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Yeast Strains and Hop Varieties Synergy on Beer Volatile Compounds

The volatile compounds produced in beers are complex and influenced by numerous factors like barley, brewing and fermentation process, hop variety and yeast strain. In this study, hop variety and yeast strain have been chosen to evaluate their influences on the volatile compound profile in beer. Malt wort was hopped with 4 different varieties of French aromatic hops and fermented with 3 different yeast strains. Volatile compounds were analysed by Stir Bar Sorptive Extraction-Gas-Chromatography (SBSE-GC-MS). Statistical analysis (ANOVA) were performed to test the influence of yeast strain and hop variety on the concentration of each of the measured compounds. Among the 39 volatile compounds detected, 9 of them were influenced only by hop variety, 2 by yeast only and the 28 remaining by both hop and yeast together. As expected, terpenes concentration in beer is directly linked to the choice of hop variety used, but it appears the yeast strains can also influence that parameter. Surprisingly, hops variety influenced the final concentration of esters, highlighting that an interaction between hops compounds and yeast metabolism remains to be investigated.

Descriptors: hop varieties, yeast strains, volatile compounds

1 Introduction

The final taste of beer is a sum of the various aromas provided by raw materials during the different steps of the beer production process. The choice of yeast strain and hop variety are often considered as determinants of the beers aromatic profile.

Among all the volatile compounds involved in the quality of beer, yeast is involved in the production of some important flavour-active compounds like isoamyl acetate, 2-phenylethanol, 4-vinylguaïacol or ethyl hexanoate as reviewed by *Carrau* et al. [4].The final concentration of these compounds is yeast-strain dependant [20, 39], diversity of brewer's yeast helps brewers to drive the aroma of beer.

In addition to yeast strain, hop variety is the other factor that brewers can also choose to strongly affect the aroma of beer [19]. Most of the flavour active-compounds brought by aromatic hops are monoterpenes, sesquiterpenes, esters and alcohols [29, 40]. Currently, only monoterpenes, especially geraniol, nerol and citronellol, are known to be influenced by yeast metabolism during fermentation [21], while sesquiterpenols, esters and alcohols are only suspected to be bioconverted by yeast.

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To investigate the influence of hop varieties and yeast strains on the volatile content of beer, four hop varieties from Cophoudal/Comptoir Agricole and two different brewer's yeast strains from the CLIB (Institut Pasteur/INRA collection) and one from the University of California, Davis (UCD) have been used to produce beer in triplicate. Following this, the beers were analysed by SBSE-GC-MS to determine their final volatile composition and to evaluate the influence of the hop variety and the yeast strain, but also the combination of both.

2 Materials and methods

2.1 Hop assays

Four French aromatic hop varieties harvested in 2013 were used to produce hopped wort, namely Strisselspalt, Aramis, Bouclier and Triskel (Cophoudal/Comptoir Agricole Hochfelden, France). Table 1 shows the linalool and α-acids composition of each hop variety.

Twenty litres of wort were brewed for each hop variety with malt extract (Bières du monde, Lille, France) and distilled water, heated to 100 °C, to obtain wort at 13 °Plato with a Braumeister 50 L (Speidel, Ofterdingen, Germany). Hop (2,1 g/L) was added in a tea bag (10 min, 100 °C). After tea bag removal, wort was quickly cooled down to 20 °C and let at room temperature during the preparation of the fermentation.

Table 1 Hop varieties composition

Compounds	Hop varieties			
	Strisselspalt	Aramis	Bouclier	Triskel
Linalool	7–8 mg/g	14 mg/g	6 mg/g	10 mg/g
α-acids	3.4 %	8.1 %	9.5 %	8.7 %

Table 2 **Results of the two ways Anova for the two factors Hop and Yeast and their possible interactions**

Compounds	Hop	Yeast	Yeast/hop
Acetate Esters			
Hexyl acetate	0.000	0.000	0.014
2-Phenethyl acetate	0.026	0.000	0.009
Isoamyl acetate	0.020	0.000	0.005
Medium Chain fatty acids			
Octanoic acid	0.000	0.000	0.038
Decanoic acid	0.000	0.000	0.000
Dodecanoic acid	0.000	0.000	0.000
Alcohols			
2-Hexanol	0.000	0.000	0.000
2-undecanol	0.000	0.000	0.000
3-methyl-2-Buten-1-ol	0.000	0.055	0.168
2-Nonanol	0.000	0.880	1.000
Esters			
2-Phenylethyl butanoate	0.000	0.000	0.000
2-Phenethyl hexanoate	0.050	0.000	0.016
Isoamyl propanoate	0.000	0.001	0.000
Methyl 4-Methyl-2-hexenoate	0.000	0.031	0.000
Methyl geranate	0.000	0.043	0.739
isobutyl butanoate	0.000	0.643	0.954
Ethyl hexanoate	0.000	0.000	0.000
Ethyl octanoate	0.000	0.000	0.000
Ethyl decanoate	0.000	0.000	0.000
Ethyl dodecanoate	0.000	0.000	0.000
Higher Alcohols			
2-Phenyl Ethanol	0.000	0.000	0.000
Isoamyl alcool	0.489	0.000	0.575
Monoterpenes			
2,7-Dimethyl-2,7-octadiene	0.000	0.060	0.417
Citronellol	0.000	0.000	0.001
Nerol	0.000	0.000	0.000
Linalool	0.000	0.086	0.021
Geraniol	0.000	0.000	0.000
Sesquiterpenols			
7-epi-amiteol	0.000	0.000	0.000
a/t-Cadinol	0.000	0.000	0.000
Hop ether	0.000	0.090	0.017
Juniper Camphor	0.000	0.447	0.438
β-Eudesmol	0.000	0.008	0.049
α-Eudesmol	0.000	0.010	0.055
Caryophyllene oxide	0.000	0.837	0.601
Terpenyl acetates			
Citronellol acetate	0.000	0.040	0.050
Geranyl acetate	0.000	0.575	0.217
Neryl acetate	0.000	0.000	0.000
Phenols			
4-Vinylguaiacol	0.000	0.000	0.000
Styrene	0.831	0.000	0.121
Other			
2,3-dihydro-benzofuran	0.041	0.000	0.014

2.2 Yeast assays

Three yeast strains were chosen to ferment the wort, one *S. cerevisiae* ale strain UCD915, and two lagers CLIB267 and CLIB279, kindly provided by *Jean-Luc Legras* from the INRA Colmar collection.

