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A Powerful Analytical Indicator to Drive Varietal Thiols Release in Beers: The “Thiol Potency”

The 3-mercaptohexan-1-ol (3MH), the 3-mercaptohexylacetate (3MHA) and the 4-mercapto-4-methylpentan-2-one (4MMP) have been identified as key aroma compounds in beers due to their appreciated olfactory notes. Hops (*Humulus lupulus*) contains substantial amount of these aroma compounds either as free form or as flavorless precursors. The chemical characterization of hops (thiols and thiol precursors) represents main advantages such as robustness, versatility but also integration in the decision-making procedure for brewers. For this purpose, we developed a Thiol Potency indicator (TP), which is based on chemical data, sensory perceptions and statistical operations. The thiol potency allowed mainly to segment hop aroma quality, choose complementary raw ingredient or drive hopping usages and fit with the needs of craft and industrial breweries. In the future, this indicator could be used as a universal label on hop packaging as aroma quality indicator and extended to other families of aroma compounds to build a standardized aroma quality index.

Descriptors: thiols, hops, hopping usages, thiol precursors, analysis

1 Introduction

Since 2006, polyfunctional thiols have been identified in beers [1] and three thiols were particularly studied due to their pleasant notes: the 3-mercaptohexan-1-ol (3MH), the 3-mercaptohexylacetate (3MHA) and the 4-mercapto-4-methylpentan-2-one (4MMP). They were described as key odorant compounds with concentrations around 250 ng/L for 3MH and from 40 to 129 ng/L for 4MMP in Ale beers [2] (Table 1). Recently, a study on 23 commercial beers (2 Ale, 8 experimental beers, 5 Belgium beers, 5 IPA and 3 Pils) [3] showed a substantial thiol level heterogeneity with no obvious link between beer styles and thiol profiles. Indeed, 3MH ranged from around 60 ng/L to 1200 ng/L whereas the 4MMP was present up to 120 ng/L; which is particularly consequent. Surprisingly, the 3MHA was not systemically present in all the studied beers as for wines.

The thiol biogenesis in beer is not well understood [6] but some investigations showed that:

- Free thiols were identified in hops at ppb levels [7], [8].
- Thiol precursors were present in hops [4], [9], malts and barley [5], [10] at ppm and ppb levels respectively and could be responsible for thiol release.
- The cysteinylated precursor of 3MH released the corresponding thiol either during bottling re-fermentation [11] or through an hypothetical dicarbonyl pathway [12].

The origin of thiols in beers is complex, partially elucidated and depends on several parameters such as the type of raw ingredients (malts, hops especially) and the brewing process (from mashing step to post-fermentation operations). Hops represents a significant source of thiol precursors either as free or bound forms [4]. Up to now, characterization of hops is essentially performed by sensorial analysis but it remains challenging because of:

- the variability of judges;
- the non-detection of flavorless compounds such as thiol precursors;
- the modification of sensory perception due to competitive interactions between aroma compounds such as geraniol which interacts with thiols [13];
- the difficulty to link hop sensory notes with the corresponding hopped-beers.

To be most representative of flavorless compounds, the analytical characterization of hops represents a good indicator for brewers to adapt hopping usages. The aim of our work was to propose a new analytical indicator focused on these three positives thiols and their corresponding precursors called “Thiol Potency (TP)” to match with decision-making procedure of brewing industry.

2 Test and results

2.1. Hop samples

Seventeen hop (*Humulus lupulus*) varieties (pellets 90), bought in local distributor, were analyzed by LC-MS/MS to quantify thiols and thiol precursors according to published methods [4], [14]. Briefly, hop pellets were extracted using an ethanolic mixture (11 % ethanol; 4 g/L of tartaric acid; pH = 3.5) for 1h at room tempera-

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ture, centrifuged and the supernatants were analyzed by LC-MS/MS. Quantification was performed by Stable Isotope Dilution Assay (SIDA) for the different analysis to ensure the best accuracy and reliability.

