Flavours and off-flavours in beer – Part 2

OVERVIEW | A group of diploma master brewer students from two different brewing courses at Scandinavian School of Brewing (SSB), Copenhagen, presented the results of their studies on the most common flavours and off-flavours found in beer in a two part series. The first part (BRAUWELT International no. 4, 2014, pp. 204-207) dealt with flavour development during the individual steps of the brewing process. This second part gives an overview of off-flavours, their causes and remedies.

THIS PAPER IS A SUMMARY of the efforts of all the students involved and is derived from the collective presentations given by each group, spanning two brewing courses. Unless stated otherwise, all material for this paper stems from the lectures and presentations from these courses.

Microbiological changes

Although the brewing process is usually subject to strict process controls, there is always the risk of contamination by undesirable microorganisms, which usually has a negative impact on the finished beer or even on the fermentation process. Contamination by bacteria or fungi, including wild yeast, can influence not only the flavour and appearance of the beer, but can also negatively impact both primary and secondary fermentation. These microorganisms overtake the brewer's yeast through consumption of the available nutrients. This lack of nutrients can prevent the brewer's yeast from multiplying or even producing alcohol. Since alcoholic fermentation is an anaerobic process, aerobic bacteria are largely prevented from multiplying under the conditions present in the oxygen-deprived environment. Another factor which reduces the risk of bacterial contamination is the addition of hops to the wort. Hops exhibit an antimicrobial effect, at least with most gram-positive bacteria. Both the flavour and antimicrobial characteristics of hops stem from the compounds referred to as terpenes, of which myrcene, β-pinene, β-caryophyllene and α-humulene are the most abundant in hops. These compounds are also often found in medicinal plants. Terpenes or terpenoids, which are essentially terpenes with additional functional groups, are major constituents in essential oils and are therefore widely used as aroma compounds for perfumes, in aroma therapy and as additives in natural flavourings. Apart from being antioxidants, terpenes and terpenoids are also known to possess qualities that are anticarcinogenic, anti-inflammatory, analgesic and anti-depressant. These are only a few examples of the broad scope of applications for terpenes and terpenoids [5].

Authors: Diploma Brewery Engineer, BSc. Helena Sørensen, the Diploma Master Brewer Class 2012/2013; supported by Axel G. Kristiansen, Director, Scandinavian School of Brewing, Copenhagen, Denmark

Neither the anti-microbial effect of hops nor the anaerobic environment of alcoholic fermentation can prevent contamination by wild yeast. There are two general kinds of wild yeast, which can be problematic for brewers: *Saccharomyces* and non-*Saccharomyces*. However, almost any yeast is considered to be wild yeast if it is not supposed to be present during fermentation [1]. The most common indicator for wild yeast contamination is higher levels of diacetyl in the finished product. Other flavour characteristics that hint at contamination with wild yeast are increased levels of phenolic, sulphury and estery compounds. Unlike bacterial contamination, wild yeast can be difficult, if not impossible, to detect by microscopic analysis as many yeasts have similar cell shape. Contamination by wild yeast has proven to be quite common, as suggested by one study discussed during the course. It found that approximately 50 percent of the breweries participating in the study provided samples contaminated with wild yeast strains, almost 60 percent of which were *Saccharomyces spp.*

Contamination is much easier to detect and identify when bacteria are the culprits rather than with wild yeast. This is largely due to the difference in appearance of bacteria and yeasts. In general, bacteria are much smaller and often shaped completely differently compared to yeasts which are almost always round. This makes it relatively easy to determine if there are indeed foreign organisms present in the yeast slurry or during fermentation through simple microscopy. One of the most common contaminants in brewing is also one that is hard to detect, namely *Megasphaera cerevisiae*, a gram-negative bacterium. Contamination by these bacteria results in increased turbidity and a sulphury, feculent aroma in beer. *Megasphaera* is difficult to detect, as it is roughly the same size and shape as brewing yeast. Other common bacterial contaminants include the gram-positive *Lactobacillus brevis* and *Pediococcus damnosus*. They both produce lactic acid, while the latter

