Dry hopping with Eureka! – Brewing tests and analyses

TRANSFER OF AROMA SUBSTANCES | In search of a characteristic hop aroma in dry hopped beers, the potential of the hop variety Eureka! was investigated in more detail in brewing tests. This investigation focused on time-dependent transfer of selected hop components.

THE EUREKA! HOPVARIETY is the outcome of a cross between the US Apollo hop variety and a male cultivar from the Hopsteiner breeding programme. Average yield of this variety ranges from 2800 and 3000 kg/ha, the variety matures late.

Eureka! has both a high alpha-acid content (17–20 %) and a high level of hop oils of up to 4 ml per 100 g of hops [1]. Fig. 1 is a sensory assessment showing strong herbaceous, resinous, spicy fruity attributes. The complex fruity aroma has manifold impressions, from tropical-citrus to dark fruit and also with an emphasis on the aroma of black currants.

Black currant in hops

The thiol 4-Mercapto-4-methylpentane-2-on (4-MMP), smelling of black currants, has been described in the literature in the Cascade hop variety for the first time [2, 3]. Since discovery of this substance in hops, a lot of research work went into the methodology for determining the volatile substance.<

According to a recently published study, it is possible to quantify this substance, using selective enrichment of the thiol, stable isotope dilution analysis and GC×GC-TOFMS technology [4].

53 different hop samples were tested. Levels of 4-MMP ranged from < 1 to 114 µg/kg. American hop varieties had the highest concentrations. The Citra hop variety (114 µg/kg) had the highest concentration, followed by Eureka! (59.1 µg/kg), Simco (51.2 µg/kg) and Apollo (28.6 µg/kg). 4-MMP could not be detected in traditional German and English hop varieties. The study also showed that hop processing such as drying or pelletising had but little influence on 4-MMP concentration though different harvesting years and hop storage conditions had a major influence.

Other hop aroma substances

Though the thiol 4-MMP, with an extremely low odour threshold value of 0.5-1.5 ng/l [5], can make an important contribution to beer aroma, other hop aroma substances play a significant part as key aroma substances in beer. Linalool is the best know aroma substance contributed by hops. Having a citrus and flowery aroma, it can be found in late hopped as well as in dry hopped beers and is significantly above the odour threshold [6, 7].

Myrcene, a monoterpene, is also involved in the overall aroma of dry hopped beers [6]. Like 4-MMP, the terpene alcohol geraniol is regarded as variety-specific. This substance is ascribed a flowery aroma. In 2009, Takoi described the ester 2-Methylbutyl-2-methylpropanoate, also referred to as 2-Methylbutyl-isobutanoate, as a key aroma substance in the Nelson Sauvin variety [8]. This compound that could be described as having an aroma of green apples and/or apricots has an average odour threshold value of 78 µg/l in beer [8].

Other hop components

In addition to aroma substances, other substances are transferred from hops to beer during the process of dry hopping. Isohumulones are known to be mainly responsible for the bitterness perceived in beer. However, the bitterness of hop-accented beers cannot be simply explained by isohumulone levels.

In order to close the gap, studies involved identification, quantification and evaluation of the intensity of bitterness of oxidised hop bitter acids such as humulinones [9–11]. Humulinones are formed by oxidation of alpha-acids (humulones) and their structure is very similar to that of isohumulones.

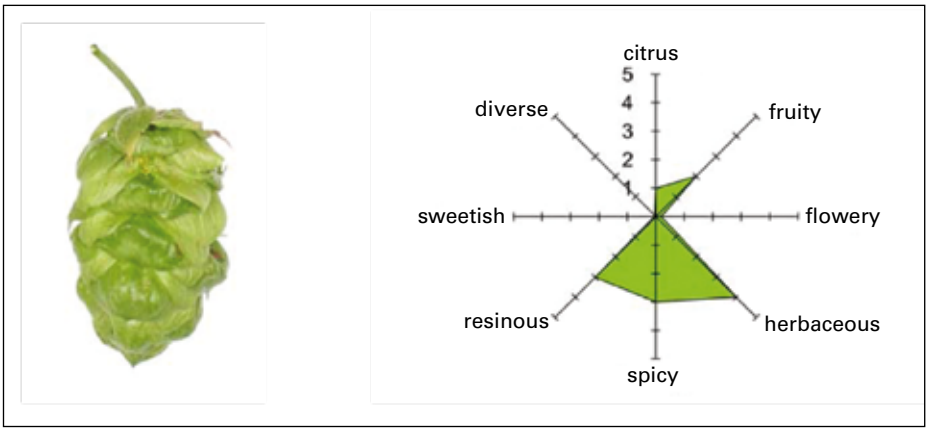

Fig. 1 Hop cone and aroma description of the Eureka! hop variety (assessment of raw hops, score 0-5)

