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Characterization of the terpenoids composition of beers made with the French hop varieties: Strisselspalt, Aramis, Triskel and Bouclier

Among the various compounds brought in to beer by hop, mono- and sesqui- terpenoids are the most studied and contribute to a certain extent to the hoppy aroma of beer. Previous studies have indicated that the concentration of these compounds in beer depends on the hop variety. The impact of new hop varieties created by the French hop growers association and their cooperative in comparison to the old variety Strisselspalt on the terpenoid content in beer has been analyzed by Stir-Bar Sorptive-Extraction-Gas-Chromatography (SBSE-GC-MS). Our results reveal that the new variety Aramis has a similar terpenoid profile to the Strisselspalt variety, both showing a similar content of sesquiterpenoids. In comparison, the new variety Bouclier was rather low in sesquiterpenoid content whereas Triskel showed the highest concentration of monoterpenoids, especially linalool.

Descriptors: hop, variety, volatile compounds, terpenoids, aroma

1 Introduction

Compared to other beverages, beer is characterized by its aroma and its bitterness. Both of these traits are influenced by the hops used. Brewers classify hops in to two groups: bittering hops, which are rich in α- and β-acids, and finishing hops or aroma hops [1].

Alsace is by far the main hop producing region in France. Alsatian hops have been used in breweries since 1770–1780 [2]. Reference to the famous Strisselspalt variety can be traced back to 1880 [2], making it one of the oldest aroma hop varieties known. Today, many international breweries continue to use Strisselspalt to produce a beer with a typical hop aroma.

In 2001 the French hop growers association and their cooperative (Hochfelden (67), France) began a breeding program using Strisselspalt as progenitor and selected new varieties based on their aroma profile, pathogen resistance and α-acids levels. This program created the new varieties Aramis, registered in 2011, and Triskel and Bouclier registered in 2012 which are proposed as both bittering and aroma-type hops.

Since 1903 and the work of *Chapman* [3], several reports have indicated the range of hop volatile compounds [3–10]. However, while it is possible to identify hundreds of compounds in hop oil [3–5], only a few of these specific compounds have been detected in beer [6–10]. Amongst them, monoterpenoids and sesquiterpenoids are considered to be associated with the "hoppy" aroma of beer [7, 8, 11, 12]. Of the many methods available to determine the concentration of these compounds in beer, the Stir-Bar-Sorptive-Extraction-Gas-Chromatography (SBSE-GC-MS) is both simple and very sensitive [13]. It has been used to measure traces of volatiles in numerous beverages [14]. In previous studies, *Kishimoto* et al. used SBSE-GC-MS to measure various terpenoids in beer made with the hop varieties Saaz, Tettnang, and Hersbrucker [6, 8] and to investigate the influence of each variety on the beer aroma.

In this study, we analyzed and compared the impact of the Strisselspalt hop variety with the newly bred Aramis, Triskel and Bouclier hop varieties on the terpenoid content in beer using SBSE-GC-MS. We also investigated the role of these compounds in flavor perception relative to their perception thresholds.

2 Materials and methods

2.1 Brewing Process

To investigate the volatile contents of different hop varieties, Strisselspalt, Aramis, Bouclier and Triskel hops were brewed in 20 hL volumes. Hop oils content, α-acids and polyphenol content of these varieties are given table 1. The wort was prepared using commercially available malts following a CMBO (Comité Malt Bière Orge) brewing process. Malt (300 kg) was brewed with water (ratio malt/water: 1/3) to obtain wort under the following brewing protocol: 0–20 min, 50 °C; 20–34 min, 50–64 °C; 34–45 min, 64 °C; 45–55 min, 64–74 °C; 55–80 min, 74 °C; 80–85 min, 74–

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78 °C. Wort was boiled (1 h, 100 °C) and after clarification, the original gravity was 12 °P. Bittering hops were added at the beginning of the boiling step to reach a bitterness of 30 EBU and aroma hops, 5 min before the end of the boiling step.

