School of Engineering
& Physical Sciences

B99BM Assignment 1

The role of yeast in the formation of aroma compounds in alcoholic beverages.

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Introduction

There are various stages during the process of brewing a beer which can contribute to the flavours and aromas active in the final product. These begin in the malt-house, are impacted by grain bill selection and preparation, are enhanced or eliminated by well-planned and practised mashing, boiling, lautering and sparging stages, influenced by hops and/or adjunct additions, unleashed during primary yeast fermentation and then refined during lagering, conditioning or maturation.

Of these stages the focus of this discussion is the fermentation process, simultaneously the name given to the entire process of brewery yeast metabolism and also the specific anaerobic, catabolic process that forms one part of the more complex combination of reactions that convert wort into beer. It is during this stage that a number of important macromolecules are excreted from the yeast cell that can greatly impact the aroma and flavour of beer (Bokulich and Bamforth, 2013).

For simplicity, the majority of the subsequent discussion centres on compounds formed by brewer's yeast (*Saccharomyces cerevisiaie*), though mention is also made latterly of the contribution of the *Brettanomyces* genus of yeast.

Of the aroma compounds formed by yeast, higher alcohols and esters comprise 99% of the bulk (Alves *et al.*, 2020), so these two compounds are given greater billing than the remaining comparatively minor constituents, with the exception that is, of the vicinal diketones (VDKs). Due to their low flavour threshold versus huge potential impact balance, and the importance placed upon them by many of the authors cited below, VDKs are considered in equal depth. The concentration ranges, flavour thresholds, potential flavour or aromatic impact and biochemical pathways that lead to the creation of each of these groups will be examined.

The metabolic roles and potential impacts on aroma of the remaining compound groups, namely: organic acids; short-chain fatty acids; aldehydes and sulphur-containing compounds, will subsequently be discussed.

Then finally, conditions that affect the growth or demise of aroma compounds will briefly be reviewed.

Higher Alcohols

Higher alcohols (also referred to as fusel alcohols or oils) are the most abundant aroma compounds present in beer (Pires *et al.*, 2014; Pires and Bránayik, 2015). Upwards of 40 higher alcohols have been identified in beer (Boulton and Quain, 2001; White, Zainasheff and White, 2010). Of these, the most important from an organoleptic perspective due to their occurrence at higher concentrations than their flavour thresholds (Renger, van Hateren and Luyben, 1992; Boulton and Quain, 2001) are: isoamyl alcohol (Suomalainen and Lehtonen, 1978; Renger, van Hateren and Luyben, 1992), n-propanol, isobutanol, (Boulton and Quain, 2001; White, Zainasheff and White, 2010) 2-methylbutanol and 3-methylbutanol (Boulton and Quain, 2001), with isoamyl alcohol comprising the largest constituent of the total higher alcohol contribution at around 40-70% (Suomalainen and Lehtonen, 1978). These compounds have a similar alcoholic taste to ethanol but can also impart a warming sensation and, solvent-like flavours to beer (White, Zainasheff and White, 2010).

Additionally, aromatic alcohols such as phenol ethyl alcohol can contribute positive qualities to beer such as floral or rose aroma (Fix, 1999; Boulton and Quain, 2001).

Table 1: important higher alcohols, with their concentration ranges in beer, flavour thresholds and flavour contributions. Adapted from (Fix, 1999).

Compound	Range (mg/L)	Threshold (mg/L)	Flavour
Isoamyl alcohol	100-110	40-130	Alcohol, banana, solvent
N-Propanol	10-40	600-800	Alcohol, rough aftertaste
Isobutanol	10-60	180-200	Alcohol, rough aftertaste
Phenol ethyl alcohol	100-200	10-80	Roses, bitter, chemical, medicinal

During fermentation yeast absorbs or synthesises amino acids from which it utilises amino nitrogen (NH_3) as a nutrient (Fix, 1999) in order to support growth and biomass production (Fairbairn *et al.*, 2017), the remaining carbon skeleton (CH_2OH)

is sent to an oxoacid pool for further processing (Fix, 1999). The formation of higher alcohols begins with 2-oxo(α-keto)acids from this pool, which are in turn decarboxylated to form an aldehyde which is subsequently reduced to its corresponding alcohol (Boulton and Quain, 2001) (Figure 1).