Yeast pre-cultures were performed on YPD medium (Yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L) at 28 °C for 24 h in 125 mL flasks under shaking. Beer wort were inoculated at initial OD_{600nm} = 0.1. Fermentation steps were performed at 15 °C in 1 L autoclaved vessels equipped with airlocks to maintain anaerobiosis, with constant stirring (250 rpm). Vessels were weighed twice daily for fermentation monitoring, and fermentation was stopped when the daily loss reached less than 1 % of the expected total loss. Each of the 4 different worts were fermented with each yeast strain in triplicate, to a total of 36 different beer combinations.

2.3 Volatile analysis

Analysis of the volatiles compounds of the 36 beers was performed using the Stir Bar Sorptive Extraction method-Liquid Desorption Gas Chromatography [6] (SBSE-LD-GC-MS). Each beer was analysed in duplicate. All reagents used were analytical grade. Stir Bars (length = 20 mm) were coated with 47 µL of polydimethylsiloxane (Twister; Gerstel, Mülheim a. d. Ruhr, Germany). The GC-MS analyses were performed with an Agilent 6890N gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5975B inert Mass Spectrometer Detector (Agilent Technologies). The gas chromatograph was fitted with a DB-Wax capillary column (60 m × 0.32 mm i.d. × 0.50 µm film thickness, J&W Scientific) with helium carrier gas (1 mL/min, constant flow). Instrument control and data processing were performed with Agilent MSD ChemStation software (G1701DA, Rev D.03.00). The mass spectra were compared with the Wiley's library reference spectral bank, Retention Index (RI) and standard when available. All compounds were semi-quantified using the ratio of their Total Ion Current peak to that of the 3-octanol (final concentration of 84 µg/L).

2.4 Statistical analyses

Statistical analyses were performed on Minitab 17.0 using Anova.

3 Results and discussion

The 36 beers produced have been analysed to investigate the influence of hop varieties, yeast strains and their potential interactions on 39 volatile compounds (Table 2).

Anova statistical analysis indicated that among the identified compounds, 9 were influenced by hop only, 2 by yeast only the 28 remaining were both influenced by hop and yeast. Most of the compounds influenced by the synergistic effect of yeast and hop (interaction yeast/hop in Table 2) are also influenced by hop and yeast separately. This is why these interaction are not discussed in this study.

A schematic representation of the conclusion of this study is presented in figure 1.

3.1 Impact of hop variety on beer volatile profile

The 9 compounds influenced by the hop variety only include three monoterpenes: linalool (1), geranyl acetate (2), 2,7 dimethyl 2,6 octadiene (3); three sesquiterpenols: hop ether (4), caryophyllenoxyde (5) and juniper camphor (6); one ester: isobutyl isobutanoate (7) and two alcohols: 2-nonanol (8) and 3-methyl-2-Buten-1-ol (9). Among all of the compounds influenced by hop variety, linalool (1) is a key contributor to hop aroma in beer [16]. Its perception threshold in beer is relatively low (27–100 µg/L [7]) and its descriptors are floral, citrus and flower. Previous studies showed that yeast was able to metabolize geraniol, citronellol and nerol but not linalool [22]. As expected, in our conditions, the concentration of this compound in beer depended only on the hop variety used. Among the four hops tested, the highest concentration of linalool was obtained with Aramis (≈ 500 µg/L rel 3-octanol, Fig. 2).

Geranyl acetate (2) is described as floral and reported to be involved in the hoppy aroma. However, as reported by *Lermusieau* et al. [26] its impact on beer aroma is still unclear. Only Aramis and Triskel hops bring geranyl acetate to beer whatever the yeast strain used (Fig. 1). This compound could be the result of the esterification of geraniol to geranyl acetate by yeast during fermentation [41] but could also come directly from hop [28, 37, 38]. Our conditions are not adapted for origin determination of this compound and need further investigation that are not the scope of this work.

Bouclier and Aramis show the highest concentration of 2,7 dimethyl 2,6 octadiene (3). This compound has already been reported in beer [1, 46] and in

