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An exploratory study on the impact of the yeast strain on hop flavour expressions in heavily hopped beers: New England IPA

In this study, the role different yeast strains might play in the flavour characteristics of heavily hopped beers was investigated. Therefore, New England Indian Pale Ale (NEIPA) style beers were brewed according to a standard brewing procedure in which all brewing parameters were kept constant with the exception of the yeast strain used for fermentation. In total, nine NEIPA beers were produced with nine different beer yeasts (seven ale yeasts (*Saccharomyces cerevisiae*) amongst which five strains are phenolic off flavour negative (POF-) and two strains are POF positive); two lager yeasts (*Saccharomyces pastorianus*, POF-). Via descriptive sensory evaluations, the beers were distinguished into several clusters which demonstrated the impact of the yeast strain on the final flavour attributes of the resulting beers. Particular yeasts strains boosted juicy and fruity flavours whereas others significantly suppressed the hop-derived aroma of the NEIPA beers in this comparative study. Analytical results point into the direction of yeast strain induced variability in the levels of (flavour-active) hop oil constituents and/or synergistic effects between yeast aromas and hop aromas as possible explanations for the flavour differences of the final beers.

Descriptors: NEIPA, *Saccharomyces* yeast, beer aroma profiles, hop volatile fingerprinting, principal component analysis

1 Introduction

New, heavily hopped beers of the Indian Pale Ale family (IPA) such as the New England IPA ('NEIPA', 'Hazy IPA', 'Juicy IPA') have emerged recently. NEIPA beers have massive hop aroma and flavour, typically expressing highly pronounced fruity, juicy, hop derived aromas. It is well known that in the production process of such heavily hopped beers the hops (e.g. dosage rate, hop variety) and the applied hopping technology (late hopping, dry hopping or a combination of both practices) play an important role in the development of pronounced and pleasant (hoppy) flavours. In the case of NEIPA beers, hops are added at different stages of the brewing process, i.e. addition to the whirlpool (late hop addition, at least 30 % of total hop dosage) and during fermentation (first addition 24 – 48 hours after pitching and subsequent additions at regular time intervals for at least 3 days). Hop addition rates typically range between 700 and 1,500 g/hL [1].

Hoppy beer flavours result from the presence of many flavour-active compounds originally present in the essential oil of the hops that are efficiently extracted into the final beer due to the practices of late and dry hopping, and the relatively high hop dosage rates used in the production process of heavily hopped beers such as IPAs. Many hop oil constituents have been proposed as key aroma components for hop aroma (i.e. the aroma of the hops as such). It is well known that monoterpene alcohols such as linalool and geraniol are associated with floral and citrussy impressions whilst particular esters (e.g. 2-methylbutyl isobutyrate, methyl 2-methylbutanoate) contribute to fruity hop aroma. The monoterpene hydrocarbon β-myrcene and oxygenated sesquiterpenoids are linked with geranium-like/citrussy and woody/spicy/herbal connotations, respectively. Finally, particular flavour hops contain volatile thiols having extremely low flavour thresholds (e.g. 4-mercapto-4methyl-pentan-2-one) and highly pronouned exotic fruit/grapefruit-like scents [2–15].

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The hop-derived beer aroma is however not identical to the aroma of the raw hops as such. In other words, the final flavour characteristics of (heavily) hopped beers can not exclusively be ascribed to aroma-impact compounds originally present in the hop essential oil. In summary, during the brewing process, the original composition of added hop oil is strongly modified due to high losses of volatile constituents and chemical and yeast catalyzed biochemical transformations of hop oil principles, leading to new, potentially flavour-active compounds or increased/decreased levels of impact components originally present. Many hop-derived volatiles contributing to the final beer flavour have been proposed in literature since decades (see e.g. [7–8, 16–24]).

Driven by the high popularity of heavily late and dry hopped beers (e.g. IPAs) research groups have focussed on the origin of hop derived beer flavours. It was found that an interplay between yeasts and hops exists in that yeasts might significantly impact the final hoppy beer flavour characteristics. Indeed, yeasts may play

Table 1 **Overview of the dry-yeasts used in the NEIPA brewing trials**

Yeast Strain		Species	Application	Aromatic properties (yeast aroma baseline)*
Ale yeast	**Code**			
SafAle S33	S33	*Saccharomyces cerevisiae*	Top fermentation, POF-	high fruity esters and tropical flavours
SafAle S04	S04	*Saccharomyces cerevisiae*	Top fermentation, POF-	moderate to low fruity character
SafAle US05	US05	*Saccharomyces cerevisiae*	Top fermentation, POF-	low intensity fermentation flavours, neutral character
SafAle K97	K97	*Saccharomyces cerevisiae*	Top fermentation, POF-	moderate to high fruity and floral notes
SafAle BE256	BE256	*Saccharomyces cerevisiae*	Top fermentation, POF-	intense fruity (banana) and floral (rose-like) notes
SafAle BE134	BE134	*Saccharomyces cerevisiae*	Top fermentation, POF+	dry, high fruitiness and pronounced phenolic flavour (spicy and clove-like aromas)
SafAle T58	T58	*Saccharomyces cerevisiae*	Top fermentation, POF+	high fruity notes (banana, apple notes) and phenolic flavours (clove-like)
Lager yeast				
SafLager S189	S189	*Saccharomyces pastorianus*	Bottom fermentation, POF-	neutral, very low fermentative fruity-floral flavours, might present sulfury notes**
SafLager S23	S23	*Saccharomyces pastorianus*	Bottom fermentation, POF-	slightly fruity, low fermentative character and might present sulfury notes**

*Results obtained under conditions comparable to the NEIPA brewing trials (15 °P all malt wort; fermentation at 23 °C); not aromatised with hops

** Aromas characteristic for lager yeast strains

a unique role during the fermentation of (heavily) hopped beers from different perspectives.

Firstly, it is well known that yeasts directly impact the beer flavour by the production of yeast strain dependent flavour-active fermentative compounds (mainly esters and higher alcohols) which can impart fruity (banana, apple, tropical fruit) and floral-like notes (roses) to the beer. The concentrations and relative proportions of those compounds found in final beers are dependent on the yeast strain and brewing (fermentation) conditions and they can impact and diversify the final beer flavour considerably [25–28]. In view of hoppy beer aroma, the aformentioned fermentation by-products can positively or negatively affect the overall perception of hop flavours [29–31].

Next, yeasts are involved in biotransformation processes of (aroma-impact) volatiles originally present in the hop essential oil. For example, the conversion of the monoterpene alcohol geraniol into β-citronellol and linalool and/or the conversion of nerol into linalool and α-terpineol has been reported several times in literature. In summary, evidence was found that several biotransformation reactions catalyzed by the yeast (i.e. reductions, translocations, *cis* to *trans* isomerisations and cyclisations of monoterpene alcohols; conversions of several hop oil esters) occur in real brewing practice and that they may have a huge impact on the intensity and quality of the final (hop derived) beer flavour [30, 32–36].

Finally, yeasts may impact hop derived beer aromas by releasing flavour compounds from volatile (e.g. geranyl esters), and non-volatile precursors (e.g. hop glycosides). For example, it is proposed that geraniol could be released from geranyl esters (geranyl acetate and geranyl isobutyrate) via yeast derived esterase activity (17, 37] and from hop glycosides via yeast derived glucoside hydrolases [33]. The presence of hop glycosides in hops, hop products and beer and their role in the development of particular hop flavours such as the kettle hoppy aroma or citrussy beer aroma has been studied by several research groups [38–46]. Evidence was found for the release of glycoconjugated flavour compounds (e.g. linalool, β-damascenone, ….) by *Saccharomyces cerevisiae* brewing yeast strains and, in particular, by non conventional yeasts belonging to the genus *Brettanomyces*.

Taken together, the role yeasts play in the development of hop derived flavours (e.g. citrussy impressions) in beer is quite complex, though extremely important for the overall beer flavour characteristics, including the subtle, but significant, differences that may occur in both intensity and quality of hoppy beer aroma.

The aim of the present study was to further explore the impact of the yeast strain on the final flavour characteristics of heavily hopped beers. To this end, a relatively new, unique beer style, i.e. New England IPA (NEIPA), has been chosen for investigation. Since to date little is known about the most appropriate yeast strain to produce NEIPA beers, we performed a comparative brewtechnical study in which nine New England IPA style beers were brewed under identical conditions. The only variable in the production process was the yeast strain used for fermentation (seven ale yeasts, two lager yeast strains). The resulting beers were evaluated via descriptive sensory analysis and the hop volatile fingerprint and fermentation profiles of the beers were determined via GC-MS.