There are two possible metabolic routes from which amino acids are derived and converted into α-ketoacids: the Ehrlich pathway or the biosynthesis pathway (Suomalainen and Lehtonen, 1978; Renger, van Hateren and Luyben, 1992; Boulton and Quain, 2001; Pires *et al.*, 2014; Pires and Brányik, 2015; Walker and Stewart, 2016)

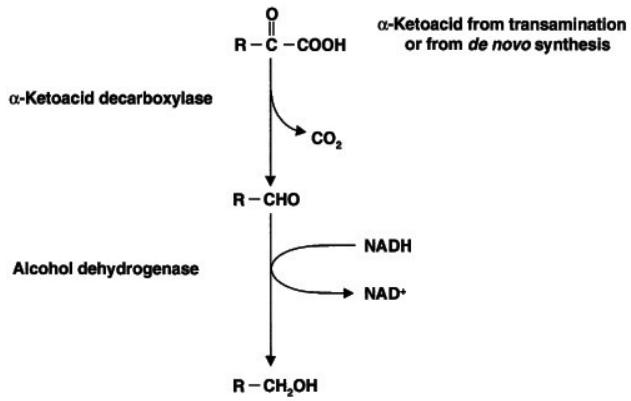

Fig.1 Generalised scheme for higher alcohol synthesis in *Saccharomyces cerevisiae*, Reproduced from (Boulton and Quain, 2001)

Ehrlich pathway

This catabolic route occurs in early fermentation when there is a high concentration of amino acids in the wort available to be assimilated by yeast (Walker and Stewart, 2016). The amino acid is transaminated, during which the amino group is transferred to its respective α-keto acid. This process involves transaminase enzymes in a Strickland reaction using glutamate/α-ketogluterate in a donor/acceptor pair (Fix, 1999; Pires *et al.*, 2014; Pires and Brányik, 2015).

Biosynthesis pathway

When there is a deficiency in available wort amino acids, the α-keto acids are derived from pyruvate (the product of an earlier the Embden-Meyerhof-Parnas pathway of carbohydrate metabolism) or acetyl-CoA as part of the anabolic *de novo* synthesis of branched-chain amino acids (BCAAs) (Boulton and Quain, 2001; Hill, 2020).

In addition to their own contribution to beer character higher alcohols also provide precursors for the synthesis of esters (Boulton and Quain, 2001).

Esters

Of the aroma compounds present in beer as a result of yeast metabolism, and despite being trace elements in comparison to other metabolites, esters are possibly the most important (Boulton and Quain, 2001; Pires *et al.*, 2014; Pires and Brányik, 2015). Over 90 esters have been identified in beer, with the three most prominent being ethyl acetate, isoamyl acetate and ethyl hexanoate (or ethyl caproate) (Fix, 1999). Other esters of note include isobutyl acetate and 2-phenylethyl acetate (Boulton and Quain, 2001). With concentrations below their flavour thresholds most esters are considered to contribute favourably to the final beer flavour (Pires and Brányik, 2015; Walker and Stewart, 2016). The flavour thresholds and concentrations of prominent esters are show in table 2.

Yeast forms esters by esterification of activated fatty acids and alcohols mediated by co-enzyme A (Renger, van Hateren and Luyben, 1992; Carrau *et al.*, 2008). Esters fall into one of two categories, acetate (organic acid) esters and ethyl (fatty acid) esters (Renger, van Hateren and Luyben, 1992; Lewis and Young, 2001; Pires *et al.*, 2014; Pires and Brányik, 2015).

Table 2: prominent esters, with their concentration ranges, flavour thresholds (found in various lager beers) and flavour profiles. Adapted from (Renger, van Hateren and Luyben, 1992; Fix, 1999; Boulton and Quain, 2001)

Compound	Range (mg/L)	Threshold (mg/L)	Flavour
Ethyl acetate	8 – 48	33	Fruity with solvent undertone
Isoamyl acetate	0.8 – 6.6	1.6	Bananas, apples
Ethyl hexoanoate (ethyl caproate)	0.1 – 1.5	0.23	Apples, aniseed
Isobutyl acetate	0.03 – 0.25	1.6	Banana, fruity
2-phenylethyl acetate	0.1 – 1.5	3.8	Roses, honey, apples

Acetate esters

Acetate esters are formed as product of alcohols (ethanol or higher alcohols) and acetyl-CoA (Renger, van Hateren and Luyben, 1992; Pires *et al.*, 2014; Pires and Brányik, 2015), catalysed by alcohol acetyl transferase (Boulton and Quain, 2001; Pires *et al.*, 2014; Pires and Brányik, 2015) (fig 2). The acetyl-CoA being formed via oxidative decarboxylation of pyruvate (Renger, van Hateren and Luyben, 1992).