Table 1 Hop analysis

	Strisselspalt	Aramis	Triskel	Bouclier
Hop oil content (ml/100g)	0.8	1.6	2.0	1.6
α-acids	3.4 %	8.1 %	9.5 %	8.7 %
Polyphenol	4.1 %	3.8 %	4.2 %	4.7 %

For the beer made with Strisselspalt, Magnum (15 % of α-acids) was substituted for Strisselspalt at the beginning of the boiling process because of the lower α-acids content of Strisselspalt (Table 2). After cooling, the fermentation was started by adding lager yeast to the cooled wort. The fermentation temperature was maintained at 12 °C until 5–6 °P when it was increased to 14 °C until the end of the maturation and then maintained at –2 °C for 5 days. Filtration was carried out with Kieselguhr and 50 g/hL of PVPP. Bottling used pilot-scale equipment and bottles were flash pasteurized. Hop recipes and standard analyses of beer are presented table 2.

2.2 Volatile compounds analysis

Analyses of the volatiles of the four beers were done by Twistaroma (Colmar, France) using the Stir Bar Sorptive Extraction method [15] adapted to our laboratory conditions, with a 1 µL injection volume. Each beer was analyzed in duplicate. All reagents 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) and helium was used as carrier gas (1 mL min^{-1}, constant flow).

Agilent MSD ChemStation software (G1701DA, Rev D.03.00) was used for instrument control and data processing. The mass spectra were compared with the Wiley's library reference spectral bank, Retention Index (RI) and standard when available. Sesquiterpenoids were semi-quantified using the ratio of their Total Ion Current peak to that of the 3-octanol (final concentration of 84 µg/L). Quantification was performed for linalool, α-terpineol, nerol, geraniol and limonene, myrcenol was quantified in geraniol equivalent. Peak areas were normalized using 3-octanol as an internal standard. Calibration factors were determined using the standard addition method and creating linear regression models. Quantification was carried out in SCAN mode.

Statistical analyses were done on MINITAB 16.0, R and the FactoMineR package [16].

Table 2 Hop recipes and beer classical analysis

Process	EBC Method	Units	Hop varieties			
			Strisselspalt	Aramis	Triskel	Bouclier
Start of the boil		g/hL	62.9 (Magnum)	71.1	48.95	58.85
Bitterness in wort	9.8	EBU	40.6	24.8	20	22
5 min before end of the boil		g/hL	160	160	160	160
Bitterness	9.8	EBU	11.4	27.2	32	30
Measurement						
Original Gravity		°P	12.0	11.7	12.4	12.2
Alcohol	9.4	%vol	4.76	4.91	5.19	5.44
Apparent Extract	9.4	°P	2.71	2.22	2.31	1.74
Real Extract	9.4	°P	4.41	3.97	4.13	3.65
Color	9.6	EBC	9.9	8.6	7.6	8.6
Bitterness in beer (target : 30 EBU)	9.8	EBU	30	38	34	32

3 Results and discussion

Beer brewed with either the old hop variety Strisselpalt or one of the newly bred hop varieties, namely Aramis, Triskel and Bouclier were analyzed by SBSE-GC-MS and the impact of hop variety on terpenoids concentration and composition was compared.

3.1 Mono and sesquiterpenoid profile of the four beers

In the four beers produced with the new varieties Aramis, Triskel and Bouclier and the old variety Strisselspalt, five mono- and seven sesqui- terpenoids were detected (Fig. 1 and Fig. 2).

All the five monoterpenoids quantified in the analyzed beers have been reported previously in both hop [22, 23] and beer [6, 7, 17–19] with concentrations in the same range as in the present study (Table 3). In the present work, neither limonene nor myrcene were detected.

Among the seven sesquiterpenoids found in this study, six were identified through their spectral signature and have already been reported in hop [7, 22–25] but one compound remained unidentified. The sesquiterpenoid γ-eudesmol was the only compound detected in all the beers.

