Barbe Rouge: impact of hop and analytical properties of

THE HOPPING METHOD MATTERS | Along with yeast, hops provide some of the key volatile compounds involved in beer typicality. This is the reason why the choice of hop variety has a major influence on beer aroma profile. As hopping methods also play an important role, this study was aimed at identifying the impact of three hopping methods, using the Alsatian Barbe Rouge hops, on the sensory and analytical properties of beers. When dry hopping beers, the level of odour-active compounds was higher than that of the other ones. Five compounds linked to red fruit and strawberry-like aromas were identified: ethyl isobutanoate, ethyl butanoate, ethyl 2-methylbutanoate, 3-methylbutyl-2-methylpropanoate and 2-phenethyl hexanoate.

HOP-DERIVED COMPOUNDS such as α-humulene (sesquiterpenes) or geraniol and linalool (monoterpenes) are known to be involved in the aroma of beer [1]. In a previous study, we were able to demonstrate that beer produced by means of dry hopping, using the Barbe Rouge hop variety, contained high concentrations of α-humulene (balsamic, wood), β-caryophyllene (clove, black pepper) and linalool (floral) and also a typical composition of esters [2]. Some of these esters, i.e. ethyl isobutanoate and ethyl 2-methyl butanoate, are reported to impart strawberry and exotic fruit aromas to beer [2] and are supposed to be responsible for the typical aroma of Barbe Rouge.

Aroma hops can be added at different stages of beer production. The most common processes are: "late hopping" (addition of hops during the latter part of the boil), "whirlpool" (addition of hops during the last whirlpool stage) and "dry hopping" (addition of hops after primary fermentation). In view of its great typicity, Barbe Rouge was chosen for evaluating the influence of the hopping method on the aroma profile of beer, especially on the compounds involved in strawberry and red fruit notes. In this context, beers supplied by Comptoir Agricole (Hochfelden, France) and produced with Barbe Rouge and each of these three hopping methods (table 1) were evaluated after Stir Bar Sorptive Extraction (SBSE), using metabolic profiling by means of Gas Chromatography–Mass Spectrometry (GC-MS) and olfactometry by means of GC-Olfactometry (GC-O) (fig. 1). Aromagrams of the 3 beers were prepared, using Nasal Impact Frequency (NIF) on the GC-O dataset, with 5 to 6 panellists per beer.

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Metabolic profiling of beers using GC-MS

A total of 208 volatile compounds were identified in late hopped, whirlpool and dry hopped beers. Of these compounds, concentrations of 179 compounds differed significantly in the three beers (ANOVA; p-value <0.05). Statistical analyses showed that late hopped and whirlpool beers had a greater similarity (only 82/179 compounds are significantly different in these two beers) than that of late hopped and dry hopped beers (166/179 compounds) and whirlpool and dry hopped beers (156/179 compounds) (fig. 2).

For this study, the total dataset was prepared in summary form using the sum of the

CHARACTERISTICS OF HOPPING METHODS (ALL BEERS: 100 % PILSNER AND 5 % ALCOHOL)

Hopping method	Hop quantities and International Bitterness Units (IBU)
Late hopping	495 g/hl (5 min before end of boiling) 45 IBU
Whirlpool	495 g/hl 45 IBU
Dry hopping	800 g/hl 45 IBU (iso-extract adjustment)

Table 1

ping method on sensory beers

compounds based on chemical family (table 2). As expected, dry hopping enhanced the positive compounds of the beer bouquet, resulting from the highest concentration of esters and terpenes [3] (table 2). Whirlpool and dry hopping methods led to evaporation and chemical or enzymatic transformation of these volatile compounds [4]. Interestingly, late hopped and whirlpool beers contained more medium-chain fatty acids (MCFA), such as hexanoic, octanoic and decanoic acids, than dry hopped beers. These compounds are associated with off-flavours but also contribute to the "bouquet" of the beer. MCFA's are formed by yeast during fermentation [5]. In whirlpool and late hopped beers, hop-derived compounds are present before fermentation whereas these compounds are present after fermentation in dry hopped beers. These results confirm that hop-derived compounds have an influence on formation of MCFA during fermentation, as has been reported previously [6].

