (Th. Geyer, article number: 7630304)
- Laboratory balance (0.1 g)
- Gaschromatograph (HP 6890) equipped with an electron capture detector (ECD)
- Column: DB-Wax 60 m x 0.32 mm x 0.5 µm (Agilent, article number: 123-7063)
- Automated sampler (MPS 2, Gerstel, equipped with Headspace-Syringe)

3. Chemicals and Materials

Chemicals:

- Deionized water
- Diacetyl (99%) CAS-number 431-03-8, Alfa Aesar, A14217.09
- 2,3-pentanedione (97%), CAS-number 600-14-6, Alfa Aesar, A13116.14
- o-phenylenediamine (for synthesis), CAS-number 95-54-5, Merck, 814538
- Hydrochloric acid, (4 mol/L), Th. Geyer, 824-1L

The following standard was used as a VDK standard:
Aqueous solution of 0.1 mg/L diacetyl and 0.1 mg/L 2,3-pentadione

4. Procedure

The GC determination of diacetyl and 2,3-pentanedione was perfomed in accordance with EBC 9.24.2 [2]

The determination of VDK by EasyDist includes the following steps:

- Preparation steps: Make sure that chiller F-314 is connected and properly functioning. Preheat the EasyDist distillation unit by running the preheating step twice. Make sure to use the constricted distillate outlet tube (043096).
- Pipette 100 mL decarbonated beer into a 300 mL sample tube.
- Start the distillation according to the parameters in Table 1. Distill until the volume is just below the calibration mark on the 25 mL volumetric flask.
- Quickly detach the volumetric flask, stopper it, and place it in a thermostatic bath at 20 °C for 15 minutes. NOTE: In case, the distillate in the volumetric flask touches outer walls of the distillate outlet tube, it should be rinsed with minimal amount of DI water to still be under the calibration mark of the volumetric flask, before placing it in a thermostatic bath. Alternatively, the constricted distillate outlet tube could also be shortened (that its lower end is just above the calibration mark of the volumetric flask to be used as a receiver) if rinsing is to be avoided.
- Make the volume up to the calibration mark with 20 °C conditioned deionized water, stopper it and mix well.
- Transfer two aliquots of 10 mL each into two suitable Erlenemeyer flasks. One aliquot serves as sample, the second as a blank sample.
- Add 0.5 mL of an o-phenylenediamine solution (10 g/L in 4N hydrochloric acid) to the sample and keep the liquid in dark for 30 minutes.
- After 30 minutes reaction time add 2.0 mL 4N hydrochloric acid to the sample and 2.5 mL of 4N hydrochloric acid to the blank sample.
- Read the absorbance (E) of the sample and the blank sample at 335 nm using a spectrophotometer.
- Subtract the blank absorbance from the sample absorbance to obtain a blank-corrected absorbance
- To calculate the VDK concentration the blank-corrected absorbance is multiplied by factor 1.2, which gives the VDK concentraiton in mg/kg [4]. As the density of beer is typically close to 1, VDK are commonly reported in mg/L rather than in mg/kg.

Table 1: Distillation parameters

Method parameters EasyDist

Steam Steps	No
Steam Power [%]	100/50/10
Level Detection	off
Distillation Time	Set until distillate reaches just below the mark on the 25 mL Volumetric flask with given steam power (approximately, 300s for steam power 10%/ 120s for steam power 50%, 90s for steam power 100%)
Instrument Settings	
MaxAccuracy Mode	On
Chiller/Tap Water	Chiller F-314
Set Temperature	10 °C

5. Results and discussion

5.1 Steam distillation by EasyDist vs Gas chromatography

Table 2: Results showing a comparison between the recovery of VDK content in an aqueous solution of VDK (containing 0.1 mg/L diacetyl and 0.1 mg/L 2,3-pentandione) on steam distillation and parallel measurements with gas chromatography

Steam Distillation by EasyDist					By Gas Chromatography		
Determination	% Steam Power	VDK [mg/L]	Mean VDK [mg/L]	% Recovery	Diacetyl [mg/L]	2,3-pentadione [mg/L]	VDK [mg/L]
1	10	0.201	0.201	100.3	0.110	0.100	0.210
2		0.201					
3		0.201					
1	50	0.199	0.198	99.1	0.110	0.100	0.210
2		0.199					
3		0.196					
1	100	0.199	0.198	99.1	0.110	0.100	0.210
2		0.197					
3		0.198					

Table 3: VDK analysis using the EasyDist at different steam power settings on a beer sample Czech Pilsner beer

Steam Distillation by EasyDist				By Gas Chromatography		
Determination	% Steam Power	Measured VDK [mg/L]	Mean VDK [mg/L]	Diacetyl [mg/L]	2,3-pentadione [mg/L]	VDK [mg/L]
1	10	0.090	0.089	0.041	0.034	0.075
2		0.089				
3		0.089				
1	50	0.095	0.091	0.040	0.032	0.072
2		0.093				
3		0.084				
1	100	0.088	0.087	0.036	0.032	0.068
2		0.085				
3		0.088				

5.2 Choice of the beer sample and comparison between two analytical methods

The distillation of VDK standards at a level of 0.1 mg/L of each diacetyl and total 2,3-pentanedione with different % steam power settings was executed to validate the efficiency of distillation. When conducting VDK distillation with a classical Parnas or Markham still, as recommended by EBC 9.24.1, a heating period of 6 minutes and a distillation time of 8-10 minutes is required to recover the VDK. As shown above, using the EasyDist flexibly at 10-100% steam power that corresponds to approx. 5-1 min distillation times, results in complete recovery of VDK.

To validate the application on EasyDist, a Czech Pilsner beer was selected. It was analyzed using different % steam power settings on EasyDist as well as using gas chromatography. Czech Pilsner beers are typically characterized by elevated VDK levels and are therefore most suitable for a methods or settings comparison. It can be seen from the results that the % steam power setting has no significant effect on the VDK results and that these results are similar to those from gas chromatographic analysis. As stated by EBC 9.24.1 both methods (GC and steam distillation) are not directly comparable, which relates to their different working principles. Moreover, the differences in the results (steam distillation vs. GC) reported here fall into the range of reproducibility R95 (R95 is an allowed tolerance when two different laboratories carry out the

analysis on the same sample with exact same method) for both 9.24.1 [3] and 9.24.2 [2] and are therefore fully satisfactory. Here, we present results for the same sample with two different methods and still obtain results under the allowed tolerance.

6. Comparison to norms and regulations

In comparison to the usage of classical Parnas or Markham stills (according to EBC method), the distillation with EasyDist can be executed more rapidly and safely. The user has great flexibility in adjusting steam power and distillation time. Also, compared to the GC analysis according to EBC [2] the steam distillation with EasyDist followed by spectrophotometric analysis for VDK content determination, is faster, easy to handle, and does not require any trained personnel without compromising quality of the results.

7. Conclusion

The determination of VDK in beer with the EasyDist provides reliable and reproducible results. In addition, it is easy to handle, and a fast method. Therefore, it is ideal for quality control of beer and in the brewing process.

8. References

Operation Manual for the EasyDist

[1] Krogerus, K., & Gibson, B. R. (2013). 125th anniversary review: diacetyl and its control during brewery fermentation. Journal of the Institute of Brewing, 119(3), 86-97
[2] EBC Analytica 9.24.2 - Vicinal Diketones in Beer: Gas Chromatographic Method, 1999
[3] EBC Analytica 9.24.1 - Vicinal Diketones in Beer: Spectrophotometric Method, 2000
[4] Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommision (MEBAK), Band II, 4 Auflage 2002.