Biotransformation: a story of yeast, hops and enzymes

Exploring new ways of achieving great flavour and aroma in beer

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Who has not heard about 'biotransformation'? Nowadays, this word has been spread around the globe and is included in the craft brewer's vocabulary – but is this complex subject really known and understood? Does it really have an impact on our beers? And more importantly, how can we benefit from it?

Biotransformation is defined as 'the chemical modification made by an organism on a compound'. Although this term is commonly used in pharmacology and toxicology, from the brewer's perspective, it refers to the interaction of two ingredients used in brewing: yeast and hops.

The role of both ingredients in biotransformation will be discussed in detail, as well as the use of exogenous enzymes to influence hop aroma and flavour in beer.

Role of hops

It is well known that hops are essential in beer production, mainly due to their contribution to aroma and bitterness. In addition to water, cellulose and various proteins, the chemical composition of hops consists of tannins (polyphenols), hop resins and hop oils, each one determining the organoleptic profile of the resulting beer (Table 1).

Hop polyphenols: Polyphenols also play an important role in the brewing process due to their contribution to non-biological haze (protein-polyphenol interaction). Although they can also be found in malt, they comprise up to 4% of the total weight of dried hop cones. Their level

Component	% of total dry weight
Resins	17
Essential oil	0.6
Tannins	4.5
Monosaccharides	2.5
Pectin	2.5
Amino acids	<0.2
Proteins	17
Lipids and wax	3.5
Ash	1
Cellulose, lignin, etc.	45

Table 1: Composition of hops[1]

depends on the hop variety, cultivation area, harvesting technique and degree of ageing[2].

Hop resins: Hop resins, as well as essential oils, are found in the lupulin glands of the female hop cone. They are mainly composed of hard resins and soft resins, where bittering acids are found. In general terms, bitterness is one of the most particular tastes that has been associated with beer, mainly due to the isomerised molecules of α-acids formed during the wort boiling process, known as iso-α-acids.

Hop oils: The quantity of essential oil present in hops is very low, but their contribution to the aroma profile in beer is significant. This is due to the low sensory threshold of the volatiles, especially thiols (sulphur group of hop oils), being detected at very low concentrations.

Dried hops contain between 0.5% and 3% w/w of oil varying between varieties[2], and these are present in a complex composition of up to 1,000 compounds, all from a wide range of chemical classes[3].

Hop oils are classified into three groups: Hydrocarbon fraction, oxygenated fraction and sulphur fraction[2]. Linalool, a monoterpene alcohol of the oxygenated fraction, has been considered a key indicator for hoppy flavour in beer for some years[4]. Nevertheless, hop aroma and flavour in beer should not be ascribed to nor associated with a single substance, since they are the result of interactions of many compounds and the synergies between them.

According to Takoi *et al*. (2010), hop oils such as geraniol and β-citronellol affect the overall flavour when linalool is present in excess, creating a synergistic effect contributing significantly to a more perceived fruity and citrusy flavour in beer[5].

Hops contain another compound which also has an important contribution on biotransformation, known as glycoside. By definition, glycosides are molecules in which a sugar molecule is bonded glycosidically to another molecule. In nature, they are commonly found in plants and, in biological terms, they play numerous roles in living organisms, such as storing chemicals or even transport carbohydrates through the plant and releasing them through hydrolysis.

In brewing, hop-derived glycosides are mainly formed by a monoterpene alcohol and a carbohydrate being, in their combined state, a molecule that has no aroma and no sweetness. Furthermore, glycosidically-bound flavour compounds of hops are considered to contribute to the hop flavour in beer[6].