2 Materials and Methods

2.1 Yeast Strains

In total, nine active dry yeasts (Fermentis, Société Industrielle Lesaffre, France) were used in this study to produce nine experimental NEIPA beers (see section 2.3). The dry yeasts used can be classified into seven ale yeasts (*Saccharomyces cerevisiae*; five POF negative (POF-) yeast strains (codes 'S33', 'S04', 'US05', 'K97', 'BE256'); 2 POF positive (POF+) yeasts; codes 'BE134', 'T58') and two lager yeast strains (*Saccharomyces pastorianus*; codes 'S189', 'S23') (see Table 1).

Table 2 Pilot-scale brews (5 hL scale): wort composition, hopping and yeast strains

Wort composition	Hops and hop regimes – (equal parts of hops/addition)	Yeasts
Pils malt (80 %), wheat malt (10 %), and oats (10 %) Original Gravity: 16 °P	Hop varieties: Simcoe, Mosaic, and Citra	SafAle S33
Original Gravity 16 °P	Hop additions: whirlpool, fermentation days 2 and 4, and maturation	SafAle S04
	Total hop amount: 1 kg/hL	SafAle US05
	(83.3 g/hl/hop/addition)	SafAle K97
		SafAle BE256
		SafAle BE134*
		SafAle T58*
		SafLager S189
		SafLager S23

* phenolic off flavour positive (POF+)

The yeast strains chosen for this study differ in their sensory baseline profile, i.e. the aromas they produce due to their metabolism (i.e. the level and relative proportion of esters, higher alcohols and phenolic volatile compounds). Although the quality and intensity of the aromas a particular yeast strain produces depends on many parameters (e.g. wort composition, pitching rate, fermentation temperature) we are able – based on multiple brewing trials, including trials with experimental non hop aromatised beers (data not shown) – to classify the yeast strains under investigation as follows: (1) highly intense fruity character: the ale yeasts 'S33', 'K97', 'BE256'; (2) moderate/low fruity character, neutral yeast aroma: ale yeasts 'S04', 'US05'; (3) low fruity character with sulfury notes: the lager yeasts 'S189', 'S23'; (4) pronounced fruity and phenolic flavours: the POF+ ale yeasts 'BE134' and 'T58'. (see also Table 1).

2.2 Hop raw materials

Hop pellets T90 varieties Citra, Mosaic and Simcoe (Yakima Chief Hops, Louvain-la-Neuve, Belgium; crop year 2017) were added at different stages of the NEIPA production process (see Table 2). The hop varieties used are hops in which the linalool and geraniol content is predominant and volatile thiols are present. It is known that the hops impart lime-like (Citra), tropical fruit-like (Mosaic) and blackcurrant/grapefruit (Simcoe) flavours to beers produced with it [15].

2.3 Pilot-scale brewing

New England IPA beers (NEIPA) were prepared in the pilot brewery of KU Leuven, Technology Campus Gent (5 hL scale). The following conditions were used: 80 kg fine milled commercial pilsner malt, 10 kg of oat and 10 kg wheat malt (disc milling under water, Meura) is mixed with 2.20 hL de-aerated reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}) and approx. 400 mL lactic acid (30 %, v/v) (precise volume to be added depends on the malt); mashing-in: temperature of 64 °C, pH of 5.4; brewing scheme: 64 °C (40 min), 72 °C (30 min), 78 °C (1 min) (rise in temp. at 1 °C/min); wort filtration: membrane assisted thin bed filter (Meura 2001); sparging with 2.5 l/kg water (extract of last runnings 3 °P and 2 °P after final compression); extract of the combined sweet wort is 16 °P (5.2 hl); wort boiling: 60 min atmospheric boiling using clean-steam injectors; at the end of boiling 0.2 ppm Zn^{2+} ions were added; wort clarification: open whirlpool and first hop addition according to table 2; after whirlpool, cooling and aeration, the wort (original gravity: 16 °P) was split into 9 fermentors of 50 L and pitched with 25 g re-hydrated dry yeasts (inoculum: dry yeasts (Fermentis, France) were re-hydrated for 1 hour in sterile R.O. water with a volume of 10 times the weight of the dry yeast – 30 min static, 30 min gentle stirring); primary fermentation at 23 °C in cilindroconical tanks (50 L tanks in temperature controlled room); Dry-hop regime according to table 2; maturation: 7 days at 12 °C; beer centrifugation (α-laval – type LAPX 404 SGP – 31GC at 1 hL/h); CO_2 saturation up to 5.6 g/L; packaging: 6 head rotating counter pressure filler (monobloc, CIMEC, Italy) using double pre-evacuation with intermediate CO_2 rinsing and foaming with hot water injection before capping (final dissolved oxygen levels: below 50 ppb- total package oxygen below 200 ppb).

2.4 Sensory evaluation of the beers

Descriptive sensory analyses of the beers were conducted by Fermentis' Beer Panel (FBP) which consists of 40 panellists. The FBP is extensively trained and involved in sensory evaluations of beer for research and development purposes.

Panel members were selected on the basis of their motivation, availability, consumption habits and health conditions and are involved in sensory evaluations on regular time intervals (at least 1x/week). The group is trained for descriptive assessments and analysis of several beer attributes such as appearance, odour, aroma, flavour, bitterness and mouthfeel. For the present investigation of the sensory characteristics of NEIPA beers, the panel was additonally trained for the description of hop flavours via commercially available total hop essential oils and hop oil fractions with hop citrussy, hop tropical, hop herbal, hop floral aromas. Next, the panel members were familiarised with the particular flavour characteristics of NEIPA beers via descriptive sensory analysis of commercially available NEIPA beers.

The experimental NEIPA beers in this study were evaluated in several sessions (20 trained panel members/session). All samples (60 mL) were coded (three- digit random numbers) and randomly and individually served in odourless glasses (200 mL). The freshly poured samples were served at 10 °C and evaluated by each panellist in separated booths at ambient temperature (18 – 20 °C). The sensory analysis was done by scoring specific descriptors for beer aroma, thereby focussing on particular fermentation related flavours (Fermentation related fruity flavour (apples, banana), phenolic flavour, sulfury flavour) and particular hop-derived aromas (e.g. 'overall Hop Odour Intensity'; 'Hop Overall Fruity', 'Hop Citrussy', 'Hop Tropical', 'Hop Herbal', 'Hop Floral'). All aromatic attributes were scored using a 8-point scale ('0': not perceived – '8': overwhelming).

2.5 Determination of fermentation by-products via headspace solid phase microextraction and gas chromatography – mass spectrometry (HS-SPME GC-MS)

Extraction of volatile esters and higher alcohols as well as 4-vinylguaiacol (4VG) was done via headspace solid phase microextraction (HS-SPME) for 30 min at 40 °C using a 65 µm PDMS/DVB fiber coating. Extracted components were separated and detected by capillary gas chromatography/mass spectrometry (CGC-MS) (Ion Trap – ITQ; Thermo Fisher Scientific, Austin, TX, USA; electron ionisation, 70 eV). The mass spectrometer was coupled to a ThermoFinnigan Trace GC (Thermo Fisher Scientific, Austin, TX, USA) equipped with a CTC-PAL auto sampler (CTC Analytics, Zwingen, Switzerland), a heated split/splitless injector with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused silica capillary column (40 m x 0.18 mm i.d. x 0.2 µm film thickness; Restek Bellefonte, PA, USA). Helium (Alphagaz 2; Air Liquide, Luik, Belgium) was the carrier gas at a flow rate of 0.8 mL/min. The inlet temperature was set at 230 °C and injection was done in the split mode (split ratio 1/10). The oven temperature was held at 40 °C for 3 min, then raised to 200 °C at 6 °C/min, followed by an increase at 15 °C/min to 250 °C (3 min iso). External calibration curves were recorded using 2-heptanol as an internal standard.

2.6 Determination of hop-derived volatiles in the beer flavour profiles via HS-SPME GC-MS

Solid phase microextractions were automated using a CombiPal autosampler (TCC Analytics, Switzerland). A volume of 5 mL of beer was pipetted into a 20 mL vial containing 1.5 g NaCl under carbon dioxide atmosphere and closed with a PTFE-coated septum. Volatile compounds were extracted by inserting a polydimethylsiloxane fibre (PDMS, 100 µm, Supelco, Bellefonte, PA, USA) into the vial headspace. Extraction temperature and time were set at 40 °C and 30 min. Before starting the actual extraction, samples were pre-incubated at 40 °C during 5 min. During pre-incubation and extraction, samples were stirred at 500 rpm.