Beers produced with Aramis and Strisselspalt showed similar trends in their terpenoid profiles. Indeed the majority of the monoterpenoid and sesquiterpenoid concentrations measured in these two beers were statistically equal (Fig. 1 and Fig. 2). Only the concentration of α-cadinol was higher in Aramis than in Strisselspalt. Moreover, α-, β- eudesmol, α-cadinol, juniper camphor

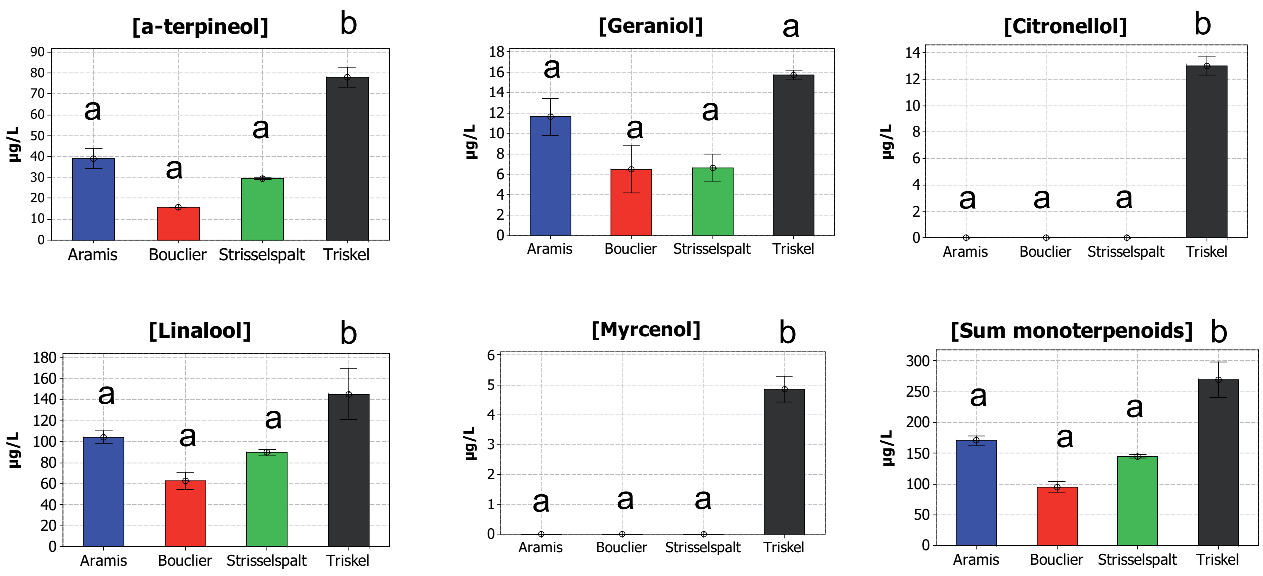

Fig. 1 **Monoterpenoid concentrations in the four beers brewed with Aramis, Bouclier, Strisselspalt and Triskel hop varieties**

Results are expressed as mean ± SEM of 2 different experiments; a: concentration statistically equal to Strisselspalt (Dunnetts' test; p-value < 0,05) b: concentration statistically different from Strisselspalt (p-value > 0,05)