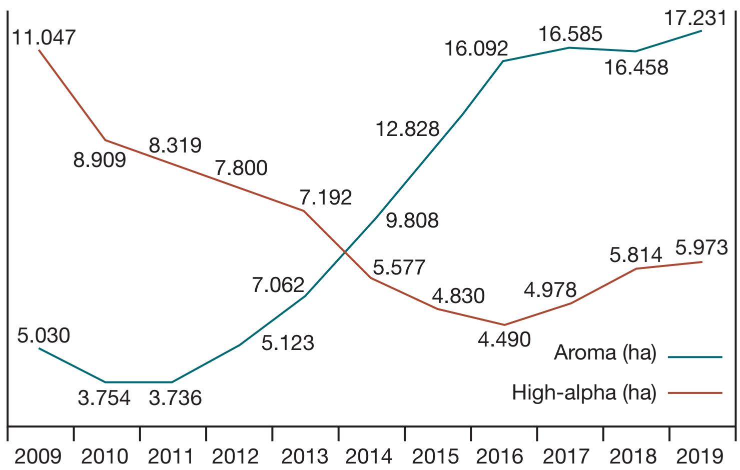

Figure 1: Hop market evolution from 2009 to 2019[7]

Since 2009, there has been a clear trend towards the growth of aroma varieties rather than the high-alpha varieties, which have decreased over the last ten years, as illustrated in Figure 1. Additionally, higher hop rates have increased over the last decade, suggesting that the hop aroma in beer is an attribute considered of a great interest[8].

This high demand for aroma hop varieties, among other production factors, led brewers to look for new and innovative ways to boost hop aroma and flavour. In other words, hop biotransformation may become a potential solution to optimise the use of hops by changing the ratio of specific aromatic compounds and, therefore, modifying the diversity of flavours and aroma in beer.

Role of yeast

No yeast, no beer. This micro-organism is responsible for transforming wort into beer, a biochemical process where alcoholic fermentation occurs, as well as the release of carbon dioxide (CO_2) and aromatic compounds.

However, yeast is much more than that. In 2003, King and Dickinson first discovered a novel biotransformation of hop compounds by yeasts that had significant impact on the formation of flavour[9]. In addition, yeast is responsible for a series of reactions modifying the structure of monoterpene alcohols

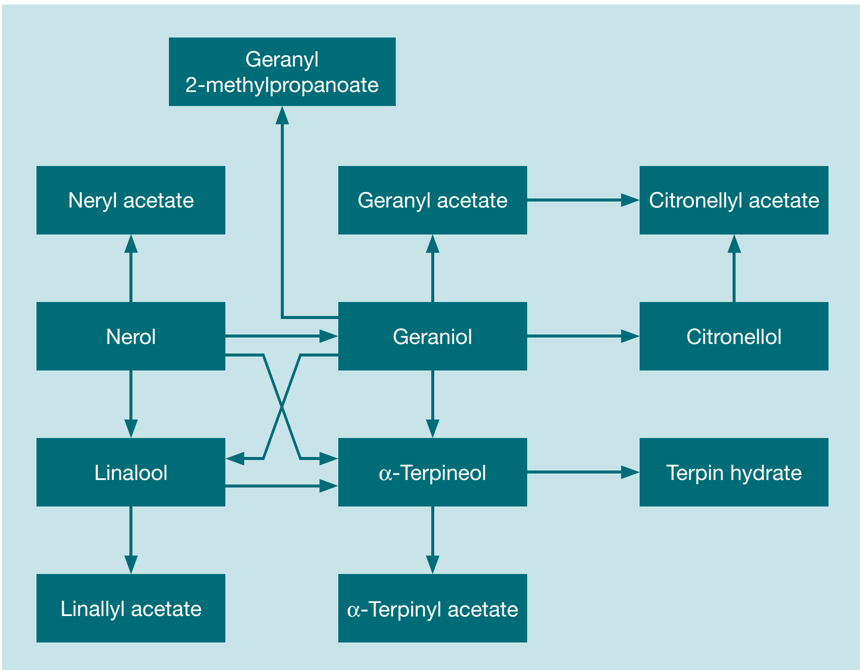

Figure 2: Proposed pathways of terpenoid biotransformation by yeasts[10]

during fermentation, which shows how complex and diverse biotransformation is (Figure 2).