Gas chromatographic operating conditions were as follows. Extracted volatiles were thermally desorbed in the heated inlet (250 °C) of the Ultra Trace gas chromatograph (Thermo Fisher Scientific, Austin, TX) for 3 min. Helium (Alphagaz 2, Air Liquide, Belgium) was used as a carrier gas at a constant flow of 1.0 mL/min. Injection was done in the splitless mode for 3 min at 250 °C. Separation of the injected compounds was performed on a 40 m x 0.18 mm i.d. x 0.2 µm (film thickness) RTX-1 capillary column (Restek Corporation, Bellefonte, PA, USA). The oven temperature program was as follows: 3 min at 35 °C, followed by a temperature increase at 5 °C/min up to 250 °C (1 min isotherm).

Mass spectrometric detection of hop oil constituents was obtained by a dual stage quadrupole MS (DSQII, Thermo Fisher Scientific, Austin, TX) operating in the electron ionisation mode (EI, 70 eV). The ion source temperature was set at 240 °C and the electron multiplier voltage was 1,525 V. Analyses were performed in full scan (m/z = 40-350). Quantitative determination was done via extracted ion chromatograms. Following fragment ions were selected: m/z = 58 (methyl ketones), m/z = 69 (geranyl esters), m/z = 70, 71, 85 (isobutyric esters); m/z = 74 (methyl esters), m/z = 88 (ethyl esters); m/z = 93 (monoterpene hydrocarbons); m/z= 69, 136, 154, (monoterpene alcohols); m/z = 93, 161, 189, 204, 220 (sesquiterpene hydrocarbons and oxygenated sesquiterpenoids). The level of the reported compounds were obtained via standard addition calibration curves of authentic reference products. 2-heptanol and dodecane were used as internal standards.

2.7 Multivariate data analysis via principal component analysis (PCA)

PCA was performed to enhance data analysis and for interpretation of the results. In this study, PCA was used for discrimination of the experimental NEIPA beers produced with different yeast strains on the basis of their organoleptic characteristics, yeast fermentation by-products, and hop oil derived volatiles, respectively. PCA

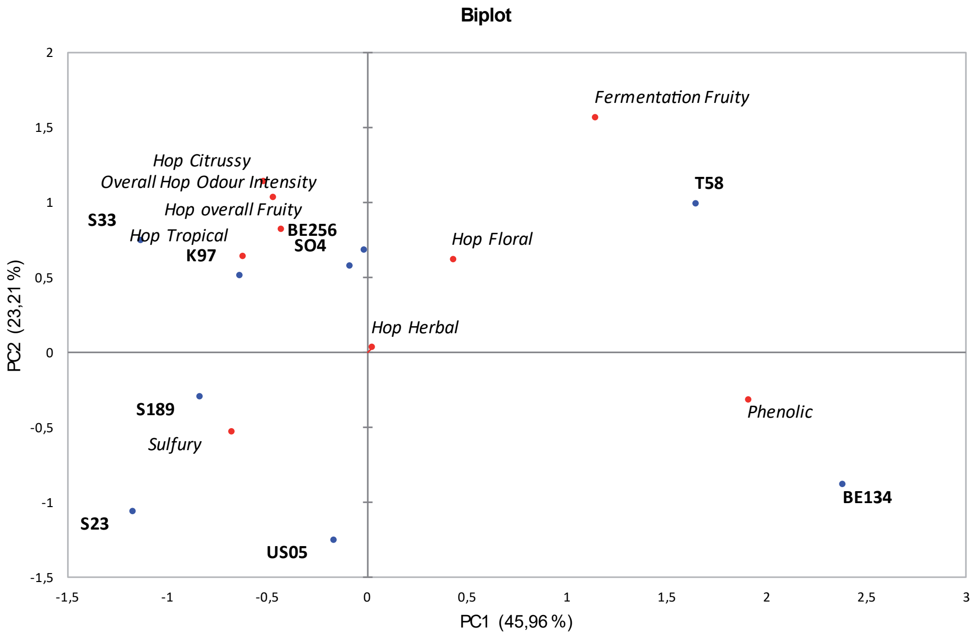

Fig. 1 **Bi-plot of principal component analysis on NEIPA beers fermented with different yeasts (objects) and sensory descriptors (variables)**

was performed by using the multivariate data analysis software package The Unscrambler X, version 10.5.1 (CAMO software AS, Oslo, Norway).

3 Results and Discussion

3.1 Sensory evaluation of the experimental NEIPA beers

Sensory evaluations of the nine experimental NEIPA beers were performed via descriptive sensory analysis as described in section 2.4. In general, the beers expressed clearly perceivable, pleasant fruity, citrussy flavours which had been expected given the high hop dosage rates, hopping technologies (late and dry-hopping) and the hop varieties used (see Table 2). However, the intensity and quality of the final (hop derived) beer flavour differed significantly and we were able to distinguish the beers on the basis of their organoleptic properties.

To visualise the diffences between the beers on the one hand, and to find correlations between the beers and the sensory descriptors on the other, principal component analysis (PCA) was performed on the sensory datamatrix with the nine beers as objects and the flavour descriptors as variables (see Fig. 1). Well separated clusters are depicted in the bi-plot:

- Cluster 1: consists of beers produced with the POF- ale yeasts ‘S33’, ‘S04’, ‘K97’ and ‘BE256’; the beers have high correlation with the hop aroma descriptors ‘Hop citrussy’, ‘Hop fruity’, ‘Hop Tropical’ and the overall intensity of hop odour;
- Cluster 2: contains the beers produced with the lager yeast strains (‘S189’ and ‘S23’) and the POF- ale yeast ‘US05’; their position at the left hand side of the plot points to similarity with the beers present in cluster 1. However, the hop flavour attributes are less characteristic and somewhat less intense for the beers in cluster 2 which could be ascribed to the perception of sulfury aromas which are inherent to the lager yeast strains (see yeast aroma base line in Table 1);
- Cluster 3: contains the beers produced with the POF+ ale yeasts (‘BE134’, ‘T58). The flavour of the beers was impacted by phenolic perceptions which suppressed the hoppy flavours considerably. Besides, the beers produced with the different POF+ yeast could be distinguished by means of a somewhat

Table 3 **Fermentation by-products in the NEIPA Beers produced with the POF- ale yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 2)**

		Ale yeasts (POF-)									
		S33		S04		US05		K97		BE256	
		mg/L	SD	mg/L	SD	mg/L	SD	mg/L	SD	mg/L	SD
No°	HIGHER ALCOHOLS										
3	n-propanol	39.8	3.8	37.1	3.5	30.8	1.6	28.7	2.5	24.6	2.1
5	2-methylpropanol	31.5	3.6	43.6	0.8	35.2	1.9	30.9	0.5	53.4	2.6
6	2-methylbutanol	13.2	0.7	16.2	0.2	12.0	0.1	17.1	0.4	20.1	0.1
7	3-methylbutanol	108	1	133	2	95	2	131	3	143	4
12	2-phenylethanol	56.4	4.2	61.1	1.5	25.8	2.2	56.1	3.1	65.1	0.3
	Sum higher alcohols	**248**	**7**	**291**	**6**	**199**	**5**	**264**	**5**	**307**	**7**
	% Alcohol group	**82.3**		**82.3**		**80.1**		**80.5**		**83.2**	
	ETHYL ESTERS										
9	ethyl butanoate	0.149	0.030	0.156	0.007	0.320	0.003	0.192	0.009	0.158	0.031
11	ethyl hexanoate	0.962	0.082	1.159	0.070	0.586	0.058	1.327	0.053	0.638	0.028
13	ethyl octanoate	0.213	0.002	0.296	0.033	0.304	0.008	0.304	0.006	0.262	0.004
15	ethyl decanoate	0.047	0.002	0.065	0.009	0.024	0.003	0.049	0.003	0.071	0.003
	sum ethyl esters	**1.371**	**0.110**	**1.676**	**0.100**	**1.235**	**0.110**	**1.871**	**0.090**	**1.129**	**0.130**
	% ethyl ester group	**0.5**		**0.5**		**0.5**		**0.6**		**0.3**	
	ACETATES										
4	ethyl acetate	48.9	2.3	57.5	2.6	46.4	2.5	59.1	3.7	56.1	2.1
8	isobutyl acetate	0.176	0.007	0.191	0.005	0.099	0.002	0.081	0.002	0.227	0.001
10	isoamyl acetate	2.42	0.05	2.52	0.07	1.37	0.04	2.32	0.13	3.41	0.22
14	phenylethyl acetate	0.569	0.090	0.584	0.095	0.205	0.027	0.442	0.043	0.807	0.122
	sum acetates	**52.1**	**1.1**	**60.8**	**1.6**	**48.1**	**2.6**	**61.9**	**3.7**	**60.6**	**2.6**
	% acetates	**17.2**		**17.2**		**19.4**		**18.9**		**16.4**	
	Total sum higher alcohols, ethyl esters, acetates	**302**	11	**353**	10	**248**	6	**328**	12	**368**	9
2	4-vinylguaiacol	0.169	0.011	0.165	0.014	0.112	0.022	0.147	0.020	0.131	0.010

lower intensity of phenolic flavour and more characteristic fermentation related fruity flavours in the beer produced with yeast strain ‘T58’.

