

Exploring the interplay of hop variety, harvest time and yeast: Sensory and chemical dynamics in beer brewing

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ABSTRACT

This study investigates the combined influence of hop variety, harvest time, and yeast strain on the chemical composition and sensory characteristics of dry-hopped beers, with the aim of identifying yeast–hop combinations that enhance sensory quality and promote more sustainable brewing practices.

Beers were produced using two *Saccharomyces cerevisiae* strains - a commercial beer strain (*Rock*) and a wine strain (*ISE77*, hereafter ‘77’) - combined with two hop varieties (*Cascade* and *Columbus*) harvested at three maturity stages.

Chemical and sensory analyses revealed that the *Rock* strain yielded beers with higher alcohol content, greater fermentative efficiency, and more consistent sensory profiles, especially when used with *Columbus* hops. In contrast, the ‘77’ strain significantly enhanced the aromatic complexity and quality of *Cascade*-hopped beers through yeast-driven biotransformation processes, particularly when early-harvested cones were used. The interaction between yeast strain and hop variety had a significant impact on perceived sweetness, sourness, and olfactory intensity.

Results suggest that yeast selection plays a pivotal role in modulating beer aroma and taste, and can be employed strategically to improve sensory quality while reducing raw material requirements, offering a promising tool for sustainable and resource-efficient brewing.

1. Introduction

Humulus lupulus L. (hop) is one of the most valued raw materials in craft brewing. In recent years, extensive research has been carried out on hops as a result of their growing importance in brewing, driven by increased consumer preference for hoppy beers, their potential health benefits (Karabın et al., 2016) and the limited understanding of their underlying chemistry. It was around 1990–2000 that research on hops in traditional beer styles gained a new boost, when highly hopped beers

became more popular, in concomitance with the United States craft beer movement (Lafontaine, Pereira, et al., 2019). Since then, the majority of research has focused on the role hops play in beer flavour (Legisa, 2021).

The quality and aroma intensity of hops in beer mainly depends on two factors: the influence of individual hop varieties (each variety has a unique chemical profile) and the timing of hop additions throughout the brewing process; when hops are added during the kettle boil, whirlpool, fermentation, and post-fermentation processes (e.g., dry hopping), hop volatiles undergo varying rates of extraction, removal and chemical

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reactions (Lafontaine, Pereira, et al., 2019; Lafontaine, Varnum, et al., 2019).

Furthermore, the chemical composition of cones modifies significantly depending on hop harvest time which, in turn, influences both beer flavour and aroma. The degree to which hop cones change during maturation varies between cultivars, affecting both volatile and non-volatile compounds. As hops mature, their total essential oil content increases. Compounds such as α -pinene, β -pinene, myrcene, limonene and linalool have been found to enhance the aromatic profile of Cascade and Willamette hops (Sharp et al., 2014).

Cone maturation also affects the concentrations of pentose-hexose monoterpene alcohol glycosides and other volatile/non-volatile compounds, which also significantly alter the hop flavour profile (Lafontaine et al., 2021). A recent study highlighted the impact of Cascade hop maturity on beer attributes: late-harvested hops tended to produce more intense citrus aroma and higher concentrations of free thiols and terpene alcohols, making these hops ideal for dry-hopping or aroma additions (Lafontaine, Varnum, et al., 2019). Consequently, important sensory differences can be perceived in beers brewed with Cascade and Willamette hops when harvested at different degrees of maturation (Sharp et al., 2014). Conversely, early-harvested hops may be better suited for bittering due to their stable humulone concentrations, which do not change significantly with maturity during the harvesting window (Lafontaine, Varnum, et al., 2019).

Beer characteristics, such as chemical composition, aroma, flavour and overall sensory profile, are also influenced by the yeast strain used in beer production. Different *Saccharomyces cerevisiae* strains, for example, can affect the levels of phenolic compounds, organic acids and antioxidants in beers (Viana et al., 2021). The US-05 yeast strain resulted in higher levels of catechins and procyanidin B1, enhancing the antioxidant capacity of beer, whereas the M15 yeast strain resulted in lower ethanol and antioxidant capacity (Viana et al., 2021).

It is known that most aromatic compounds in beers are intermediate metabolites or by-products of yeast metabolism (Capece et al., 2018; Iorizzo et al., 2021). Different flavour profiles (fruity, floral, spicy, or hoppy) also depend on the yeast strain used, as each strain is characterised by different levels of esters and terpenes production. A study by Kumar et al. (2023) found that beers fermented with one of twelve different yeast strains exhibited distinct volatile organic compound profiles: beers fermented with WLP730, OTA29, SPH and WB06 strains had the highest levels of 4-vinylguaicol, which contributed to their spicy flavour, whereas beer fermented with the W3470 yeast exhibited high levels of nerol, geraniol and citronellol, resulting in a distinctly "hoppy" character.

For many of the chemical compounds in beer, it is difficult to determine how their presence influences taste and aroma perception as they exist in such low concentrations. Sensory evaluation of beer can, therefore, be an essential tool for understanding the interactions between the various contributing factors.

Sensory evaluation is, in fact, a commonly used method for assessing beer aroma and flavour. This method is defined as the science used to obtain, measure, assess, and interpret the reactions of tasters to specific characteristics of food, beverages, or other non-food products, as experienced through human senses (Stone et al., 2020). Aspects such as freshness, quality, craftsmanship, balance, conformity to style or brand and overall drinkability can be assessed. This method, used singly or alongside other tools, is widely used in breweries as it provides a fairly accurate reflection of the consumer's experience of beer, even though it may not be as objective or precise as laboratory tests.

This study aims to evaluate the effects of hop variety, time of hop harvest (hereafter, harvest stage) and yeast strain on the sensory characteristics of beer. In particular, a panel of experts evaluated the aspect, taste and aroma of 12 different beers, each characterised by a combination of two hop varieties (Cascade and Columbus), three cone-harvest stages (early, optimal and late) and two yeast strains (the wine strain ISE77 and the commercial strain Rock).

2. Material and methods

2.1. Experimental design

For beer production, a wort was prepared using Malt Extract Super Light (Mr. Malt, Italy) following manufacturer's instructions. The water used in this study (Luna-Smart, Lecco, Italy) was purchased from the supermarket, and was characterized by the following chemical profile: pH 7.61, EC ($\mu\text{S}/\text{cm}$) 372, DS (mg/L) 264, Na^+ (mg/L) 5.1, K^+ (mg/L) 2.08, Ca^{2+} (mg/L) 50.0, Mg^{2+} (mg/L) 17.5, Cl^- (mg/L) 8.1, NO_3^- (mg/L) 10.7, SO_4^{2-} (mg/L) 51.1. Beer production was conducted at laboratory scale using food-grade vessels and 5-L fermentation tanks under controlled conditions.

The unhopped wort extract was first diluted with water to 13.3 °Plato /1.053 OG, then divided into 5-liter tanks, after which 6 g/L of hop lyophilized cones were added (dry-hopping) to each tank. Twelve batches of Ale type beer were produced, with each beer characterised by a combination of two yeast strains, two hop varieties and three cone-harvest stages (Table 1).

Thoroughly, two different *Saccharomyces cerevisiae* yeasts (inoculum size 10^6 cell/ml) were separately employed: *S. cerevisiae* ISE 77 strain (hereafter, '77') belonging to the Culture Collection of Oenological and Viticultural Environment (CREA-CMVE, Asti); and the commercial strain 'Rock' (Brewline, Italy). Each strain was employed on two hop cultivars (cv.): cv. Cascade (aroma hop), and cv. Columbus (bittering hop). The cones were selected on the basis of three harvest stages: early harvest (T1-24/08/2023), optimal harvest (T2-31/08/2023) and late harvest (T3-15/09/2023). Following harvesting, cones were immediately lyophilized and vacuum packed at -20°C until ready for use.