Authors: Dr. Christina Schmidt and Dr. Martin Biendl, Hopsteiner – Simon H. Steiner, Hopfen, GmbH, Mainburg; Dr. Stefan Hanke, Bitburger Braugruppe GmbH, Bitburg; Dr. Klaas Reglitz and Dr. Martin Steinhaus, Leibniz Institute for Food Systems Biology,TU Munich, Freising; Germany

The authors Algazzali and Shellhammer reported in 2016 that humulinones contain 66 per cent of the relative intensity of bitterness compared to isohumulones [11].

Xanthohumol, a substance imparted by hops, has a mild bitterness [12]. Depending on hop variety, xanthohumol concentration can reach up to one per cent. Prenylflavonoids, including xanthohumol as well as 6- and 8-prenylnaringenine, are increasingly the subject of research as various positive physiological and pharmacological properties are ascribed to compounds of this substance class [13–16].

Brewing tests

The base beer is an industrial Pilsner beer brewed in the Bitburger test brewery. Hops were added at the beginning of boiling using pellets of the Perle and Hallertauer Tradition hop varieties and an ethanol extract of the Hercules, Hallertauer Magnum and Hallertauer Taurus hop varieties. The beer was centrifuged but not filtered, had an original gravity of 11.6 %, 35 bittering units, an alcohol content of 5.0 per cent by volume and a pH value of 4.5.

Dry hopping took place in cylindroconical tanks (10 hl) at –1 °C. The beer was evenly distributed into four tanks. Eureka! hops was then added in the form of pellets type 90 (2016 harvest). 250 g of hops were added per hl of beer. Dry hopping took place statically. After a contact time of 24 h, the hop sediment in tank 1 was withdrawn. Contact time with Eureka! was two, four and eight days in tanks 2, 3 and 4. Subsequently, 50 per cent of beer were removed from all tanks and filtered using kieselguhr (100 g/hl). The other 50 per cent were not filtered. The base beer was processed accordingly. The filled beers were stored at 4 °C and analysed immediately after the brewing test.

Behaviour of hop aroma substances in beer

The method EBC 9.49 [17] was used to determine hop aroma substances except for 4-MMP. Thiol concentrations in beer were determined in line with the method described in reference 4. More details can be found in reference 18. Fig. 2 shows the results of reliable quantification of hop aroma substances using the Headspace-Trap GC-MS technology. Both filtered and unfiltered beers showed a clear increase in the aroma-active substances myrcene, linalool, geraniol and 2-Methylbutyl-isobutanoate during dry hopping.

Main transfer of these four compounds had taken place after a contact time of two days. The highest myrcene level of 1287 µg/l was determined in the unfiltered beer after a contact time of four days. A clear drop after filtration was noted for myrcene only and not for the other aroma substances. Pfeifer and Cocuzza [19] had previously described a drop in myrcene concentration after filtration. Linalool levels were comparable in the beers after a contact time of two, four and eight days. Concentrations of myrcene and linalool analysed clearly exceeded the odour threshold values of 9–1000 µg/l of myrcene and 2–80 µg/l of linalool described in the literature [5]. These two compounds thus make a contribution to overall aroma of beers tested. Geraniol levels in beers ranged from 26 µg/l (1 day) and 38 µg/l (4 days) and were clearly lower than linalool concentrations determined. Geraniol levels of 4 µg/l also clearly exceed-