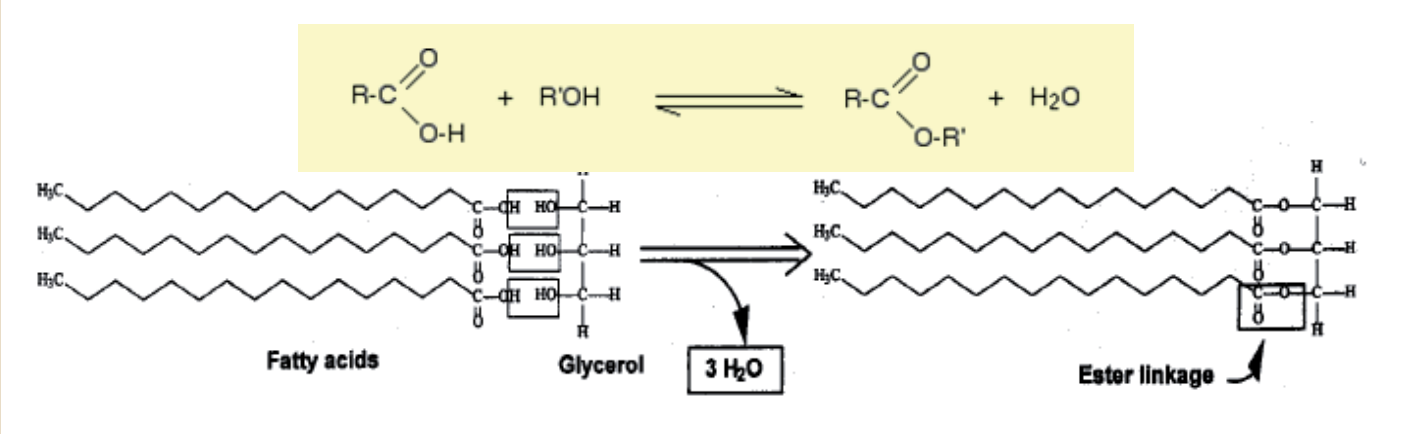

Fig. 1 Esterification of fatty acids [1]

also increases the viscosity and produces diacetyl. Another common gram-negative bacterial contaminant is *Pectinatus cerevisiiphilus*, which produces acetic acid as well as the same sulphury/feculent aromas as *Megasphaera*.

Brewing yeast is adapted to life in an alcoholic environment, and most wild yeast strains are maintained at a minimum level purely due to the increasing alcohol content of the fermenting brew. It is best to limit the "recycling" of yeast or the number of yeast generations, in order to further reduce the risk of contamination, i.e. the possibility that the growth of wild yeast will surpass that of the brewing yeast. However, because brewing yeast is highly optimised for beer as a growth medium, it possesses an advantage over competing most wild strains, thus lessening the risk of contamination from the outset.

Primary contamination

Throughout the process, from the fermentation vessel to the packaged product, there are two principal categories of contamination: primary and secondary. Primary contamination occurs between wort cooling and the BBTs and includes the wort, the pitching yeast (from yeast storage tanks, propagation vessels, etc.), fermentation vessels, maturation tanks, filtration line, the BBTs and all of the relevant piping. For beer filtration, it is imperative that both the CO_2 and the de-aerated water used for purging the filter are sterile. Processing aids, such as kieselguhr and filter sheets, are ideal hiding places for bacteria, wild yeast and moulds and should therefore be thoroughly cleaned and inspected regularly. To prevent contamination from water, treatment by means of a UV filter effectively destroys any bacteria that may be present there. The UV light alters the bacteria to such an extent that they cannot survive. Prior to beer filtration, 85 °C water should be circulated through the filter for at least 30 minutes, in order to disinfect the filter, possibly even circulating the water through the CIP plant or dedicated heater to maintain the temperature. Recovered CO_2 should also undergo sterile filtration to avoid any potential transfer of bacteria. When it comes to pipes, blind spots caused by sharp bends in the pipes should be avoided as such areas are highly prone to contamination, especially by bacteria. Bacterial biofilms are always a concern as they are difficult to eliminate, which can result in a continual re-contamination of the affected areas. Biofilm removal often requires vigorous mechanical cleaning or harsh chemicals, such as chlorinated caustic, applied at regular intervals, e.g. once every two months, in order to effectively remove any build-up of biofilm.

Primary contamination should be the chief concern of any brewery, as contamination in either of these areas could lead to an entire batch having to be discarded.

Secondary Contamination

Secondary contamination is associated with the processes from the BBTs to the packaged product. In these cases, 35 percent of the contamination stems from the sealer, 25 percent from the filler, while the remaining 30 percent is divided equally between failure by the 'empty/full bottle inspector' (EBI/FBI), bottle washer (through contaminated water dripping into cleaned bottles) and from the area around the filler and sealer [1]. Unless there is a systematic process error, secondary contamination could be considered less critical as there is less beer waste, and especially if a tunnel pasteurizer is utilised in the final step of production. If a flash pasteurizer is used, secondary contamination may cause damage to the brewery's reputation. However, if there is a systematic process error involved, the entire batch of packaged product may need to be recalled, which would represent considerable cost to the brewery since the product may already be in the consumers' hands.