Interestingly, although the beers were heavily hopped, the intrinsic sensory aroma properties of the different yeast strains come to expression in the final organoleptic characteristics of the beers. This follows in first instance from the positioning of the beers produced with the lager yeast strains (‘S189’, ‘S23’; lower left side of the PCA-plot) and the POF+ yeast strains (‘right side of the PCA bi-plot) and their correlation with sulfury and phenolic notes respectively. Furthermore, the beers produced with the yeast strains having a sensory base line profile that is highly intense in fruity aroma are postively correlated with intensity and quality of hoppy flavour expressions (‘hop citrussy’, ‘hop fruity’, ‘hop tropical)’. This probably points to synergestic effects between hop and (fruity) yeast aromas, resulting in increased overall fruity (hop) flavour expressions. This is highly desired in the case of the NEIPA type beers under investigation in this study and demonstrates that the aforementioned yeast strains are probably the best choice in view of maximal fruity-juicy flavour expressions in this type of beers. Obviously, it can not be recommended to use POF+ strains when aiming at the production of heavily hopped beers with pronounced hop fruity flavours since this desirable flavour attribute is significantly suppressed by compounds produced by the yeast metabolism (phenolic flavours, e.g. 4VG) (see *Montandon* et al. [31]).

3.2 Chemical-analytical characterisation of the yeast and hop related aromatic profiles

To gain insight into the origin of the sensory flavour characteristics of the final beers all nine experimental NEIPA beers were analysed for typical yeast fermentation by-products and hop related volatiles via HS-SPME GC-MS.

3.2.1 Yeast fermentation by-products

Quantitative GC-MS profiling of yeast fermentation by-products, i.e. esters and higher alcohols, was performed on the nine NEIPA beers as described in section 2.5. In total, 14 volatiles were assigned and grouped according to their chemical compound class, i.e. higher alcohols, ethyl esters, acetates (see Table 3 (POF- ale yeasts) and Table 4 (POF+ ale yeasts and POF- lager yeasts)).

Table 4 **Fermentation by-products in the NEIPA Beers produced with the POF+ ale yeasts and POF- lager yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 2)**

		Ale yeasts (POF+)				**Lager yeasts (POF-)**			
		BE134		**T58**		**S189**		**S23**	
		mg/L	**SD**	**mg/L**	**SD**	**mg/L**	**SD**	**mg/L**	**SD**
No°	**HIGHER ALCOHOLS**								
3	n-propanol	32.7	2.7	32.1	2.6	13.8	0.5	12.9	0.8
5	2-methylpropanol	35.6	2.3	38.8	2.2	18.0	1.1	14.6	0.9
6	2-methylbutanol	11.8	0.1	16.2	0.2	10.4	0.0	9.2	0.1
7	3-methylbutanol	127	3	152	2	113	4	117	3
12	2-phenylethanol	45.9	2.0	47.6	0.3	35.8	2.9	35.6	1.4
	Sum higher alcohols	**253**	**8**	**287**	**7**	**191**	**6**	**189**	**6**
	% Alcohol group	**75.9**		**80.7**		**78.4**		**78.8**	
	ETHYL ESTERS								
9	ethyl butanoate	0.402	0.020	0.247	0.004	0.135	0.022	0.153	0.008
11	ethyl hexanoate	0.733	0.026	0.828	0.063	0.566	0.018	0.588	0.004
13	ethyl octanoate	0.378	0.012	0.313	0.014	0.246	0.021	0.266	0.011
15	ethyl decanoate	0.072	0.011	0.061	0.005	0.028	0.004	0.034	0.003
	sum ethyl esters	**1.584**	**0.100**	**1.448**	**0.100**	**0.974**	**0.120**	**1.042**	**0.130**
	% ethyl ester group	**0.5**		**0.4**		**0.4**		**0.4**	
	ACETATES								
4	ethyl acetate	74.8	3.8	62.9	2.5	49.9	1.4	47.9	1.5
8	isobutyl acetate	0.229	0.011	0.226	0.000	0.073	0.001	0.063	0.001
10	isoamyl acetate	3.21	0.23	3.46	0.13	1.48	0.08	1.47	0.06
14	phenylethyl acetate	0.640	0.060	0.497	0.086	0.367	0.048	0.375	0.053
	sum acetates	**78.9**	**3.7**	**67.1**	**2.6**	**51.8**	**1.8**	**49.8**	**2.1**
	% acetates	**23.7**		**18.9**		**21.2**		**20.7**	
	Total sum higher alcohols, ethyl esters, acetates	**333**	12	**355**	9	**244**	5	**240**	5
2	4-vinylguaiacol	0.262	0.020	0.201	0.010	0.118	0.010	0.114	0.010

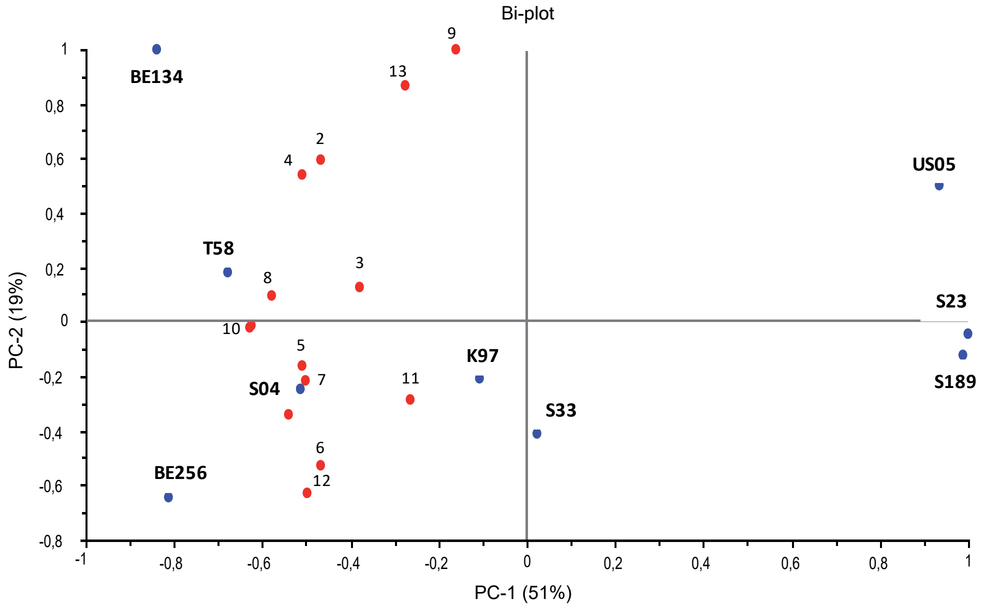

Fig. 2 **Bi-plot of principal component analysis on NEIPA beers fermented with different yeasts (objects) and fermentation by-products (variables). Numbering of the variables in accordance with the numbering in tables 3–4**

The total level of the higher alcohols, ethyl esters, and acetates ranges from 240 mg/L (beer ‘S23’) to 368 mg/L (beer ‘BE256’). This clearly demonstrates the different performances of the yeast strains in producing volatiles. The higher alcohols group is predominant, accounting for 76–83 % of the total content of the fermentation by-products. The acetate group accounts for 16–24 % of the total content, whilst the ethyl ester group is only a minor fraction accounting for less then 1 %.