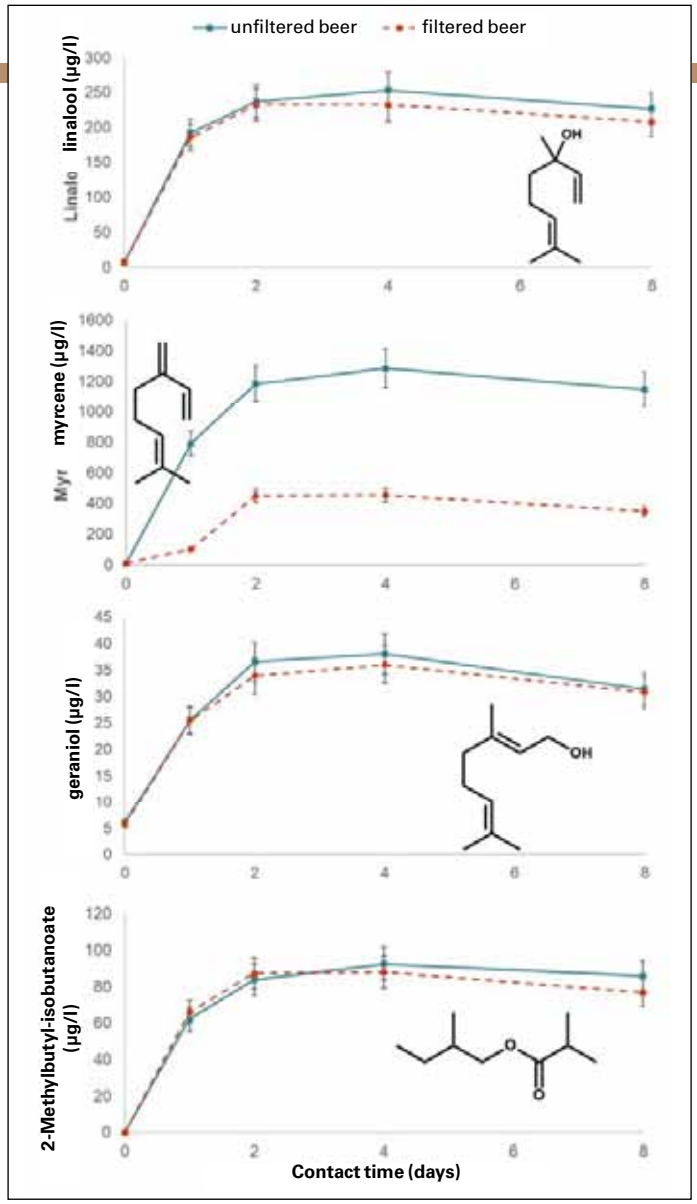

Fig. 2 Concentration of hop aroma substances in µg/l (±standard variation of two measurements) in unfiltered and filtered beers (base beer and beers with different contact times)

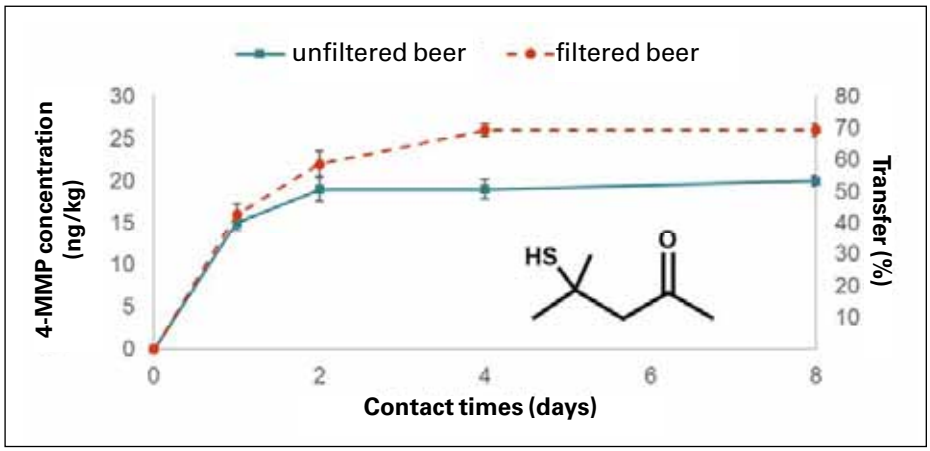

Fig. 3 Behaviour of 4-MMP during dry hopping in filtered and unfiltered beer (±standard variation of three measurements) [18]

ed the odour threshold [2]. The substance 2-Methylbutyl-isobutanoate described as having a fruity fragrance could be quantified in concentrations ranging from 66 µg/l (1 day of dry hopping) and 92 µg/l (4 days of dry hopping). The odour threshold value in beer determined in the literature is 78 µg/l [8]. 2-Methylbutyl-isobutanoate thus contributes to the overall aroma of beers after a contact time of two, four and eight days. Fig. 3 summarises the behaviour of the compound, 4-MMP, smelling of black currants. It could be shown for this substance that main transfer had taken place after two days and that concentrations rose slightly between day 2 and day 8. Transfer ranged between 50 and 70 per cent. Concentrations determined were between 16 and 22 ng/kg, surpassing the odour threshold value of 0.5 to 1.5 ng/kg. A contribution of 4-MMP to the overall aroma of these dry hopped beers was thus obvious. It came as a surprise that the filtered beers had higher 4-MMP levels.

