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A unifying approach to wheat beer flavour by chemometric analyses. Could we speak of 'terroir'?

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ABSTRACT

Raw materials are recognized to affect the sensory profile of 'Blanche' craft beers and their 'terroir'. Two common wheat (*Triticum aestivum,* L.) were harvested in three experimental fields with different pedo-climatic conditions and altitudes, and then used for beer production. The taste and flavour of wheat beers were analysed by sensory (panel and consumer test) and SPME GC-MS analyses. Panel dataset was processed by multivariate statistical analyses: a principal component analysis (PCA) revealed that formulation was the main source of variation of sensory profile in wheat beers and a generalized Procrustes analysis (GPA) showed how wheat origin affected the sensory profiles of wheat craft beers based on the consensus among panelists. Moreover, a partial least-squares discriminant analysis (PLS-DA) on VOCs permitted to discriminate and characterize beers selected by a panel-driven approach. By comparing panel and VOCs results, it was possible to highlight that higher altitudes of wheat cultivation determine an increase of pleasant notes such as fruity and herbal. A PCA on consumer test data confirmed that formulation was the main factor affecting liking scores and that the preferences were affected by age, involvement and frequency of use. An internal preference map combining panel and consumer data suggested that the majority of preferences are driven by a few key sensory attributes. Differences in liking among the considered beers revealed two main consumer groups.

1. Introduction

The concept of 'terroir' refers to the special characteristics of a region or a place and combines different factors related to the specific growth habitat of a crop (e.g. vine), such as environmental factors, including pedological and climatic conditions, as well as agronomic practices, which impart a unique quality to this crop (Cross et al., 2011). The effect of terroir implies that food products (e.g. wine) derived from crops grown in a particular region have unique characteristics, incapable of being reproduced outside that area, even if the variety, agronomic practices and processing techniques are the same. The concept of terroir that historically has been adopted by winemakers (van Leeuwen, 2022), recently has also been extended to beers (Yool and Comrie, 2014). Some recent studies (Van Holle et al., 2017, 2021; Carbone et al., 2020; Morcol et al., 2020) were carried out on beers to evidence the effect of terroir but they were exclusively aimed to investigate the origin of hops, whereas the geographic combination of variations in yeast, hops, malt (or cereals) and water produce, a 'taste of the place' that one can term the 'terroir' of beer (Matthews, 2016).

Wheat is a prime material commonly used for specialty beer production (Blanche or Witbier, and Saison). Wheat is grown on more land area than any other commercial crop. Although the crop is most successful between the latitudes of 30° and 60°N and 27° and 40°S, wheat can be grown beyond these limits, from within the Arctic Circle to higher elevations near the equator. As far as elevation is concerned, wheat is grown over a wide range of altitudes from sea level to more than 3000 m a.s.l. (Curtis, 2002). In Italy, the cultivation of wheat at high altitudes is practised in the Alps and Apennine Mountain areas (Bell, 1987), where farmers expanded the agricultural land.

Differences in pedoclimatic conditions, resulting from cultivation in different places and at different altitudes, seem much more important than wheat genetics in influencing the volatiles profile of kernels (De Flaviis et al., 2021, 2022a); the place of wheat cultivation could thus impart to this crop distinctive quality traits.

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The results of recent studies showed that it is possible to classify wheat according to their origin (species, variety, area of cultivation) based on their flavour profile (De Flaviis et al., 2021, 2022a, 2023) and that differences in wheat variety and cultivation site could affect the volatile organic compounds (VOCs) profile of wheat beers (De Flaviis et al., 2022b, 2022c). These results confirm those of other authors (Alvim et al., 2017) who reported that the volatile profile of beer is more influenced by raw materials, such as malt and hops, and the method of production than by the kind of fermentation.

The volatiles that contributed to the aroma of beer are known to derive mainly from malted barley (due to the barley itself and the thermal treatment during malting), hops and yeasts as well as beer maturation and ageing (Priest and Stewart, 2006). In traditional beer, barley malt is the main component of beer, on the contrary, wheat beer is constituted of wheat (either raw unmalted or malted) to barley malt ratio of 50%, thus it is still an open question whether the sensory profile of wheat beer could be significantly affected by the origin of wheat.

Our goal is to use a unifying statistical approach that integrates data from both chemical and sensory analyses in assessing the effect of formulation (wheat variety and origin) on wheat beer flavour. To this purpose Blanche (or Witbier) style craft beers were produced using wort made of raw wheat (*Triticum aestivum* L.) of two varieties cultivated in fields sited at different altitudes (70, 500, and 1200 m a.s.l.) added to malt in amounts of 25% and 40%. As a first step sensory data were processed by shape analysis and only the samples that were successfully discriminated for their quality attributes were processed with further analyses. This 'sensory driven approach' was carried out throughout the manuscript, both for sensory and chemical data, in order to unravel as much information as possible from the beers discriminable by expert panelists. As a second step, the effect of wheat origin based on their VOCs profile was evaluated using a partial least-squares discriminant analysis (PLS-DA) approach. Thus, a further shape analysis was computed in order to compare human and instrumental responses. Eventually, the effect of altitude of cultivation on VOCs profile and flavour of the beers was studied by PLS-R. Finally, differences in liking among the considered beers were also studied by internal preference mapping, which unifies sensory preference and descriptive data.

2. Material and methods

2.1. Grain samples

Two different cultivars of common wheat (*Triticum aestivum* L.) were cultivated in randomized plots located in the Abruzzo region (Italy) under organic conditions. The farms were sited in Corropoli (F1 = 70 m a.s.l.), Introdacqua (F2 = 500 m a.s.l.) and Rocca Pia (F3 = 1200 m a.s.l.), presenting different soil and climatic characteristics. F1 is characterized by dry summers and mild, wet winters; F2 is characterized by harsher winters with temperatures that frequently reach negative values, and summers are characterized by strong temperature changes; F3 is located in the Cinquemiglia Plain, one of the major Italian uplands, with winters among the harshest in all the Apennines. The seeds sown were a modern variety Vittorio (registered in 2004), supplied by Agroservice S.p.A. (S. Severino Marche, Italy) and an ancient local one Solina (cultivated in Abruzzo at least since the sixteenth century), supplied by Az. Agricola De Santis (Introdacqua, Italy). All the grains (n = 6) were harvested at physiological maturity between July and August (2020) based on the elevation of the fields. Moisture content of the grains was measured and was lower than 13%. Samples were stored in plastic bins at a controlled temperature (20 ± 1 °C) until the production of beers.