Each beer was produced in triplicate. Fermentations were carried out at 22°C and weight loss was measured. At the end of the fermentation period, the beers were bottled and 6 g of sugar were added for the second fermentation.

2.2. Beer chemical analysis

All beer samples were analysed using BeerFoss™ FT Go without prior degassing or filtration. Measured parameters included: alcohol content; specific gravity (SG), i.e. the density of beer at standard temperature and pressure (Chlup, 2013); density; pH; apparent degree of fermentation (ADF); real degree of fermentation (RDF), which measures the extent to which sugar in wort has been converted into alcohol (attenuation); calories and extracts.

Sensory analysis

Tests were conducted by a panel of 12 experts (10 men, 2 women) aged 18-50, all members of the Union Birrai Beer Tester (UBT) association and official judges in national and international beer competitions.

Table 1

Twelve beers used in the study with their relative yeast strain ('Rock' and '77'), hop variety ('Cascade' and 'Columbus') and harvest stage (T1, early harvest; T2, optimal harvest; T3, late harvest).

Beer	Yeast	Hop variety	Harvest stage
RoCasT1	Rock	Cascade	T1
RoCasT2	Rock	Cascade	T2
RoCasT3	Rock	Cascade	T3
77CasT1	77	Cascade	T1
77CasT2	77	Cascade	T2
77CasT3	77	Cascade	T3
RoColT1	Rock	Columbus	T1
RoColT2	Rock	Columbus	T2
RoColT3	Rock	Columbus	T3
77ColT1	77	Columbus	T1
77ColT2	77	Columbus	T2
77ColT3	77	Columbus	T3

Panelists had extensive prior experience in beer sensory evaluation, including aroma recognition, defect identification, descriptor usage and intensity scaling. Prior to the study, panelists participated in a targeted calibration phase focused on the specific beer samples under investigation. This phase included two steps: 1) descriptor generation and standardization, conducted through guided consensus sessions where panelists evaluated a preliminary set of beers representing the experimental conditions; 2) calibration and alignment, in which assessors evaluated reference samples to ensure consistent use of scales and descriptors prior to formal data collection.

Although the gender distribution was unbalanced (10 men, 2 women), expert sensory panels are considered analytical instruments in which training and calibration minimize demographic effects.

This sensory evaluation was conducted in accordance with ethical standards for research involving human participants.

Two sensory evaluation methods were employed: a quantitative descriptive analysis (QDA) and a Check-All-That-Apply (CATA) evaluation. The QDA aimed to characterise the qualitative aspects of beers by assessing 18 attributes (Table 2), based on the AIS list (Italian Sommelier

Table 2

List of sensory attributes evaluated by the 12 panellists, divided into those used for the quantitative descriptive analysis (QDA) and those employed in the Check-All-That-Apply analysis (CATA). For the 18 QDA attributes, the ID code used in Fig.s, type of sensory characteristic and score range used by panellists to characterise each beer are reported.

QDA				CATA
Attribute	ID	Type	Score	Attribute
Colour (of foam)	col	Visual	1 (white), 2 (ivory), 3 (rosé), 4 (beige)	Menthol
Aspect (of foam)	app	Visual	1 (coarse texture), 2 (intermediate t.), 3 (fine t.)	Tea
Persistence (of foam)	per	Visual	1 (evanescent), 2 (intermediate), 3 (persistent)	Unripe fruits
Limpidity (of beer)	lim	Visual	1 (veiled), 2 (clear), 3 (crystal clear)	Ripe fruits
Aromatic intensity	ar. int	Olfactory	From 1 (moderate) to 10 (extremely intense)	Red berries
Aromatic complexity	ar. com	Olfactory	From 1 (moderate) to 10 (wide)	Floral
Aromatic quality	ar. qua	Olfactory	From 1 (acceptable) to 5 (excellent)	Tropical fruits
Sweetness	swe	Gustatory	From 1 (dry) to 4 (sweet)	Citrusy
Bitterness	bit	Gustatory	From 1 (absent) to 4 (extremely perceptible)	Spicy
Sourness	sou	Gustatory	From 1 (absent) to 4 (extremely perceptible)	Vegetal
Sapidity	sap	Gustatory	From 1 (absent) to 4 (extremely perceptible)	Woody
Alcoholic strength	alc	Gustatory	From 1 (low) to 3 (high)	Sweet
Carbonation	car	Gustatory	From 1 (low) to 3 (high)	Vinous
Softness	sof	Gustatory	From 1 (low) to 3 (high)	Lactic
Dryness	dry	Gustatory	From 1 (low) to 3 (high)	Acetic
Gustatory-olfactory intensity	go. int	Gust.-olf.	From 1 (moderate) to 10 (extremely intense)	Ethereal
Gustatory-olfactory persistence	go. per	Gust.-olf.	From 1 (moderate) to 10 (extremely persistent)	Sulphurous
Gustatory-olfactory quality	go. qua	Gust.-olf.	From 1 (acceptable) to 5 (excellent)	Cheesy
				Hay

Association). An overall final judgment was also recorded, with judges attributing a value to each beer ranging from 1 (acceptable) to 5 (excellent). For CATA sensory evaluation, panellists were given a list of 19 predefined descriptors (Table 2) selected on the basis of preliminary tests and the literature. Each panellist was instructed to identify all the attributes they perceived in each sample (Ares & Jaeger, 2023).

Each panellist received a random 50 mL sample of each beer and, to eliminate carry-over effects, was supplied with mineral water and dry unsalted breadsticks for palate cleansing between samples.

2.4. Statistical analysis

To determine which values effectively discriminated between experimental conditions, chemical data for each parameter was subjected to one-way analysis of variance (ANOVA) using XLSTAT software.

Attributes evaluated for the QDA were analysed using partial redundancy analysis (p-RDA) and linear mixed-effect models (LMMs). A p-RDA was carried out to test the role of the hop variety, harvest stage and yeast strain on the 18 sensory attributes of beers (scaled at zero-mean and unit-variance). The entire dataset was used (three replicates per beer per panellist). The constrained component included hop variety (two-level factor), harvest stage (three-level factor) and yeast strain (two-level factor), while the conditional component included the panellist (12-level factor). The variance inflation factor (cut-off value of 3) was computed to exclude potential linear dependencies among predictors. Permutation-based tests (no. permutations=10000) were carried out to test the significance of the model, terms and canonical axes.

LMMs were carried out to assess the effect (single and combined) of hop variety, harvest stage and yeast strain on the olfactory, gustatory and gustatory-olfactory beer attributes. The final evaluation supplied by panellists was also analysed. For each characteristic, the model included the following fixed effects: hop variety (two-level factor), harvest stage (three-level factor), yeast (two-level factor) and their two- and three-way interactions. The models, fitted with restricted maximum likelihood (REML), were compared using the Akaike information criterion. For all models, “panellist” was selected as the random structure. A top-down model selection was then applied using the ML method (maximum likelihood), removing step-by-step non-significant terms to obtain a final model retaining only significant fixed effects ($p < 0.05$) (Crawley, 2012; Zuur et al., 2009). The estimates of the effects of factors tested and their significance were obtained fitting the final model by REML. During each step, model residuals were visually checked to verify linear model assumptions. Partial redundancy analysis and LMMs were performed in R version 4.0.2 (R Core Team, 2020) with the package *vegan* (Oksanen, 2015) and *nlme* (Pinheiro et al., 2020); LMMs graphic outputs and post-hoc pairwise comparisons were produced using *ggeffects* (Lüdtke, 2018) with Šidák p -value adjustment.

Sensory data obtained from the CATA evaluation was analysed using frequency counts for each descriptor. Cochran's Q test was applied to determine significant differences in the frequency of selected attributes among beer samples. Correspondence analysis (CA) was performed to visualize the relationship between the samples and sensory descriptors. Statistical analysis was carried out using XLSTAT, setting significance at $p < 0.05$.