To avoid secondary contamination, disinfection of all equipment such as conveyors, fillers, sealers, crowners, crown corks, floors under machines etc., is of great importance. Care should also be taken to avoid contamination of any liquids, such as conveyor lubricants and recycled detergents, as these also represent possible sources of contamination. If recycled bottles are used, it would be more pertinent to use a double-end bottle washer as opposed to single-end. This would avoid possible re-contamination of clean bottles by dirty bottles and also provides more efficient cleaning. Using sterile water for the final rinse also reduces the risk of re-contamination and could even be employed if the finished product and bottles are sent through a tunnel pasteurizer. As the clean bottles make their way towards the filler, the conveyors on which they travel should be covered to prevent any debris from falling into the bottles, regardless of whether there are bottle inspectors along the conveyor system. As the filler and sealers are responsible for 60 percent of secondary contamination, it is important to factor in frequent foam cleaning of the filler and filler tubes, monitoring crown capping and can seaming on a regular basis (each batch) and even creating a slight positive pressure on the space where the filler/sealer is locat-

OVERVIEW OF FLAVOURS, THEIR CAUSES AND REMEDIES

Meilgaard Flavour no.	Flavour term	Associated fault	Suggested remedy
0111	Spicy	Formed during beer maturation	Hygiene
0121	Plastic	Wild yeast during fermentation or infected CO_2 supplies	Hygiene, clean CO_2 supply
0130 group	2-phenylethyl acetate (roses, honey)	Produced by yeast during fermentation	To decrease: decrease wort gravity, increase aeration, decrease the fermentation temperature
0130 group	Ethyl butyrate (tropical fruits)	Produced by yeast during fermentation, yeast strain-dependent. Can also originate from poor brewhouse hygiene	To decrease: Select other yeast strain, lower the fermentation temperature. To increase: Select other yeast strain, lower the level of saturated fatty acids, increase the fermentation temperature
0130 group	Ethyl octanoate (fruity, sweet, waxy)	Produced by yeast during fermentation	To decrease: decrease wort gravity, increase aeration, decrease temperature
0131	Isoamyl acetate	High primary fermentation temperature	To decrease: Select other yeast strain, decrease the fermentation temperature
0132	Ethyl hexanoate (red apples, anise seed)	Produced by yeast during primary and secondary fermentation, high content of fatty acids, strain-dependant	To decrease: Select other yeast strain, decrease the wort gravity, increase aeration, decrease the fermentation temperature
0133	Ethyl acetate (nail polish remover)	Yeast stress during high gravity fermentation, contamination of wild yeast.	Select other yeast strain, decrease wort gravity, increase wort aeration, lower the fermentation temperature, agitation/ mixing during fermentation, increase pressure during fermentation
0150	Acetaldehyde (green bruised apples)	Precursor from malt, precursor to alcohol, oxidation of alcohol during fermentation, stuck fermentation	Long maturation, re-pitching of fresh yeast for stuck fermentation to increase attenuation
160	Flowery rose	Strecker degradation by thermal stress	Lower thermal stress from: wort boiling (more efficient cooling in the whirlpool and shorter boiling times), pasteurisation and storage conditions
0230	Grassy	Poor quality malt, poor storage of malt, too fine milling	Ensure optimal storage conditions, less fine milling
0310	Grainy	Too fine grist, raw barley, high sparging temperature	Less fine milling, lower the sparging temperature, avoid oversparging
0330	Worty	Low RDF, stuck fermentation	Re-pitch with viable yeast
0423	Smoky	Originates from raw materials, occa-sionally infection during fermentation	Avoid excessive use of dark, smoked malts. Hygiene during fermentation
0500 group	Bromophenol	External contamination of packaging materials	Check recycled cardboard for packaging in contact with beer
0500 group	Chloroanisole	External source	Can originate from malt, kieselguhr or moulds
No number	Indol	Infection of water, wort or syrup, sometimes associated with DMS	Hygiene, particularly the wort chiller and sugar tanks
No number	Fusel alcohol (warming sensation, floral)	By-product of amino acid degradation, high levels of zinc	To decrease: increase pitching rate, lower the fermentation temperature, avoid oxygen after pitching, decrease zinc addition
0500	Phenolic (4VG, cloves, spicy)	Contamination by some *Saccharomyces* and *Brettanomyces*, roasted/smoked malt, wheat	Hygiene and pasteurisation. Avoid mashing in at a low temperature, ensure sufficient wort evaporation
0504	Chlorophenol (medici-nal, antiseptic)	Contamination of brewing liquor or packaging materials	Monitor chlorine levels in final rinsing water. Can also originate from moist packaging materials
0600	Butyric (baby vomit, rancid)	Contamination with lactic acid bacteria or *Pediococcus*	Locate the source of contamination and improve hygiene and process control; can origin from malt, wort and syrups
No number	Caprylic (goat, waxy, fatty acid, rancid)	Yeast autolysis	Improve yeast health or use fewer generations of yeast, remove yeast immediately and review the cause
0613	Iso-valeric (old cheese/ sweaty feet)	Oxidised/old hops or certain strictly anaerobic bacteria (*Pectinatus*)	Store hops in cold, dry, cool environment and use opened packages quickly. Packaging hygiene for anaerobic bacteria