2.2. Production of wheat craft beers

Nine samples of craft beers (Table 1) were produced and bottled in a pilot plant (BeerStudioLAB, Giulianova, TE, Italy) integrated with a Programmable Logic Controller (PLC). The samples under investigation were: (i) a control beer (C) using 100% barley malt; (ii) wheat beers using a blend of 75% barley malt and 25% of unmalted Solina (25S) or Vittorio (25V) wheat; (iii) wheat beers using a blend of 60% barley malt and 40% of unmalted Solina (40S) or Vittorio (40V) wheat.

Other ingredients were the same for the production of all the craft beers to avoid a further source of variation: Hallertau Hersbrucker hops characterized by 2.8% alpha-acids (Hopsteiner, New York, NY, USA), tap water (SO_4^{2-} < 10 mg L^{-1}, Cl^- < 5 mg L^{-1}), Italian barley malt characterized by 200 °WK diastatic activity (Agroalimentare Sud S.p.A., Melfi, PZ, Italy), and neutral dry ale yeast (SafAle US-05, Fermentis by Lesaffre, Marcq en Baroeul, France).

Just before the mashing step, barley malt and raw wheat were dry-ground into a roller mill, to retain as much as prime material volatile compounds as possible. The infusion was obtained in stainless steel tank (Braumeister 20l PLUS, Speidel Tank - und Behälterbau GmbH, Ofterdingen, Germany) initially set at 43 °C. The mashing profile for all the experimental beers was originally optimized for 40% wheat beers considered the worst case scenario from a brewery point of view, and then set as follows: the temperature was raised from 43 to 51 °C and held for 15 min for protein rest; from 51 to 64 °C and held for 30 min for β-amylase rest; from 64 to 72 °C and held for 20 min for α-amylase rest. The final temperature was then raised to 78 °C, and held for 10 min. The PLC system ran the temperature program, and periodic checks were carried out to control the process parameters (temperature and pH). After mashing, a filter basket was used to separate the draff, and then, in order to dissolve the remaining sugars, hot water was poured for about 15 min. Afterwards the wort was boiled and, after 60 min, 20 g of hops pellets was added. After boiling, the wort was cooled down at 20 °C and transferred to a conical fermenter (The grainfather, Bevie Handcraft Auckland, New Zeland). The dry yeast was rehydrated and inoculated in the wort (1 g L^{-1}) for the fermentation step. When the final gravity of the wort reached the value of 1.9 °P (corresponding to at least 7 day at 20 °C), the fermenter temperature was set at 4 °C. The beers were bottled when no yeast was detectable in the purge, and 3.5 g L^{-1} of dextrose monohydrate was added for secondary fermentation, carried out for 20 days at 20 °C. About 21 ± 1 L of each type of beer was obtained. Samples were stored at 18 ± 1 °C until analysis.

Table 1
Formulation of craft wheat beers produced in pilot plant.

Test ID	Pilsen barley malt (%)	Wheat (%)	Wheat varieties	Farm location	Farm coordinates	Farm altitude (m a.s.l.)
C	100	0	–	–	–	–
25SF2	75	25	Solina	Introdacqua	42°02′ N, 13°53′E	500
25VF2	75	25	Vittorio			
40SF1	60	40	Solina	Corropoli	42°49′ N, 13°51′ E	70
40VF1	60	40	Vittorio			
40SF2	60	40	Solina	Introdacqua	42°02′ N, 13°53′E	500
40VF2	60	40	Vittorio			
40SF3	60	40	Solina	Rocca Pia	41°56′ N, 13°58′ E	1200
40VF3	60	40	Vittorio			

2.3. Physical-chemical measures of wort and beers

Beer original extract (°P) was measured on a densimeter (EasyDens, Anton Paar GmbH, Austria). Fermentable sugars (g L^{-1}), alcohol (% v/v), total SO_2 content (ppm), color (EBC units), bitterness (IBU), total polyphenols (mg L^{-1}), residual starch (ppm), free amino nitrogen (FAN; mg L^{-1}) and pH were measured by CDR BeerLab® (FOODLAB®, Florence, Italy). The determination of colour, bitterness, total polyphenols and pH were conducted according to EBC Analytica methods 9.6, 9.8, 9.11 and 9.35 (European Brewery Convention, 2010). The real and apparent attenuation was calculated according to Mascia et al. (2014). Physical-chemical measures were conducted in duplicate.

2.4. SPME GC–MS analysis

The SPME GC-MS analysis was performed as reported by De Flaviis et al. (2022b) without modifications. VOCs identification was carried out by mass spectra matching with the standard NIST14 library, considering a minimum match factor of 700 as a confirmatory value (Sparkman et al., 2011). The identification was complemented by using the Automated Mass spectral Deconvolution and Identification System (AMDIS) software. TurboMass 6.1.0 software was used to calculate the semi-quantitative data normalized using the external standard peak area. GC-MS analysis was conducted in duplicate by using two different beer bottles opened just before analysis.

2.5. Sensory analyses

Consumer and panel tests were carried out in a laboratory designed according to ISO 8589 (1988) and equipped with individual booths and white illumination (D_{65}) and different beer samples (25 mL) were provided to participants at a temperature of service of 6 ± 2 °C with unsalted crackers and still water to serve as palate cleansers. In both sensory analyses, a three-digit random number code was assigned to each beer to have double-blind testing conditions aimed to reduce biases. To avoid the alone and the carry-over effect, the order of serving samples was randomized. The judges tested each sample the same number of folds in different orders during each session. Participants were asked not to have any food or beverage other that plain water at least 30 min before the session. The sensory analyses were planned and carried out in the same week of the GC-MS analysis.

2.5.1. Panel test

A panel of six initiated assessors (2 women and 4 men aged 25–51 years), who were highly experienced with the beverage, was selected for beer sensory characterization. The assessors are beer producers, beer retailers and consultant for beer-making process and periodically collaborate as judges in beer awards competitions. Before analysis, a brief training was organised to define the attributes and vocabulary based on literature and the terminology listed by Analytica- EBC/ASBC (Briggs et al., 2004). Finally, sixteen attributes were chosen and implemented in the sensory schedule. Four of them were flavour descriptors (malt, wheat, hop, and fermented), three were appearance descriptors (clarity, color and foam retention), four were taste descriptors (bitterness, sweet, sour, and taste persistence), three were mouthfeel descriptors (astringency, metallic and warmth) and two were kinaesthetic descriptors (carbonation and body). The scale ranged from 0 (low intensity) to 10 (high intensity). All samples were served immediately after the bottle opening in 80 mL TEKU glass. The test was conducted in duplicate in different days. The sessions were always carried out in the evening to avoid the influence of environmental factors.