3. Results

3.1. Beer chemical analysis

Alcohol content differed between beers fermented with different yeast strains: ‘Rock’ beers showed higher alcohol levels (5.9–6.1 % ABV) compared to ‘77’ beers (5.1–5.5 % ABV) regardless of hop variety or harvest stage (Table 3). However, alcohol content tended to be higher in Cascade beers compared to Columbus ones, with a significant difference at T2 and T3 harvest stages. For Cascade beers, alcohol content increased progressively with cone-harvest stage, reaching a significantly

Table 3

Mean ($n = 3$) of chemical parameters measured in each of the 12 analysed beers: alcoholic content (ABV), real extract (% w/w), real degree of fermentation, final gravity and pH. For each chemical parameter, different letters indicate significant differences ($p < 0.05$) between beers.

Beer	Alcohol (ABV)	Real extract (% w/w)	Real degree of fermentation	Final gravity	pH
<i>RoCasT1</i>	5.97 ab	3.91 d	71.30 a	1.007 e	4.30 d
<i>RoCasT2</i>	6.05 a	3.86 de	71.80 a	1.006 e	4.42 cd
<i>RoCasT3</i>	6.09 a	3.76 de	72.47 a	1.006 e	4.49 bc
<i>77CasT1</i>	5.40 d	4.97 bc	63.70 cd	1.012 bc	4.67 a
<i>77CasT2</i>	5.41 d	4.81 c	64.53 bc	1.011 cd	4.69 a
<i>77CasT3</i>	5.59 c	4.79 c	65.37 b	1.011 d	4.69 a
<i>RoColT1</i>	6.01 ab	3.69 e	72.60 a	1.006 e	4.49 bc
<i>RoColT2</i>	5.90 b	3.83 de	71.50 a	1.007 e	4.42 cd
<i>RoColT3</i>	5.90 b	3.83 de	71.40 a	1.007 e	4.37 cd
<i>77ColT1</i>	5.36 d	5.04 b	63.17 cd	1.012 b	4.59 ab
<i>77ColT2</i>	5.15 e	5.34 a	60.87 e	1.014 a	4.63 a
<i>77ColT3</i>	5.29 de	5.05 b	62.83 d	1.012 b	4.65 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

higher level at T3.

The specific gravity (SP) of beer samples showed an inverse relationship with alcohol content for beers fermented with '77' (Table 3), being lowest in *77CasT3* (1.011) and highest in *77ColT2* (1.014). In contrast, SP in 'Rock' beers showed no significant differences. A similar trend was observed for the real extract (RE) (Table 3), which considers the original gravity of the wort, the extent of fermentation (attenuation) and the final alcohol level of the beer. In detail, RE quantifies the solids remaining in the beer after fermentation, including unfermented sugars and other components (Chlup, 2013). The *77ColT2* beer exhibited the highest RE value (5.3 % w/w), while *RoColT1* recorded the lowest. Beers fermented with '77' showed significant differences between those with Columbus (higher RE) and those with Cascade, regardless of harvest stage. However, RE in 'Rock' beers was not significantly different.

The Real Degree of Fermentation (RDF) was higher in 'Rock' beers (71.3–72.6 %), regardless of hop variety or harvest stage (Table 3). Beers fermented with '77' yeast showed lower fermentative efficiency: *77CasT3* reached 65.4 % RDF, whereas Columbus beers did not exceed 63 %, with a 60 % minimum at T2.

The highest pH values were recorded in '77'-fermented beers, with no significant differences between hop varieties. In 'Rock' beers, the greatest difference was between *RoColT1* (4.48) and *RoCasT1* (4.29).

3.2. Sensory analysis

Partial redundancy analysis on sensory characteristics of beers (Fig. 1) revealed a significant variation depending on hop variety (pseudo- $F_{1,416}=18.720$, $p=0.001$), harvest stage (pseudo- $F_{2,416}=2.066$, $p=0.002$) and yeast (pseudo- $F_{1,416}=2.587$, $p=0.007$). However, only a very small amount of variance was explained by the constrained component (3.6 % based on adjusted R^2), which was lower than the amount explained by the panellists (26.0 % based on adjusted R^2), leaving 68.2 % of variation unexplained by factors considered in this study. Hop variety appeared to be associated with the first axis (RDA1), whereas harvest stage and yeast were associated with the second axis (RDA2). The first and second axis explained 3.7 % and 0.6 % (based on adjusted R^2), respectively, of the variance in sensory characteristics recorded by the panel. Cascade-hopped beers were perceived to have a higher aromatic quality, complexity, gustatory-olfactory quality, sapidity and alcoholic strength than Columbus-hopped beers. The latter were considered drier and characterized by a more persistent foam. Finally, beers with the '77' strain or T3 cones were evaluated as more limpid, fizzier, softer, more bitter and as having a more persistent foam compared to beers with 'Rock' strain and T1 or T2 harvest stages.

With regard to beer attributes (olfactory, gustatory and gustatory-olfactory) and the final evaluation on beer characteristics, ANOVA output of LMMs are reported in Table 4.

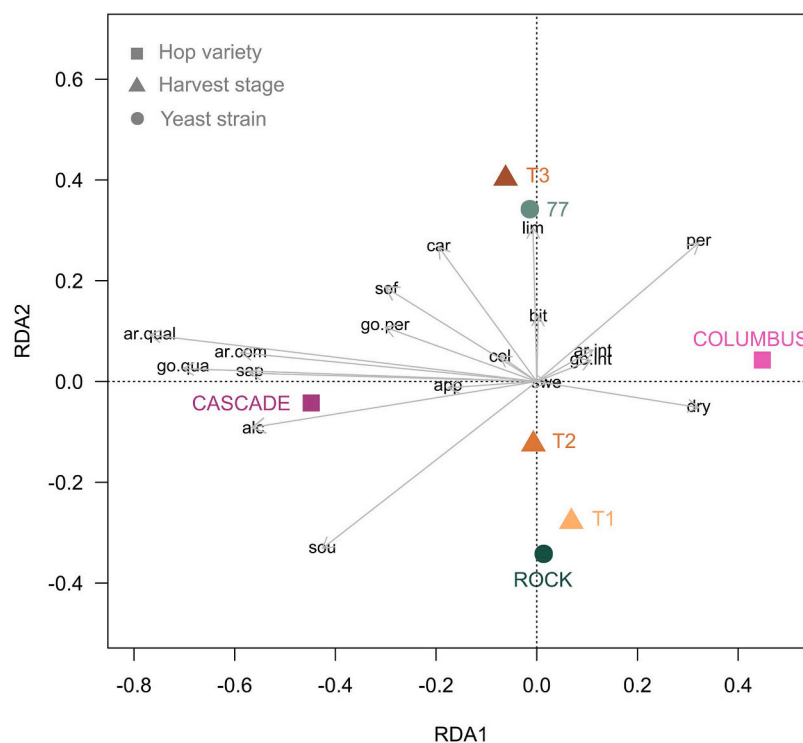


Fig. 1. Partial RDA correlation biplot of variation in 18 sensory characteristics of beers hopped with a different hop variety (Cascade and Columbus), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77' and 'Rock'). Attribute abbreviations are reported in Table 2.

Table 4

Results of linear mixed-effect models on factors influencing olfactory, gustatory and gustatory-olfactory attributes of beers: hop variety (H), harvest stage (S) and yeast strain (Y). The significant terms and interactions ($p < 0.05$) retained in the final model are reported (when three-way interaction is significant, non-significant two-way interactions are kept). Variance structures included in the final model are also specified, where 'Ide' indicates that the variance of residuals is allowed to have different spread among the levels of the categorical variable. "Panellist" was set as the random structure in all models.