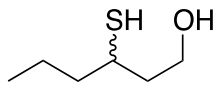
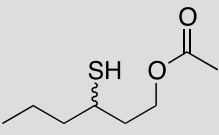
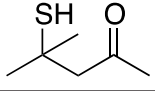
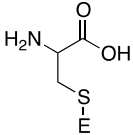
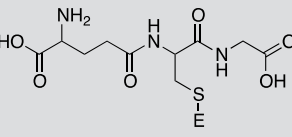
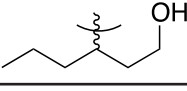
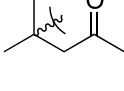
2.2. Analytical characterization of hops

From more than 10 years, lots investigations have been conducted to characterize the amount of free thiols and precursors in hops [2], [4], [7–9]. From sensory point of view, several hop cultivars as Nelson Sauvin and Cascade analyzed by GC-olfactometry, are well known to exhibit rhubarb, blackcurrant bud and grape fruit notes. These odors have been linked with the occurrence of both 4MMP and 3MH in hops [15] already widely used by brewers to impart bitterness and a citrus-like aroma to beer. By comparison with two bitter varieties (Nelson Sauvin and Nugget. These thiols occurred in hops at ppb levels and might be affected by several field treatments like the copper treatments in field [16], storage conditions, the cultivation location and the harvest year [8]. In addition, a recent benchmark of 53 hop cultivars highlighted that 4MMP ranged from <1 to 114 ug/kg, with the highest values reported for US varieties such as Citra, Eureka, Simcoe and Apollo.

Because hop contained both free and bounded form of thiols, it is particularly interesting to quantify all these latter compounds. In table 2, we presented the classical analysis report of 17 hop samples with both concentration of free thiols and the corresponding precursors. These results confirmed that the most important source of 3MH was the G3MH and that 4MMP had two distinct origins as already reported [4]. We already showed that the US hop varieties exhibited higher 4MMP levels than US ones whereas this statement could be moderated for 3MH. In this dataset, there was no clear correlation between free thiols and corresponding thiol precursors ($R^2 = 0.24$) as already reported for grapes [17]. Some tendencies showed that more 3MH there is, more thiol precursors there are. However, it is not possible to conclude about this dataset due to the ignorance of agricultural practices and manufacturing transformation process that could affected thiols in hops. Concerning the 4MMP levels, it is difficult to conclude about any correlation due to the absence of thiol precursors in most of hop samples.

Analytical characterization provides standardized and extremely interesting results for brewers but not helpful for the decision-making procedure because the information remains complicated and unclear. Armed with this observation, we propose to simplify the understanding of chemical data through a novel analytical indicator called the “Thiol Potency” or TP. This index, only based on analytical data and statistical operations, aimed to be global

Table 1 Sensory descriptors and perception thresholds of 3MH, 3MHA and 4MMP in beers and their corresponding precursors in hops and malts.

THIOLS	Chemical structures	Sensory descriptors	Perception Threshold (ng/L)
3MH		Rhubarb, Exotic, Citrus	55
3MHA		Passion fruit	4
4MMP		Blackcurrant bud, box tree, catty	1.5
THIOL PRECURSORS	Chemical structures	Concentration in Hops (ug/kg) [2], [4]	Concentration in Malts (ug/kg) [5]
Cysteinylated precursors		Cys3MH: 129–1140 Cys4MMP: 0–39	Cys3MH: 0–6.8 Cys4MMP: nd
Glutathionylated precursors		G3MH: 1283-19056 G4MMP: 0–3.5	G3MH: 0–698 G4MMP: nd
E=Electrophiles		3MH precursors	
		4MMP precursors	

and adapted to the decision-making procedure of craft and industrial breweries.

2.3 The “thiol potency” as a novel indicator

In a first step, we decided to focus our innovative analytical concept especially on 3 thiols (3MH, 3MHA and 4MMP) with aim to be generalized to other aroma compounds in further investigations as a standardized analytical aroma index. For the moment, the thiol potency considered only all known thiol sources and no other aroma compounds susceptible to exhaust the sensory perception, to be more standardized and robust.