2.5.2. Consumer test

Fifty-five healthy individuals (53% women and 47% men, ages ranging from 25 to 63 years) of European ethnicity were recruited in the campus of the *University of Teramo* (Teramo, Italy). The inclusion criteria were: regular beer consumption, interest, and availability of time to participate in the study, while pregnancy was considered an exclusion criterion. The participants were informed about the samples to test (beer), and the risk associated with alcohol intake, as well as the possible presence of priority allergens listed in Annex II of Regulation (EU) 1169/2011. Written informed consent to participate in beer testing was obtained for all subjects. The limited number of participants was due to difficulty in assessors recruiting for different sessions, restricted volume of pilot plant production, and COVID-19 restrictions. The number of participants was deemed sufficient for the study since it was demonstrated that even less participants were enough for discrimination purpose (Márquez et al., 2013). Before tasting, consumers were asked to fill a form with the informed consent to address a set of questions on their sex, age, and familiarity with beer, as described by frequency of use, self-assessed knowledge and involvement. Participants were informed that the questionnaire was anonymous, that their personal data would have been treated according to the EU regulation, that personal information would have not been used for any commercial or non-commercial purposes, and that sensitive information would have been published in aggregate form.

The respondents were asked to evaluate the frequency of use on a 1–5 discreet scale (from less than once a week to once a day), whilst their self-knowledge and involvement were tested on a 1–5 Likert-type scale by using the questions proposed by Perito et al. (2019).

Each beer was tested and rated individually without going back, by using a LAM (labelled affective magnitude) scale on a 100-point bases (Cardello and Schutz, 2004). Every participant chose one phrase on the scale that best represents him/her overall opinion. Different sensory sessions were performed with all the 55 consumers. In each session three samples and a commercial lager beer (R) were served. The latter, a market Italian leader, was used as a reference sample to evaluate the ability of the assessors to discriminate among different beer styles. Participants were instructed to swallow the sample and allowed to rest from 5 to 10 min between beers to help them avoid fatigue due to palate saturation caused by the flavours found in beers as well as the presence of alcohol that could affect their sensorial perception. The sessions were always carried out in the late morning to avoid the influence of environmental factors.

2.6. Statistical analysis

Consumer data were centred within each session per participant before any further statistical analysis in order to obtain a common relative scale. One-way ANOVA was computed to evaluate statistical significance ($p < 0.05$) in each VOCs, and mean differences were evaluated by LSD post-hoc test. Friedman test, a non-parametric alternative to ANOVA test, was used to evaluate significant ($p < 0.05$) differences for liking scores in consumer test, where the assumption of normality was not acceptable.

Panel test data were preliminary analysed by mixed-model ANOVA considering panel and session as random factors for each variables separately. Principal component analysis (PCA) based on the covariance matrix was carried out on the complete panel dataset to evaluate the panelist consensus. In addition, a PCA based on the correlation matrix was performed on the consumer and panel scores to reduce the dimensionality of the dataset. Moreover, a generalized Procrustes analysis (GPA) was used to transform several multidimensional configurations (judges) so that they become as much alike as possible. Data were scaled, rotated and/or reflected by using Commaunder method. GPA was also performed to compare GC-MS results with panel scores, treating GC-MS data as a further configuration.

Partial least-squares (PLS) analyses were used as supervised methods in regression (PLS-R) and discriminant analysis (PLS-DA). Jackknife method, using 5 groups, was chosen for cross-validation and permitted to identify the significant number of latent variables (LV) by minimizing the predicted residual error sum of squares (PRESS). Centred and standardized explanatory variables were processed by the algorithm. Only VOCs with a variable importance in projection (VIP) greater than one was chosen and further discussed.

PLS-R was performed both on the panel and VOCs dataset, and altitude was chosen as a unique continuous dependent regression variable. Subsequently, PLS-DA was chosen in order to classify and predict beer samples using VOCs as dependent variables. Variables identified as VIP were capable of discriminating wheat beers and were evaluated based on the weight (w*) on the relative LV. PLS-DA was visualized using the w*/c space on the two calculated LVs that explains the maximum R^2Y. Moreover, a further PLS-R was computed to understand the importance of psychographic variables towards overall consumer liking. Internal preference mapping was computed by using the PLS method and standardizing X and Y datasets.

Statistics were performed using XLSTAT 2021 software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France) while internal preference mapping was computed using ConsumerCheck (Version: 2.3.1, freely available on https://consumercheck.co/downloads/, last accessed on May 24, 2022).

3. Results and discussion

Being this study focused on the effect of wheat amount and type and sensory properties of wheat beer, flavourings, such as coriander, orange, and bitter orange (typically used for Blanche or Witbier – style craft beers), were avoided and a commercial 'neutral' yeast US-05 was used due to its ability to produce well-balanced beers with a very clean, and crisp palate (Canonico et al., 2016). Moreover, the hops (Hallertau Hersbrucker) was chosen due to its delicate hops-like aroma profile (Steinhaus et al., 2007) as reported in the Hopslist wheat beer style guide (www.hopslist.com/hops/aroma-hops/hersbrucker/, accessed on May 24, 2022) and already used by other authors (Benedetti et al., 2016).

3.1. Craft beer characterization

In Table 2 the main physical-chemical parameters of beers and wort were reported. In general, all samples presented the typical characteristics of Belgian Ale (Blanche or Witbier) style beer (Beer Judge Certification Program, 2015). These results suggested that, from a technological perspective, the craft beers were similar, as expected by the experimental plan, and the differences are due mainly to malt/wheat ratio and wheat origin, rather than process parameters.

As shown by the real and apparent attenuation values, fermentation progressed to a greater extent in C beer, and to a lesser extent in the 40SF3, 40VF3, 40VF2, and 40SF1 samples. These findings were in accordance with parameters measured in wort, such as original extract, FAN, and fermentable sugars, which significant decrease with the increase of wheat amount. Conversely, the residual starch is positively correlated with wheat addition. These results are reasonable, since the barley malt undergoes to germination step producing fermentable sugars and nitrogenous materials (e.g. amino acids, peptides), which promote the fermentation process, whereas nutrients provided by unmalted wheat are more difficult to metabolize (Pires et al., 2014; Faltermaier et al., 2014).