Type	Attribute	Fixed term	df (N, D)	F-value	p-value	Variance structure
Olfactory	Aromatic intensity	Hop (H)	1,409	2.97	0.085	Ide(S)
		Harvest stage (S)	2,409	1.45	0.236	
		Yeast (Y)	1,409	3.28	0.071	
		$H \times S$	2,409	4.16	0.016	
		$H \times Y$	1,409	2.51	0.114	
		$S \times Y$	2,409	6.23	0.002	
		$H \times S \times Y$	2,409	4.69	0.010	
	Aromatic complexity	H	1,409	6.53	0.011	Ide(H)
		S	2,409	1.83	0.162	
		Y	1,409	5.84	0.016	
		$H \times S$	2,409	3.96	0.020	
		$H \times Y$	1,409	2.76	0.097	
		$S \times Y$	2,409	5.27	0.006	
		$H \times S \times Y$	2,409	7.17	<0.001	
	Aromatic quality	H	1,413	81.59	<0.001	Ide(H), Ide(S), Ide(Y)
		S	2,413	0.93	0.395	
		Y	1,413	11.12	<0.001	
		$H \times S$	2,413	6.36	0.002	
		$H \times Y$	1,413	17.33	<0.001	
		H	1,409	0.32	0.570	
		S	2,409	2.12	0.122	
Gustatory*	Sweetness	Y	1,409	1.56	0.213	Ide(H), Ide(S)
		$H \times S$	2,409	2.13	0.120	
		$H \times Y$	1,409	0.57	0.451	
		$S \times Y$	2,409	3.57	0.029	
		$H \times S \times Y$	2,409	3.80	0.023	
		H	1,409	11.54	<0.001	
		S	2,409	0.61	0.542	
	Sourness	Y	1,409	2.66	0.104	Ide(H)
		$H \times S$	2,409	0.48	0.620	
		$H \times Y$	1,409	1.28	0.259	
		$S \times Y$	2,409	2.11	0.122	
		$H \times S \times Y$	2,409	4.00	0.019	
		H	1,419	42.79	<0.001	
		S	2,414	1.47	0.230	
	Sapidty	Y	1,414	5.59	0.019	Ide(H)
		$H \times S$	2,414	3.47	0.032	
		H	1,419	15.70	<0.001	
		S	2,409	1.81	0.165	
		Y	1,409	0.03	0.862	
		$H \times S$	2,409	7.92	<0.001	
		$H \times Y$	1,409	0.26	0.613	
Gustatory-Olfactory	Alcoholic strength	$S \times Y$	2,409	2.92	0.055	Ide(H), Ide(S), Ide(Y)
		$H \times S \times Y$	2,409	4.30	0.014	
		H	1,409	2.65	0.104	
		S	2,409	1.96	0.143	
		Y	1,409	0.18	0.675	
		$H \times S$	2,409	7.64	<0.001	
		$H \times Y$	1,409	1.82	0.178	
	Carbonation	$S \times Y$	2,409	0.72	0.486	Ide(H), Ide(S)
		$H \times S \times Y$	2,409	3.99	0.019	
		H	1,413	87.47	<0.001	
		S	2,413	0.85	0.430	
		Y	1,413	4.69	0.031	
		$H \times S$	2,413	8.21	<0.001	
		$H \times Y$	1,413	10.68	0.001	
Final evaluation	Softness	H	1,413	74.64	<0.001	Ide(H)
		S	2,413	2.55	0.080	
		Y	1,413	3.91	0.049	
		$H \times S$	1,413	8.94	<0.001	
		$H \times Y$	2,413	13.13	<0.001	

* The attribute "bitterness" is not reported since no significant effects of investigated factors were detected

3.2.2. Olfactory attributes

With regard to aromatic intensity (Fig. 2a), a significant three-way interaction ($p=0.010$) between hop variety, harvest stage and yeast strain was found (post-hoc output in Table S1).

The *RoCasT1* beer showed the highest aromatic intensity, being higher compared to *RoCasT2* and *RoCasT3* (non-significant contrasts). The opposite pattern was observed with the '77' strain, with lower values in *77CasT1* vs *77CasT2* and *77CasT3* (non-significant contrasts). For Columbus beers, the maximum intensity was recorded with the '77' strain, at both T1 and T3 harvest stages, whereas 'Rock' beers exhibited similar values across all harvest stages. Overall, '77'-fermented beers displayed variable aromatic intensity depending on the hop variety and harvest stage, whereas Rock-fermented beers exhibited a similar response pattern across hop varieties (Fig. 2a).

Aromatic complexity (Fig. 2b) was generally higher in beers hopped with Cascade ($p=0.011$) and in 'Rock'-fermented beers ($p=0.016$). Panel's perception, however, significantly differed between beers depending on the combination of hop variety, cone-harvest stage and yeast strain (three-way interaction $p<0.001$; post-hoc output in Table S2). With cv. Cascade, aromatic complexity tended to be lower when T1 hops and '77' yeast was used, whereas the opposite trend was observed with 'Rock'. With cv. Columbus, aromatic complexity was generally higher with T3 hops using both yeast strains (significant contrast only between *77ColT3* vs *T2*).

The aromatic quality (Fig. 2c, d) was generally higher for beers brewed with Cascade vs Columbus ($p<0.001$), with the perception modulated by the yeast used and the harvest stage (interaction hop $\times$ yeast $p<0.001$ and hop $\times$ harvest stage $p=0.002$; post-hoc output in Table S3). Cascade-hopped beers displayed the highest quality when fermented with the '77' yeast, whereas Columbus beers exhibited the opposite pattern. Furthermore, while the aromatic quality of beers hopped with cv. Cascade was slightly enhanced by T1 hops, early-harvested cones lowered the quality of Columbus beers compared to those with T2 and T3 hops.

3.2.3. Gustatory parameters

All gustatory attributes were influenced by the investigated factors, either singly or combined, with bitterness being the only parameter showing no significant differences across beers (Table 4).

Sweetness perception (Fig. 3a) varied according to hop variety, harvest stage and yeast strain (three-way interaction $p=0.023$; post-hoc output in Table S4). For *77Cas* beers, sweetness tended to be highest with T1 hops, whereas for *RoCas* it tended to peak with T3 hops. For Columbus beers no particular pattern was observed.

Sourness (Fig. 3b) was generally higher in Cascade vs Columbus beers ($p<0.001$), although perception differed according to hop variety, harvest stage and yeast strain (three-way interaction $p=0.019$; post-hoc output in Table S5). Within the same hop variety, '77'-fermented beers did not significantly differ between harvest stages. Interestingly, despite significant differences in pH, Cascade beers were perceived as more acidic than those hopped with cv. Columbus. In addition, *77Cas* beers were not considered sourer than *RoCas* beers (except for *RoCasT1*) despite notable differences in pH values (Table 3). Furthermore, acidity in 'Rock'-fermented beers (Table 3) varied depending on the hop harvest stage and variety: in *RoCas* values were highest with T1 hops (significant contrast between *RoCasT1* vs T3), whereas in *RoCol* with T2 hops (non-significant contrasts with T1 and T3). Sourness evaluation of 'Rock'-fermented beers matched pH values, with the highest sourness for *RoCasT1* and the lowest for *RoColT1* (Fig. 3b). However, for '77'-fermented beers, perceived sourness did not align with analytical pH values: panellists rated *77Cas* beers significantly sourer than *77Col*, despite pH values suggesting the opposite trend.

Sapidity (Fig. S1a) and alcoholic strength (i.e. warmth sensation; Fig. S1b) were higher in Cascade vs Columbus beers ($p<0.001$), regardless of harvest stage or yeast strain. Despite the c. 0.5 % ABV difference between *RoCas* (6.0 % ABV) and *77Cas* (5.5 % ABV) (Table 3), no difference in warmth perception occurred.