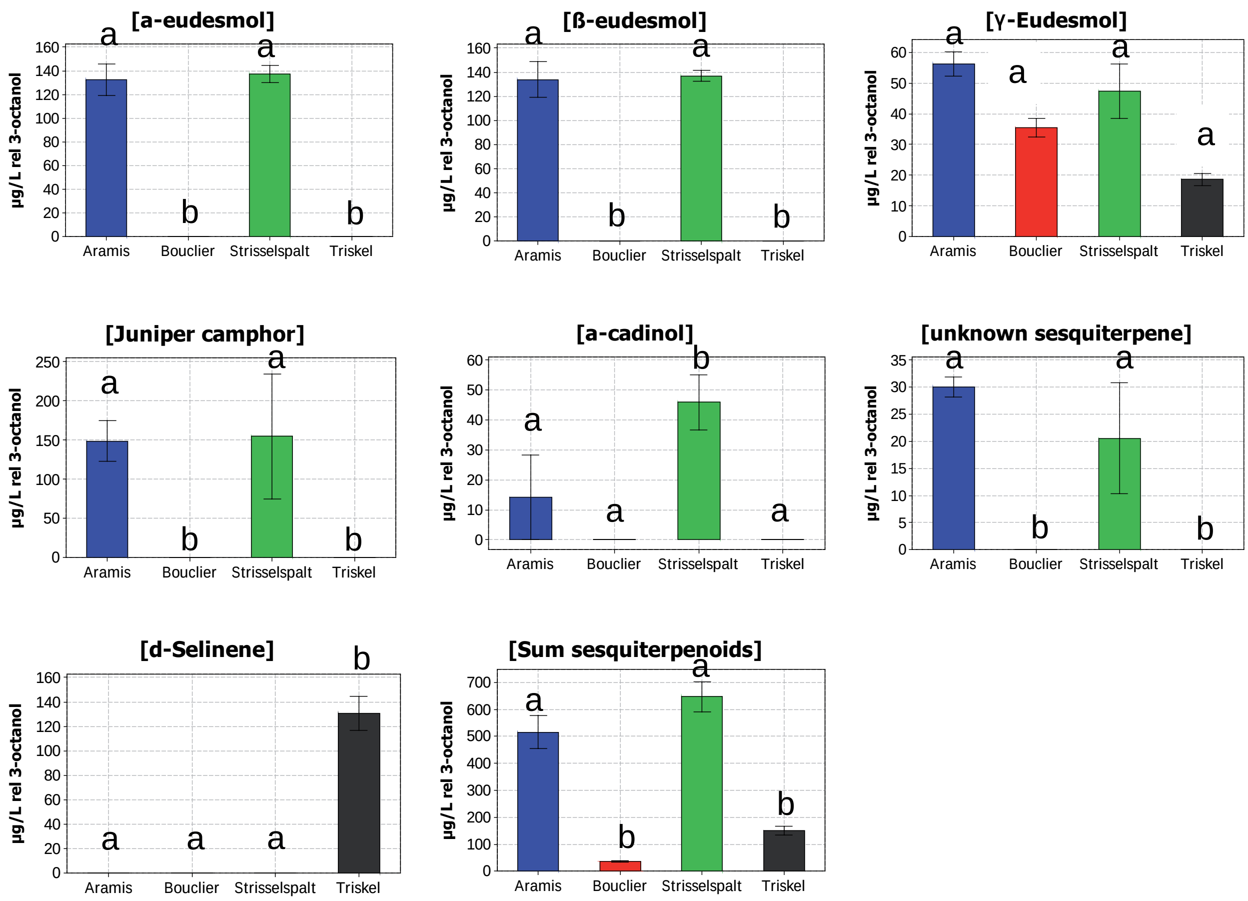

Fig. 2 **Sesquiterpenoid concentrations in the four beers brewed with Aramis, Bouclier, Strisselspalt and Triskel hop varieties**

Results are expressed as mean ± SEM of 2 different experiments. a: concentration statistically equal to Strisselspalt (Dunnetts' test; p-value < 0,05) b: concentration statistically different from Strisselspalt (p-value > 0,05)

Table 3 Odor Activity Values (OAV), thresholds, minimum and maximum concentrations in beer; presence in hop and odor quality of the volatile compounds influenced by the hop variety in beer