Moreover, the beer with the highest polyphenol content was the control sample (C), followed by 25% wheat beers, and 40% wheat beers, these results are in accordance with those reported by Yorke et al. (2021). In fact, an increase in wheat proteins promotes a decrease in polyphenol content, indeed, during fermentation wheat proteins interact with polyphenols forming insoluble precipitates (Depraetere et al., 2004). Within 40% wheat beers, a significant difference was found between varieties, and in particular, Vittorio wheat showed higher polyphenol content compared to Solina wheat regardless of the experimental fields (F1, F2, F3).

3.2. Panel test

The panelists tasted all the craft beers in two different sessions and evaluated them by using both fixed attributes and facultative ones, available in a vocabulary list previously defined. Twelve of them were selected to deeper describe the sample beers: alcoholic, fruity, floral, spicy, herbal, balsamic, nutty, roasted, phenolic, lactic, diacetyl, yeast and honey. Often, the attribute "fruity" was followed by specifications i.e. banana, apricot, pear, citrus, apple and peach, which were added to the dataset, leading to a total of 19 facultative attributes. All the attributes were statistically analysed through a preliminary mixed-model ANOVA, revealing no significance ($p > 0.05$) in session or panelist factors.

3.2.1. PCA results

Since a total of 36 variables were evaluated by the panel, a PCA was computed on the attribute means to reduce dimensionality to a few

Table 2
Physical–chemical analysis of wort and wheat beers.

											F	F	F
		C	25SF2	25VF2	40SF1	40VF1	40SF2	40VF2	40SF3	40VF3	WA	VAR	EF
Beer	pH	4.32 a	4.21 bc	4.22 bc	4.27 ab	4.24 b	4.24 b	4.17 c	4.24 b	4.27 ab	ns	ns	ns
	Fermentable sugars (g L^{-1})	0.1 a	0.1 a	0.1 a	0.1 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	ns	ns	ns
	Alcohol (% v/v)	6.2 a	5.8 ab	5.9 ab	5.1 c	5.7 b	5.8 ab	5.5 b	5.9 ab	5.9 ab	ns	ns	ns
	Total Polyphenols (mg L^{-1})	125 a	96 b	100 b	82 d	90 c	84 d	89 c	85 d	90 c	17.17**	40.50**	ns
	Color (EBC units)	6 a	6 a	6 a	6 a	6 a	5 a	5 a	5 a	6a	ns	ns	ns
	Bitterness (IBU)	5 a	8 a	5 a	7 a	8 a	7 a	8 a	7 a	7 a	ns	ns	ns
	Total SO_2 content (ppm)	1.3 a	1.0 b	1.0 b	1.1 b	1.0 b	1.0 b	1.1 b	1.1 b	1.1 b	ns	ns	ns
	Apparent extract (°P)	1.7 a	1.9 a	1.9 a	1.9 a	1.9 a	1.9 a	1.9 a	1.9 a	1.9 a	ns	ns	ns
	Real extract (°P)	3.5 a	3.5 a	3.6 a	3.4 a	3.6 a	3.6 a	3.5 a	3.4 a	3.4 a	ns	ns	ns
	Apparent attenuation (%)	85.2 a	82.8 b	83.1 b	81.6 c	83.1 b	83.1 b	82.3 bc	81.8 c	81.8 c	ns	ns	ns
	Real attenuation (%)	69.6 a	68.5 b	67.9 b	67.0 c	67.9 b	67.9 b	67.2 bc	67.3 bc	67.3 bc	ns	ns	ns
Wort	pH	5.42 ab	5.43 ab	5.49 a	5.38 b	5.41 ab	5.42 ab	5.42 ab	5.43 ab	5.41 ab	2.16*	ns	ns
	Original extract (°P)	11.5 a	11.1 b	11.2 b	10.3 c	11.2 b	11.2 b	10.7 bc	10.4 c	10.4 c	ns	ns	ns
	Fermentable sugars (g L^{-1})	98 a	91 b	93 b	84 cd	87 c	88 c	88 c	85 cd	83 d	1.02**	ns	ns
	Residual starch (ppm)	0.22 e	0.44 bc	0.35 d	0.68 a	0.38 d	0.40 c	0.48 bc	0.53 b	0.42 c	ns	ns	ns
	Color (EBC units)	7 a	6 a	6 a	5 a	5 a	5 a	5 a	5 a	5 a	ns	ns	ns
	FAN (mg L^{-1})	55 a	53 a	52 a	45 b	47 b	48 b	45 b	44 b	45 b	35.51***	ns	ns

(25) 25% wheat amount; (40) 40% wheat amount; (C) Control sample; (V) Vittorio wheat; (S) Solina wheat; (F1) 70 m a.s.l; (F2) 500 m a.s.l.; (F3) 1200 m a.s.l.; (WA) wheat amount; (VAR) variety; (EF) experimental fields; (FAN) free amino nitrogen; (F) Fisher test; (ns) not significant. Significance level $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

components that explained the majority of the variance. In Fig. 1a a biplot shows the scores and loadings along the first two principal components, which explained the 47.2% of the total variance. The figure describes the data structure in terms of variance, and permits the separation of beers based on their wheat amount, since only 25VF2 and 25SF2 have positive and negative values along the first and second components respectively. The separation based on wheat addition may be due to different formulations of craft beers, since it has been widely reported that wort nutrients such as FAN and fermentable sugars affect the aroma profile (Pires et al., 2014). Given that, the main effect on the sensory profile was the wheat percentage used, and there is not any evident effect of wheat origin.

Thus, to unveil hidden information, and to avoid an additional source of variation, further statistical analyses were computed on samples with 40% of wheat (n = 6). Reducing the dataset implied the removal of diacetyl, cherry, and peach attributes, identified only in 25% wheat beers and control beer.