Carbonation (Fig. S1c, d) was generally higher in Cascade vs Columbus beers ($p=0.002$) and in beers with '77' vs 'Rock' yeast

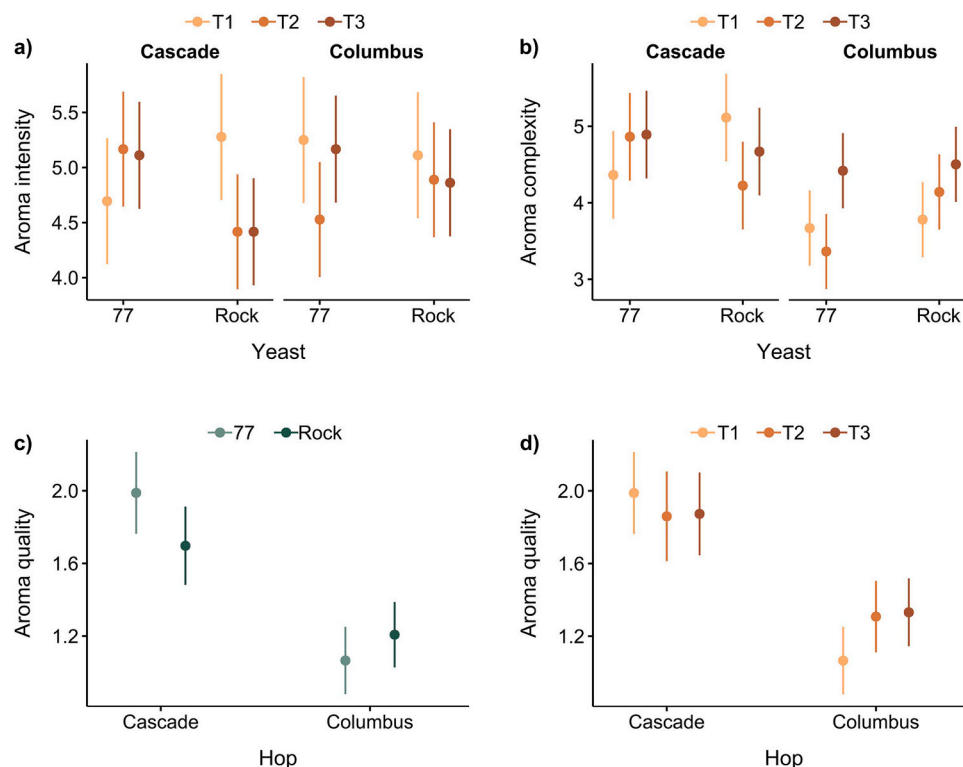


Fig. 2. Olfactory attributes (mean $\pm$ 95 % CI) perceived in beers obtained from different combinations of hop variety (Cascade and Columbus), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77' and 'Rock'): **a)** aromatic intensity, **b)** aromatic complexity, **c)** and **d)** aromatic quality. Only significant effects are reported ($p<0.05$).

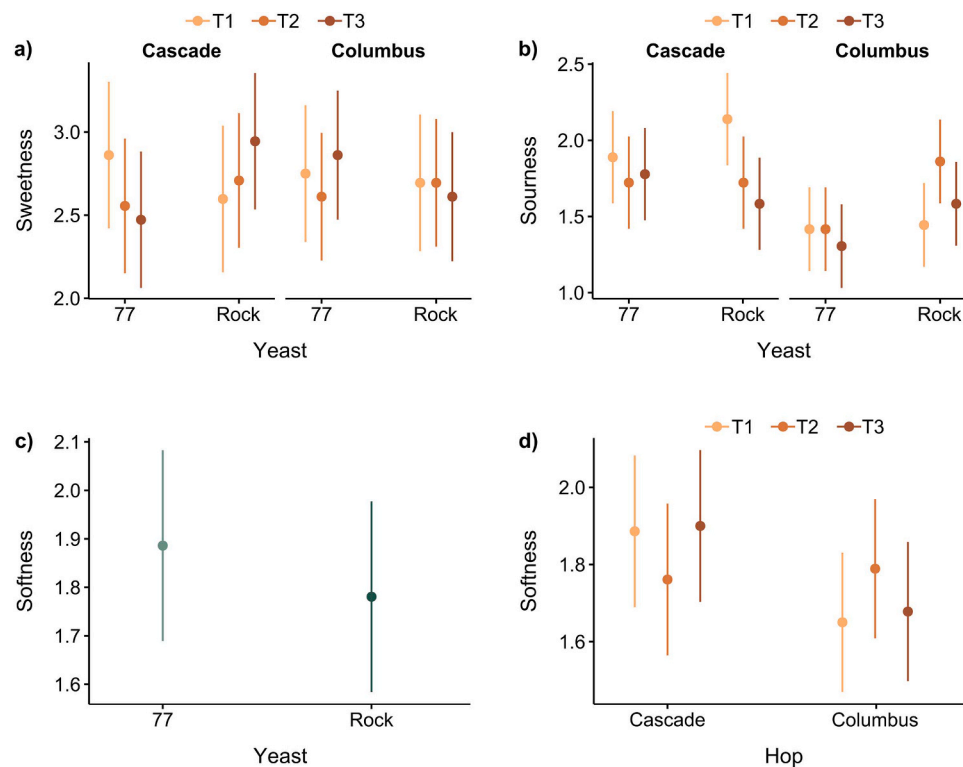


Fig. 3. Gustatory attributes (mean $\pm$ 95 % CI) perceived in beers obtained from different combinations of hop variety (Cascade and Columbus), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77' and 'Rock'): **a)** sweetness, **b)** sourness, **c)** and **d)** softness. Only significant effects are reported ($p < 0.05$).

($p = 0.036$). Carbonation of Cascade and Columbus beers depended on the yeast type and cone-harvesting date (interactions hop $\times$ yeast

$p = 0.014$ and hop $\times$ harvest stage $p = 0.003$; post-hoc output in Table S6).

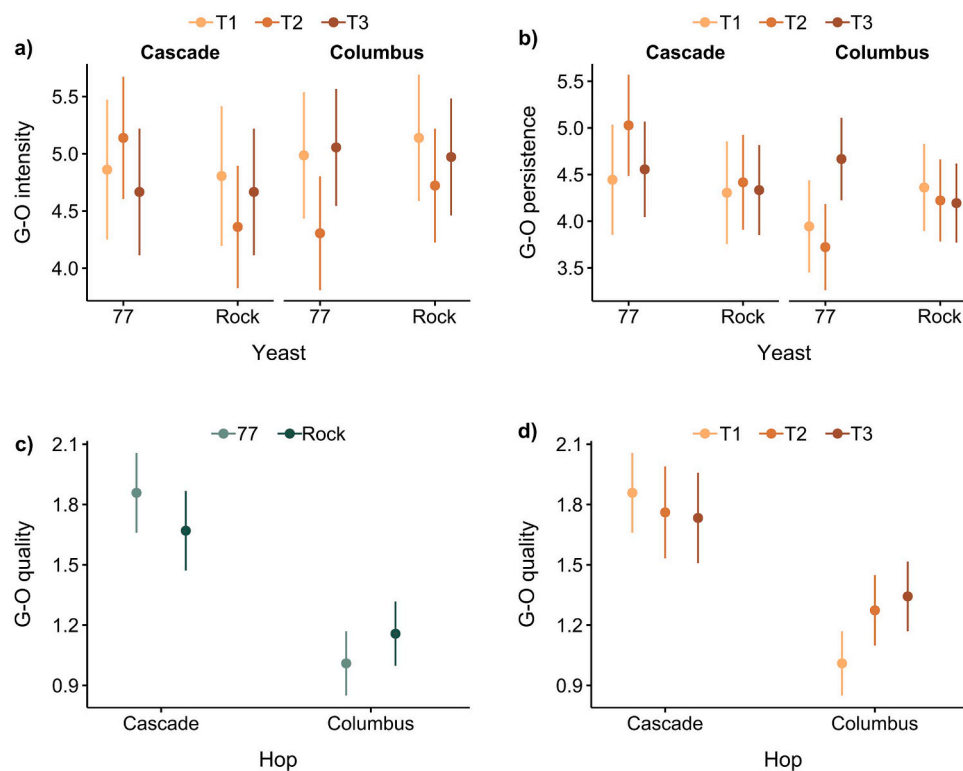


Fig. 4. Gustatory-olfactory (G-O) attributes (mean $\pm$ 95 % CI) perceived in beers obtained from different combinations of hop variety (Cascade and Columbus), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77' and 'Rock'): **a)** G-O intensity, **b)** G-O persistence, **c)** and **d)** G-O quality. Only significant effects are reported ($p < 0.05$).