Olfactometry analysis and correlation with volatile compounds analysis

The three beers were investigated using GC-O analyses to evaluate the impact of hopping methods on odour-active compounds and to identify compounds involved in red fruit aromas imparted by Barbe Rouge. More than 70 odour-active compounds were identified and described by panellists. However, only the impressions determined by at least 50 % of panellists were retained to draw the aromagram (NIF > 50 %) (fig. 3). A total of 19, 27 and 38 odour-active compounds respectively were found in late hopped, whirlpool and dry hopped beers and identified using their mass spectra, retention indices and odour properties according to the literature. Among these odour-active compounds, some result from

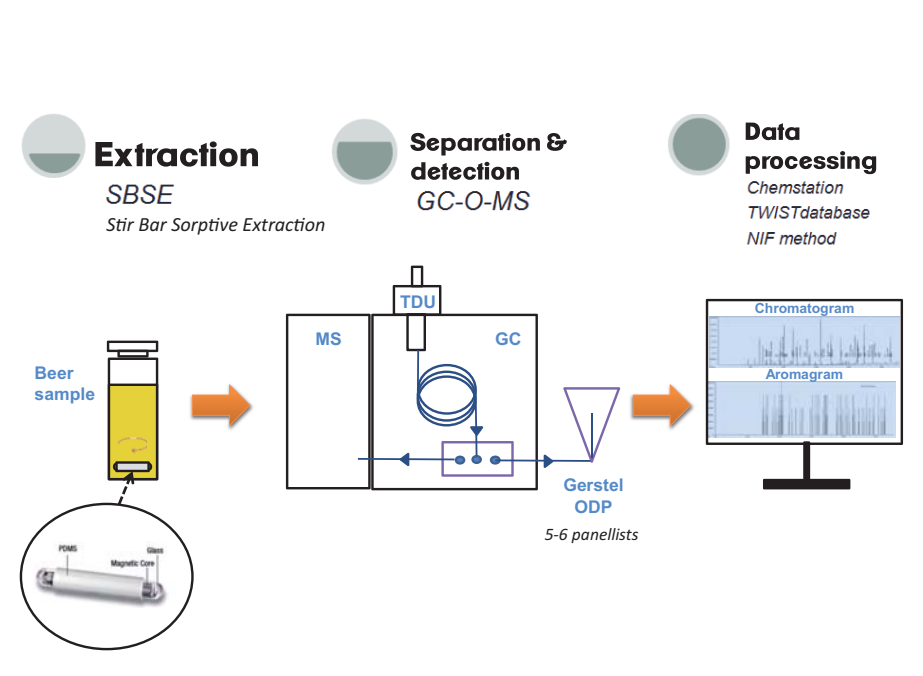

Fig. 1 Analytical protocol of the three beers (TDU:Thermal Desorption Unit; ODP: Olfactory Detection Port; NIF: Nasal Impact Frequency)

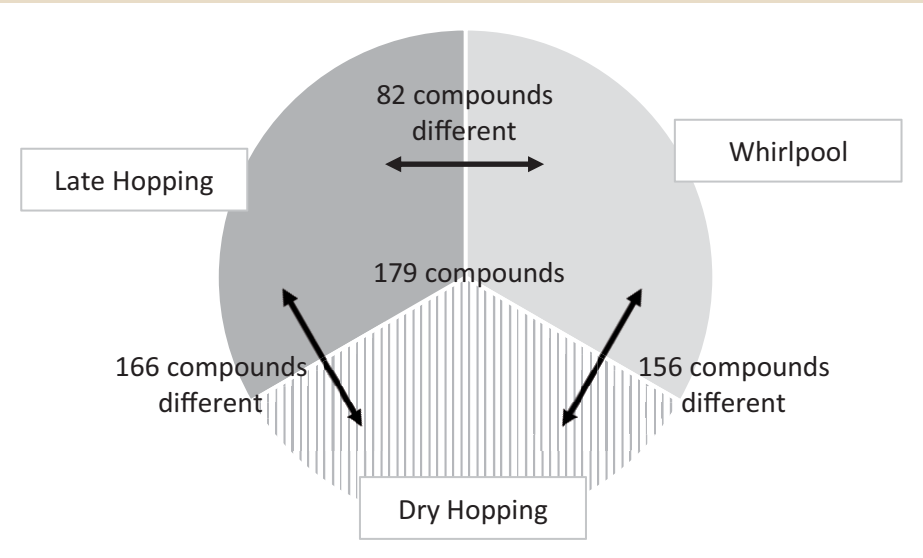

Fig. 2 Comparison of concentrations of volatile compounds in the beers according to hopping method

VOLATILE COMPOUND CONCENTRATION OF THE THREE BEERS BASED ON CHEMICAL FAMILY (µG/L REL 3-OCTANOL) (LH: LATE HOPPING, W: WHIRLPOOL, DH: DRY HOPPING)