Following this approach, a new PCA was computed (Fig. 1b), and the first two PCs explained the 68.2% of the total variance. The PC2 (explaining the 26.7% of total variance) permitted to discriminate beers made with wheat cultivated in two different experimental fields (F1 = 70 and F2 = 500 m a.s.l.). The highest loadings (>0.7) observed along the PC2 were clarity, sweet, banana, characterizing F1 (+), and floral and wheat, characterizing F2 (−). Additionally, the first two PCs were not able to separate the beers produced by using wheat cultivated in mountain area (F3). By exploring the PCA, 40VF3 and 40SF3 are explained by the fourth PC ($\cos(t)^2$ of 0.954 and 0.944 respectively) that explained only the 9.5% of the total variance. The profile of F3 samples in the radial graphs showed higher circularity and roundness than those of other fields (Supplementary Fig. S1); this indicates that F3 samples had a flavour profile less characteristic and more balanced than the others, and thus they scored close to the centre of the PCA plot. In general, altitude may affect in several ways the quality of wheat such as nutrients content. Indeed, the wheat cultivated at 1200 m a.s.l. (F3) showed the lowest protein content (see Section 3.5), probably due to stricter environmental conditions, resulting in different VOCs metabolism of yeasts (Pires et al., 2014) compared to other wheat cultivated at lower altitudes.

3.2.2. *Consensus approach*

Two statistical approaches were used to understand the consensus among panelists. Firstly, a PCA based on covariance matrix (raw scores data), was computed by using all the dataset, and permitted to show the scores of the six panelists and to highlight, through confidential ellipses, a few outlier scores and higher density close to the axes centre (Supplementary Fig. S2). Panelist #4 and Panelist #1 were the most extremist (the largest ellipse), whereas panelist 6# was the most conservative (the narrowest ellipse).

From this picture, it is not possible to understand which sample or samples generated more accordance considering the judgements for this reason, a GPA was computed, and a consensus map (Fig. 2a and b) was constructed to analyse the response of every panelist separately (configurations). Fig. 2a and b shows the GPA scores of each panelist along the first three GPA factors explaining the 89.54% of the total variance. The samples 40VF1, 40SF2, and 40VF2 were the beers with more consensus along the first two factors since the panelists scores do not show overlapping with those of other samples, whilst the sample 40SF1 was the beer with more consensus along the third factor. Beers made with wheat cultivated in mountain area (F3) resulted very similar among them and are located at the centre of the axes of the first three factors; this result is in accordance with PCA data (Fig. 1b). Subsequently, the consensus centroids for samples and attributes were plotted along the first three factors in Fig. 3a and b. The panel was able to describe 40VF1, 40SF2, 40VF2, and 40SF1 individually and different attributes characterized different samples, this indicates a strong interaction between genotype (variety) and environment (altitude) for wheat used in beer formulation. In particular, the 40VF1 beer sample was described with sweet, lactic, malt and banana notes, relatively more alcoholic, with a fuller body and higher clarity; the 40VF2 beer sample was described as carbonated and with nutty, apple and herbal notes; the 40SF1 beer sample was described with fermented, yeast, phenolic, spicy, apricot and banana notes and with a higher level of metallic and astringency; 40SF2 beer sample was described with pear, citrus and balsamic notes and with a higher level of bitterness.

3.3. *Panel driven analysis of VOCs profile by PLS-DA*

Being the aim of this work to obtain useful information on the flavour profile of craft wheat beer as described by VOCs analysis and the human sensory system, all the beers were previously analysed by SPME GC-MS (De Flaviis et al., 2022b) and the obtained VOCs dataset was used for further statistical analysis.

The wheat beers consensually discriminated by all the panel members (40VF1, 40SF2, 40VF2, and 40SF1) showed 30 VOCs of which 7 alcohols, 2 aldehydes, 14 esters, 1 ketone, 3 organic acids, 1 terpene and 2 other compounds not classified in the previous chemical groups (Table 3). A supervised multivariate statistical approach was used in order to better analyse the VOCs dataset, in particular a PLS-DA was

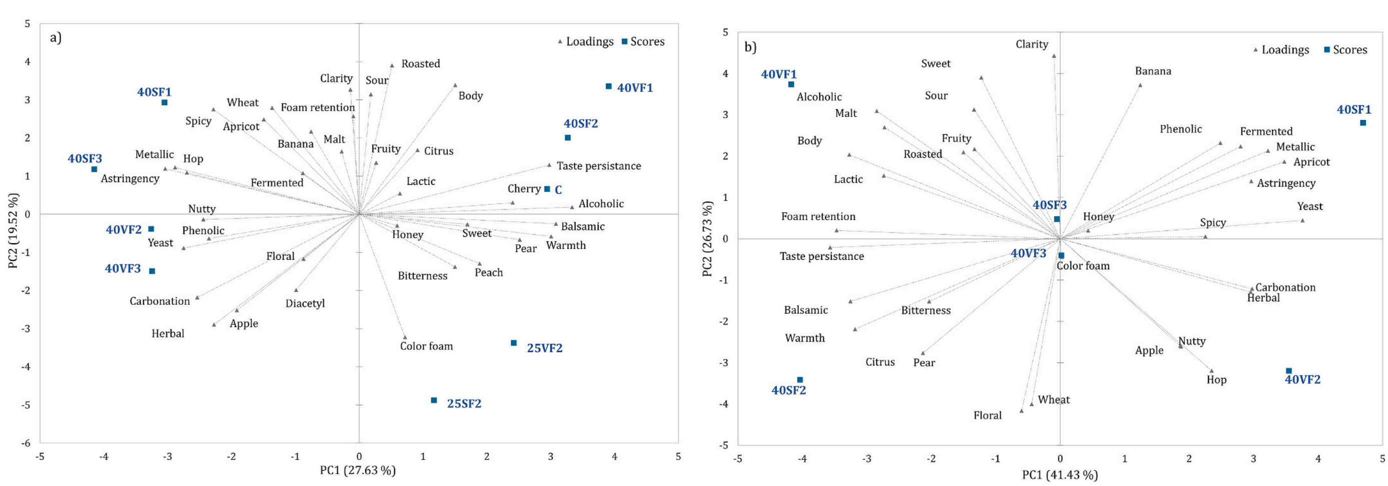

Fig. 1. PCA bi-plots obtained by using the panel dataset. All the craft wheat beers (a) and only the 40% wheat beers (b) were used as sample scores. Score nomenclature: (25) 25% wheat amount; (40) 40% wheat amount; (C) Control beer sample; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua (F3; 1200 m a.s.l.); Rocca Pia.