Beer softness was influenced by hop variety (Cascade>Columbus, $p=0.003$), yeast ('77'>'Rock', $p=0.019$; Fig. 3c) and harvest stage $\times$ variety ($p=0.032$; post-hoc output in Table S7). Cascade-hopped beers tended towards lower smoothness with T2 hops, while the opposite pattern was perceived for Columbus beers (Fig. 3d).

Finally, dryness differed only between hop varieties ($p<0.001$), the driest being Columbus beers (Fig. S1e).

3.2.4. Gustatory-Olfactory Parameters

Gustatory-olfactory (G-O) attributes were all influenced by the investigated factors (Table 4). In particular, a significant three-way interaction hop variety $\times$ harvest stage $\times$ yeast was detected for intensity ($p=0.014$) and persistence ($p=0.019$).

G-O intensity (Fig. 4a) of Cascade-hopped beers was highest for 77CasT2 and lowest for RoCasT2 (post-hoc output in Table S8). For Columbus beers the trend was reversed: T2 beers generally less intense, regardless of the yeast used. Moreover, the highest intensity was generally perceived with T1 hops, apart from 77CasT2.

The persistence of G-O intensity in '77'-fermented beers varied significantly based on hop variety and harvest stage (Fig. 4b) with the highest values for 77CasT2 and 77ColT3 (post-hoc output in Table S9). In contrast, with 'Rock' yeast, perception seemed consistent across beers, regardless of harvest time or hop variety.

G-O quality was greater in Cascade vs Columbus beers ($p<0.001$) and in '77' vs 'Rock' beers ($p=0.031$) with significant interactions found between hop variety and yeast strain ($p=0.001$; Fig. 4c) and between hop variety and cone maturation ($p<0.001$; Fig. 4d). For Cascade-hopped beers the quality tended to be higher when fermented with '77' yeast compared with RoCas, whereas for Columbus-hopped beers the pattern was opposite. Hop variety and harvest stage interaction showed that ColT2 and ColT3 beers had a significantly higher G-O quality compared to ColT1 (significant contrasts; post-hoc output in Table S10), whereas Cascade beers exhibited a similar quality across different harvest stages.

3.2.5. Final beer evaluation

The beers that received the best ratings in terms of olfactory and taste-olfactory quality also scored highest in the final evaluation. Scores were generally higher for Cascade beers ($p<0.001$) and for '77'-fermented beers ($p=0.049$) (Table 4). A significant interaction emerged between yeast $\times$ hop variety ($p<0.001$; Fig. 5a) and between variety $\times$ harvest stage ($p<0.001$; Fig. 5b) (post-hoc output in Table S11).

For Cascade-hopped beers, there was a tendency towards higher scores for beers fermented with '77' vs 'Rock' strains, whereas for Columbus-hopped beers there was the opposite pattern ('Rock'>'77'). Cascade-hopped beers brewed with T1 hops exhibited the highest ratings, while for Columbus beers the best ratings were with T3 hops.

3.2.6. Panel test and CATA analysis

Panel analysis revealed that judges were able to distinguish between olfactory parameters (Fig. S2), with the least variability, i.e. greater agreement between judges, being perceived in vegetal, vinous, cheesy and sulphurous notes.

The CATA analysis assessed attributes which were most strongly associated with each beer (Fig. 6) with the biplot showing beers and sensory attributes across two main dimensions, F1 (54.83 %) and F2 (15.74 %), together explaining 70.57 % of the total variance. By checking the proximity of beers with specific attributes (i.e. stronger association), the analysis revealed that 77CasT2 and 77CasT3 are closely linked to sweet fruits, ripe fruits and floral notes while RoCasT1 are associated with ethereal and lactic attributes. Similarly, 77ColT3 is strongly associated with sulphuric notes and 77ColT2 with vegetal attributes. The biplot shows an evident separation based on the different strains, highlighting the influence of yeast on sensory perception. The harvest stage also plays a role in differentiating the beers (see 77ColT1 and 77ColT2, Fig. 6).

4. Discussion

This study aimed to evaluate whether hop variety, cone-harvest time and yeast strain modify the sensory characteristics of beer and, if so, to assess their impact. Analytical analyses revealed the superior fermentative efficiency (RDF) of the Rock strain, which is also evident in parameters such as alcohol content, specific gravity, real extract, and pH. (Table 3). As previously noted, 'Rock' is a commercial beer yeast strain, whereas ISE77 ('77') is a wine yeast strain, thus is less suitable for fermenting beer wort. Genome-wide studies by Gonçalves et al., (2016) not only demonstrated that beer yeast strains are substantially distinct from wine strains, but also evidenced which selection of yeasts optimised maltose utilisation in beer brewing. It is known that not all yeast species metabolize all the four main sugars present in wort, i.e. maltose, maltotriose, glucose and fructose (Cubillos et al., 2019). For instance, Pos-tigo et al. (2021) observed that *Saccharomyces* wine strains are unable to ferment maltotriose, thus do not utilise all the maltose present in wort. This could explain the lower alcohol content we observed in beers fermented with the '77' strain.

Interestingly, Cascade-hopped beers fermented with '77' (77Cas) exhibited higher RDF than Columbus-hopped beers fermented with the same yeast (77Col). This difference was probably the result of a phenomenon known as "hop-creep": during dry-hopping, the enzymes in hops can alter beer composition, with hydrolysis transforming non-fermentable destrins into fermentable sugars, making them available for fermentation (Bruner et al., 2021; Kirkpatrick & Shellhammer, 2018b; Steyer et al., 2017). With live yeast, these newly released fermentable sugars can lead to over-attenuation, resulting in higher alcohol content and reduced residual sugar levels. Cascade hops have, in fact, been found to exhibit higher enzymatic activity compared to other

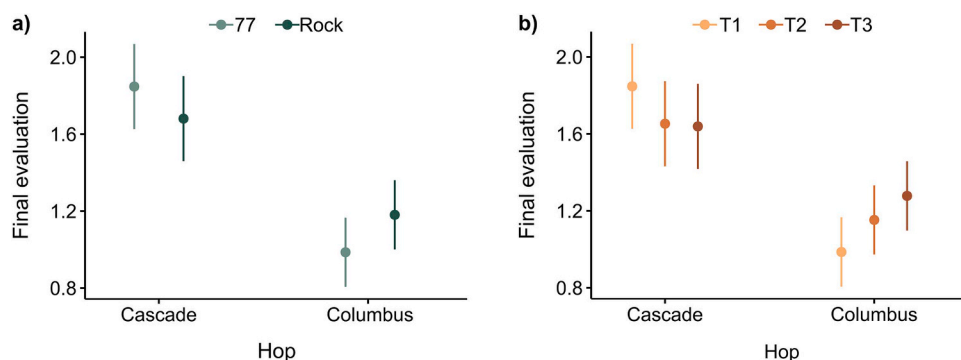


Fig. 5. Final evaluation (mean $\pm$ 95 % CI) of beers obtained from different combinations of hop variety (Cascade and Columbus), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77' and 'Rock'). Only significant effects are reported ($p < 0.05$).