To be the most integrative as possible, the “thiol potency” is based on:

- Absolute concentrations of each target compounds obtained by Stable Isotope Dilution Assay to be the most reliable as possible.
- Perception thresholds of each aroma compounds in beer matrix.
- Basic statistical operations.

We proposed to evaluate the thiol potency for 1kg of hops and 1 hL of beer to be standardized. This indicator can be calculated for both free and bound forms but also with a most global approach as follows:

$$\text{Free 3MH potency} = \left(\frac{[3MH + 3MHA]}{\text{Perception threshold}_{3MH}} \right) \times MW_{3MH}$$

$$\text{Bound 3MH potency} = \left(\frac{[Cys3MH + G3MH]}{\text{Perception threshold}_{3MH}} \right) \times MW_{3MH}$$

$$\text{3MH potency} = \left(\frac{[3MH + 3MHA] + [Cys3MH + G3MH]}{\text{Perception threshold}_{3MH}} \right) \times MW_{3MH}$$

with:

- [3MH] and [3MHA]: the concentration of 3MH and 3MHA expressed in µM/kg.
- Perception threshold of 3MH in beer: 55 ng/L [18].
- MW_{3MH}: the molecular weight of 3MH which is equal to 134 g/mol.

The fraction numerator represents either the free or the bound 3MH sources that are weighted by the 3MH perception threshold in beer. Consequently, the thiol potency was expressed as Odour Activity Value (AOV). In addition, the free/bound ratio is a powerful indicator to help brewers in hopping usages and especially for the 4MMP.

The same equations could be used to evaluate the 4MMP potency as follows:

$$\text{Free 4MMP potency} = \left(\frac{[4MMP]}{\text{Perception threshold}_{4MMP}} \right) \times MW_{4MMP}$$

$$\text{Bound 4MMP potency} = \left(\frac{[Cys4MMP + G4MMP]}{\text{Perception threshold}_{4MMP}} \right) \times MW_{4MMP}$$

$$\text{4MMP potency} = \left(\frac{[4MMP] + [Cys4MMP + G4MMP]}{\text{Perception threshold}_{4MMP}} \right) \times MW_{4MMP}$$

with:

- [4MMP]: the concentration of 4MMP expressed in µM/kg.
- Perception threshold of 4MMP in beer: 1.5 ng/L [8].
- MW_{4MMP}: the molecular weight of 3MH which is equal to 132 g/mol.

Finally, the sum of both 3MH and 4MMP potencies lead us to calculate an integrative thiol potency as follows:

$$\text{Thiol potency} = \left(\frac{[3MH + 3MHA] + [Cys3MH + G3MH]}{\text{Perception threshold}_{3MH}} \times MW_{3MH} \right) + \left(\frac{[4MMP] + [Cys4MMP + G4MMP]}{\text{Perception threshold}_{4MMP}} \times MW_{4MMP} \right)$$

The use of thiol potency index simplifies the analysis report considerably (Table 3) for brewers and becomes most adapted to the decision-making procedure of craft and industrial breweries. Indeed, a simple look at the Table 3 showed that 3MH came essentially from thiol precursors whereas the 4MMP could have distinct origins.

The thiol potency indicator represents several technical advantages but also opens avenue for integrated innovations in brewing field in the years ahead such as:

- Standardized analytical index (for 1 kg of hops and 1 hL of wort/beer).
- Easily understandable and updatable (in case of new thiol precursor identified for instance).
- Could be referenced on each hop package (but also malts) as for % of alpha acids.
- Could be related to a recommended hopping usage.
- Could be linked to a specific yeast strains able to release thiols.