The sensory characteristics imparted by compounds from the higher alcohol group are generally described in terms of ‘alcohol’ (n-propanol, isobutanol, 2- and 3-methylbutanol), ‘fruity’ (banana: 2-methylbutanol and 3-methylbutanol), ‘floral’ (rose-like: 2-phenylethanol), and ‘solvent-like (isobutanol, 2-methylbutanol) [26,28]. The threshold values of the higher alcohols are relatively high (65–800 ppm), and are considered to give pleasant aromas when present in optimal levels. However at high levels they are associated with strong, pungent smell and taste [28]. The NEIPA beers produced with the lager yeast strains contain significantly lower higher alcohol levels (190 mg/L) compared to the beers produced with the ale yeast strains (concentration range: 248–307 mg/L), except for the POF- ale yeast US05 beer (199 mg/L). Besides, significant variations are also observed in the group of the ale yeast beers. At the levels reported here only 3-methylbutanol exceeds its threshold level which is reported at 70 ppm.

High impact flavour-active esters are however found in the ethyl ester and acetates group. In particular, ethyl hexanoate (apple, aniseed), ethyl octanoate (fruity, apple), ethyl acetate (solvent-like, fruity), isoamyl acetate (banana, apple, pear), phenylethyl acetate (roses, honey, sweet) are commonly reported in review articles as yeast derived high flavour-impact compounds in beer [25–26, 28]. In the NEIPA beers investigated in this study, the levels of the aforementioned compounds exceed their generally accepted threshold values, with the exception of phenylethyl acetate and ethyl octanoate. This means that most of the typical fermentation related esters and acetates directly contribute to the flavour of the NEIPA beers thereby imparting characteristic (fruity) flavours. Moreover, high variability is observed in the levels of these compounds between the NEIPA beers. For example, the level of isoamyl acetate ranges between 1.5 mg/L (beers produced with the lager yeast strains) and 3.5 mg/L (ale yeast strain ‘BE134’). In general, the ale yeast strains (except ale yeast US05) produce significant higher levels of fruity flavour-impact compounds when compared to the lager yeast strains. Moreover, the absolute levels and relative proportions of the fruity esters clearly differ between beers produced with the ale yeasts, which demonstrates the variable intrinsic flavour profiles of each particular yeast strain.

In order to visualize the data displayed in table 3 and table 4, PCA was performed on a datamatrix consisting of the nine NEIPA

Table 5 **Levels of monoterpene alcohols in the NEIPA Beers produced with POF- ale yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)**

		Ale yeasts (POF -)									
		S33		S04		US05		K97		BE256	
		µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD
No°	MONOTERPENE ALCOHOLS										
1	linalool	100	4	106	6	106	3	104	6	110	6
2	p-menth-1-en-4-ol	2.8	0.2	2.9	0.3	2.5	0.2	2.5	0.3	2.5	0.3
3	α-terpineol	7.3	0.4	8.7	0.3	8.7	0.4	8.1	0.3	8.8	0.4
4	nerol	1.4	0.1	1.5	0.1	1.4	0.1	1.9	0.2	1.3	0.1
5	β-citronellol	16.5	2.2	10.8	0.5	8.2	1.2	19.5	1.5	11.1	0.5
6	*trans*-geraniol	98.8	6	115	5	119	3	105	4	109	6
	Sum monoterpene alcohols	**227**	11	**245**	14	**245**	7	**241**	14	**243**	13

beers as objects and fermentation-related volatiles as variables. Figure 2 displays the result of the analysis by plotting the first two principal components (PC1 and PC2), which together explain 70 % of the total variance. The beers produced with the lager yeast strains ('S189', 'S23') and the POF- ale yeast 'US05' (right side of the PCA bi-plot) are well separated from the other beers (left side of the bi-plot). Furthermore, the beers produced with the POF+ ale yeast strains ('BE134', 'T58') are distinguished from the beers produced with the POF- yeast strains ('BE256', 'S04', 'S33', 'K97') on account of 4VG, ethyl acetate, ethyl butanoate, and ethyl octanoate that are more characteristic for the POF+ ale yeast beers.

Interestingly, the separation of the beers on account of their fermentation related volatile fingerprint correlates well with the general basic sensory profiles of the different yeast strains (Table 1) and the result of sensory analysis of the nine NEIPA beers (Fig. 1). In other words, the base aroma profile of the yeast strains, i.e. the intrinsic flavour potential of the particular yeast strains, comes clearly to expression in the heavily hopped beers under investigation.

3.2.2 Hop-derived volatiles

In total, 41 hop volatiles were assigned upon analysis of all experimental NEIPA beers (see Tables 5–10). The volatile constituents were further classified into different chemical compound classes, that is monoterpene alcohols (6), esters (neryl and geranyl esters (6), isobutyric esters (5), miscellaneous esters (10)), ketones (6), and monterpene hydrocarbons (8). For the sesquiterpene hydrocarbon and oxygenated sesquiterpenoids group, the total levels were reported. Measurements of hop derived volatile thiols are not included in this study.

3.2.2.1 Monoterpene alcohols

The predominant volatiles in the group of the monoterpene alcohols are linalool and geraniol (see Tables 5 and 6). Their concentrations range from 100–119 µg/L and 84–119 µg/L, respectively. At these concentrations, a significant contribution of both components in terms of (rose-like) floral flavours can be expected in all of the experimental NEIPA beers (reported thresholds values for linalool and geraniol: 1–3 µg/L and 4–7 µg/L respectively [15]. Next, β-citronellol levels range from 6.4 µg/L (POF+ ale yeast beer 'BE 134') – 19.5 µg/L (POF- ale yeast beer 'K97'). At these concentrations, a direct contribution of β-citronellol to the hop derived aroma (lime-like citrus flavour) of most of the beers can be expected since its threshold is around 10 µg/L [33]. Moreover, as reported by *Takoi* et al. [47], the three aforementioned monoterpene alcohols act synergistically which may shift the resulting flavour into lime-like characteristics, depending on the actual levels and relative proportions of the compounds. It is unlikely that α-terpineol and nerol significantly contribute to the aroma of the experimental NEIPA beers since their estimated threshold values in beer are relatively high (350–2000 µg/L [47,48]).

Although the total level of the monoterpene alcohol group does not show highly pronounced variability, significant differences are observed for the levels of individual compounds between the beers. For example, the beers produced with the lager yeast strains ('S189', 'S23') contain higher linalool levels compared to the other beers, whilst the beers 'S04' and 'US05' have significant higher geraniol levels than the beers 'S23' and 'S33'.

Interestingly, high variation is observed in the β-citronellol levels of the beers produced with the different yeast strains. Clearly, the beers 'BE134' and 'T58' (both POF+ ale yeasts) contain the lowest β-citronellol levels (6.4 and 7.5 µg/L, respectively), whereas the lager strain beers ('S189', 'S23') have relatively high levels of this particular compound (15.9 and 13.8 µg/L, respectively). It has been reported in literature that β-citronellol is mainly produced during the fermentation and could result from the biotransformation of geraniol by yeast enzymes [32-33, 35]. However, it is also hypothesised only recently that the increase of β-citronellol during fermentation could be ascribed to de novo production by the yeast rather than by the reduction of geraniol [30]. Thus, the above observations point to yeast induced variability of the actual level of particular key-odorant monoterpene alcohols in the experimental NEIPA beers.