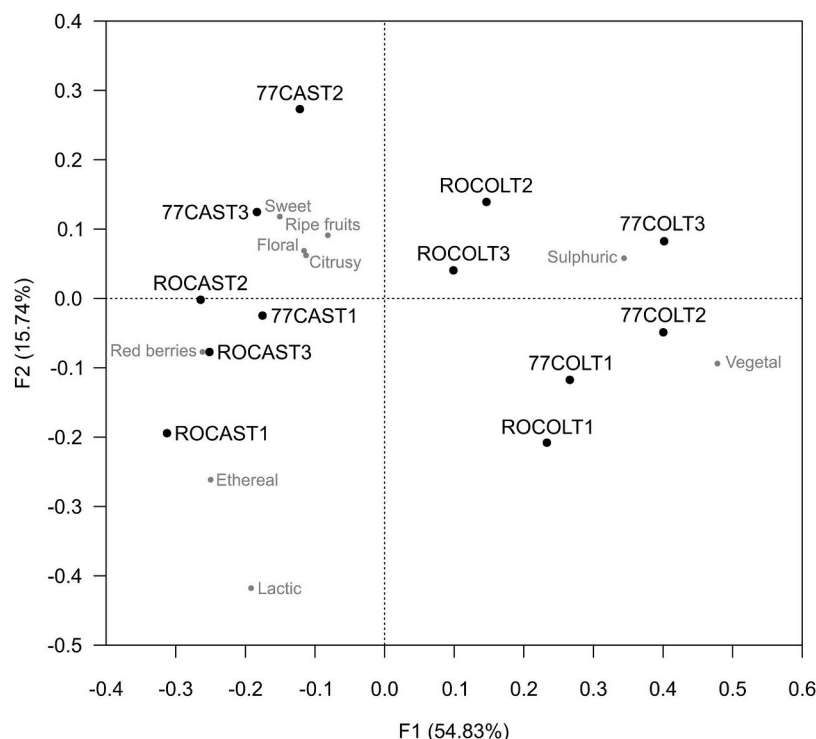


Fig. 6. CATA analysis biplot representing beers samples (in black) and sensory attributes (in grey) across two main dimensions. Beer samples are characterized by different combinations of hop variety (Cascade, CAS; and Columbus, COL), cone-harvest stage (early, T1; optimal, T2; late, T3) and yeast strain ('77', 77; and 'Rock', RO).

hop varieties (Kirkpatrick & Shellhammer, 2018a).

Concerning beer pH, this is influenced by various factors during the brewing process: the composition of the brewing water, the ingredients used and the type of fermentation (Coote & Kirsop, 1974, 1976). Since the same wort was used to produce all the beer samples used in our experiment, any variations in pH was probably the result of the fermentation process, during which pH is reduced by means of organic acid excretion and the consequent uptake of basic amino acids (Coote & Kirsop, 1974, 1976; Li & Liu, 2015). Therefore, there seems to be a correlation between lower pH values and a greater degree of fermentation.

While it is relatively easy to understand macroscopic changes during fermentation (e.g. pH or acidity) changes in aromatic composition are more difficult to assess, since the perception of flavour in food and beverages is a complex, multisensory experience involving taste, smell, sound and sight. For this reason, the establishment of linear relationships between chemical-physical parameters and sensory attributes is particularly challenging.

The panel assessment of olfactory parameters (aroma intensity, complexity and quality) reflected the way in which the investigated hop varieties are employed in brewing, with Cascade-hopped beers rated higher than Columbus-hopped beers. Furthermore, significant interactions between hop variety, harvest stage and yeast strain emerged, such as the higher aroma intensity perceived in RoCasT1 and 77ColT1. It has already been demonstrated that hop-derived aroma intensity and quality can increase during cone maturation (Bailey et al., 2009). In particular, citrus aromas can be an indicator of dry-hop aroma development for cv. Cascade since the quality of dry-hop aroma of these hops shifts from herbal to citrusy during ripening (Lafontaine, Varnum, et al., 2019). Yeast metabolism may, thus, influence the beer aromatic profile when hops are harvested early, which could explain the results observed for T1 beers (Fig. 2). Moreover, these findings align with evaluations of aroma complexity and quality, with Cascade hops receiving higher ratings due to their fruity, floral, and citrusy aromas. Despite these

results being expected, it is particularly noteworthy that the perceived olfactory parameters were influenced by the yeast strain employed, with the aroma quality of cv. Columbus achieving higher scores when fermented with 'Rock'.

At the sensory level, parameters directly linked to the chemical analyses of beers, i.e. sweetness, sourness and alcoholic strength, did not always fully correspond with analytical values, suggesting that multiple factors influence the perception of a single parameter. Factors influencing sensory perception are complex and include interactions between the main flavor components. Beer flavor is, in fact, the result of the combination of a large number of volatile components and the contribution of carbonation, ethanol, bitterness (from hop acids) and sweetness on the other (Meilgaard, 1982), which also influence its mouthfeel and appearance. Flavor is perceived by the detection and integration of stimuli from the gustatory, olfactory and trigeminal systems, and the interactions between these stimuli can considerably modify sensory perception (Clark et al., 2011; Ickes & Cadwallader, 2017; Verhagen & Engelen, 2006). For instance, the perception of sweetness was highest in RoCasT3, even though, according to the analytical data, this beer exhibited the highest alcohol production and fermentative efficiency, resulting in the lowest residual sugar content. However, several factors are involved in the perception of sweetness. Hop variety can affect sweetness both directly, through hop's bittering substances (iso-alpha acids), and indirectly, via its aromatic contribution. The type of yeast strain can also produce a distinct aromatic profile (e.g. fruity esters or spicy phenols) impacting sweetness perception (Techakriengkrai et al., 2004), and the composition and interaction of volatile compounds may also generate sweetness which can be perceived by the panellists (Dietz et al., 2021). Lastly, by providing a warmth sensation, alcohol content may enhance sweetness perception. In the case of Cascade hops, interactions with the two yeast strains seem to affect how the panellists perceived the sweetness of the beer, especially when cone maturation was considered. The sweetness of Columbus-hopped beer, on the other hand, appeared to depend more on the hop variety itself than on other

variables.

No significant differences were observed in the perception of bitterness, which could be explained by the small scale of brewing in our experiment. As [Postigo and colleagues \(2021\)](#) noted, brewery-scale fermentation yields higher bitterness concentrations compared to the smaller one-litre experimental scale. Additionally, other studies have suggested that α -acid molecules in hops can adhere to yeast cell walls, removing these molecules from the beer and thus reducing bitterness ([Laws et al., 1972](#); [Popescu et al., 2013](#)).

The evaluation of sourness in beers further illustrates how aromatic compounds influence taste-olfactory perception. Interestingly, beers brewed with Cascade hops were perceived as sourer than those brewed with Columbus hops, despite pH data. For beers hopped with cv. Columbus, the perceived sourness reported by panellists closely corresponded to the measured pH values, whereas Cascade-hopped beers fermented with the '77' yeast strain exhibited a trend which could not be completely explained by analytical parameters. In detail, the sourness perceived in 77CasT1 was second only to RoCasT1, despite the former's significantly higher pH compared to all other beers fermented with 'Rock'. The dealignment of the perceived sourness with the pH value, has been previously described by [Gloess and colleagues \(Gloess et al., 2013\)](#), in a study on sensory and instrumental analysis of coffee. In their research, the authors observed no relationship between pH or titratable acidity and the perceived acidity in taste or aftertaste; specifically, the coffee brew perceived as the least acidic was also the one exhibiting the highest pH value.