Volatile compounds	OAV Stris	Ara	Tris	Bouc	Min-max in beer	Threshold (µg/L)	reported in hop	Odor quality
Monoterpenoids								
α-terpineol	0.10	0.13	0.26	0.05	0.00–0.25[a]	300[f]	j	lilas, floral[n]
Citronellol	0.00	0.00	**2.61**	0.00	0.20–18[a]	5[g]	j	green, citrus, fresh[n]
Geraniol	0.18	0.32	0.44	0.18	0.03–2.50[a]	36[h]	j	geranium,rose, floral[n]
Linalool	**3.33**	**3.86**	**5.39**	**2.32**	0.04–17.41[b]	27[h]	j	citrus, floral, sweet, grape-like[o]
Myrcenol					Detectablec	NT	j	fresh, floral[p]
Sesquiterpenoids								
α-Eudesmol					(1–100µg/L)d	NT	k	
β-Eudesmol	0.01	0.01	< 0.001	< 0.001	0.001–0.006[e]	10 000[i]	k	sweet, fruity, herbaceous[q]
δ-Selinene						NT	l	
γ-Eudesmol						NT	m	
Junipercamphor						NT	m	
α-cadinol						NT	j	fruity[r]

NT: No Threshold value; Stris: Strisselspalt ; Ara: Aramis; Tris: Triskel ; Bouc: Bouclier; OAV: Odor Activity Value : concentration/perception threshold a: [7, 17] ; b: [6, 7, 17, 18]; c: [19]; d: [17]; e: [7, 8]; f: [20]; g: [10]; h: [17]; i: [21] ; j: [22, 23]; k: [7, 22, 24]; l: [22]; m: [22, 25]; n: [26]; o: [27]; p: [28]; q: [8]; r: [29]

and an unknown sesquiterpene were only detected in these two beers.

Beer produced with Triskel showed a different terpenoid profile with the presence of citronellol, myrcenol and δ-selinene which were not detected in the three other beers.

Triskel hop variety was the highest producer of monoterpenoids in beer followed in order by Aramis, Strisselspalt and Bouclier varieties (Fig. 1). The beer produced with Bouclier showed the lowest concentration of mono- and sesqui-terpenoids. The beer made with this variety was not distinguished by any specific compound.

3.2 Principal Component Analysis

Mono- and sesqui- terpenoid concentrations were analyzed by Principal Component Analysis (PCA) (Fig. 3). More than 88 % (PC1: 64 %; PC2: 24 %) of the variance observed in the four beers could be explained by this analysis.

The first axis (PC1) of the PCA separated varieties according to their concentrations of monoterpenoids relative to sesquiterpenoids. Triskel hop produced high concentrations of monoterpenoids, but ~~a~~ low concentrations of sesquiterpenoids. In contrast, Aramis and Strisselpalt hops gave beers with higher concentrations of sesquiterpenoid compounds and lower concentrations of monoterpenoid compounds (Fig. 3).

The second axis (PC2) separated the hop varieties according to their total concentration of both mono- and sesqui- terpenoids. Bouclier produced less of these compounds in comparison to the three other varieties (Fig. 3).

3.3 Influence of the variety in the aroma profile

Strisselspalt and Aramis hop varieties emerged as high producers of sesquiterpenoids in beer in comparison to Triskel and Bouclier. Unfortunately, perception thresholds of these compounds in beer are not known, except for β-eudesmol (10 000 µg/L [21]). However the concentrations of this compound in the beers in our study (Fig. 1), and as reported in the literature, were less than one µg/L suggesting that this compound may have little or no effect on the aroma of beer. Nevertheless, *Goiris* et. al. [11] showed that the sesquiterpene fraction of hop oil is associated with the "spicy or herbal hoppy beer character". Thus the results here, demonstrating that Strisselspalt and Aramis hop varieties are high producers of sesquiterpenoids, could suggest that they may contribute to such spicy/herbal flavors in beer.