	Late hopping		Whirlpool		Dry hopping		Student test		
	Mean	SD	Mean	SD	Mean	SD	DH vs W	DH vs LH	W vs LH
Acids	1555.4	61.1	1637.0	74.3	1121.9	50.7	***	***	
Alcohols	2677.8	82.5	2680.9	41.4	3431.6	50.5	***	***	
Aldehydes	10.2	4.8	9.1	2.2	8.4	3.2			
Esters	9093.7	146.4	9630.7	519.1	11762	59.7	***	***	
Lactones	7	1.3	3.9	3.4	0	0		***	
Norisoprenoids	4.8	0.5	5	0.2	5	0.3			
Phenols	39.6	1.4	46.7	2.7	45.1	19			***
Pyridine	2.3	0.5	1	1.2	1.9	1.3			
Pyrrole	2.1	0.1	1.4	1.2	2.7	0.2			
Terpenes	330.5	9.2	404.0	13.3	846.4	47	***	***	***
Thiol	26.4	5.8	13.9	2.4	19.9	4.8			***
Total	13751.3	155.8	14433.6	581.2	17251.4	159.6	***	***	

****: p-value <0.05*

Table 2

fermentation such as isoamylacetate and 2-phenylethylacetate, malt (beer ageing) such as β-damascenone [7], others are breakdown products of hop α- and β-acids such as 3-methyl butanoic acid [8] or are typically found in hops such as β-myrcene, linalool, citronellol and geraniol.

Fruit aromas, in particular red-fruit and strawberry-like aromas attributed to esters, were also determined by half of the panellists (NIF > 50 %) and tentatively identified as ethyl isobutanoate (7.48 min; A), ethyl butanoate (8.62 min, B), ethyl 2-methylbutanoate (8.91 min; C), 3-methylbutyl-2-methylpropanoate (11.37 min; D) and 2-phenethylhexanoate(25.44min;E)(table 3, fig. 3). Compounds A, B and C are known to be a key aroma substance of strawberry [9-11], implying the fact that these compounds are involved in the typicality of the Barbe Rouge variety. These five compounds only co-existed in the dry hopped beer with a relatively high NIF (> 66 % except for compound E) (table 3). It might thus be inferred that this is the most appropriate method for revealing all red-fruit aromas derived from Barbe Rouge. However, the whirlpool beer was described as having a higher NIF (NIF = 80, 60 and 80 % for compounds A, B and C respectively) (table 3), highlighting that the whirlpool can also be used to enhance the strawberry aroma when adding Barbe Rouge. Late hopped beer was described as having a low NIF (60 %) only in relation to compounds D and E, implying that this method may not be the most appropriate for Barbe Rouge.