Table 2 Analysis report of 17 hop samples (nd: not detected)

Hop cultivars	Thiols (ug/kg)			Thiol Precursors (ug/kg)			
	3MH	3MHA	4MMP	Cys-3MH	G3MH	Cys-4MMP	G4MMP
Apollo	11.1	nd	7.5	382	7340	0.02	0.06
Bravo	20.1	nd	0.5	240	5901	0.02	0.03
Calypso	15.5	nd	0.1	1905	14421	0.03	0.03
Cascade	10.5	2.8	2.4	456	13498	nd	nd
Citra 1	24.2	nd	28.4	394	4821	0.03	0.03
Citra 2	16.0	4.7	43.5	376	5209	nd	nd
Eureka	11.0	nd	17.5	326	7595	0.04	0.06
Hallertau Cascade	15.8	6.0	1.0	142	3418	nd	nd
Hallertau Hallertauer Tradition	2.3	1.5	0.2	444	10637	nd	nd
Hallertau Herkules	6.3	2.3	0.3	0	5993	nd	nd
Hallertau Nugget	nd	4.4	0.6	333	6252	nd	nd
Hallertau Perle	2.1	1.5	0.6	455	15467	nd	nd
LES-Nugget	2.2	3.4	0.3	488	8753	nd	nd
Saaz 1	4.7	1.5	0.5	431	9184	nd	nd
Saaz 2	2.3	0.9	0.3	532	20678	nd	nd
Saaz 3	4.4	1.0	0.2	890	19890	nd	nd
Simcoe	22.5	nd	13.5	646	8981	0.03	0.01

2.4 What is the added value of the “thiol potency”?

2.4.1 For hop manufacturers

Hops is a powerful source of flavors due to substantial amount of aroma compounds in lupulin glands. For 10 years, interest in positive odorant thiols is still growing but there are few data in literature dealing with thiol precursors such as cysteinylated and glutathionylated conjugates (Table 1). Indeed, the cysteinylated precursors of 3MH was identified for the time in Cascade hops in 2012 [2]. More recently, we identified and quantified both cysteinylated and glutathionylated precursors of 3MH and 4MMP in several hop varieties [4] (harvests 2014-2015) at substantial amounts around mg/L.

For 1 kg of brewing raw ingredients, hops represent the most important possible source of thiols under either precursor form for 3MH or free thiols for 4MMP [19]. The use of thiol potency allowed the hop manufacturer to:

- Segment their products from aromatic potential point of view.
- Open the avenue for specific blending hops.
- Valorise hop by-products.
- Track the hop quality from year-to-year.

The thiol potency can be differently exploited according to user specific needs: either with analytical or hops as descriptors. In this context, analytical data are obviously the best descriptors for hop producers to rank their products (Figure 1). For this purpose, it is necessary to normalize the data to obtain a visible chart just with a simple look. In our study, Citra and Eureka contained the most important sources of 4MMP either as free thiol or as thiol precursors that could be globalized under the 4MMP potency indicator. By contrast, 3MH is present at substantial amounts in Cascade, Hallertau Cascade, Hallertau Perle, Saaz, Citra and Calypso hop varieties.

In the future, the thiol potency will be extended to:

- several types of brewing raw ingredients (malts, cereals but also other food matrices).
- other molecules to generate a standardized aroma quality index which will be able to aggregate different families of aroma compound.

Table 3 Repartition of free and bound thiols on the 17 studied hop samples. The 3MH and 4MMP potencies are expressed as Odour Activity Values (OAV) for 1kg of hop and 1 hL of wort/beer

Hop cultivars	% free 3MH	% bound 3MH	3MH POTENCY (OAV)	% free 4MMP	% bound 4MMP	4MMP Potency (OAV)
Apollo	0	100	48	40	60	12
Bravo	1	99	38	7	93	4
Calypso	0	100	108	1	99	5
Cascade	0	100	86	100	0	2
Citra 1	1	99	34	80	20	24
Citra 2	1	99	36	100	0	29
Eureka	0	100	49	57	43	20
Hallertau Cascade	2	98	22	100	0	1
Hallertau Hallertauer Tradition	0	100	69	100	0	0
Hallertau Herkules	0	100	36	100	0	0
Hallertau Nugget	0	100	41	100	0	0
Hallertau Perle	0	100	98	100	0	0
Nugget	0	100	58	100	0	0
Saaz 1	0	100	60	100	0	0
Saaz 2	0	100	130	100	0	0
Saaz 3	0	100	129	100	0	0
Simcoe	1	99	61	68	32	13