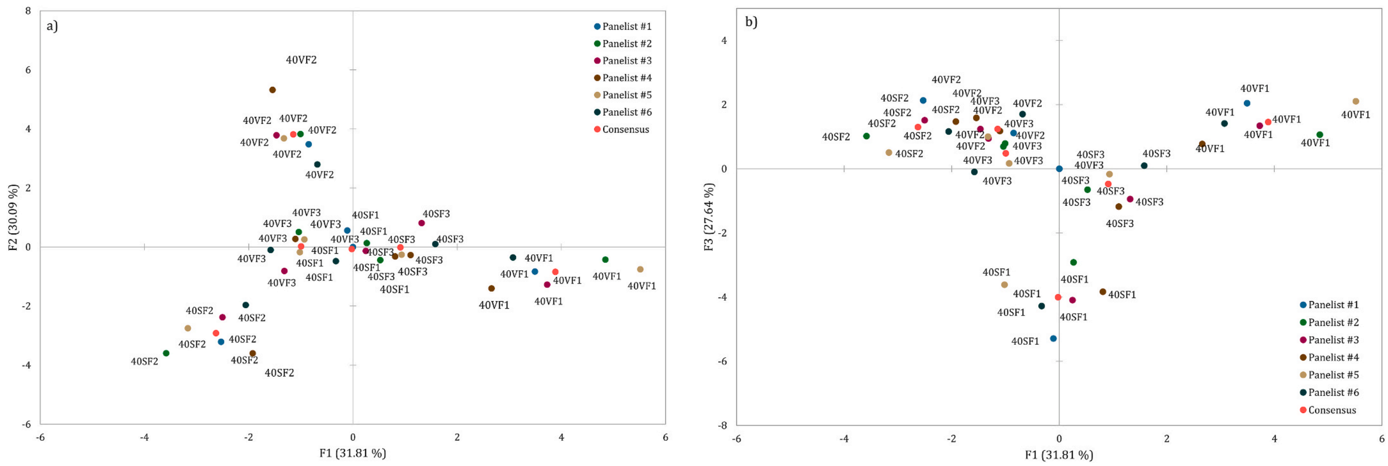

Fig. 2. Consensus maps, presented by configuration (six panelists), obtained through GPA by using the panel dataset, along the first and second factors (a), and the first and third factors (b). Score nomenclature: (40) 40% wheat amount; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua (F3; 1200 m a.s.l.); Rocca Pia.

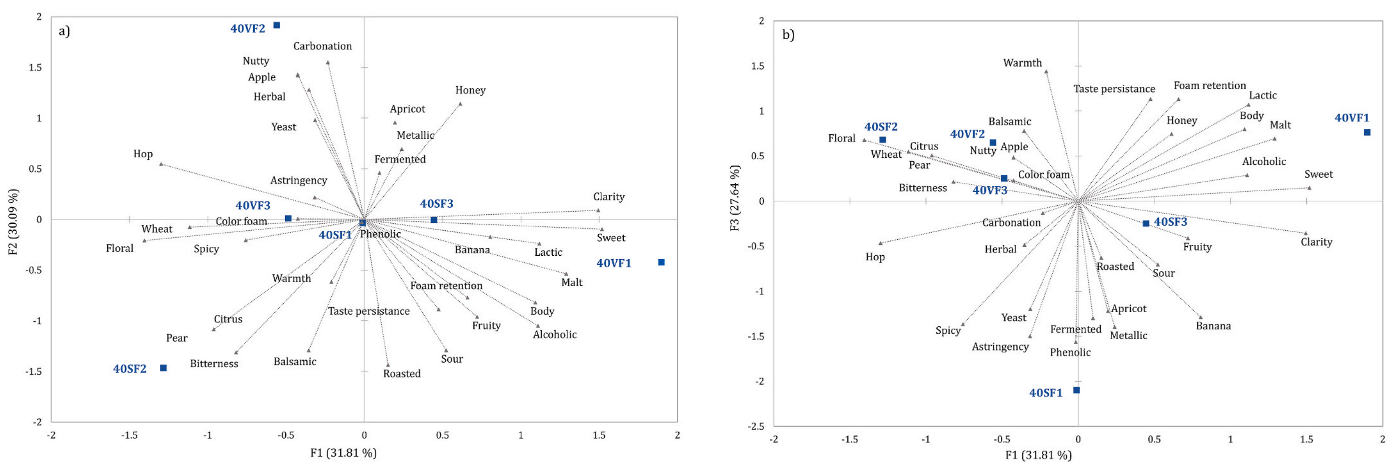

Fig. 3. GPA bi-plots of centroid coordinates by using the panel dataset, along the first and second factors (a), and the first and third factors (b). Score nomenclature: (40) 40% wheat amount; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua (F3; 1200 m a.s.l.); Rocca Pia.

performed on 40VF1, 40SF2, 40VF2 and 40SF1 samples as a categorical predictor (y) with a 100% correct classification. Three calculated LVs permitted to discriminate sample beers among them based on the VOCs profile as shown by Fig. 4a and b, where the variable weights (w*) were plotted together with samples scores (c vectors). Fifteen VOCs were computed as VIPs (Supplementary Table S1) and further discussed.

Just two calculated LVs, which explain the 32% and 31% of R^2X and R^2Y respectively, were sufficient to discriminate samples 40SF1 and 40VF1 from samples 40SF2 and 40VF2. On other hand, the third LV that explains 33% of R^2Y permitted to discriminate 40VF2 from 40SF2. Variable weight (w*) of selected VIPs permitted to understand the contribution of the variable to the sample prediction as depicted in Fig. 4a and b, where also the odour type (www.thegoodscentscompany.com) was reported.

In particular, the 40VF1 beer sample was characterized by 1-Decanol (fatty, waxy, floral, orange, sweet, clean, watery), 4-Vinylguaiacol (spicy, clove, carnation, phenolic, peppery, smoky, woody, powdery), Ethyl decanoate (sweet, waxy, fruity, apple, grape, oily, brandy) and 2-Methyl-1-butanol (roasted, ethereal, fusel, alcoholic, fatty, greasy, winey, whiskey, leathery, cocoa); 40VF2 beer sample was characterized by 2-Ethylhexanol (citrus, fresh, floral, oily, sweet), 2-Phenylethanol (floral, rose, dried rose), 2-Nonanone (fruity, fresh, sweet, green, weedy, earthy, herbal), 3-Methyl-1-butanol (fermented, fusel, alcoholic, whiskey, fruity, banana) and Styrene (sweet, balsam, floral, plastic); 40SF1 beer sample was characterized by 2-Nonanone (fruity, fresh, sweet, green, weedy, earthy, herbal), 4-Vinylguaiacol (spicy, clove, carnation, phenolic, peppery, smoky, woody, powdery) and Decanal (sweet, aldehydic, waxy, orange, peel, citrus, floral); 40SF2 beer sample was characterized by Styrene (sweet, balsam, floral, plastic), Decanoic acid (unpleasant, rancid, sour, fatty, citrus) and Ethyl dodecanoate (sweet, waxy, floral, soapy, clean).