According to Liu (2015), biotransformation of oxygenated terpenes would at least partially explain the hoppy aroma differences between the raw hops and the finished beer, as well as some other volatiles found in beer[10]. A more recent study by Sharp *et al*. (2017), also concluded that brewing yeasts (*Saccharomyces* spp.) exhibit a broader range of abilities to hydrolyse glycosides than was previously thought.

Other studies have revealed the importance of the yeast in biotransformation, based on the catalysis of glycosidic bonds in the production of hoppy beers.[10, 11, 12, 13, 21]

During fermentation, where cells are highly active, yeast naturally secretes β-glucosidase enzymes extracellularly, these being responsible for the hydrolysis reaction. Figure 3 shows the mechanism for cleavage of a non-aromatic compound into a glucose and linalool, obtaining more aromatic compounds and fermentable sugars derived from a lynalyl glycoside molecule.

The release of terpenes from glycosides is not the only interaction between yeast and hops, there are other examples as shown below:

Esterification: By definition, esters are a bond between a carboxylic acid and an alcohol. It has been reported in research studies that yeast has esterase activity, resulting in the esterification of a number of hop compounds. For example, geraniol and citronellol, which are naturally found in hops, are transformed into their acetate forms, geranyl acetate and citronellyl acetate, respectively[9].

Thiols: Also known as mercaptans, thiols are a family of aroma compounds naturally found in hops, either free or in the form of inodorous non-volatile precursors, which can be released with the enzyme β-lyase.

Nowadays, they are gaining popularity due to their contribution to aroma in beer, despite their low concentration (less than 1% of hop oils), as well as the low perception threshold (parts per trillion or ng/L). As stated by Shellhammer, "Thiols are 10,000 times more potent than geraniol, but they are only there in hops in tiny amounts"[15].

The three most abundant thiol molecules in brewing are 3SH (3-sulfanylhexan-1-ol, also known as 3MH), which gives notes of citrus and grapefruit, its acetate form 3SHA (also known as 3MH-A), which gives passion fruit notes and 4MSP (4-methyl-4-sulfanylpentan-2-one, also known as 4MMP), which is responsible for blackcurrant aroma[16].

During fermentation, *Saccharomyces cerevisiae* is able to uptake and cleave the precursors to release the free thiols, such as 4MSP, through β-lyase activity (Figure 4). In addition, yeasts have different capacities to reveal volatile thiols depending on their genetic background and their corresponding enzymatic activities[17]. Thiols have been well known in wine research for decades compared to beer, which reflects that more studies are needed to understand their great potential in beer flavour.

Other interactions: Yeast and hop interactions may also result in undesirable effects, which mostly depend on the dry-hopping regime during fermentation. The addition of hops in post-fermentation is practiced in many breweries, but yeast may not then contribute to biotransformation. A brief summary of considerations in regards to the dry-hopping time is shown in Table 2.

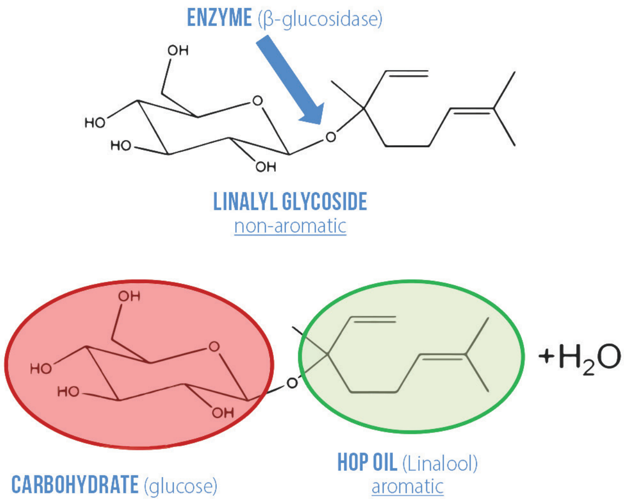

Figure 3: Lynalyl glycoside hydrolysis releasing a fermentable sugar and a terpene[14]