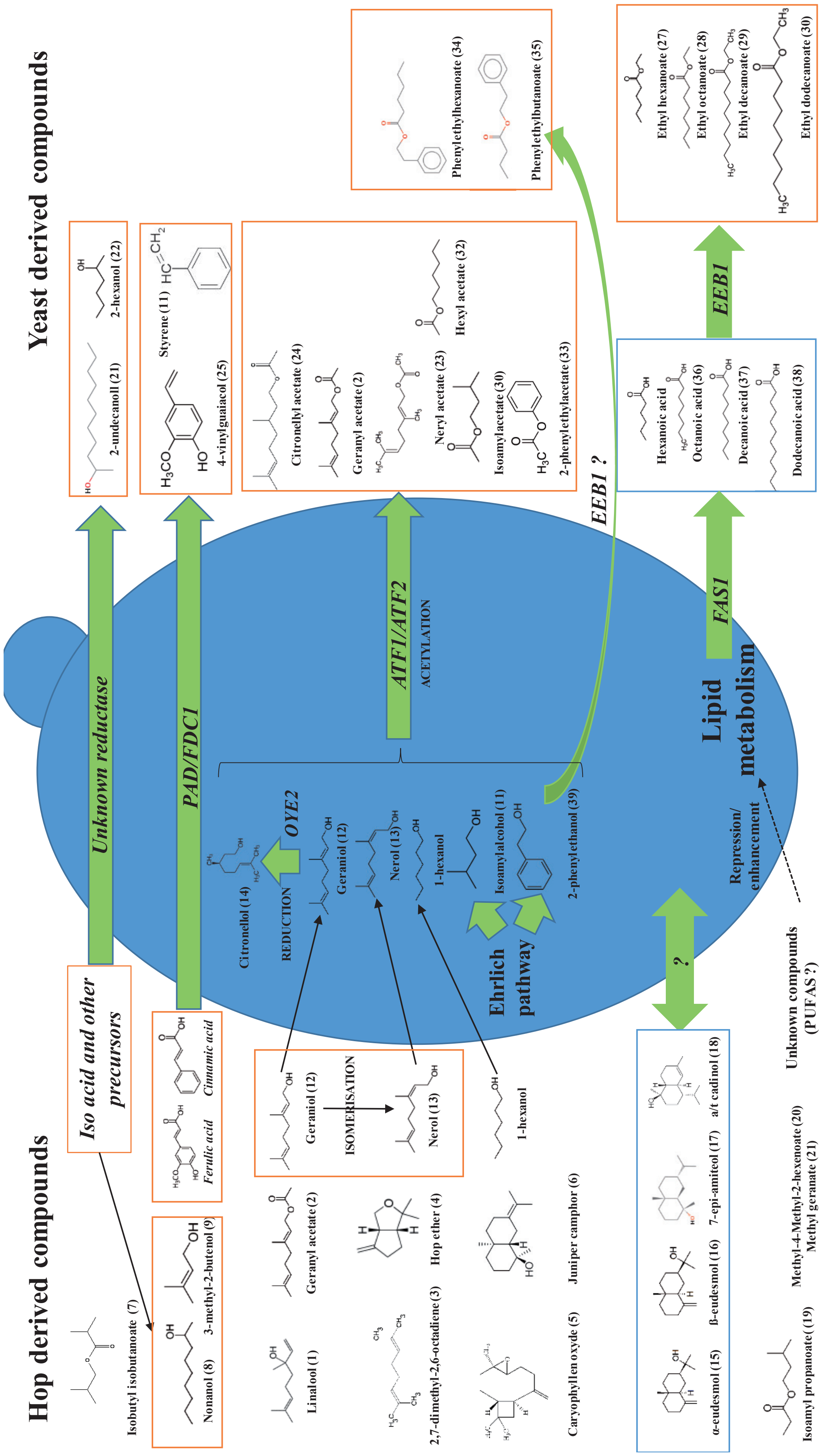

Fig. 1 **Schematic representation of the interaction between hop and yeast volatile compounds during the fermentation of beer (green arrows: yeast enzymatic reactions)**

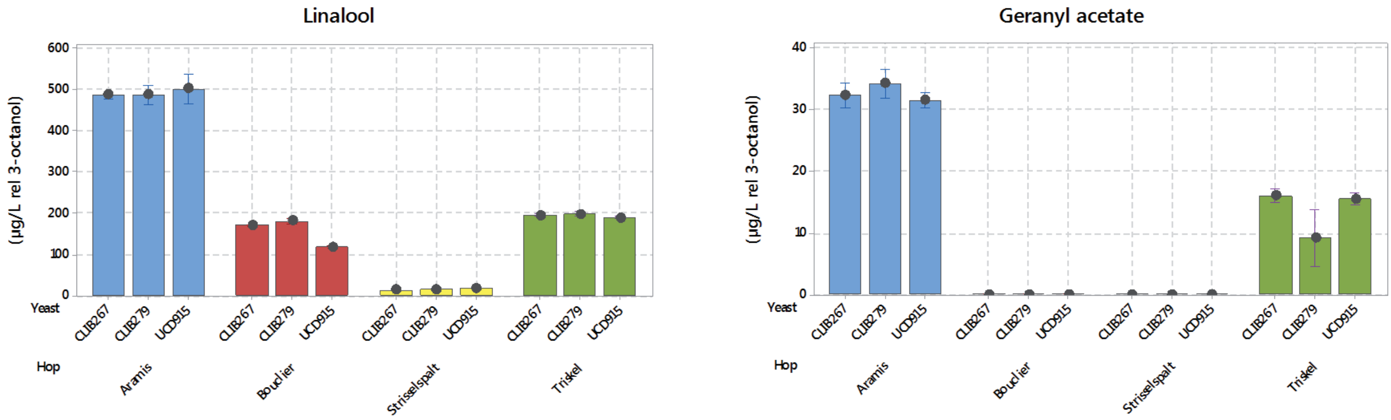

Fig. 2 **Graphical representation of the concentration of linalool and geranyl acetate (µg/L rel 3-octanol) in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915**

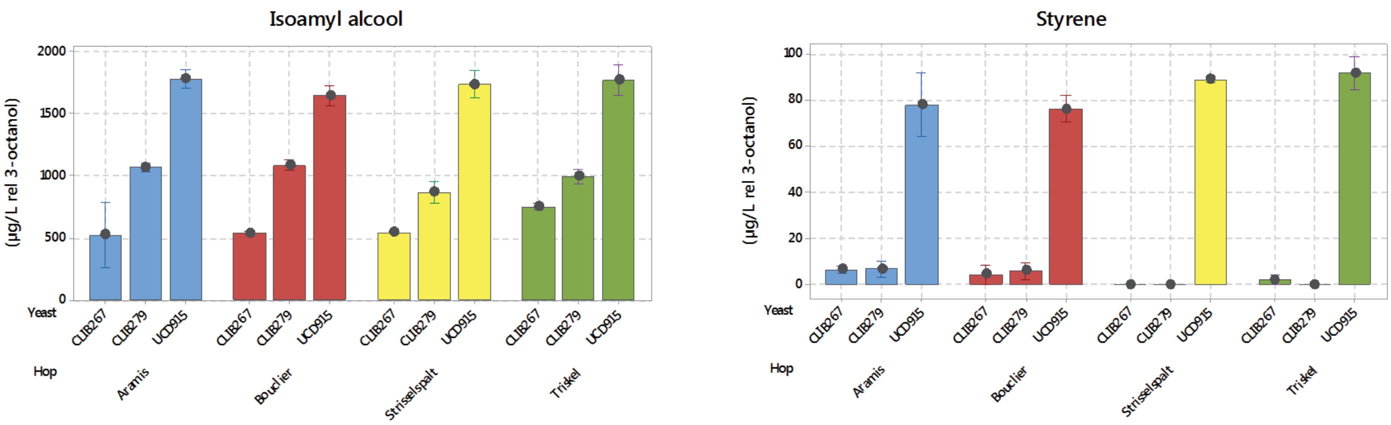

Fig. 3 **Graphical representation of the concentration of Isoamyl alcohol and Styrene (µg/L rel 3-octanol) in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915**

hop [27], but to our knowledge, there is no data about its influence on the aroma of beer.

Aramis appears to be the highest producer of hop ether (4) in our conditions. This aroma-active compound founds in hop oil in previous study [44]. Its odour impact on beer has been evaluated by *Lam* et al. [25] who determined that this compound was not a major contributor to hop aroma. Its influence on beer remains to be elucidated.

Caryophyllen oxyde (5) brought by hop [28, 45] was known to contribute to the spicy aroma of beer [11] and was involved in the "Noble" character of hop varieties. This compound can be the result from the oxidation or hydrolysis of β-caryophyllen during the boiling step [15]. Aramis and Bouclier bring the highest concentration of this compound in beer in this study (Table 3 and Table 4).