Taken together, the actual levels of flavour-active monoterpene alcohols in the final beers depend on many variables, such as the initial content present in the hops, but also yeast induced enzymatic processes which may result in reductions, cyclisation and release from volatile and non-volatile precursors. The aim of our study was

Table 6 **Levels of monoterpene alcohols in the NEIPA Beers produced with POF+ ale yeasts and POF- lager yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)**

		Ale yeasts (POF+)				Lager yeasts (POF-)			
		BE134		T58		S189		S23	
		µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD
No°	**MONOTERPENE ALCOHOLS**								
1	linalool	107	2	110	3	119	4	118	3
2	p-menth-1-en-4-ol	2.6	0.2	2.6	0.2	2.6	0.2	2.6	0.3
3	α-terpineol	8.3	0.3	8.3	0.2	9.4	0.4	9.0	0.3
4	nerol	1.4	0.1	1.8	0.2	2.3	0.2	1.8	0.3
5	β-citronellol	6.4	0.3	7.5	0.3	15.9	1.3	13.8	1.2
6	*trans*-geraniol	104	2	101	3	104	4	84	4
	Sum monoterpene alcohols	**230**	5	**232**	6	**253**	11	**229**	7

Table 7 Levels of esters in the NEIPA Beers produced with POF-ale yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)

		Ale yeasts (POF-)									
		S33		S04		US05		K97		BE256	
		µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD
No°	**ESTERS**										
	NERYL and GERANYL ESTERS										
9	neryl acetate	1.85	0.08	2.47	0.20	1.60	0.42	2.05	0.10	2.33	0.20
10	geranyl acetate	5.89	0.04	5.62	0.08	2.80	0.10	6.94	0.70	5.96	0.10
11	neryl propanoate	1.03	0.07	1.15	0.33	0.42	0.05	1.17	0.05	0.97	0.05
12	geranyl propanoate	1.09	0.10	0.26	0.05	0.11	0.01	1.19	0.10	0.46	0.19
13	neryl isobutyrate	2.70	0.12	3.35	0.06	1.39	0.07	3.07	0.32	2.25	0.37
14	geranyl isobutyrate	7.38	0.50	6.35	0.27	1.72	0.41	7.97	0.57	4.02	0.32
	Sum neryl and geranyl esters	**19.9**	1.0	**19.2**	1.0	**8.0**	2.0	**22.4**	2.0	**16.0**	2.0
	ISOBUTYRIC ESTERS										
15	isobutyl isobutyrate	42.0	2.1	36.5	2.6	27.5	3.2	42.1	1.1	33.9	3.5
18	isoamyl isobutyrate	51.9	3.2	34.3	1.1	28.0	3.5	53.7	2.0	36.6	2.5
19	2-methylbutyl isobutyrate	54.9	3.1	36.9	2.9	34.7	5.2	53.1	2.2	41.5	2.3
16	butyl isobutyrate	2.6	0.1	2.3	0.3	2.1	0.3	2.8	0.1	2.0	0.2
23	hexyl isobutyrate	7.2	0.3	3.9	0.2	3.4	0.2	7.5	0.2	4.5	0.3
	Sum isobutyric esters	**159**	8	**114**	6	**96.0**	7.0	**159**	6	**118**	7
	MISCELLANEOUS										
7	*cis*-methyl geranate	1.5	0.1	1.1	0.1	0.9	0.1	1.1	0.1	0.9	0.1
8	*trans*-methyl geranate	65.7	4.2	62.4	3.8	60.0	3.4	66.8	4.2	58.6	3.2
17	2- and 3-methylbutyl propanoate	5.1	0.2	3.3	0.2	2.6	0.3	5.2	0.2	3.5	0.3
20	methyl 4-ethyl-4-pentenoate	9.3	0.4	8.6	0.8	6.2	0.6	9.3	0.4	7.5	0.6
21	2-methylbutyl 2-methylbutyrate	1.8	0.2	1.5	0.1	1.3	0.1	1.9	0.2	1.5	0.1
22	2-methylbutyl 3-methylbutyrate	5.0	0.2	4.2	0.2	3.4	0.1	5.1	0.3	4.0	0.2
24	methyl octanoate	0.16	0.05	0.11	0.02	0.07	0.01	0.09	0.01	0.10	0.02
25	ethyl heptanoate	0.77	0.02	0.89	0.02	0.66	0.09	0.77	0.06	0.85	0.02
26	ethyl *trans*-4-decenoate	12.4	0.6	12.8	0.4	5.6	0.2	14.4	0.7	13.1	0.4
27	ethyl *cis*-4-decenoate	3.9	0.3	4.4	0.4	5.1	0.5	5.0	0.2	3.2	0.2
	Sum miscellaneous esters	**106**	6	**99**	5	**86**	5	**110**	6	**93**	4

not to unravel underlying mechanisms but to generate analytical data in support of the results of sensory evaluations. The results however point to the impact of the yeast strain on the final level of flavour-active monoterpene alcohols in the produced beers and, consequently, to the impact the yeast strain may have on the hop flavour expression in beer. Supported by the moderate correlation (R = 0.685) between the geraniol and β-citronellol content for the beers produced with POF- ale yeasts, we could speculate that biotransformations of monoterpene alcohols, in particular geraniol into β-citronellol, occured here.

3.2.2.2 Hop-derived Esters

A high number of hop-related esters (21) was detected in the volatile fraction of the experimental beers. They were grouped in different chemical compound classes, i.e. geranyl esters, isobutyric esters and a group labeled 'miscellaneous' comprising several saturated, unsaturated branched esters and methyl esters (see Table 7 and Table 8, see page 35). The composition of the different chemical compound classes is further discussed below.

Geranyl esters

Geranyl esters (e.g. geranyl acetate, geranyl isobutyrate) have been considered as aroma impact compounds for the floral hop aroma of beer. Besides, several studies reported the hydrolysis of geranyl esters during the fermentation as a source of free geraniol in finished beers [16-17, 37, 48]. We were able to detect both neryl and geranyl esters in the NEIPA beers. Interestingly, the levels of geranyl acetate and, in particular, geranyl isobutyrate, show high variability between the beers. Geranyl isobutyrate levels are significant lower in the beers brewed with the lager yeast strains ('S189', 'S23') and the POF- ale yeast US05 (average level: 1.70 µg/L) in comparison with the beers produced with

Table 8 **Levels of esters in the NEIPA Beers produced with POF+ ale yeasts and POF- lager yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)**

		Ale yeasts (POF+)				Lager yeasts (POF-)			
		BE134		T58		S189		S23	
		µg/L	SD	µg/L	SD	µg/L	SD	µg/L	SD
No°	ESTERS								
	NERYL and GERANYL ESTERS								
9	neryl acetate	2.21	0.20	2.04	0.20	2.99	0.10	2.62	0.20
10	geranyl acetate	6.38	0.22	5.81	0.22	5.98	0.19	5.58	0.19
11	neryl propanoate	0.92	0.04	1.13	0.06	0.86	0.06	0.54	0.06
12	geranyl propanoate	0.93	0.06	0.95	0.09	0.16	0.02	0.13	0.04
13	neryl isobutyrate	1.62	0.21	1.88	0.30	1.96	0.12	1.21	0.01
14	geranyl isobutyrate	3.65	0.39	4.32	0.58	1.94	0.11	1.40	0.10
	Sum neryl and geranyl esters	**15.7**	2.0	**16.1**	2.0	**13.9**	2.0	**11.5**	2.0
	ISOBUTYRIC ESTERS								
15	isobutyl isobutyrate	40.4	1.4	43.0	2.6	40.0	1.7	39.4	2.0
18	isoamyl isobutyrate	51.1	2.4	49.9	1.2	39.2	1.5	38.2	1.6
19	2-methylbutyl isobutyrate	55.9	4.5	54.9	3.1	43.3	2.6	43.4	3.1
16	butyl isobutyrate	2.8	0.2	3.0	0.1	2.6	0.1	2.5	0.1
23	hexyl isobutyrate	6.1	0.3	6.3	0.2	4.4	0.2	4.2	0.3
	Sum isobutyric esters	**156**	7	**157**	7	**129**	6	**128**	6
	MISCELLANEOUS								
7	*cis*-methyl geranate	0.8	0.1	1.0	0.1	1.1	0.1	1.0	0.1
8	*trans*-methyl geranate	59.8	2.8	66.6	4.3	72.7	3.4	65.8	3.1
17	2- and 3-methylbutyl propanoate	5.6	0.2	5.7	0.1	3.8	0.1	4.1	0.2
20	methyl 4-ethyl-4-pentenoate	9.4	0.4	10.5	0.4	9.4	0.3	9.7	0.2
21	2-methylbutyl 2-methylbutyrate	1.5	0.1	1.5	0.1	1.6	0.1	1.5	0.1
22	2-methylbutyl 3-methylbutyrate	4.1	0.2	4.0	0.1	4.3	0.2	3.7	0.2
24	methyl octanoate	0.20	0.01	0.10	0.01	0.11	0.01	0.13	0.01
25	ethyl heptanoate	0.82	0.02	0.80	0.03	1.1	0.02	1.1	0.02
26	ethyl *trans*-4-decenoate	11.6	0.2	11.3	0.6	14.2	0.8	11.3	0.7
27	ethyl *cis*-4-decenoate	5.6	0.3	4.4	0.2	17.4	0.3	12.8	0.3
	Sum miscellaneous esters	**99**	4	**106**	7	**126**	6	**111**	5

the ale yeasts ‘S33’, ‘S04’, ‘K97’ (average level: 7.2 µg/L). The beers with the POF+ yeast strains contain intermediate levels (4.0 µg/L). At these levels, a direct impact to the flavour of the experimental NEIPA beers is not expected because of the relatively high treshold levels of the neryl and geranyl esters (e.g. threshold geranyl isobutyrate: 200–450 µg/L [16-17]). Given the fact that hop aromatising was done in the same way, hydrolysis of geranyl isobutyrate during fermentation has been reported in literature, and similar levels are observed for related yeast strains (e.g. lager yeasts and POF+ ale yeast) in our study, this observation points once again to the impact of the yeast strain on the hop derived beer flavour profile.