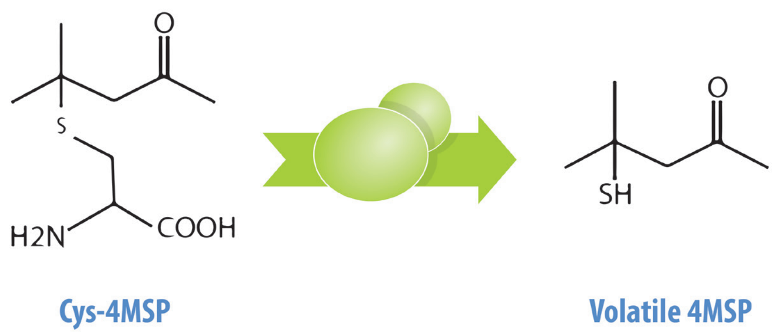

Figure 4: Example of conversion from an inodorous precursor into a volatile thiol by yeast[17]

Dry-hopping time	
Start of fermentation	**Near the end of fermentation**
Reduction of hop oils by CO_2 stripping	More hop oils presence due to less CO_2 stripping (counter pressure)
Hop aroma masked by other fermentation compounds (e.g. POF+ yeast)[13]	Reduction of dissolved oxygen by active yeast
Essential oils being adsorbed by the yeast cell membrane, and extracted from beer when flocculation occurs	Higher hop oil solubility when ethanol content increases
Source: based on Sharp *et al.* (2016)18	

Table 2: Effects of dry-hopping depending on the addition point

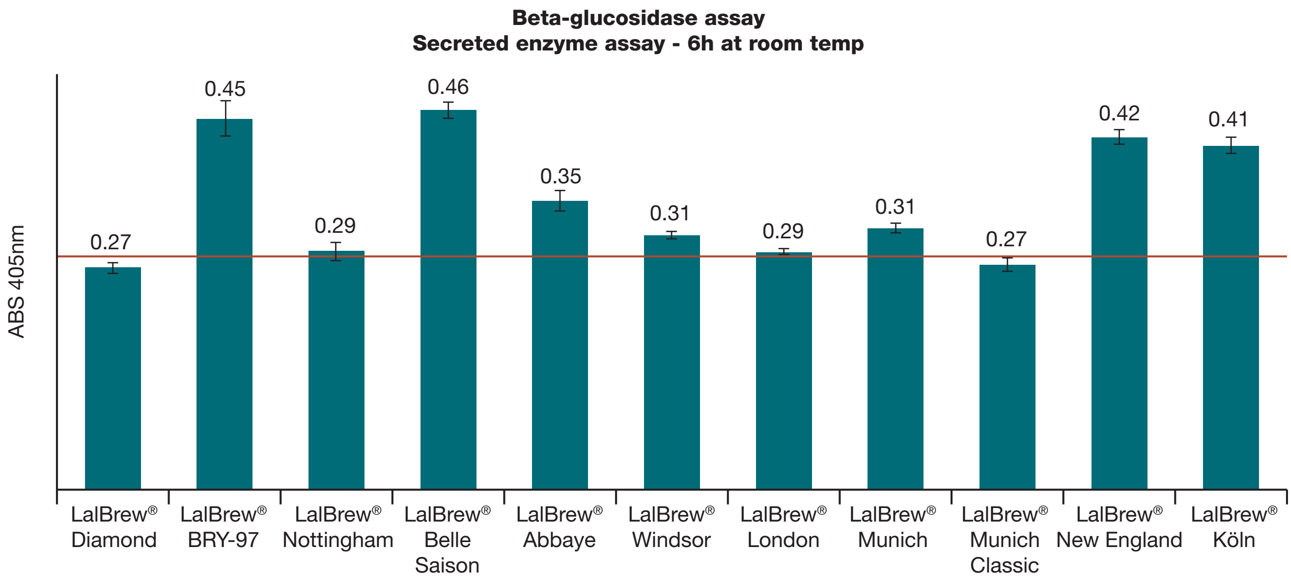

Figure 5: Characterisation of β-glucosidase activity in Lallemand Brewing yeast strains[14]

Sometimes biotransformation can be confused with another interaction where yeast and hops are involved, known as the 'hop creep' effect. This phenomenon is basically detailed as the refermentation observed in a fully attenuated beer after dry-hopping, and has its first studies published in 1893 by Brown and Morris.