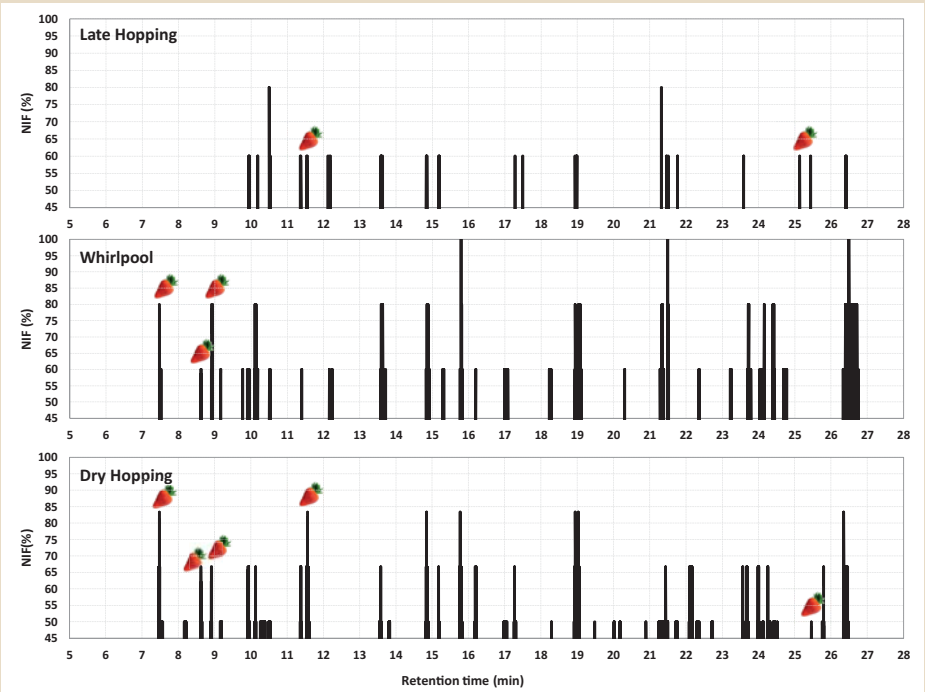

Fig. 3 Aromagrams of late hopped, whirlpool and dry hopped beers

Finally, all descriptors given by the panellists during the olfactometry analysis were used to compile the sensory profile of the three beers according to different aroma categories (fig. 4). Late hopped beer is the most vegetal, whirlpool beer the most citrus-like and dry hopped beer the most floral. In addition, whirlpool and dry hopped beers have been described as having the most pronounced red berry aroma.

Conclusion

In this study, volatile and odour-active compounds from late hopped, whirlpool and dry hopped beers hopped with Barbe Rouge were compared using GC-MS and GC-O. The three hopping methods resulted in three different beers with typical Barbe Rouge aromas. GC-MS analyses showed similar volatile profiles for late hopped and

ODORANT COMPOUNDS IDENTIFIED IN THE THREE BEERS (LH: LATE HOPPING, W: WHIRLPOOL, DH: DRY HOPPING) (RT: RETENTION TIME)

RT (min)	Panellists' descriptors	Nasal Impact Frequency (%)			Compounds identification (odorant published in the literature)
		LH (n=5)	W (n=5)	DH (n=6)	
7.48	Red fruit, strawberry		80.0	83.3	Ethyl isobutanoate (fruit, sweet, strawberry) (A)
8.19	Chemical, solvent			50.0	Isobutyl acetate (fruit, floral, banana)
8.62	Red fruit, strawberry		60.0	66.7	Ethyl butanoate (fruit) (B)
8.91	Strawberry		80	66.7	Ethyl 2-methyl butanoate (fruit, apple) (C)
9.16	Fruit		60.0	50.0	Ethyl isopentanoate, butyl acetate (fruit, floral)
9.77	Burnt, plastic		60,0		Unknown
9.93	Brewery, vegetal*	60.0	60.0	66.7	Geranic oxide* (herbal, rosemary), hexanal* (green, grassy)
10.12	Banana*	60.0	80.0	66.7	Isoamyl acetate* (banana, fruit, sweet)
10.27	Old hops, underwood			50.0	Unknown
10.51	Green, grass, hay*	80.0	60.0	50.0	b-myrcene* (spicy)
11.37	Fruit, strawberry	60.0		66.7	3-Methylbutyl-2-methylpropanoate (sweet, fruit) (D)
11.54	Unpleasant, old hops	60.0		83.3	Isoamyl alcohol (alcoholic, malty, fusel)
12.16	Fruit	60.0	60.0		Ethyl hexanoate (fruit)
13.57	Almond, roasted*	60.0	80.0	66.7	Acetol* (nutty), 3-methyl-2-butenol* (herbaceous)
13.82	Unpleasant, old hops			50.0	Unknown
14.84	Green, grass, vegetal*	60.0	80.0	83.3	Trans-3-Hexenol* (green, moss)
15.18	Floral, rose, mushroom	60.0		66.7	Ethyl octanoate (fruit, floral)
15.79	Earthy, forest, hops		100.0	83.3	Unknown
16.20	Mushroom, moss		60.0	66.7	Furfural (alkane, sweet, floral)
17.01	Unpleasant, plastic		60.0	66.7	Propanoic acid (pungent, rancid, soy)
17.29	Floral, roasted	60.0		66.7	Linalool (floral, citrus, fruit)
17.49	Unpleasant, white flower	60.0			1-Octanol (green, floral, rose)
18.26	Soap, rancid		60.0	50.0	Butanoic acid (rancid, cheesy)
18.98	Old hops, cheese*	60.0	80.0	83.3	3-methyl butanoic acid* (rancid, cheesy)
19.48	Hot milk, vanilla			50.0	Unknown
20.31	Floral, soap, wood		60.0	50.0	Citronellol (rose, green)
20.90	Tobacco, soap, floral, lemon, wax			50.0	Isogeraniol (rose), nerol (floral), Ethyl-2-phenylacetate (honey, fruit)
21.31	Old garage, soap, floral, lemon*	80.0	80.0	50.0	Hexanoic acid* (cheesy, rancid), 2-phenylethylacetate* (floral, rose), geraniol* (rose, floral) (coelution ?)
21.49	Fruit, apple sauce, rose*	60.0	100.0	66.7	β-Damascenone* (floral, tobacco)
21.74	Floral, roasted	60.0		50.0	Benzyl alcohol (floral, fruit), guaiacol (burnt, smoky)
22.13	Burnt, floral			66.7	Ethyl dihydrocinnamate (floral, fruit, sweet)
22.32	Floral, rose, white flower		60.0	50.0	2-Phenylethanol (rose, floral, honey)
22.73	Rose, fruity			50.0	α-Calacorene (wood)
23.23	Roasted, smoky		60,0		Phenol (medicinal, smoky)
23.58	Roasted, burnt, candy, red fruit*	60.0		66.7	4-Ethylguaiacol* (spicy, clove, smoky), furaneol*(caramel, burnt)
23.71	Dust, powdery, burnt		80.0	66.7	Ethyl tetradecanoate (wax)
24.00	Floral, coconut		60.0	57.9	g-Nonalactone (coconut, peach)
24.14	Urine		60.0	66.7	p-Cresol (phenolic, cattle, medicinal)
24.29	Vanilla, honey, caramel		80.0	50.0	1,10-di-epi-Cubenol
24.44	Caramel, leather			66.7	Unknown
24.74	Rubber, burnt		60.0		Nonanoic acid (fatty, rancid)
25.14	Floral, fruit	60.0			Ethyl cinnamate (honey, floral)
25.44	Fruit, red fruit, marshmallow	60.0		50.0	2-Phenethyl hexanoate (fruit) (E)
25.78	Roasted, spicy			66.7	4-vinyl guaiacol (spicy, clove, smoky)
26.42	Unpleasant, motor oil*	60.0	100.0	83.3	Decanoic acid* (rancid, soapy)