Ethyl esters

Ethyl esters are formed as a product of a condensation reaction between ethanol and a fatty acyl-CoA (Pires *et al.*, 2014; Pires and Brányik, 2015). The acyl-CoA formation in this case depends upon fatty acid synthesis (Renger, van Hateren and Luyben, 1992). For example, ethyl hexanoate is formed by the reaction between ethanol and Hexanoyl-CoA, mediated by ethanol hexanoyl transferase (Pires *et al.*, 2014; Pires and Brányik, 2015) (fig.2).

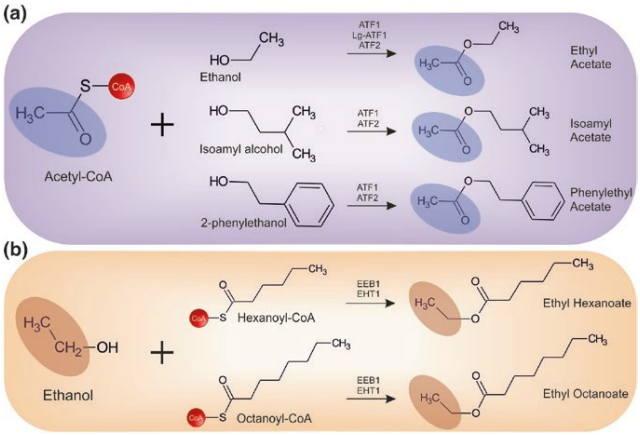

Fig 2: A scheme of the chemical reactions involving (a) acetate ester, and (b) ethyl ester biosynthesis. Reproduced from (Pires *et al.*, 2014; Pires and Brányik, 2015)

Vicinal Diketones

The term vicinal diketones (VDKs) is used to refer to specific carbonyl compounds, formed as by-products of amino acid synthesis, the most important of which being diacetyl (2,3-butanedione) and 2,3-pentanedione, respectively (Boulton and Quain, 2001; Bamforth, 2009, 2012, 2018; Krogerus and Gibson, 2013; Pires and Brányik, 2015). Both diacetyl and 2,3-pentanedione have very low flavour thresholds though the latter is 10 times greater than the former so generally has a lesser potential impact on beer (Pires and Brányik, 2015). These VDKs impart a butterscotch, buttery or honey flavour/aroma on beers and with the exception of some very specific Czech Pilsner style beers, this is largely considered to be an undesirable contribution (Bamforth, 2009, 2012, 2018; Pires and Brányik, 2015). The thresholds are listed in table 3. Aside from highlighting inefficiencies in the fermentation process, high volumes of diacetyl can be indicative of microbial contamination by either *Lactobacillus* or *Pediococcus* spp. (Boulton and Quain, 2001; Bamforth, 2012).

Table 3: vicinal diketones, along with acetoin, their thresholds and flavour contributions. Adapted from (Fix, 1999)

Compound	Threshold (mg/L)	Flavour
Diacetyl	01.-0.15	Buttery, butterscotch
Acetoin	1.0	Fruity, musty
2,3-pentanedione	1.0	Honey

The formation of diacetyl and 2,3-pentanedione occurs extracellularly as a result of spontaneous oxidated decarboxylation of α-acetolactate or α-acetohydroxyl acid which has been excreted from the yeast cell during the biosynthesis of either valine, in the case of diacetyl, or isoleucine, in the case of 2,3-pentanedione (Boulton and Quain, 2001; Pires and Brányik, 2015).

During a standard fermentation with a sufficient amount of vigorous, healthy yeast (Bamforth, 2012, 2018) the VDKs are re-absorbed into the yeast and reduced to diols which have a much higher flavour threshold with little significant impact on beer (Bamforth, 2012, 2018; Pires and Brányik, 2015). Diacetyl is firstly reduced to acetoin by the acetoin reductase enzyme ((Pires and Brányik, 2015) and then subsequently to 2,3-butanediol. 2,3-pentanediome is similarly reduced to 2,3-pentanediol (Boulton and Quain, 2001; Pires and Brányik, 2015)(Figure 3). There are commercially available bacterial enzymes, such as Novozymes Maturex®, that can be added at the start of fermentation to transform the α-acetolactate directly to acetoin, hence bypassing diacetyl formation in the first place (Bamforth, 2012, 2018).