OVERVIEW OF FLAVOURS, THEIR CAUSES AND REMEDIES

Meilgaard Flavour no.	Flavour term	Associated fault	Suggested remedy
0620	Diacetyl (butterscotch, rancid butter)	Cooling fermenter before reaching attenuation or later contamination by bacteria	Review fermentation temperatures, improve yeast viability and growth, determine if caused by bacterial contamination and locate the source
No number	Lactic acid (sour)	Contamination by lactic acid bacteria	Locate source of contamination and improve hygiene
No number	Mousy	*Brettanomyces* wild yeast infection	Hygiene, pasteurisation
0700	H_2S (rotten eggs)	Contamination by wild yeast or bacteria, high sulphate in wort	Locate source of contamination and improve hygiene, determine source of sulphate
0710	SO_2 (Burnt matches)	High levels of nitrogen during fermentation, low nutrients (sugars), low lipids	Review level of fermentable sugars at the start of fermentation
0722	Mercaptan	Contamination by bacteria, UV-radiation	Locate source of contamination and improve hygiene, use UV-resistant packaging
0724	Light-struck (skunky)	UV-radiation from sunlight or UV-lamps (supermarkets)	Use isomerised hop extracts, use UV-resistant, enclosed cartons for packaging or brown glass bottles
0725	Autolysed (meaty)	Autolysed yeast in contact with beer	Remove and crop yeast when fermentation is finished
0729	Onion	Autolysed yeast	Crop yeast when attenuation is reached
0730	DMS sweet corn, cooked vegetables	Malt quality, too low boiling intensity, infection in fermentation	Use malt with less DMS-precursor, increase boiling time for efficient evaporation, avoid condensation in kettle exhaust chimney
0733	DMSO (garlic)	Malt quality, boiling intensity	Change malt, increase boiling time for greater evaporation
0810	Catty	Oxidation of final product	Monitor dissolved oxygen and adjust filling/packaging process accordingly
0820	T-2-N (papery)	Oxidised, stale	Avoid oxygen when filtering, packaging and in pipes and storage tanks
0828	Strecker aldehydes (bready)	Kilning, mashing, wort boiling and fermentation	Avoid oxygen in beer processing, packaging and in pipes and storage tanks
0830	Leathery	Oxidised, formed from reactions of precursors during beer storage	Avoid oxygen when filtering, packaging and in pipes and storage tanks
0840	Mouldy	Contamination of raw materials by fungus or bacteria, damp cellars	Dry the cellars, hygiene
0841	Earthy	Microorganisms from water or damp cellars	Check cellar walls, hygiene
0842	Musty (cellar-like, old)	Improperly stored barley/malt	Ensure optimal conditions in storage facilities
0910	Acetic aid (vinegar)	Contamination of acetic acid bacteria or acetic acid producing wild yeast	Locate the source of contamination and improve hygiene and process control
0920	Sour (citric acid, lemon)	Contamination by lactic acid bacteria or *Pediococcus*	Locate source of contamination and improve hygiene
1003	Vanilla	Degradation of some phenolic compounds, degradation of barley cell wall, aging, contamination by wild yeast	Locate source of wild yeast contamination and improve hygiene
1100	Salty	Origins from malt or brewing water	Reduce NaCl additions
1310	Alkaline (detergent)	Contamination with caustic cleaning detergents	Locate the source and review cleaning processes
1330	Metallic (iron, blood, copper)	Contamination of metals in brewing liquor, from equipment or from lower grade kieselguhr	Locate the source of contamination, use iron-free kieselguhr
1340	Astringent	Too fine milling, too high sparging temperature, too high pH of water and excessive sparging. Polyphenols from malt or hops	Higher cut-off gravity, lower the sparge water temperature (maximum 80 °C), lower water pH to avoid tannin extraction. Check malt and hops from polyphenols
1410	Body (thick, sweet)	Low RDF, stuck fermentation, high OE	Re-design beer style, lower OE, higher RDF
1411	Body (watery)	Thin body, bland flavour	Dilution rate too high for high gravity beer