When the brewing tests were repeated (results not shown here), the same effect was noted. Release of 4-MMP from precursors during filtrations might be a possible explanation. In the context of this study, this assumption was not verified.

Behaviour of hop bitter substances in beer

Concentrations of bitter substances in dry hopped beers were analysed also using the method EBC 9.47 [20]. The calibration standards listed below were used for the analyses:

- ICE-4 for alpha-acids (detection at 270 nm);
- ICS-Hum 1 for humulinones (detection at 270 nm);
- ICS-X1 for xanthohumol (detection at 370 nm).

The picture for alpha acids and humulinones was similar to that of hop aroma substances. Main transfer of substances from hops to beer took place after a contact time of two days. The highest concentrations of alpha-acids were measured in the beers after a contact time of two and four days. Concentrations amounted to 11 mg/l. Concentration of humulinones at maximum 3 mg/l was clearly lower. Filtration did not show any influence on concentrations measured. The behaviour of xanthohumol was different. The test resulted in an increase in the substance of 0.3 mg/l, though only after a contact time of four days.

Sensory assessment of beers

The focus of the descriptive sensory assessment of filtered beers was exclusively on hop aroma. The attributes citrus, fruity, flowery, herbaceous, spicy, resinous, sweetish and diverse were evaluated on a scale of 1 to 5. The individual categories contain further descriptions of the respective attributes, details of which can be found in reference [21].

The category fruity can, for example, be differentiated further into honey melon, maracuja, apricot, banana, pear or berry.

In these investigations, black currant was added to the differentiation of the category fruity. The sensory panel trained in hop-accented beers assessed all filtered beers in one session. Before the session, beers were stored at ambient temperature. In order to illustrate the differences more clearly, only assessments for beers with a contact time of one day and eight days are shown (fig. 5).

Scores of individual assessments were averaged. The dry hopped beer after a contact time of one day was assessed as fruity, particularly tasting of black currant, and citrus whereas the beer with a longer dry hopping time as herbaceous and spicy and less citrus and fruity. Though analytical data of key aroma substances such as linalool, geraniol, 4-MMP and also 2-Methylbutyl-isobutanoate, known for their fruity, citrus and flowery attributes, had comparable levels in these beers, sensory assessment resulted in clear differences between the two beers in terms of citrus and fruity attributes. The high concentration of myrcene in beer with a contact time of eight days seems to mask the fruity impression of this beer.

Conclusion

The Eureka! hop variety has a high potential for use in dry hopping of beers. Time-dependant transfer of the hop aroma substances myrcene, linalool, geraniol, 2-Methylbutyl-isobutanoate and 4-MMP as well of the hop bitter substances alpha-acids and humulinones had already been largely finished after a contact time of two days. Further increase in levels in beer, having undergone longer dry hopping times, was small indeed.

Sensory assessment resulted in clear differences as a function of contact time. An increase in the hop aroma substance myrcene during a longer dry hopping time presumably led to masking of the fruity attribute in the beer. It is thus essential to investigate overall aroma including all possible interactions between individual aroma substances. When focusing on a fruity beer aroma, emphasising a black currant aroma, a contact time of two days when dry hopping with Eureka! is recommended. Should the focus be towards herbaceous and spicy attributes, a longer contact time with this hop variety is required.