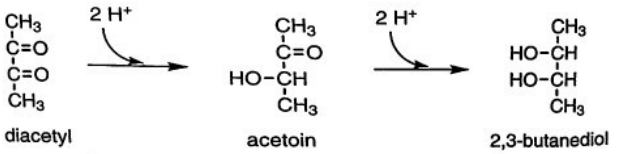

Fig 3: Pathway of enzymatic reduction of diacetyl by yeast cells. Reproduced from (Fix, 1999)

Other Compounds

Organic Acids

Organic acids include pyruvate, citrate, malate, acetate, succinate, lactate and 2-oxoglutarate and, aside from pyruvate itself, the majority of these are derived directly from pyruvate or from the tricarboxylic acid cycle (Boulton and Quain, 2001). In most fermentations, these acids are precursors for various metabolic pathways and are consumed, those that remain in the final beer are usually in concentrations below their flavour thresholds (White, Zainasheff and White, 2010). These acids have flavours ranging from sour, vinegary, vomit and barnyard animals (Boulton and Quain, 2001; White, Zainasheff and White, 2010).

Whilst organic acids can impact aroma and flavour in their own right, their primary importance are the roles they play in the formation of VDKs (α-acetolactate & α-acetohydroxylate) (Boulton and Quain, 2001), in the formation of acetate esters (White, Zainasheff and White, 2010; Pires *et al.*, 2014; Pires and Brányik, 2015) and in the reduction of pH levels (Boulton and Quain, 2001).

Short-Chain Fatty Acids

Short-chain fatty acids are released into beer as by-products of the hydrolysis of acyl-CoA derivatives during *de novo* lipid synthesis (Boulton and Quain, 2001; Carrau *et al.*, 2008). The fatty acids synthesized by yeast are largely the same and in similar proportions regardless or irrespective of the raw materials or beverage being produced (Suomalainen and Lehtonen, 1978). They have the potential to adversely impact foam performance (Boulton and Quain, 2001) as well as introduce qualities of bitterness, astringency and rancidity to beer (Alves et al., 2020). In the case of caproic and capryllic acids their aroma contribution is described by Boulton and Quain (2001) as “goat-like”.

Aldehydes

Of the 200 or so carbonyl compounds detected in beer and with the exception of VDKs (discussed above), aldehydes including acetaldehyde are of importance to the flavour and aroma of beer (Boulton and Quain, 2001). Though their levels in beer are normally well below their flavour thresholds, higher levels can be

indicative of non-standard fermentation performance (Boulton and Quain, 2001). The aromas associated with aldehydes, which are more generally present in wort than in beer, are described as 'grassy', 'fruity', 'green leaves' and 'cardboard' depending on the compound (Boulton and Quain, 2001).

Otherwise, aldehydes contribute to the formation of other aroma compounds as intermediates in the formation of higher alcohols (Suomalainen and Lehtonen, 1978), and acetaldehydes are intermediates of ethanol or acetate (Boulton and Quain, 2001).

Sulphur-containing Compounds

Yeast produces sulphur-containing compounds in large quantities during fermentation, however, under healthy conditions they are generally volatile enough to be driven from the solution along with CO_2 (White, Zainasheff and White, 2010). The most significant sulphur compounds are hydrogen sulphide (H_2S), sulphur dioxide (SO_2) and dimethyl sulphide (DMS) (Boulton and Quain, 2001; White, Zainasheff and White, 2010; Bokulich and Bamforth, 2013).

Hydrogen sulphide is normally released during fermentation alongside CO_2, however, should it remain the potential aroma contribution is one of rotten eggs (White, Zainasheff and White, 2010).

As well as potentially impacting flavour, SO_2 contains antioxidant properties and protects against flavour deterioration ((White, Zainasheff and White, 2010; Bokulich and Bamforth, 2013). The aroma that sulphur dioxide can contribute to beer is described as being similar to a burnt match (White, Zainasheff and White, 2010).

Boulton and Quain (2001) state H_2S and SO_2 can arise from yeast metabolism by two routes: "First, from the dissimilation of complex organic molecules such as sulphur-containing amino acids and vitamins, and second, from assimilatory reactions involving inorganic sulphur-containing nutrients".

Whilst wort-based DMS is normally eliminated by the boil, its oxidized version DMSO is not, however, yeast has the ability to reduce DMSO back to DMS (Boulton and Quain, 2001; White, Zainasheff and White, 2010) and with it can introduce 'canned corn' or 'cooked cabbage' aromas into the beer (White, Zainasheff and White, 2010).