Isobutyric esters

Isobutyric esters are present in relatively high levels in hop essential oils and have been considered as marker compounds to distinguish between hop varieties [49]. The transfer rates of the isobutyric esters from hops to beer is high (up to 80 % in dry-hopped beers [50]) and, consequently, significant levels can be found in late and dry hopped beers.

Isobutyl isobutyrate, isoamyl isobutyrate and 2-methylbutyl isobutyrate are the predominant compounds in the hop related ester fraction of the experimental NEIPA beers (see Table 7 and Table 8). At the reported levels they may impart fruity character (apple, apricot-like) to the beers. The content of the esters in the different beers varies significantly, which is apparent from the total level of isobutyric esters ranging from 96 µg/L (beer ‘US05’) to 159 µg/L (beers ‘S33’ and ‘K97’). The high variation could possibly be explained by transesterifications of hop oil esters during fermentation leading to new, potentially flavour-active compounds as hypothesised by Takoi et al. [51] recently. To date, the knowledge on this particular item is far from complete and requires further investiga-

Table 9 **Levels of ketones, monoterpene and sesquiterpene hydrocarbons and oxygenated sesquiterpenoids in the NEIPA Beers produced with POF- ale yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)**

		Ale yeasts (POF-)									
		S33		**S04**		**US05**		**K97**		**BE256**	
		µg/L	**SD**	**µg/L**	**SD**	**µg/L**	**SD**	**µg/L**	**SD**	**µg/L**	**SD**
No°	**KETONES**										
28	2-nonanone	2.62	0.14	3.48	0.10	1.82	0.32	2.70	0.36	2.29	0.10
29	2-decanone	2.85	0.18	3.27	0.07	2.04	0.20	3.02	0.27	2.45	0.03
30	2-undecanone	20.1	0.6	20.6	1.4	12.9	0.2	21.2	0.4	16.3	0.0
31	2-dodecanone	1.67	0.13	1.81	0.17	0.93	0.17	1.87	0.29	1.26	0.07
32	2-tridecanone	5.14	0.47	6.55	0.34	2.48	0.66	5.50	1.37	3.72	0.45
	Sum methyl ketones	**32.4**	1.0	**35.7**	2.40	**20.2**	1.00	**34.3**	0.60	**26.0**	0.10
33	β-damascenone	7.8	0.7	9.5	0.1	7.5	0.5	6.4	0.5	7.3	0.8
	MONOTERPENE HYDROCARBONS										
	α-pinene	3.4	0.4	2.5	0.2	1.3	0.2	3.9	0.4	1.7	0.2
	β-pinene	3.0	0.4	2.0	0.1	1.0	0.1	3.4	0.4	1.2	0.1
34	β-myrcene	295	38	200	18	106	8	342	48	132	12
	limonene	5.4	0.4	3.6	0.3	2.8	0.1	6.0	0.5	3.2	0.1
	cis-ocimene	0.3	0.1	0.2	0.0	0.1	0.0	0.2	0.0	0.2	0.0
	trans-ocimene	0.6	0.1	0.5	0.1	0.4	0.1	0.7	0.1	0.4	0.1
	γ-terpinene	0.5	0.1	0.3	0.1	0.2	0.0	0.5	0.1	0.2	0.1
	terpinolene	1.2	0.1	0.9	0.1	0.8	0.1	1.2	0.1	0.7	0.1
	Sum monoterpene hydrocarbons	**309**	40	**210**	19	**113**	9	**358**	50	**140**	13
	SESQUITERPENE HYDROCARBONS										
	Total level	162	6	315	2	42	3	144	7	111	3
	OXYGENATED SESQUITERPENOIDS										
	Total level	34	2	32	3	27	2	38	1	36	3

tion. The esterification reactions and the extent to which they occur are however (at least partly) explained by yeast esterase activity. It can therefore be presumed that the yeast strains in our study differ in esterase activity. This once again points to the impact of the yeast strain on the final (hoppy) beer flavour.

Miscellaneous esters

The group of miscellaneous esters (Table 7, Table 8) contains volatiles which are originally prestent in hop essential as well as volatiles which originate from the transformation of hop oil constituents during fermentation [10, 33]. Ethyl heptanoate, ethyl *cis*- and *trans*-4-decenoate are volatiles which have been reported as biotransformation products from the precursors methyl heptanoate and methyl 4-decenoate, respectively. The total level of the miscellaneous ester group ranges between 86 µg/L (beer 'US05') and 126 µg/L (beer 'S189'). The significant differences in the total level are mainly ascribed to the varying levels of the predominant volatile compound *trans*-methyl geranate .

3.2.2.3 Ketones, mono- and sesquiterpene hydrocarbons, sesquiterpenoids

Further indications for (yeast-induced) differences between the beers are found in the ketone, terpene hydrocarbon and oxygenated sesquiterpenoid group (see Table 9 and Table 10, see page 37). The most striking observation is the high variability of the β-myrcene level between the beers. β-myrcene is the predominant compound in hop essential oil and is regarded as a flavour-impact compound in hops and dry hopped beers (estimated threshold in beer: 13 – 100 µg/L; resinous, citrussy, geranium-like flavours). The β-myrcene levels in the NEIPA beers in our study range between 75 µg/L (lager yeast 'S23') and 295 µg/L (ale yeast 'S33'). At these levels, a contribution of β-myrcene to the overall beer flavour is very likely and, moreover, the significant differences in the levels can also provoke flavour differences between the beers. According to *King* and *Dickinson* [32], the major terpene hydrocarbons in hop essential oil (i.e. β-myrcene, α-humulene and β-caryophyllene) are however not transformed by the yeast during fermentation. In view of the potential impact the yeast might have, the highly fluctuating β-myrcene levels might be explained by variable adsorptions of this highly apolar volatile compound to the yeast biomass of which the characteristics are yeast strain dependent [20, 30, 32]. By extension, this assumption could also explain the high variability in total content observed for the monoterpene and sesquiterpene hydrocarbon group.

3.2.2.4 Principal component analysis

To further explore and gain insight into the data set in tables 5 – 10, PCA was performed on a data matrix comprising nine NEIPA beers produced with the different yeast strains as objects and all

Table 10 **Levels of ketones, monoterpene and sesquiterpene hydrocarbons and oxygenated sesquiterpenoids in the NEIPA Beers produced with POF+ ale yeasts and POF- lager yeasts (mean of triplicate analyses; SD: standard deviation; No°: Compound number in accordance with numbering in Fig. 3)**

		Ale yeasts (POF+)				**Lager yeasts (POF-)**			
		BE134		**T58**		**S189**		**S23**	
		µg/L	**SD**	**µg/L**	**SD**	**µg/L**	**SD**	**µg/L**	**SD**
No°	**KETONES**								
28	2-nonanone	3.35	0.18	3.91	0.00	3.54	0.01	3.56	0.10
29	2-decanone	2.96	0.12	3.50	0.06	3.33	0.01	3.15	0.04
30	2-undecanone	16.4	0.3	18.8	0.9	18.8	0.4	15.7	0.2
31	2-dodecanone	1.06	0.06	1.19	0.12	1.21	0.07	0.82	0.01
32	2-tridecanone	2.47	0.35	3.06	0.52	2.83	0.19	1.84	0.10
	Sum methyl ketones	**26.3**	0.10	**30.5**	1.50	**29.7**	0.60	**25.0**	0.40
33	β-damascenone	8.4	0.7	10.6	0.9	14.0	0.5	11.9	0.5
	MONOTERPENE HYDROCARBONS								
	α-pinene	1.8	0.2	1.5	0.1	1.7	0.1	0.7	0.1
	β-pinene	1.5	0.1	1.6	0.1	1.3	0.1	1.0	0.1
34	β-myrcene	124	8	128	14	123	15	75	4
	limonene	3.3	0.1	3.7	0.3	3.9	0.2	3.2	0.2
	cis-ocimene	0.2	0.0	0.3	0.0	0.3	0.0	0.3	0.0
	trans-ocimene	0.5	0.1	0.6	0.1	0.6	0.1	0.6	0.1
	γ-terpinene	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1
	terpinolene	0.8	0.1	0.8	0.1	0.9	0.1	0.8	0.1
	Sum monoterpene hydrocarbons	**133**	9	**137**	15	**132**	16	**82**	5
	SESQUITERPENE HYDROCARBONS								
	Total level	38	2	106	3	44	2	44	2
	OXYGENATED SESQUITERPENOIDS								
	Total level	39	3	40	2	52	1	37	3

assigned volatiles in the monoterpene alcohol, ester, ketone group and β-myrcene as marker compound for the terpene hydrocarbon group, as variables. Figure 3 displays the result of the analysis by plotting the first two principal components (PC1 and PC2), which together explain 69 % of the total variance.