Table 1

ed, in order to prevent contaminants in the surrounding air from entering the packaging area.

Flavour attributes produced during fermentation

The main flavour attributes produced during fermentation are due to the esterification of fatty acids with alcohol, i.e. alcolysis of acyl coenzyme A, which is part of yeast metabolism.

During this reaction, which is illustrated in figure 1, the fatty acid combines with the alcohol molecules, forming ester linkages. Some of the most common esters found in beer are isoamyl acetate (banana/pear drops), ethyl hexanoate (red apples, anise seed), ethyl butyrate (tropical fruits like mango and pineapple) and ethyl acetate (nail varnish). The level of various esters depends heavily on the yeast strain used and the fermentation temperature. A 'rule of thumb' dictates that the quantity of all esters produced increases with fermentation temperature, which is especially true for isoamyl acetate. In recent years, following the increase in high gravity brewing, it has also become evident that this has a profound effect on the quantity of the esters produced, due to the added stress factor for the yeast. Other stress factors include insufficient aeration of the wort and low natural mixing of the yeast during fermentation, which also increases the levels of esters produced. During maturation or secondary fermentation, many of the esters and general flavour compounds produced during primary fermentation are broken down, while new flavour compounds are produced. One of the compounds reduced during maturation is diacetyl. This compound imparts a sweet, butterscotch-like flavour, although some would describe it as rancid butter, which is most definitely an off-flavour in lager beers. During the first few days of primary fermentation, the level of diacetyl in the fermenting wort increases dramatically, reaching a peak concentration between 48 and 72 hours into fermentation (at least this is the author's own observation while investigating the fermentation profiles from two large breweries). After the peak has been reached, the yeast slowly re-absorbs the diacetyl, converting it to the intermediate product acetoin and ultimately to 2,3-butanediol [1]. Other flavours produced during maturation are fusel alcohols, some of which often have a rose-like, floral character, often described as an "aged" flavour.

Oxidation

One of the biggest culprits when it comes to producing unwanted flavours in beer is oxygen. High levels of dissolved oxygen in finished beer facilitate the development of many staling compounds, which were present in the beer in their often non-compromising precursor forms.

During fermentation, the yeast is initially provided an aerobic environment and uses up dissolved oxygen during the initial growth phase. It is the subsequent process of transferring beer from the tanks through a series of hoses, connections and valves through to packaging that is responsible for oxygen picked up and dissolved in the finished beer.

During filling, oxygen can come from air trapped inside the bottle, cans or filler tubes. However, through employing inert gases, such as carbon dioxide or nitrogen, for flushing the filler bowl and filler valves before filling, the potential for O_2 uptake is reduced drastically. When capping bottles or seaming cans, it is also of the utmost importance that oxygen is eliminated by flushing the entire seaming/crowning compartments with inert gas, which is a slightly risky approach due to the potential for leakage into the immediate surroundings where people are working. During bottling, the preferred method for minimizing oxygen uptake is 'jetting', in which a brief stream of sterile water is squirted directly into the filled bottle, causing the contents to foam up, effectively pushing any oxygen in the headspace out of the bottle just before it is sealed. The crown cap itself also poses a risk because oxygen may still enter through the closure.

To monitor oxygen levels in the various fermentation and storage tanks, in-line oxygen meters should be installed to continuously register the quantity of dissolved oxygen, in order to give an immediate indication of any potentially problematic situations. To avoid oxygen in piping systems, oxygen-free water can be pumped through the system, effectively absorbing any oxygen that may be present. If kieselguhr filtration is used, the kieselguhr should be de-aerated prior to filtration, e.g. by pumping inert gas such as carbon dioxide or nitrogen through it. Sufficiently high liquid pressure upstream from the filter pump will also prevent any oxygen from dissolving in the product. A general 'rule of thumb' states that dissolved oxygen, DO, should be kept below 0.2 ppm to avoid premature staling of the finished product.