Juniper camphor (6) has been previously reported in beer produced with Aramis and Strisselspalt [40] and was reported to be present in hop oil [2,9]. Its influence in beer aroma is not yet defined. This compound is only detected in beer hopped with Aramis (Table 3 and Table 4).

Isobutyl isobutanoate (7) has been reported in hop oil [12,31] and beer [2]. It displays a relatively low threshold perception at 36 µg/L [3] and with a positive description for the aroma of beer, ie pineapple. Aramis is the highest producer of this compound during our experiments.

2-nonanol (8) and 3-methyl-2-buten-1-ol (9) are 2-alkanols which have been reported in hop oil [10,37] and beer [2]. According to Hashimoto [17,18], they are the results of degradation of iso-α-acids of hop during the oxidation of beer. This reaction leads also to the formation of 2-alkanone which could be further reduced to 2-alkanols by yeast [47]. Their respective influences on the final aroma of beer are not known. Aramis is the highest producer of these compounds even if its α-acids is not the highest.

3.2 Impact of yeast strain on beer volatile profile

The two volatile compounds only influenced by yeast strains are isoamyl alcohol (10) and styrene (11) (Table 2). Isoamyl alcohol (10) concentration is statistically equivalent in beer whatever the hop variety chosen but its concentration is different depending on the yeast used in fermentation. As a by-product of the metabolism of yeast during fermentation (Fig.1) this compound is known to be dependent on the yeast strain [2,20,39]. Lermusieau et al have shown that unhopped and hopped beer (with Saaz and Challenger) have the same factor of dilution (linked to the concentration of the compounds) for isoamyl alcohol in GC-O experiment [26].

Table 3 **Concentration (µg/L relative to 3-octanol) of volatile compounds in beer hopped with Strisselspalt and Bouclier and fermented with CLIB 267, CLIB279 and UCD915**

Hop varieties		Strisselspalt						Bouclier					
Yeast strains		**CLIB267**		**CLIB279**		**UCD915**		**CLIB267**		**CLIB279**		**UCD915**	
Compounds	**N°**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**
Acetate Esters													
Hexyl acetate[1,2]	32	2.4	29%	3.5	25%	8.4	29%	12.7	5%	19.2	19%	15.5	14%
2-Phenethyl acetate[1,2]	33	784.8	9%	744.9	26%	2115.4	38%	938.4	6%	1248.6	5%	1624.0	4%
Isoamyl acetate[1,2]	31	194.3	11%	605.3	30%	1810.9	44%	699.3	10%	3150.4	9%	2901.4	5%
Medium Chain fatty acids													
Octanoic acid[1,2]	36	205.3	18%	362.6	35%	1169.7	48%	807.7	40%	1811.4	17%	2066.5	14%
Decanoic acid[1,2]	37	1258.9	9%	1411.1	34%	3713.3	38%	1559.9	4%	3141.5	4%	8366.5	3%
Dodecanoic acid[1,2]	38	0.0		53.4	183%	4.9	67%	252.0	162%	2657.4	6%	1284.3	10%
Alcohols													
2-Hexanol[1,2]	22	0.0		0.0		34.0	70%	16.0	4%	4.7	173%	0.0	
2-undecanol[1,2]	21	0.0		0.0		0.0		19.8	3%	13.9	13%	8.5	8%
3-methyl-2-Buten-1-ol[1]	9	0.0		0.0		0.0		3.3	87%	0.0		4.6	18%
2-Nonanol[1]	8	0.0		0.0		0.0		7.7	87%	3.3	173%	7.2	87%
Esters													
2-Phenylethyl butanoate[1,2]	34	16.0	35%	3.8	13%	6.5	40%	98.7	6%	23.6	3%	16.4	9%
2-Phenethyl hexanoate[1,2]	35	837.7	21%	119.4	12%	0.0		1434.8	9%	75.7	13%	41.6	9%
Isoamyl propanoate[1,2]	19	0.9	35%	1.5	14%	0.0		4.5	87%	5.2	95%	5.6	4%
Methyl 4-Methyl-2-hexenoate[1,2]	20	5.5	16%	6.3	5%	9.0	18%	91.0	7%	95.7	7%	78.9	5%
Methyl geranate[1,2]	21	0.0		0.0		0.0		13.9	15%	16.2	19%	14.4	11%
isobutyl butanoate[1]	7	0.0		0.0		0.0		3.8	89%	4.7	15%	4.6	15%
Ethyl hexanoate[1,2]	27	119.8	13%	99.6	9%	697.8	64%	603.4	5%	463.0	7%	1060.8	6%
Ethyl octanoate[1,2]	28	112.1	15%	196.1	21%	549.4	46%	570.7	2%	941.0	7%	856.7	5%
Ethyl decanoate[1,2]	29	31.0	35%	38.1	37%	157.3	51%	146.1	3%	316.2	8%	455.6	6%
Ethyl dodecanoate[1,2]	30	3.4	29%	9.6	69%	20.8	43%	39.6	10%	140.2	10%	82.0	7%
Higher Alcohols													
2-Phenyl Ethanol[1,2]	39	254.4	19%	207.8	41%	491.4	42%	939.1	5%	863.4	18%	730.3	20%
Isoamyl alcool[2]	10	547.3	6%	868.9	18%	1735.9	37%	540.2	3%	1085.0	7%	1647.8	9%
Monoterpenes													
2,7-Dimethyl-2,7-octadiene[1]	3	6.1	87%	13.0	36%	0.0		84.0	49%	42.5	8%	38.1	28%
Citronellol[1,2]	14	0.0		0.0		0.0		23.9	8%	18.8	6%	11.7	173%
Nerol[1,2]	13	0.0		0.0		0.0		0.0		0.0		1.2	173%
Linalool[1]	1	39.9	4%	43.4	12%	43.6	7%	154.3	3%	147.1	3%	150.7	4%
Geraniol[1,2]	12	4.8	21%	4.2	14%	5.3	7%	12.0	1%	7.7	4%	11.5	14%
Sesquiterpenols													
7-epi-amiteol[1,2]	17	0.0		0.0		0.0		0.0		0.0		0.0	
a/t-Cadinol[1,2]	18	0.0		0.0		0.0		0.0		28.4	16%	0.0	
Hop ether[1,2]	4	0.0		0.0		0.0		4.6	173%	13.8	8%	9.1	87%
Juniper Camphor[1]	6	0.0		0.0		0.0		0.0		0.0		0.0	
β-Eudesmol[1,2]	15	38.5	11%	32.6	22%	0.0		9.9	173%	0.0		0.0	
α-Eudesmol[1,2]	16	35.7	5%	34.3	24%	0.0		0.0		0.0		0.0	
Caryophyllene oxide[1]	5	19.3	3%	21.0	20%	18.6	26%	69.5	19%	63.7	41%	54.4	15%
Terpenyl acetates													
Citronellol acetate[1,2]	24	0.0		0.0		0.0		15.7	9%	15.1	10%	14.5	16%
Geranyl acetate[1]	2	0.0		0.0		0.0		17.2	14%	15.5	3%	10.4	5%
Neryl acetate[1,2]	23	0.0		0.0		0.0		10.3	5%	17.22	15%	15.46	3%
Phenols													
4-Vinylguaiacol[1,2]	25	19.6	9%	15.2	13%	573.0	67%	17.9	14%	15.1	9%	411.1	10%
Styrene[2]	11	0.0		0.0		88.9	70%	4.3	173%	6.0	104%	76.0	13%
Other													
2,3-dihydro-benzofuran[1,2]	26	0.0		0.0		88.8	86%	0.0		0.0		19.8	87%