As previously detailed, glucose molecules are released through the hydrolysis of glycosides, although this does not present a problem from the brewer's perspective, since they are metabolised during fermentation. However, the 'hop creep' effect also implies the liberation of glucose molecules through the action of amylolytic enzymes, naturally found in hops, which have the ability to degrade unfermentable carbohydrates (dextrin material) into fermentable sugars.

In craft brewing, most beers are not filtered". meaning that they have some yeast cells still in suspension in the final product. Therefore, the combination of free glucose and yeast cells could result in fermentation within a packaged beer leading to over-carbonated beers, presence of diacetyl and higher alcohol content, therefore presenting a quality issue in hoppy beers.

As recently suggested by Stokholm and Shellhammer (2020), there are several factors that could promote or reduce the 'hop creep' effect in order to overcome this problem in dry-hopped beers, including recipe design (wort composition), yeast strain selection (high or low flocculent), its suspension during dry-hopping, and dry-hop method (contact time and temperature), among others[19].

The potential for yeast and hop interactions has gained more interest in recent years, to the point where some commercial yeast suppliers have worked to better understand the potential value of commercially available yeast strains in influencing biotransformation and impacting beer aroma and flavour.

An example of this is shown in Figure 5, where commercially-available brewing yeast strains were characterised by their β-glucosidase activity, through a secreted enzyme assay at room temperature. Nevertheless, there are also cell-associated enzymes, which may have a contribution as well on biotransformation – when yeast and hops are in contact during fermentation. In regard to top or bottom fermenting strains, Sharp *et al.* (2017) concluded that there was no indication that either lager or ale yeasts exhibited higher activities than the other[12].

The use of exogenous β-glucosidase enzymes

In the same study conducted by Sharp *et al.* a total number of 80 yeast strains were tested and ranked according to their ability to transform hop compounds[12]. Although the results were not very encouraging, the addition of pure enzymes was also studied, showing a more interesting potential for biotransformation than enzymes secreted naturally by yeast.

Referring to one of his studies, Shellhammer reported that "Free hop aroma is probably about 90% of the total hop flavour in beer and the glycosides are probably just 10%". However, he also mentions that a significant portion of the aroma from hops can be released from these glycosides[15].

More recently, Meiners and Cavanna (2020) also tested the use of commercial enzymes, such as β-glucosidase and β-lyase, in order to boost biotransformation of terpenes and thiols, respectively[20]. The results showed that the use of enzymes presents a real possibility to alter the aroma and/or flavour profile of IPAs, although recipes should be adjusted in regards to hop varieties and amounts.

More research is needed to understand the complexity of the reactions involved when using exogenous enzymes and their impact on the organoleptic profile beer. Besides, it is also important to take into account that many other factors which could play a role, such as the selection of the yeast strain, hop crop year, variety and oil composition, its point of addition, yeast-hop contact time/temperature, among others.

To sum up, the universe of biotransformation is indeed fascinating, and the proper selection of yeast strains, hop varieties and its usage, as well as the addition of exogenous enzymes, brings more opportunities for brewers to explore new ways of achieving great flavour and aroma in beers.

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References and additional reading

1. Bamforth, C. W. (2004). *Beer: Health and Nutrition* (1st Ed.). Wiley-Blackwell Publishing.

2. Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). *Humulus lupulus - A story that begs to be told. A review*. J. Inst. Brew., 120(4), 289-314. doi:10.1002/jib.160

3. Eyres, G., and Dufour, J.-P. (2009). *22 - Hop essential oil: Analysis, chemical composition and odor characteristics*. Editor(s): V. R. Preedy. In *Beer in health and disease prevention* (239-254). Elsevier Inc. doi:10.1016/B978-0-12-373891-2.00022-5

4. Hanke, S. (2009). *Linalool - A key contributor to hop aroma*. MBAA - Global Emerging Issues.

5. Takoi, K. *et al*. (2010). *The contribution of geraniol metabolism to the citrus flavour of beer: synergy of geraniol and β-citronellol under coexistence with excess linalool*. J. Inst. Brew., 116(3), 251-260. doi:10.1002/j.2050-0416.2010.tb00428.x

6. Kollmannsberger, H., Biendl, M., & Nitz, S. (2006). *Occurence of glycosidically bound flavour compounds in hops, hop products and beer*. Monatsschrift fur Brauwissenschaft, 59, 83-89.