Our results indicated that Triskel hop variety produced the highest content of linalool in beer among the four hops tested, with an Olfactive Activity Value (OAV = concentration divided by the perception threshold) of 5.39 (Table 3). Since monoterpenoids, and especially linalool, have been described in many other studies as responsible for hop floral aroma in beer [6, 10, 30], Triskel could

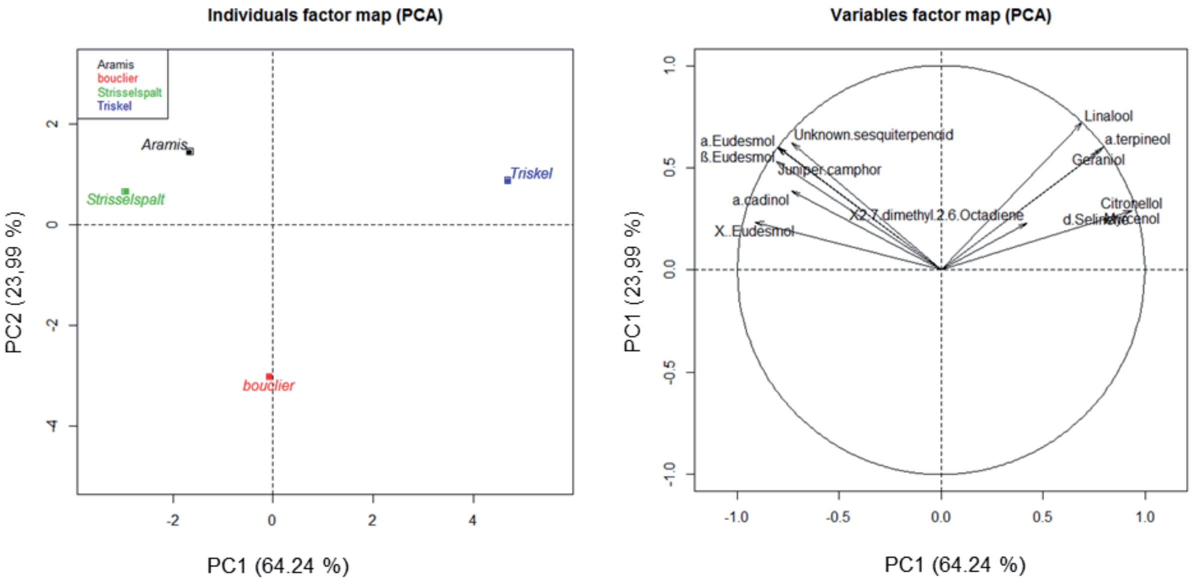

Fig. 3 **Principal Component Analysis (PCA) of mono- and sesqui- terpenoids in the four beers brewed with Strisselspalt, Aramis, Bouclier and Triskel hop varieties**

be considered as the most floral variety we tested. Moreover, Triskel was also the only variety tested which contributed citronellol to the beer and it is notable that both linalool and citronellol have been described as conferring an overall positive aroma to beer [26, 27].

Linalool, as the main contributor to the floral aroma of beer, has been detected in all the four beers analyzed at concentrations between 62 and 145 µg/L (Fig. 1). In comparison with the concentration usually quoted in the literature (mean = 37.7 µg/L; n = 27 [6–8, 18, 31]), these high concentrations are notable. Indeed, it confirms a high aromatic potential for these new varieties created by the French hop growers association and their cooperative in beer flavor improvement.

4 Conlusion

According to our data, the new variety Bouclier appears to be the least aromatic hop tested, yielding low concentrations of both mono- and sesqui-terpenoids in beer. We showed also that Aramis and Strisselspalt have similar terpenoid profiles. As they contributed sesquiterpenoids, these varieties could be used to give a spicy or herbal note to beer. Triskel, as a high producer of monoterpenoids, especially linalool, could bring a floral note to beer. Finally, our data showed that each variety gave a high concentration of linalool to beer. The three new varieties have a concentration in α-acids close to other known bittering hops and can, therefore, be used as both aroma-giving and as bittering hops.

Acknowledgments

The authors are grateful for the assistance of Dr. Ing. V. Ledouce, Dr C. Marcic and Dr P. Darby.

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Received 01 September 2013, accepted 28 November 2013