[1] compounds influenced by hop variety (p-value < 0.05); [2] compounds influenced by yeast (p-value < 0.05)

Table 4 **Concentration (µg/L relative to 3-octanol) of volatile compounds in beer hopped with Triskel and Aramis and fermented with CLIB267, CLIB279 and UCD915**

Hop varieties		Triskel						Aramis					
Yeast strains		**CLIB267**		**CLIB279**		**UCD915**		**CLIB267**		**CLIB279**		**UCD915**	
Compounds	**N°**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**	**Mean**	**Err**
Acetate Esters													
Hexyl acetate[1,2]	32	16.0	11%	19.9	27%	30.4	5%	16.1	9%	26.2	19%	34.3	28%
2-Phenethyl acetate[1,2]	33	1221.1	14%	953.2	26%	2291.9	19%	988.6	17%	891.8	20%	1839.5	17%
Isoamyl acetate[1,2]	31	1132.5	13%	2790.3	28%	5920.2	9%	829.4	13%	2860.3	13%	5766.6	5%
Medium Chain fatty acids													
Octanoic acid[1,2]	36	1623.0	16%	2247.6	19%	4124.5	2%	1244.8	3%	2088.0	9%	3997.6	7%
Decanoic acid[1,2]	37	1670.5	26%	1892.2	36%	8788.1	11%	638.8	3%	2409.0	17%	9507.9	17%
Dodecanoic acid[1,2]	38	0	0	735.8	82%	2455.9	8%	109.0	39%	1460.0	51%	2333.1	8%
Alcohols													
2-Hexanol[1,2]	22	0.0		0.0		1.3	173%	9.5	3%	10.3	15%	11.2	18%
2-undecanol[1,2]	21	16.5	21%	10.8	8%	13.9	19%	67.4	5%	43.6	23%	63.1	35%
3-methyl-2-Buten-1-ol[1]	9	7.1	46%	6.0	25%	4.2	92%	9.8	18%	4.4	87%	12.1	45%
2-Nonanol[1]	8	6.9	12%	5.3	22%	6.4	16%	32.7	87%	32.5	7%	35.9	8%
Esters													
2-Phenylethyl butanoate[1,2]	34	103.2	32%	20.6	26%	24.9	24%	167.5	10%	23.1	12%	23.2	12%
2-Phenethyl hexanoate[1,2]	35	1244.4	28%	74.5	22%	65.7	23%	1301.1	19%	70.6	22%	0.0	
Isoamyl propanoate[1,2]	19	10.8	10%	14.0	42%	20.2	14%	33.1	7%	29.4	1%	35.1	10%
Methyl 4-Methyl-2-hexenoate[1,2]	20	51.7	8%	48.7	14%	59.3	4%	128.6	1%	129.3	3%	153.2	1%
Methyl geranate[1,2]	21	35.2	23%	32.7	2%	39.4	2%	75.7	7%	77.2	12%	88.9	13%
isobutyl butanoate[1]	7	6.9	88%	6.1	87%	5.0	87%	22.8	7%	21.7	6%	25.4	4%
Ethyl hexanoate[1,2]	27	744.5	14%	571.9	11%	1999.8	18%	758.3	7%	548.2	9%	1249.8	10%
Ethyl octanoate[1,2]	28	797.7	18%	985.9	21%	1891.1	8%	438.6	14%	830.0	5%	1389.9	6%
Ethyl decanoate[1,2]	29	182.3	29%	195.1	28%	812.5	12%	46.3	15%	159.5	34%	703.1	18%
Ethyl dodecanoate[1,2]	30	26.3	35%	56.8	60%	129.2	15%	12.4	9%	58.8	66%	108.8	7%
Higher Alcohols													
2-Phenyl Ethanol[1,2]	38	1516.3	9%	862.4	10%	1103.9	18%	1962.5	17%	961.4	15%	1458.3	18%
Isoamyl alcool[2]	10	751.3	6%	994.5	10%	1771.7	12%	525.0	87%	1070.4	5%	1796.5	4%
Monoterpenes													
2,7-Dimethyl-2,7-octadiene[1]	3	35.6	50%	29.8	55%	28.5	37%	56.0	7%	49.0	88%	18.0	94%
Citronellol[1,2]	14	35.5	18%	0.0		0.0		52.5	10%	41.4	19%	55.3	13%
Nerol[1,2]	13	0.0		0.0		0.0		8.0	24%	7.1	23%	10.2	20%
Linalool[1]	1	149.3	2%	146.1	2%	168.9	1%	342.2	6%	343.2	5%	352.3	5%
Geraniol[1,2]	12	12.0	3%	7.6	13%	10.1	18%	31.9	2%	19.2	2%	31.6	2%
Sesquiterpenols													
7-epi-amiteol[1,2]	17	105.2	6%	71.7	87%	0.0		108.8	13%	117.3	11%	28.7	173%
a/t-Cadinol[1,2]	18	0.0		17.0	11%	13.2	16%	0.0		84.0	13%	44.6	90%
Hop ether[1]	4	11.3	3%	11.6	17%	14.5	10%	23.2	9%	23.7	7%	23.5	10%
Juniper Camphor[1]	6	0.0		0.0		0.0		126.0	16%	129.3	12%	157.7	44%
β-Eudesmol[1,2]	15	0.0		0.0		0.0		163.8	14%	177.7	12%	151.2	15%
α-Eudesmol[1,2]	16	11.7	37%	11.6	26%	8.9	5%	168.5	15%	188.2	13%	161.1	16%
Caryophyllene oxide[1]	5	53.0	8%	47.3	7%	57.5	13%	63.1	15%	72.0	22%	73.8	27%
Terpenyl acetates													
Citronellol acetate[1,2]	24	8.6	87%	11.1	19%	19.0	8%	15.7	24%	23.9	26%	23.0	19%
Geranyl acetate[1]	2	16.1	12%	9.2	88%	15.5	11%	32.4	11%	34.2	12%	32.6	7%
Neryl acetate[1,2]	23	0.0		0.0		0.0		0.0		0.0		0.0	
Phenols													
4-Vinylguaiacol[1,2]	25	31.3	39%	21.0	7%	573.2	7%	27.6	10%	18.9	20%	486.6	5%
Styrene[2]	11	2.2	173%	0.0		91.6	13%	6.4	41%	6.7	88%	78.7	18%
Other													
2,3-dihydro-benzofuran[1,2]	26	0.0		0.0		94.8	32%	0.0		0.0		90.1	98%