3.4. Consensus between panel and VOCs analysis

Assessors and instrumental (GC-MS) responses were compared with a further GPA in order to understand if there is a correspondence between the two analytical approaches. Fig. 5 shows the consensus map of the four wheat beers discriminated by assessors along the first two factors that explain the 70.9% of the total variance. Since no overlapping among samples was present, it could be assumed that SPME GC-MS behaves as a panelist for beer aroma profile discrimination. So, an attempt of comparison between the odour types of VOCs and panel attributes was carried out. Interestingly, several communal odour descriptors were found between the two analytical methods by comparing loadings computed in GPA (Fig. 3a and b) and weights in PLS-DA (Fig. 4a and b), briefly: sweet, fruity, and alcoholic notes

Table 3
Volatile compounds (VOCs) detected in selected beers.

Chemical group	Compound	Average RT	40SF1	40SF2	40VF1	40VF2
Alcohols	1-Propanol, 2-methyl	2.57	0.071 c	0.078 bc	0.087 ab	0.091 a
	1-Butanol, 3-methyl-	3.34	0.526 a	0.530 a	0.568 a	0.611 a
	1-Butanol, 2-methyl-	3.37	0.133 c	0.151 b	0.171 a	0.155 b
	2-Ethylhexanol	7.86	0.001 c	0.020 b	0.001 c	0.028 a
	1-Octanol	8.66	0.013 a	0.010 b	0.012 a	0.011 ab
	2-Phenylethanol	9.52	0.327 a	0.287 a	0.293 a	0.338 a
	1-Decanol	12.36	0.003 a	0.003 a	0.003 a	0.000 b
Aldehydes	Nonanal	9.32	0.006 a	0.002 b	0.003 b	0.002 b
	Decanal	11.2	0.003 a	0.001 b	0.002 ab	0.001 b
Esters	Ethyl acetate	2.51	0.068 ab	0.064 b	0.078 a	0.074 ab
	2-Methylpropyl acetate	3.73	0.006 a	0.005 ab	0.004 b	0.005 ab
	Ethyl butyrate	4.06	0.010 a	0.010 a	0.011 a	0.010 a
	3-Methylbutyl acetate	5.16	0.227 a	0.197 ab	0.191 b	0.196 ab
	Ethyl hexanoate	7.3	0.200 a	0.164 b	0.152 b	0.162 b
	Ethyl heptanoate	9.15	0.002 b	0.003 a	0.003 ab	0.003 ab
	Heptyl acetate	9.42	0.003 a	0.000 b	0.000 b	0.000 b
	Ethyl octanoate	10.99	0.812 b	1.005 a	0.966 ab	0.947 ab
	Ethyl 2-phenylacetate	11.87	0.001 a	0.000 b	0.000 b	0.000 b
	2-Phenethyl acetate	12.08	0.096 a	0.066 b	0.064 b	0.062 b
	Ethyl nonanoate	12.75	0.018 a	0.004 b	0.001 b	0.000 b
	Ethyl 9-decenoate	14.3	0.015 b	0.021 ab	0.024 ab	0.030 a
	Ethyl decanoate	14.43	0.036 b	0.066 a	0.066 a	0.052 ab
	Ethyl dodecanoate	17.56	0.000 c	0.004 a	0.002 b	0.002 b
Ketone	2-Nonanone	9.05	0.004 a	0.000 c	0.000 c	0.002 b
Organic acids	Pentanoic acid	6.83	0.004 a	0.003 a	0.003 a	0.003 a
	Octanoic acid	10.58	0.147 a	0.112 ab	0.095 b	0.113 ab
	Decanoic acid	13.85	0.003 a	0.003 a	0.002 a	0.002 a
Others	Styrene	5.48	0.031 ab	0.039 a	0.027 b	0.034 ab
	4-Vinylguaiacol	13.08	0.005 a	0.003 b	0.004 ab	0.002 b
Terpene	β-Linalool	9.23	0.007 a	0.006 a	0.005 a	0.005 a

Data on column with different letters were statistically different at p level <0.05.

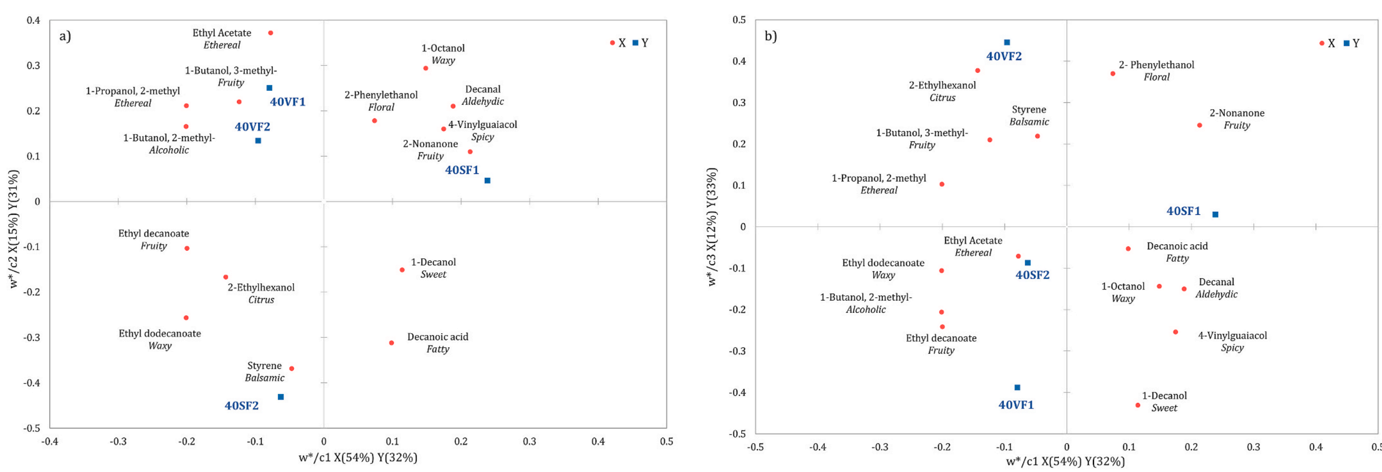

Fig. 4. Scatter plot of sample scores (c) and VOC weights (w*) on the first and second LVs (a) and on the first and third LVs (b) calculated by PLS-DA using four selected panel-driven beers as categorical variable. VOC labels report the main odour type (www.thegoodscentscompany.com). Score nomenclature: (40) 40% wheat amount; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua.

characterized sample 40VF1; fruity and herbal notes characterized sample 40VF2; phenolic, spicy and fruity characterized sample 40SF1; citrus and balsamic characterized sample 40SF2.