References

1. Variety data sheet Eureka!: https://www.hopsteiner.com/variety-datasheets/Eureka/
2. Kishimoto, T; Wanikawa, A.; Kono, K.; Shibata, K.: "Comparison of the Odor-Active Compounds in Unhopped Beer and Beers Hopped with Different Hop Varieties"; J. Agric. Food Chem., vol. 54, 2006, pp. 8855–8861.
3. Steinhaus, M.; Wilhelm, W.; Schieberle, P.: "Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis"; Eur. Food Res. Technol., vol. 226, 2007, pp. 45–55.
4. Reglitz, K.; Steinhaus, M.: "Quantitation of 4-Methyl-4-sulfanylpentan-2-one (4MSP) in Hops by a Stable Isotope Dilution Assay in Combination with GC×GC-TOFMS: Method Development and Application to Study the Influence of Variety, Provenance, Harvest Year, and Processing on 4MSP Concentrations", J. Agric. Food Chem., vol. 65, 2017, pp. 2364–2372.
5. Biendl, M.; Engelhard, B.; Forster, A.; Gahr, A.; Lutz, A.; Mitter, W.; Schmidt, R.; Schönberger, C.: "Hopfen – Vom Anbau bis zum Bier", Fachverlag Hans Carl, Nuremberg, 2012.
6. Fritsch, H. T.: "Einfluss des Hopfens auf wertgebende Aromastoffe in Pilsner-Bieren sowie in Zwischenstufen des Brauprozesses"; doctoral the-

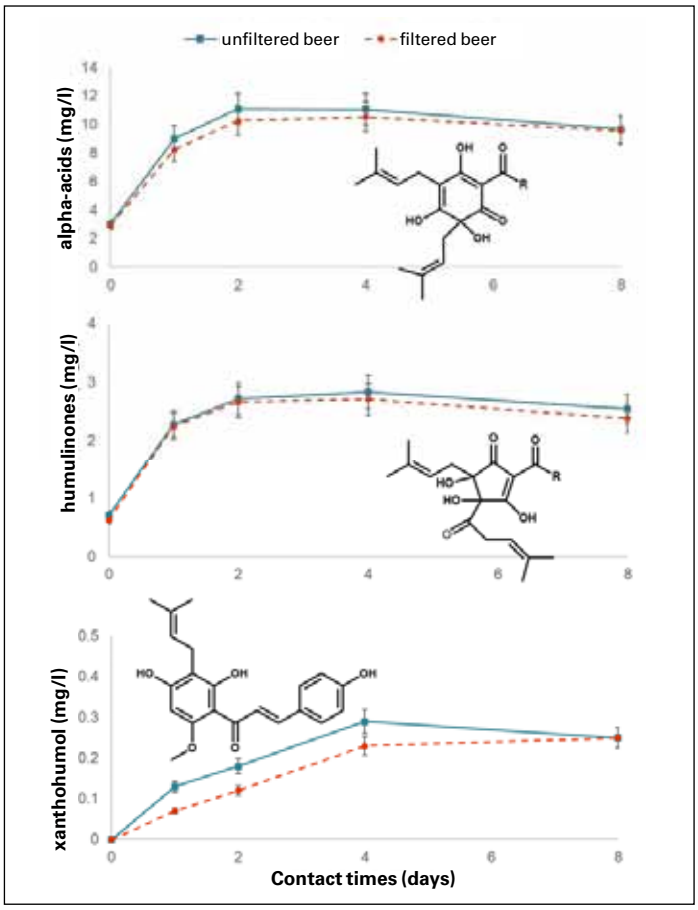

Fig 4. Concentration of hop bitter substances in mg/l (±standard variation of two measurements) in unfiltered and filtered beers (base beers and beers with different contact times