Brettanomyces yeasts

Of the non-*S. cerevisiae* yeasts (or those derived from *S. cerevisiae*) used in beers, alcohol-tolerant *Brettanomyces* strains are quite common, particularly in the production of Saisons or barrel-aged sour beers. Yeasts from the *Brettanomyces* genus are capable of metabolising the sugars that remain post-primary fermentation with *S. cerevisiae* (Stewart, Russell and Anstruther, 2018) and are able to thrive in conditions that *S. cerevisiae* would consider adverse (White, Zainasheff and White, 2010). Of the flavour compounds discussed above, *Brettanomyces* produces very high levels of fatty acids and esters (Stewart, Russell and Anstruther, 2018) as well as a phenol called 4-vinylguaiacol (4 VG) (White, Zainasheff and White, 2010). This compound is produced by yeast from the decarboxylation of ferulic acid by the enzyme ferulic acid decarboxylase. Most *S. cerevisiae* strains have a natural mutation in the phenolic off-flavour gene, such that they are incapable of producing 4-vinylguaiacol (White, Zainasheff and White, 2010).

Brettanomyces yeasts can produce aromas and flavours such as barnyard, horse blanket and sweat (White, Zainasheff and White, 2010), though my own contribution would be that 'Brett' beers are funky, though not necessarily in a cool or hip way. According to Bokulich and Bamforth (2013), 4 VG imparts a spicy or clove-like character.

Fermentative Controls

There are a number of factors of a brewery fermentation that impact directly or indirectly on the formation of the aroma compounds discussed above. The control of which are key to consistent beer quality (Lewis and Young, 2001)

Of the numerous factors those collectively considered to be most important (from the cited sources) can be summarised as:

- Appropriate selection of yeast strain (Boulton and Quain, 2001; Bamforth, 2012, 2018)
- Yeast condition (age, virility etc) (Bamforth, 2012, 2018)
- Yeast pitching rates (Bamforth, 2012, 2018)
- Wort composition (Free Amino Nitrogen levels, sugar levels etc.) (Fix, 1999; Boulton and Quain, 2001; Lewis and Young, 2001; Bamforth, 2018)
- Temperature during fermentation
- Availability of molecular oxygen (Fix, 1999; Boulton and Quain, 2001; Lewis and Young, 2001; Bamforth, 2018)
- Hydrostatic Pressure (Bamforth, 2018)

As a general rule, fermentative conditions which encourage yeast growth encourage the formation of most aroma active metabolites (Lewis and Young, 2001), however exceptions can be seen with esters and fatty acids, which generally have the inverse effect (Boulton and Quain, 2001; White, Zainasheff and White, 2010). For example, higher temperatures, or greater availability of molecular oxygen encourage yeast growth and result in increased concentrations of higher alcohols (Boulton and Quain, 2001), higher initial concentrations of VDKs (as well as improved conditions for VDK reduction)(Krogerus and Gibson, 2013) and potentially increased levels of organic acids. In both of these scenarios, the opposite trend has been observed of fatty acids and their esters (Boulton and Quain, 2001; Bamforth, 2018).

Bamforth (2018) points out that higher levels of hydrostatic pressure also lowers ester formation, and as such fermenter shape is important. High volume, conical fermenters have less surface contact with the wort and therefore exert greater hydrostatic pressure than shallower, open-top fermentation vats.

When considering diacetyl in particular, additional methods of control exist: such as a 'diacetyl rest' which involves increasing the fermentation temperature by 1 or 2°C mid-fermentation (Bamforth, 2012, 2018; Krogerus and Gibson, 2013); "Kräusening", which involves introducing a large amount of young, virile yeast at the end of primary fermentation which can

mop up not only its own VDKs but those of the primary yeast (Bamforth, 2012, 2018); or passing 'green' beer through packed bed reactor containing immobilised yeast (Bamforth, 2012, 2018; Krogerus and Gibson, 2013).

Sulphur compounds are more prevalent in lagers than in ales due to the lower fermentation temperatures leading to a less vigorous fermentation (White, Zainasheff and White, 2010).

Finally, as many of the aroma compounds above can be formed by microbial contamination, adequate sanitisation is, as always, key during every step of brewing (Fix, 1999; Bamforth, 2012).

Conclusion

The various metabolic reactions that occur within and outwith the yeast cell during the fermentation stage of brewing have been shown to be of great importance on the balance of aromas and flavours, and as a result the final quality of beer.

Understanding how to encourage growth or degradation of the various compounds as desired, and the implications of key factors such as choice of suitable yeast (genus and strain); choice of suitable fermentation vessel; temperature control; availability of molecular oxygen at the start of fermentation; measuring and potentially manipulating wort composition etc. are evidently crucially important to the consistent production of quality beer.

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