[1] compounds influenced by hop variety (p-value < 0.05); [2] compounds influenced by yeast (p-value < 0.05)

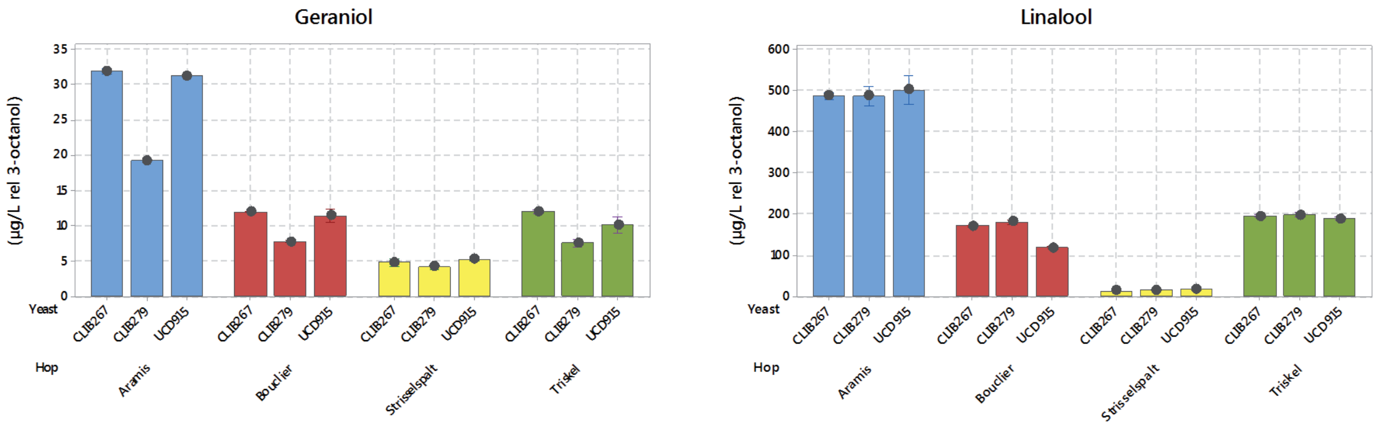

Fig. 4 Graphical representation of the concentration of geraniol and linalool (µg/L rel 3-octanol) in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915

This observation confirms that isoamyl alcohol brought by hop in beer is negligible compared to those produced by yeast. In our condition, UCD915 reaches the highest concentration of isoamyl alcohol (≈1700 µg/L rel 3-octanol, Fig. 3).

According to *Schwarz* et al. [35], styrene (11) is a sweet-smelling fluid considered to be toxic for humans. In our conditions, beers produced by the yeast strain UCD915 contained high concentration of styrene (60 to 105 µg/L rel 3-octanol) whereas beers produced by the two other yeast strains present a lower concentration for this compound (0 to 8 µg/L rel 3-octanol). Styrene is produced by the same pathway than 4-vinylguaiacol (4VG) (25), by transformation of cinnamic and ferulic acids into styrene and 4VG respectively (Fig.1). Two processes for their production have been described in beer [36]: during wort boiling by thermal decarboxylation and during fermentation by enzymatic process if the yeast strain are "*POF+*" (Phenol Off-Flavor, Fig.1). The genes *PAD1* and *FDC1* of yeast *Saccharomyces cerevisiae* are involved in this process. Yeast strains that are able to decarboxylate cinnamic and ferulic acids are *POF+*. The styrene concentration that we observed strongly suggests that the UCD915 yeast strain is *POF+* (Fig. 3) and would be in adequation with observations with Weissbier strains [14].

3.3 Impact of hop variety and yeast strain on beer volatile profile

Among 39 compounds measured in this experiment, 28 are influenced by both the hop varieties and yeast strains. Fifteen of these compounds are known to be hop-derived compounds, 3 monoterpenes, *ie* geraniol (12), nerol (13), citronellol (14); 4 sesquiterpenols *ie* α-eudesmol (15), β-eudesmol (16), 7 epi amiteol (17), a/t cadinol (18), 3 esters, *ie* isoamylpropanoate (19), Methyl-4-methyl-2-hexenoate (20), methyl geranate (21), 2 alcohols, *ie* 2-undecanol (22), 2-hexanol (23), 2 terpenyl acetates, *ie* neryl acetate (24), citronellyl acetate (25) and one various compounds 2,3 dihydrobenzofuran (26). Thirteen of these compounds are yeast-derived compounds esters, *ie* ethyl hexanoate (27); ethyl octanoate (28); ethyl decanoate (29); ethyl dodecanoate (30); isoamylacetate (31); hexyl acetate (32); 2-phenethyl- acetate (33); 2-phenylethyl butanoate (34); 2-phenethyl hexanoate (35); three medium chain fatty acids : octanoic (36), decanoic (37) and dodecanoic acids (38), and 2-phenylethanol (39).

3.3.1 Hop-derived compounds

3.3.1.1 Monoterpenes

Geraniol (12) is an odour-active compound in beer [24] and is known to be hop variety dependent [11, 13, 19, 23, 24, 42, 43]. Yeast is able to transform geraniol into citronellol (14) [21, 41, 43] and to acetylate geraniol into geranyl acetate [41], but also other geraniol-derived compounds like nerol (13) and citronellol (14). We show here that hop and yeast together influenced the final composition of the beer. The maximum concentration which can be obtained is with the combination Aramis and CLIB267 (30 µg/L) (Fig.4).

3.3.1.2 Sesquiterpenols

Sesquiterpenols concentration in beer is linked to the hop variety [40], however their influences on beer aroma are poorly understood.