sis, TU München, 2001.
7. Fritsch, H. T.; Schieberle, P.: "Identification Based on Quantitative Measurements and Aroma Recombination of the Character Impact Odorants in a Bavarian Pilsner-type Beer", J. Agric. Food Chem., vol. 53, 2005, pp. 7544–7551.
8. Takoi, K.; Degueil, M.; Shinkaruk, S.; Thibon, C.; Kurihara, T.; Toyoshima, K.; Ito, K.; Bennetau, B.; Dubourdieu, D.; Tominaga, T.: "Specific Flavor Compounds Derived from Nelson Sauvin Hop and Synergy of these Compounds", BrewingScience, vol. 62, 2009, pp. 108–118.
9. Taniguchi, Y.; Matsukura, Y.; Ozaki, H.; Nishimura, K.; Shindo, K.: "Identification and Quantification of the Oxidation Products Derived from α-Acids and β-Acids During Storage of Hops (Hulumus Lupulus L.)"; J. Agric. Food Chem., vol. 61, 2013, pp. 3121–3130.
10. Maye, J. P.; Smith, R.; Leker, J.: "Humulinone Formation in Hops and Hop Pellets and Its Implications for Dry Hopped Beers", MBAA TQ, vol. 53, 2016, pp. 23–27.
11. Algazzali, V.; Shellhammer, T.: "Bitterness Intensity of Oxidized Hop Acids: Humulinones and Hulupones"; J. Am. Soc. Brew. Chem., vol. 74, 2016, pp. 36–43.
12. Intelmann, D.: "Molekulare, psychophysikalische und rezeptorbasierte Studien zum Bittergeschmack von Bier", doctoral thesis, TU München, 2011.
13. Miranda, C. L.; Elias, V. D.; Hay, J. J.; Choi, J.; Reed, R. L.; Stevens, J. F.: "Xanthohumol improves dysfunctional glucose and lipid metabolism in diet-induced obese C57BL/6J mice"; Archives of Biochemistry and Biophysics, vol. 599 (2016), pp. 22–30.
14. Venturelli, S.; Burkard, M.; Biendl, M.; Lauer, U. M.; Frank, J.; Busch, C.: "Prenylated chalcones and flavonoids for the prevention and treatment of cancer", Nutrition, vol. 32, 2016, pp. 1171–1178.
15. Ferk, F.; Mišík, M.; Nersesyan, A.; Pichler, C.; Jäger, W.; Szekeres, T.; Marculescu, R.; Poulsen, H. E.; Henriksen, T.; Bono, R.; Romanazzi, V.; Al-Serori, H.; Biendl, M.; Wagner, K.-H.; Kundi, M.; Knasmüller, S.: "Impact of xanthohumol (a prenylated flavonoid from hops) on DNA stability and other health-related biochemical parameters: Results of human intervention trials", Mol. Nut. Food Res., vol. 60, 2016, pp. 773–786.
16. Van Breemen, R. B.; Yuan, Y.; Banuvar, S.; Shulman, L. P.; Qiu, X.; Ramos Alvarenga, R. F.; Chen, S.-N.; Dietz, B. M.; Bolton, J. L.; Pauli, G. F.; Krause, E.; Viana, M.; Nikolic, D.: "Pharmacokinetics of Prenylated Hop Phenols in Women Following Oral Administration of a Standardized Extract of Hops", Mol. Nut. Food Res., vol. 58, 2014, pp. 1962–1969.
17. Analytica EBC 9.49, "Hop aroma components in beer by Headspace-Trap Gas Chromatography (HT-GC)", 2018, accessed online on 23 October 2018.
18. Reglitz, K.; Lemke, N.; Hanke, S.; Steinhaus, M.: "On the Behavior of the Important Hop Odorant 4-Mercapto-4-methylpentan-2-one (4MMP) during Dry Hopping and during Storage of Dry Hopped Beer", BrewingScience no. 11/12, vol. 71, 2018, pp. 96–99.
19. Peifer, F.; Cocuzza, S.: "Einfluss der Filtration auf hopfengestopfte Biere"; Brauindustrie no. 10, 2018, pp. 34–37.
20. Analytica EBC 9.47, "Iso-α-acids and reduced iso-α-acids (Rho, Tetra, Hexa) in beer by HPLC", 2010, accessed online on 23 October 2018.
21. Schmidt, C.; Cocuzza, S.: "Well-rounded sensory evaluation", BRAUWELT International no. 2, 2014, pp. 116–118.

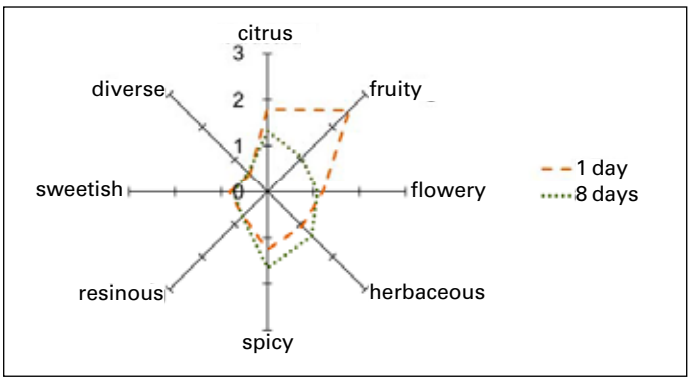

Fig. 5 Sensory assessment of dry hopped filtered beers after contact times of 1 day and 8 days with Eureka! (score 0-5)