The bi-plot of PCA clearly visualises differences and similarities between the beers on the one hand, and correlations between beers and particular hop oil volatiles on the other. As a result, we are able to distinguish four groups of beers in that high similarity in the analytical hop oil fingerprint was found for the beers produced with (1) the POF- ale yeasts ‘S33’ and ‘K97’, (2) the lager yeast strains ‘S189’ and ‘S23’, (3) the POF+ ale yeasts (‘T58’, ‘BE134’) and POF- ale yeast ‘S04’, and to a lesser extent, (4) the ale yeasts ‘US05’ and ‘BE256’ respectively.

Clearly, beers ‘S33’ and ‘K97’ are rich in hop volatiles amongst which geranyl isobutyrate (14), neryl propanoate (11), geranyl propanoate (12), neryl isobutyrate (13), 2-methylbutyl 2-methylbutyrate (21), 2-methylbutyl 3-methylbutyrate (22), hexyl butyrate (23), 2-dodecanone (31), 2-tridecanone (32), β-myrcene (34) are more characteristic compared to the other beers. The hop derived volatile fingerprint of the beers produced with the lager yeast strains contains lower levels of hop derived volatile esters compared to beers ‘S33’ and ‘K97’ and has linalool (1), α-terpineol (3), ethyl heptanoate (25), ethyl *cis*-4-decenoate (27), and β-damascenone (33) as more characteristic compounds in the profile. Clearly the group consisting beers ‘US05’ and ‘BE256’ contains the least characteristic volatiles.

In conclusion, the analytical hop volatile fingerprints of the nine NEIPA beers allow us to discriminate the beers. Since the yeast strain used in the production of the beers was the only variable, it can be proposed that the differences arise from the impact the yeasts have on the composition of the hop volatile fraction of the final beers. Although no repetitions of the fermentations with the yeasts were performed, the observed differences in hop volatile profiles of the beers are significantly higher than one can expect from inter brew-variability for a particular beer [52]. The positioning of genetic related yeast strains in the same clusters (e.g. the 2 lager yeast strains and the POF+ yeast strains) further indicates that the observed analytical changes in the hop volatile profile are indeed yeast induced.

3.3 Comparison of sensory and analytical profiles

The separation of the beers on account of their fermentation related volatile fingerprint (Fig. 2) correlates well with the general basic sensory profiles of the different yeast strains (Table 1) and the result of sensory analysis of the nine NEIPA beers (Fig. 1). This demonstrates that the intrinsic flavouring potential of the particular yeast strains used for fermentation, can come clearly to expression,

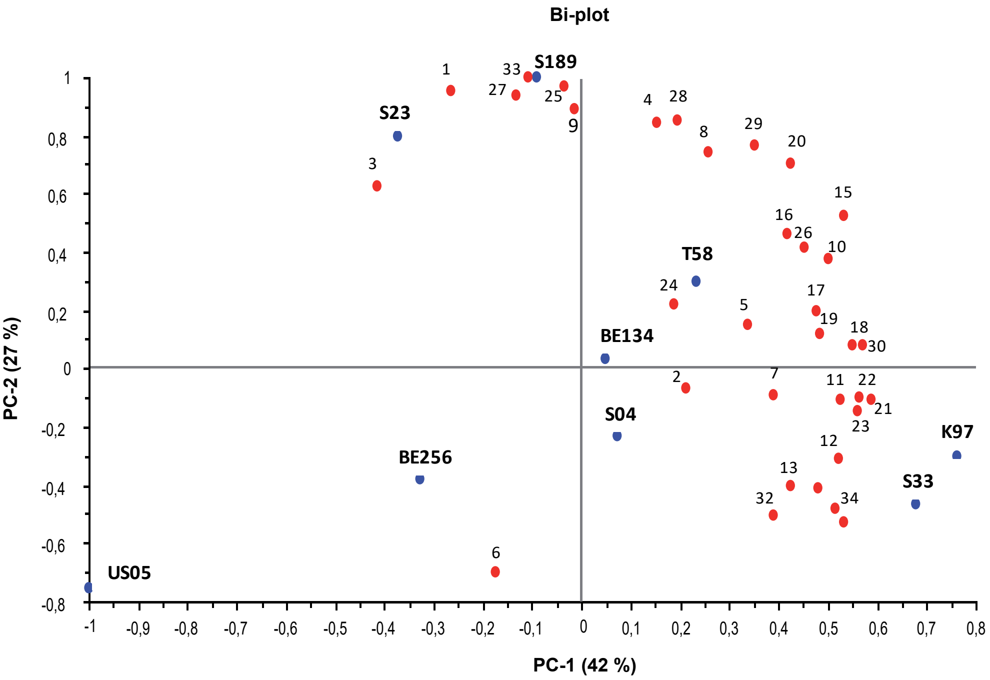

Fig. 3 Bi-plot of principal component analysis on NEIPA beers fermented with different yeasts (objects) and hop derived volatiles (variables). Numbering of the variables in accordance with the numbering in tables 5–10

even in heavily hopped beers such as the experimental NEIPA beers under investigation.

When comparing the results from the analytical hop oil fingerprinting (Fig. 3) with the sensory profiling (Fig. 1) some similarities are observed. For example, the beers produced with the yeast strains ‘K97’ and ‘S33’ have highly intense fruity, hop related flavours (‘hop citrussy’, ‘hop tropical’, ‘hop overall fruity’, ‘overall hoppy intensity’) from a sensory perspective and are found to be the beers with the highest level and number of hop volatiles with fruity odour characteristics. This demonstrates that the hop flavour comes to expression in these particular beers. The interplay between the hop flavours and the fruity esters from fermentation (see section 3.2.1) could further explain the high correlation of those beers with overall fruity perception. A correlation between sensory and analytical data is also found for the beers produced with the lager yeast strains ‘S23’, ‘S189’ in that they cluster and have less intense fruity flavours.

However, the analytical hop profiles do not always correlate with sensory impressions and thus are not always an accurate predictive analytical tool in view of hop aroma expression in the final beers. This is apparent from the position of the beers produced with the POF+ yeast strains ‘T58’ and ‘BE134’ in figure 1 and figure 3. Indeed, intense hoppy flavours are to be expected in the beers on analytical grounds, however, these flavours are clearly suppressed by highly flavour-active compounds (phenolic compounds) produced by these particular yeast strains.

4 Conclusions

New England IPA style beers brewed under the same conditions with the yeast strain as only variable were sensorially and analytically distinguished. The quality and intensity of fruity flavours in the final beers differed, depending on the yeast strain used for fermentation. Neutral yeast strains producing relatively low levels of flavour-active fermentation compounds are not necessarily the best in view of hoppy aroma intensity of the produced beers, whilst yeasts producing significant levels of volatiles with fruity character can boost hop fruity flavours.

Furthermore, analytical results point into the direction of particular fermentation by-products and yeast strain dependend alterations of the hop volatile fingerprint as explanations for the differences in both quality and intensity of the (fruity) flavours of the experimental NEIPA beers. In conclusion, the role fermentation flavouring components produced by the yeasts play in the overall flavour perception of heavily hopped beers must not be underestimated. The level and or variety of fruity compounds derived from fermentation as a consequence of the metabolism of a given yeast must be considered as an elementary factor for overall fruity flavours in hopped beer. Moreover, the quality and intensity of the fruity flavour can also be influenced by yeast strain induced alterations of the level of flavour-active hop derived volatiles.

Yeasts are a very effective raw material for further beer flavour diversification. By thoughtfully choosing yeast strains brewers can accentuate the fruity flavour profile of heavily hopped beers with tropical and citrus aromas.

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