We describe here that α- and β-eudesmol (16-17), 7 epi amiteol (18), a/t cadinol (19) concentration (Table 1) are influenced by yeast. Beer fermented with ale yeast UCD915 is the less concentrated in sesquiterpenols, (Table 2 and Table 3). Our experiments also confirm that Aramis and Strisselspalt lead to higher sesquiterpenols production than Triskel or Bouclier [40] (Table 3 and Table 4).

3.3.1.3 Esters

Isoamylpropanoate (19), methyl-4-methyl-2-hexenoate (20) and methyl geranate (21) have been reported in hop oil [2, 28, 29, 48] but their influences on beer aroma remain unclear. In our conditions there is a slight influence of yeast strain on both compounds while a strong influence of hop variety (Fig. 5). Surprisingly, isoamyl propanoate is not detected in beer produced with the yeast UCD915 using Aramis. Aramis can be considered as the highest producer of these three esters in beer (Table 1 and Fig. 5).

3.3.1.4 Alcohols

As previously stated, 2-hexanol (22) and 2-undecanol (21) could be considered as the result of yeast-mediated 2-alkones, derived from iso-α acid from hop, reduction. It is not clear why and how 2-nonanol is influenced by yeast strain while 2-hexanol and 2-un-

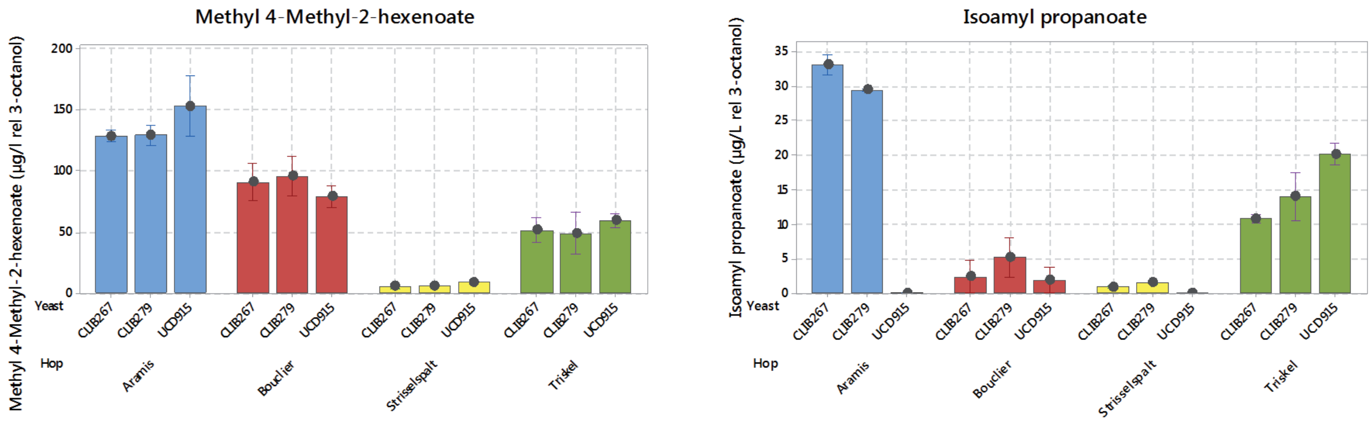

Fig. 5 **Graphical representation of the concentration of methyl-4-methyl-2-hexenoate and isoamylpropanoate (µg/L rel 3-octanol) in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915**

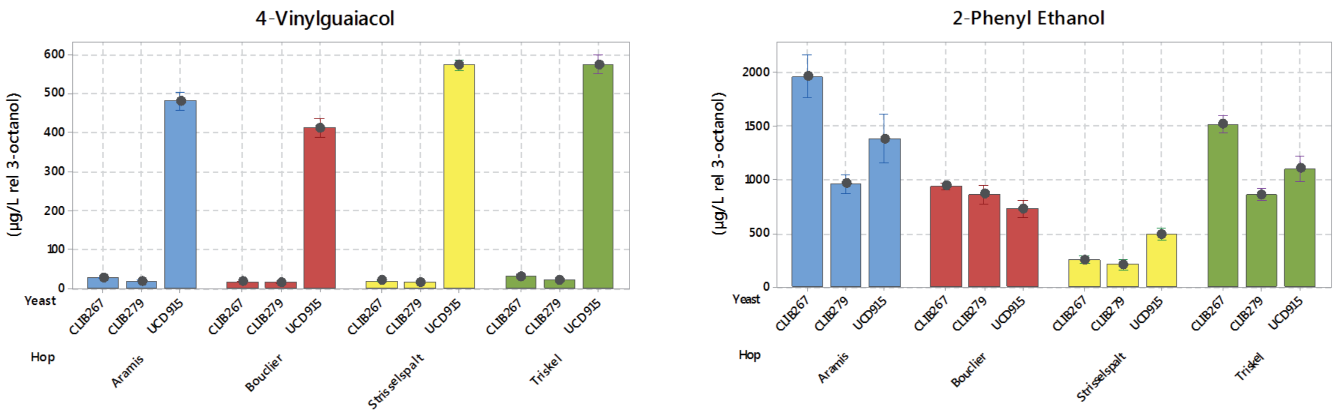

Fig. 6 **Graphical representation of the concentration of 4-vinylguaïacol (µg/L rel 3-octanol) and 2-phenylethanol in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915**

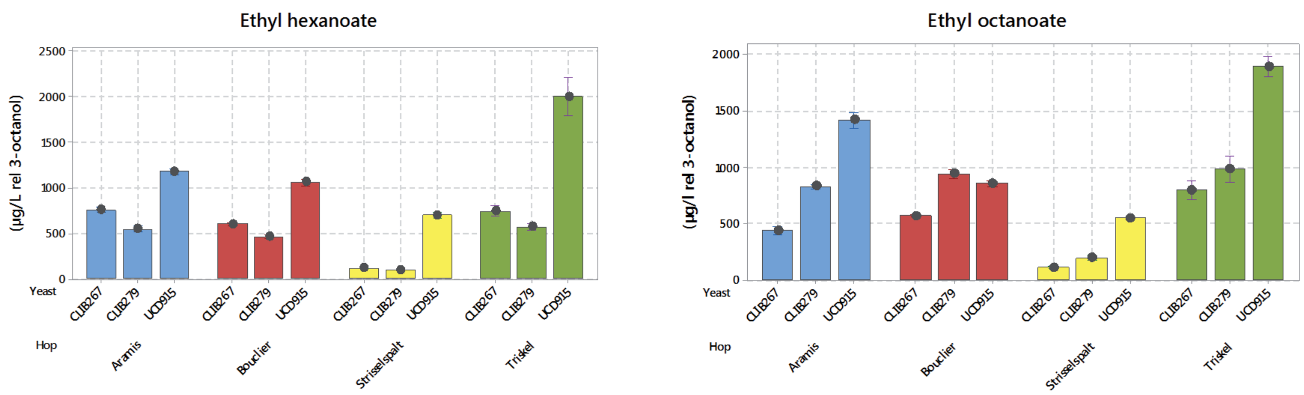

Fig. 7 **Graphical representation of the concentration of ethyl hexanoate and ethyl octanoate (µg/L rel 3-octanol) in beers produced with Aramis, Bouclier, Strisselspalt and Triskel as aromatic hop and fermented with yeast CLIB267, CLIB279 and UCD915**