Strecker aldehyde oxidation

Of the Strecker aldehydes found in finished beer, 85 percent are derived from the wort production process, and the final 15 percent are caused by Strecker degradation of the remaining Strecker amino acids (valine, leucine, iso-leucine, methionine and phenylalanine) in the packaged product [1].

The perception of Strecker aldehydes varies with both the beer style and the specific Strecker aldehyde. Phenylalanine, the most abundant of Strecker amino acids in finished beer, is converted to phenyl acetaldehyde, which gives a flowery, rose-like character to beer. This is usually considered to be an aged flavour and therefore not positive. By comparison, the Strecker aldehyde 3-methyl butanal lends a malty, chocolate-like flavour and is usually considered positive. Despite some positive flavours, Strecker aldehydes are usually related to staling, and generally form the background for other staling compounds, such as trans-2-nonenal. Strecker degradation increases with oxygen uptake and thermal stress, i.e. pasteurization, boiling and excessively high holding temperatures in the whirlpool.

In any effort to avoid thermal stress in the finished product, a flash pasteurizer could be used instead of a tunnel pasteurizer, because flash pasteurization requires shorter holding times. However, with flash pasteurization there is the added risk of contamination at the filling line. To completely avoid thermal stress, it would also be possible to sterile filter the beer, although the method is far more time consuming and is associated with the same risk of contamination at the filling line as flash pasteurization. Storage of the finished beer is also an important factor with regard to staling, as beer stored at 30 °C will stale 25 times faster than beer stored at 0 °C.

T-2-N-type oxidation

The aldehyde trans-2-nonenal, or T-2-N, which is responsible for the cardboard-like staling flavour, originates from the malt itself, through lipid oxidation of linoleic and linolenic acid. During wort production, the unsaturated aldehyde binds to malt proteins and is then carried through the brewing process in the bound form, only to be re-

leased during storage of the packaged beer. The release of T-2-N is catalysed by higher temperatures, i.e. the higher the storage temperature, the more the protein/T-2-N complex reacts with the dissolved oxygen present in the beer, prompting the release of unsaturated T-2-N. One interesting point is that T-2-N-type oxidation seems more prominent in pale beers with a low alcohol content as opposed to Strecker degradation, which occurs more in dark beers with high alcohol content. Recent research into barley breeding has resulted in the development of barley which is defective in the synthesis of lipoxygenase, the enzyme responsible for T-2-N oxidation, among other things. Having a defect in the genes that express the lipoxygenase enzyme means the oxidation of linolenic and linoleic acids does not take place, and that T-2-N staling is prevented.

"Lightstruck" Flavour

The final off-flavour to be discussed in this paper is 'lightstruck' or 3-methylbut-2-ene-1-thiol, caused by the vitamin riboflavin reacting with iso-humulone, one of the alpha acids in hop. The reaction is catalysed by UV light. This off-flavour, often described as "skunky", occurs when natural light penetrates translucent beer containers and can take anywhere between a minute to hours to be produced, depending on the colour and thickness of the glass or plastic in question. To avoid the possibility of producing 3-methylbut-2-ene-1-thiol in the finished product without changing the container, it is possible to use isomerised kettle extracts, since these have increased resistance to the degradation of iso-humulone by UV light, unlike 'raw' hop pellets, whole hops and non-isomerised extracts.

Overview of flavours, their origins and possible remedies

Table 1 gives an overview of the off-flavours, their origins and remedies as they were discovered and presented in the two brewing courses. ■

Literature

1. SSB Lecture presentations and in-class notes from Diploma Master Brewer Class 2012/2013.
2. Moreira, N.; Mendes, F.; Guedes de Pinho, P.; Hogg, T.; Vasconcelos, I: "Heavy sulphur compounds, higher alcohols and ester production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must", International Journal of Food Microbiology, 2008, 124(3), pp. 231-238.
3. Landaud, S.; Helinck, S.; Bonnarme, P: "Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food", Applied Microbiology and Biotechnology, January 2008, volume 77, issue 6, pp. 1191-1205.
4. Vermeulen, C.; Lejeune, I.; Tran, T. T.; Collin, S.: "Polyfunctional Thiols in Fresh Lager Beers", J. Agric. Food Chem., vol. 54, no. 14, 2006.
5. Course: "Fundamentals of Beer Brewing and Wine Making", Copenhagen University, Autumn 2012.
6. Meilgaard Flavour wheel