2.4.2 For brewers

According to the literature, 3MH in beer is mainly originating from thiol precursors contained in both hops and malt. For hops, more

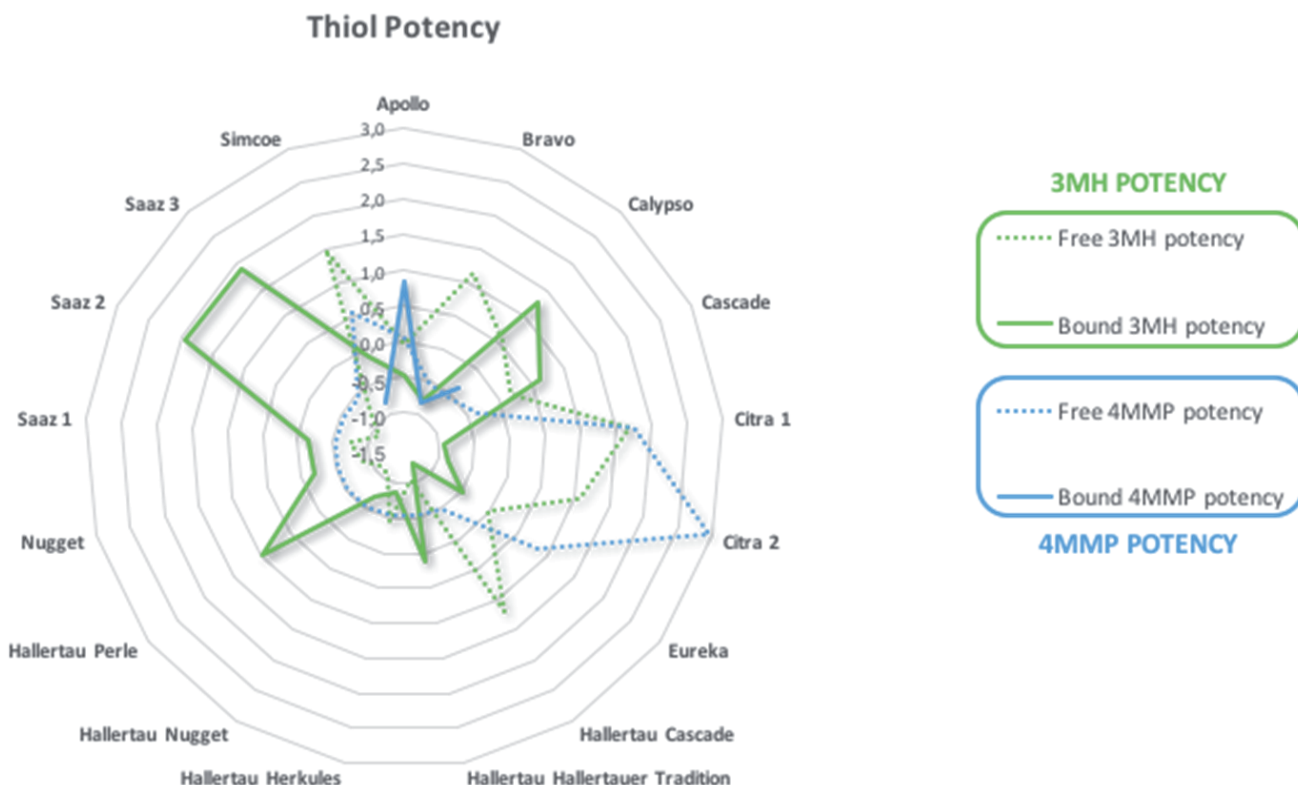


Fig. 1 Characterization of 17 hop cultivars using thiol potency as aromatic quality parameter (Data were normalized)