7. Barth-Haas (2019). *Hop market update Barth-Haas Group* (Last update Nov. 27th 2019).

8. Lafontaine, S. and & Shellhammer, T. (2019). *How hoppy beer production has redefined hop quality and a discussion of agricultural and processing strategies to promote it.* MBAA TQ, 56(1), 1-12. doi:10.1094/TQ-56-1-0221-01

9. King, A. J., and Dickinson, J. R. (2003). *Biotransformation of hop aroma terpenoids by ale and lager yeasts*. FEMS yeast research, 3, 53-62. doi:10.1016/S1567-1356(02)00141-1

10. Liu, S.-Q. (2015). *Impact of yeast and bacteria on beer appearance and flavour*. Editor(s): A. E. Hill. In *Brewing Microbiology* (357-374). Woodhead Publishing Series in Food Science, Technology and Nutrition. doi:10.1016/B978-1-78242-331-7.00017-4

11. Takoi, K., Koie, K., Itoga, Y., Katayama, Y., Shimase, M., Nakayama, Y., & Watari, J. (2010). *Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer*. Journal of agricultural and food chemistry, 58, 5050-8. doi:10.1021/jf1000524

12. Sharp, D. C., Steensels, J., & Shellhammer, T. H. (2017). *The effect of hopping regime, cultivar and β -glucosidase activity on monoterpene alcohol concentrations in wort and beer*. J. Inst. Brew., 123, 185-191. doi:10.1002/jib.418

13. Opstaele, F., De Rouck, G., Janssens, P., & Montandon, G. (2020). *An exploratory study on the impact of the yeast strain on hop flavour expressions in heavily hopped beers: New England IPA*. BrewingScience. 73, 26-40. doi:10.23763/BRSC20-04OPSTAELE

14. Lallemand (2017). *Best Practices: Biotransformation.* Retrieved from www.lallemandbrewing.com/wp-content/uploads/2017/03/LAL-bestpractices-Biotransformation-digital-1.pdf

15. Bullen, C. (2019, January 2). *An uncharted wilderness - Understanding hop compound biotransformation*. Retrieved June 6, 2020, from *Good Beer Hunting*: www.goodbeerhunting.com/blog/2018/12/19/an-uncharted-wilderness-understanding-hop-compound-biotransformation

16. Hieronymus, S. (2018, Sep 19). *The complex case of thiols*. Retrieved Jun 7, 2020, from *Craft Beer & Brewing* Website: www.beerandbrewing.com/the-complex-case-of-thiols

17. Lallemand (2019). *Sauvy™ - The thiolic yeast.* Internal communication, Lallemand Oenology.

18. Sharp, D., Vollmer, D., Shellhammer, T. (2016). *Recent advances in controlling flavor and aroma in hoppy beers*. Craft Brewers Conference . Philadelphia, Pennsylvania.

19. Stokholm, A. and Shellhammer, T. H. (2020). *Hop Creep - Technical Brief*. Brewers Association. Retrieved from www.brewersassociation.org/educational-publications/hop-creep-technical-brief

20. Meiners, L. and Cavanna, M. (2020). *Using exogenous enzymes to boost biotransformation*. MBAA TQ, 57(1), 33-36. doi:10.1094/TQ-57-1-0330-01.

21. Praet, T., Van Opstaele, F., Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2012). *Biotransformations of hop-derived aroma compounds by Saccharomyces cerevisiae upon fermentation*. Cerevisia, 36(4), 125-132. doi:10.1016/j.cervis.2011.12.005