decanol are (Table 4). To our knowledge there is no data on the influence of these compounds on the aromatic profile of beer. Both of these compounds are influenced by hop variety and yeast strain (Table 4).

2-phenylethanol (39), a higher alcohol and flavour active compound in beer, is known to be influenced by yeast and hop variety. Since it is an Erhlich pathway by-product (Fig. 1), its concentration is linked to the yeast strain. Besides, it has been shown by *Gros* et al. [26] that this compound can also be brought in beer by hop. In our case, Aramis and Triskel wort fermented with CLIB267 appears to be the combination bringing the highest concentration of 2-phenylethanol.

3.3.1.5 Various compounds

4 vinylguaiacol (4VG) (26) is a volatile compound with typical phenolic and clove aroma and is often found in Weissbier. This

compound is the result of the decarboxylation of ferulic acid by the enzymatic machinery of yeast (Fig.1). The yeast UCD915 is *POF+* and this hypothesis was confirmed by the high concentration of 4VG produced by this yeast in our conditions. Ferulic acid is a precursor of this compound and is provided by malt and hop. The malt used in our experiments remained unchanged in all 36 hop/yeast combinations. We conclude that the small variations of 4VG observed are linked to ferulic acid content of the hops. In our conditions, Triskel and Strisselspalt give the highest concentration of 4VG in beer (Fig.6).

3.3.2 Compounds deriving from yeast metabolism

Ethyl hexanoate (27); ethyl octanoate (28); ethyl dodecanoate (29); ethyl decanoate (30); isoamylacetate (31); hexyl acetate (32); 2-phenethylacetate (33); 2-phenylethylbutanoate (34); 2-phenethylhexanoate (35) are volatile compounds derived from yeast metabolism during fermentation. Most of them being flavour active, they could then contribute to the overall flavour of beer [31].

Surprisingly, production of these yeast derived compounds are influenced by the hop variety. Ethyl esters and acetate esters are significantly higher when Triskel hop was used (Fig.7). Neither of these esters (compounds 28–36) nor the precursors of these esters (1-hexanol, hexanoic, octanoic (36), decanoic (37), dodecanoic acids (38), 2-phenylethanol (39)) are known to be present in hop [5], or at best at very small concentrations (data not shown). However, it is well known that these compounds are produced by yeast during fermentation [34, 49] and are tightly linked to yeast genes involved in their production [20, 39]. As expected UCD915 (ale), produces high amount of acetate esters and ethyl esters compared to CLIB267 and 279 (lager yeasts) [33]. The same trends were also observed for medium chain fatty acids (compounds 36–38).

It has been shown that synthesis of esters can be repressed by presence of unsaturated fatty acids during fermentation [30, 32]. Interestingly, presence of unsaturated fatty acids have been reported in hop by *Demireva* et al. [8]. We propose that hop varieties contain different concentrations of these compounds which could influence in an indirect manner the production of esters by yeast. However there is a lack of data on the variability of these kind of compounds in hop. This hypothesis needs to be further investigated by measuring these unsaturated fatty acids in hops.

4 Conclusion

Hop varieties and yeast strains are often considered as key elements to distinguish beers. On one side, hop varieties are known to influence the concentration of various volatile compounds like monoterpenes and sesquiterpenols bringing floral and wood notes to the beer. On the other side, yeasts are known to be responsible for the productions of various esters giving fruity notes to the beer.

Some publications reported the influence of yeasts on specific volatile compounds brought by hop in beer [22] but none of them have evaluated the influence of different yeast strains combined with the use of different hop varieties on the volatile content of beer.

In this study, we evaluated the influence of yeast on the volatile compounds brought by hop but also to study the influence of hop on the volatile compounds produced by yeast.

We confirm here that some hop volatile compounds like linalool, geranylacetate, 2,7 dimethyl 2,7 octadiene, hop ether and juniper camphor are not metabolized by yeast during the fermentation. Nonetheless, some of them, including terpenols and sesquiterpenols, are influenced by yeast strains, for instance geraniol, neryl acetate, citronellol, b-eudesmol, 7 epi amiteol, a/t cadinol, isoamylpropanoate, methyl-4-methyl-2-hexanoate, 2,3 dihydrobenzofuran, 2-undecanol, 2-hexanol and 4 vinylguaiacol.

Through different enzymes, yeasts are able to acetylate (*ATF1/ATF2*), reduce (*OYE2*) or decarboxylate (*PAD*) most of the volatiles or precursors of the volatiles found in this study. Mutation in enzyme gene(s) involved in this pathway is one way of explaining variability in yeast strains impact on volatile compounds. In other cases, like for 4VG, it could also depend on the concentration of the precursor provided, which is, in this case, hop-dependent.

Among all the volatile compounds produced by yeast, it has been shown in this study that compounds like ethyl esters, acetate esters and medium chain fatty acids are dependent on the yeast strain but also on the hop variety used. This interaction between hop variety and yeast strain could lead to better control the production of yeast-derived compounds in beer.

Hop can be used to monitor volatile compounds, for example, here we show that Aramis can be used to bring monoterpenol (floral) and sesquiterpenol (woody, spice), whereas Triskel and Strisselspalt increase the final concentration of 4-vinylguaiacol (clove) when the appropriate yeast strain is used, finally we demonstrate here that Bouclier can bring neryl acetate (floral) in to the beer.

We propose here that a separate view of yeast and hop input for beer flavouring might be too restrictive. The intricacy of yeast/hop combination allows for a more complex and subtle control of the beer produced and should be assessed for an advanced understanding of beer design.

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