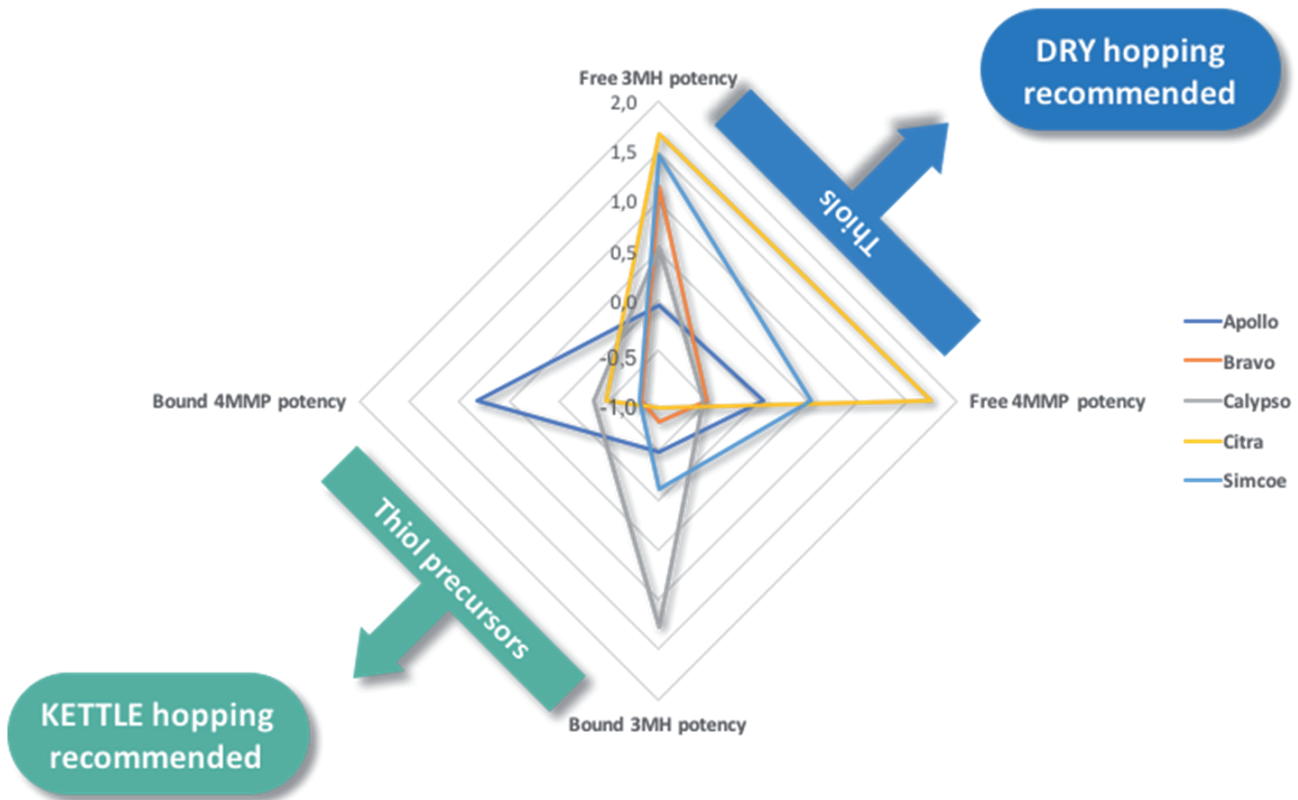


Figure 2: The thiol potency as a smart tool to recommend hopping usages and much more in future (Data were normalized)

than 99% of 3MH is present as thiol precursors; highlighting the importance of kettle hopping to cleave these precursors during fermentation. The 4MMP has two distinct origins in hops: free form mainly in lupulin glands [8] and thiol precursors [4]. According to the hop variety, the 4MMP/4MMP precursors ratios could be highly modified and hopping usages also.

For brewers, the thiol potency should be particularly efficient to:

- drive hopping usages according to targeted beer thiol profile (Kettle Vs. Dry Hopping as shown in Figure 2). Indeed, Calypso is particularly rich in 3MH precursors and more adapted for a

kettle hopping to cleave precursors during fermentation. By contrast, Eureka should be most suited for dry hopping because of its substantial amounts of both 4MMP and 3MH.