** NIF > 50% in the three beers*

Table 3

whirlpool beers. However, olfactometry highlighted the fact that whirlpool and dry hopped beers contained more odour-active compounds than late hopped beer. Of these compounds, five of them linked to red fruit and strawberry-like aromas were identified as ethyl isobutanoate, ethyl butanoate, ethyl 2-methylbutanoate, 3-methylbutyl-2-methylpropanoate and 2-phenethyl hexanoate. In our investigation setup, dry hopping and whirlpool were the most efficient hopping methods to enhance red fruit aroma in beer using the Barbe Rouge variety, compared to late hopping. This study highlights the unique aroma qualities of Barbe Rouge and shows that the combination of hop varieties and hopping methods can be crucial for maximising the aroma impact of hops on beer. ■

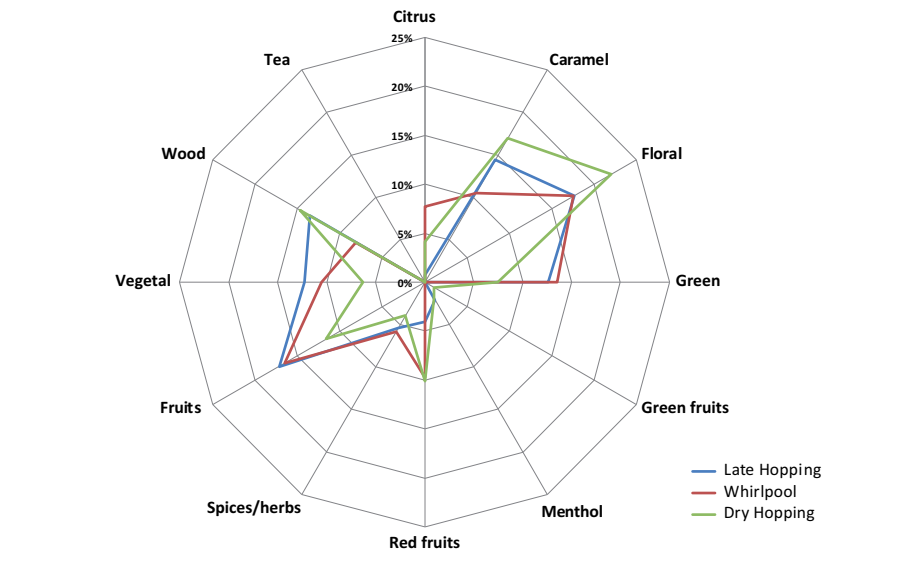

Fig. 4 Flavours according to hopping method

Acknowledgement

The authors would like to thank Jean-Marie Kessler (Meteor Brewery, Hochfelden, France) and the CAMBAP team (Illkirch, France) for their involvement in GC-O analyses.

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