The interaction between hop-derived bittering, other flavour compounds and yeast-produced esters has been shown to influence beer taste and mouthfeel. Citrus and floral notes, for example, are associated with "smooth bitterness" and can contribute to the perception of sweetness and sourness ([Dietz et al., 2022](#)). In our experiment, panellists distinctly perceived citrus and fruity aromas in beers brewed with 77Cas ([Fig. 6](#)). The generally higher perception of sweetness attributed to the Cascade hops appears to have influenced the panellists' evaluation of beer alcoholic strength, described as a "warming" sensation. Beers flavoured with cv. Cascade were perceived as warmer compared to those flavoured with cv. Columbus, regardless of harvest date or yeast strain used. This suggests the perception of sweetness and warmth are connected, as revealed in previous studies where the addition of sweeteners was found to enhance the perception of warmth while, in a similar way, increased ethanol levels amplified the perception of sweetness ([Dietz et al., 2021](#)). The observed interaction in this study may, therefore, be the result of ethanol activating certain nerve fibres sensitive to sugar, as suggested by [Scinska et al. \(2000\)](#). Sensory attributes such as softness and dryness also appear to be closely tied to hop variety and cone maturation stage ([Fig. 3](#) and [Fig. S1](#)). Overall, beers hopped with cv. Cascade exhibited greater softness compared to those with cv. Columbus, the latter being perceived as drier ([Fig. 3](#)).

Regarding gustatory-olfactory (G-O) intensity, persistency and quality, the panel's evaluation was similar to the corresponding olfactory parameters. For example, in beers hopped with Cascade, the highest olfactory intensity perception was in 77CasT2 beers, while with Rock it was in RoCasT1.

In beers hopped with Columbus, the trend was reversed, with T2 hop beers being generally less intense, regardless of the yeast used.

In general, the G-O quality of Cascade-hopped beers was considered higher than that of Columbus-hopped beers, particularly in beverages fermented with '77' yeast. When considering Columbus-hopped beers alone, G-O quality was judged superior in beverages fermented with 'Rock' yeast. The panel's final, overall evaluation of the investigated beers depended mainly on hop variety and yeast type, with Cascade-hopped beers generally receiving higher ratings compared to Columbus-hopped beers. Several studies have demonstrated that different hop varieties naturally differ in their essential oil composition and volatile compound profiles, resulting in distinct sensory characteristics in beer ([Inui et al., 2013](#)). These differences are mainly related to

the qualitative and quantitative variability of key aroma compounds such as monoterpenes (e.g., myrcene, linalool, geraniol), sesquiterpenes (e.g., humulene, caryophyllene), and sulfur-containing volatiles. For instance, Cascade hops are typically associated with citrus, floral, and slightly spicy notes due to their relatively high levels of linalool and geraniol, whereas Columbus hops are characterized by resinous, piney, and earthy aromas, related to their higher concentrations of myrcene and α -acids, and these differences support their distinct aromatic profiles and technological behaviors during brewing.

Overall, our data indicates a strong interaction between hop variety and yeast strain. In fact, although the greater olfactory pleasantness of beers hopped with cv. Cascade was expected, it was interesting to see that Columbus-hopped beers fermented with '77' were considered less pleasant than beers fermented with 'Rock' yeast. The differences attributed to yeast could be related to the higher presence in '77' of enzymes which augment the expression of aromatic precursors of Cascade hops, especially those from thiolic molecules which impart a variety of desirable beer flavours and aromas ([Kumar et al., 2023](#); [Molitor et al., 2022](#)). In cv. Columbus, the lower performance of '77' might be explained by the presence of molecules that lead to the production of a sulphuric odour ([Fig. 6](#)). On the other hand, 'Rock' yeast showed consistent characteristics, which chiefly depended on the variety, giving both cv. Cascade and cv. Columbus beers similar overall ratings, especially when cv. Columbus was late-harvested. As can be seen from the biplot ([Fig. S2](#)) and the CATA analysis ([Fig. 6](#)), the addition of 'Rock' moved the olfactory perception of Columbus-hopped beer closer to the fruity attributes area, whereas with the use of the '77' strain (77Col) beers were located in the area of sulphurous and vegetal attributes.

5. Conclusions

Findings from the current experiment highlight the importance of the interaction between yeast strain, hop variety and cone-harvest stage on the sensory characteristics of beer. While the differences among beers produced with various hop varieties were expected, there is limited literature available which evaluates the flavour and aroma perceptions of a trained panel regarding the specific interaction between cone maturity and yeast strain.

The 'Rock' yeast, a commercial strain selected for beer production, demonstrated significant potential in ensuring the presence of consistent sensory notes in beers, even when hops are not harvested at their optimal stage. This was evidenced by the ability of 'Rock' yeast, when used with cv. Columbus hops, to shift the panel's perception from predominantly vegetal and sulphurous aromas towards more fruity notes, despite the fact that cones of this hop variety, especially when very mature, are known for their pungent aroma which comes from sulphuric compounds.

The ISE77 ('77') yeast strain, on the other hand, exhibited a notable potential to enhance both the aromatic and flavour profiles of hops (such as cv. Cascade) with a high content of aromatic precursors, even when these are harvested before the optimal stage.

Another significant finding concerned the panel's perception of warmth, sweetness and sourness even in those beers with a lower alcohol content (0.5 % ABV less when compared with other beers). Such an outcome could mean that brewers might be able to produce beers with lower alcohol content without sacrificing the tactile effects ensured by alcohol.

It appears, therefore, that the choice of yeast could provide a useful tool for brewers, extending far beyond its influence on beer style. Our results show that selecting the right yeast can improve the quality of beer brewing even when raw materials are suboptimal, so that the best possible result is achieved (depending on the brewing objectives) even with reduced raw material usage. One way of making beer brewing more sustainable, therefore, would be by exploiting biotransformation to enhance the product's aroma while using less hop cones. Since hops

typically account for the largest portion of raw material costs, reducing the quantity needed for brewing could also limit their environmental impact. Future studies integrating the determination of free amino nitrogen (FAN) or detailed amino acid profiles throughout the fermentation process would enable a deeper understanding of yeast nitrogen assimilation and its contribution to flavour development. In this context, the present results should be regarded as an initial step, paving the way for further investigations required to clarify how these biochemical interactions influence the beer brewing process and to provide brewers with scientifically grounded knowledge and new opportunities for process optimisation.

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Ethical statement

Ethical review and approval were not required for this study in accordance with national legislation and institutional requirements. Sensory evaluation was carried out by trained expert panellists who voluntarily participated after signing a written informed consent form. No personal or sensitive data were collected, and all participants were fully informed about the study objectives and their right to withdraw at any time. All assessors voluntarily participated in the tasting sessions after receiving full disclosure of the study objectives, procedures, and potential risks. Prior to participation, each panellist provided informed consent by signing a consent form acknowledging their understanding of the study and their right to withdraw at any time without penalty. No coercion was applied to encourage participation, and participants' personal data were treated confidentially in compliance with privacy protection standards. The study did not involve any vulnerable populations. (Copies of all signed consent forms have been collected and are available as individual PDF files for verification.)

Ethical statement

Participants gave informed consent via the statement “I am aware that my responses are confidential, and I agree to participate in this survey” where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

CRedit authorship contribution statement

Tina Lino: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Tai Gladys Whittingham Forte:** Writing – review & editing, Writing – original draft, Software, Investigation, Data curation. **Margherita Rodolfi:** Writing – review & editing, Formal analysis. **Antonella Costantini:** Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Martina Galaverni:** Formal analysis, Data curation. **Gianina Forestello:** Investigation. **Katya Carbone:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Christos Tsolakis:** Investigation. **Laura Pulcini:** Investigation. **Federica Bonello:** Investigation, Formal analysis. **Maurizio Petrozziello:** Investigation, Data curation. **Deborah Beghe:** Writing – review & editing, Formal analysis. **Matteo Marieschi:** Formal analysis. **Andriani Asproudi:** Investigation. **Vasiliki Ragkousi:** Investigation. **Maria Carla Cravero:** Investigation, Data curation. **Tommaso Ganino:** Writing – review &

editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tommaso Ganino reports financial support was provided by University of Parma Department of Food and Drug. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2026.101729](https://doi.org/10.1016/j.afres.2026.101729).

Data availability

Data will be made available on request.

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