- choose complementary raw ingredients (hops vs. malts): Malts contained only 3MH precursors at ug/L range and represent a potential non-negligible source of 3MH. The systematic characterization of both malts and hops could help brewers to adjust their brewing conditions (hopping usages, type of yeast).

2.4.3 For the brewing industry

To fit with worldwide brewing industry, the thiol potency indicator

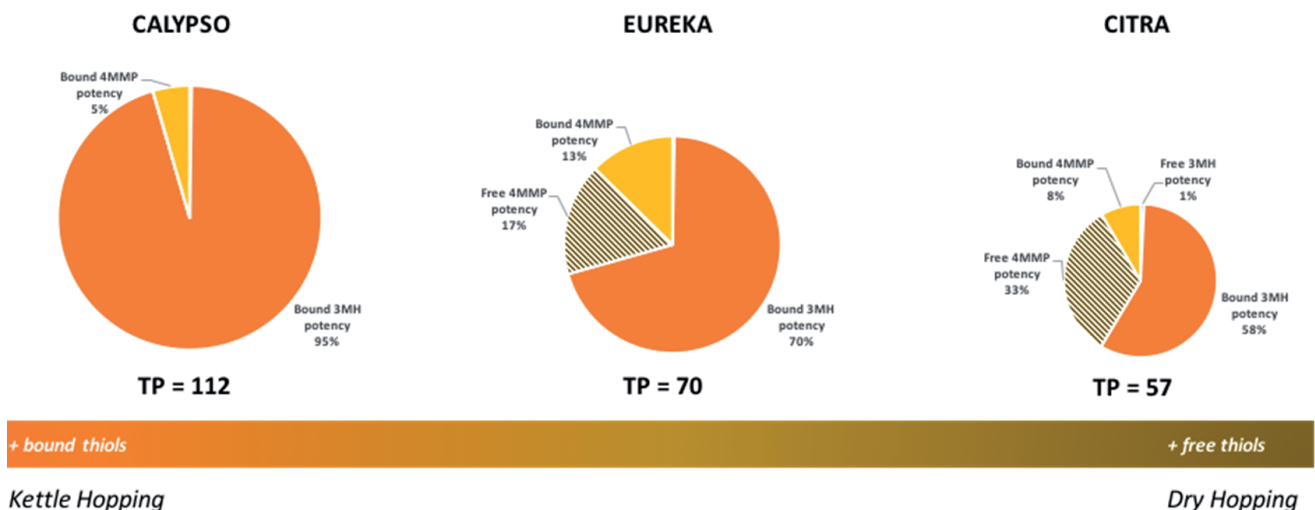


Figure 3: Thiol potency (expressed as OAV) as a universal quality label that could be put on each hop packaging

aimed to clearly inform users about raw ingredient aroma quality.

We can imagine that thiol potency indicator could be referenced on hop package as a quality label (Figure 3). In a quick look, it could be easy to discriminate hop cultivars according to their thiol sources and so, to adapt hopping usages. Indeed, Calypso is particularly recommended for kettle hopping due to the large amount of thiol precursors and traces of free thiols. In contrast, Eureka contained substantial amounts of free thiols that could be efficiently transferred to beer during dry-hopping. At last, Citra is the most versatile hop that can be used during kettle hopping as well as dry-hopping due to similar amounts of thiol and thiol precursors.

The progressive use of thiol potency to characterize hop quality should help the people working in the brewing field to optimize their processes.

3 Conclusions

The chemical characterization of hops is vital to obtain robust and standardized data from year to year. Today, the analysis report does not fit with brewing industry needs due to the result complexity. To have operational data, we developed an innovative analytical index focused on varietal thiol sources that we called the "thiol potency". This indicator, only based on analytical data, is mainly adapted for hop producers to segment their products but also for brewers to drive hopping usages.

In further work, the thiol potency will be extended to other aroma compound families contained in brewing raw ingredients (hops, malts, spices) to have a standardized aroma quality index, most adapted to the specific needs of brewers. That is why we need the feedback of people working in the brewing world to customize and upgrade our analytical indicator as best as possible.

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