Fruity aroma was the characterizing attribute more frequent in beers for both panel and GC-MS analyses, and, as reported by Viejo et al. (2019), it may contribute to a higher aroma, flavour and overall liking.

3.5. *Effect of altitude of cultivation on VOCs and panel data*

The craft beer obtained with wheat cultivated in mountain areas, which were placed at the centre of PCA and GPA plots and were not discriminated among them, were the most appreciated by involved and expert judges, thus further statistical analysis on the effect altitude on both VOCs and panel data were carried out.

To understand if a specific linear combination of VOCs and panel attributes may continuously predict the altitude of wheat used in beer production, independently of wheat concentration and varieties, a PLS1-R was used. Since PLS-R is a linear multivariate model, a log-transformation on altitude (y) was applied as suggested by Wold et al. (2001), in order to improve model prediction capabilities, thus suggesting that the main difference in determining aroma profile lay between the wheat obtained from experimental fields sited at 70 (F1) and 500 m a.s.l. (F2) rather than between 500 (F2) and 1200 (F3).

The model depicted five LVs that explained an overall variance of 97.5% and 86.0% of R^2Y and R^2X respectively. Fig. 6 shows the predicted versus observed values (Log_{10} m a.s.l.) calculated by the model,

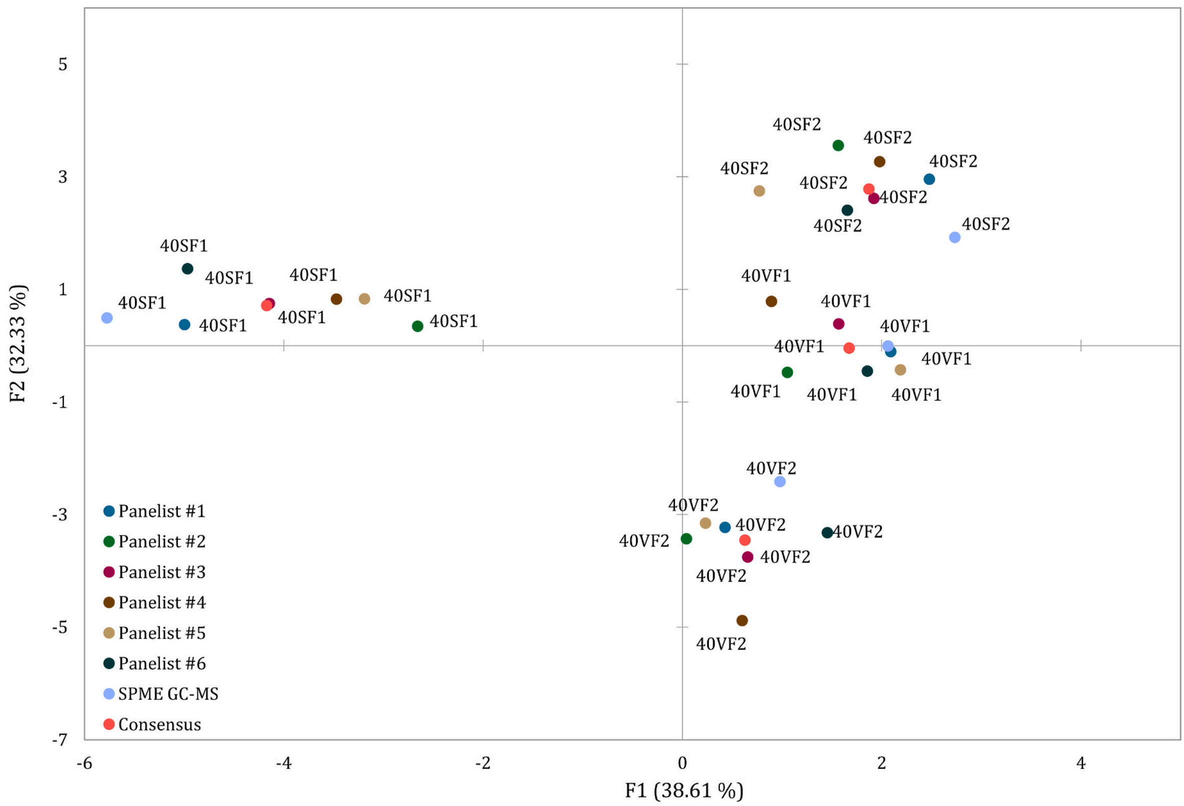

Fig. 5. Consensus map, presented by configuration (six panelists and GC-MS analysis), obtained by GPA using four selected panel-driven beers. Score nomenclature: (40) 40% wheat amount; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua.

demonstrating a good classification efficiency without overlapping in the three altitude levels ($R^2 = 0.975$; RMSE = 0.08; RMSE% = 3.1).

Table 4 lists the VIP values of the most important VOCs for the model together with the relative β-coefficient. Ten VOCs were considered VIPs (the 32% of total compounds identified), and further discussed. Ethyl 9-decenoate (fruity odour), 2-Ethylhexanol (medium citrus odour), Decanoic acid (medium fatty odour), Ethyl decanoate (medium waxy odour), Octanoic acid (medium fatty odour), Ethyl dodecanoate (medium waxy odour), Ethyl octanoate (medium waxy odour), and Nonanal (high aldehydic odour) had a positive standardized regression coefficient whereas Ethyl nonanoate (medium waxy odour) and Heptyl acetate (medium green odour) had a negative standardized regression coefficient. This discrimination is mainly due to different esters deriving from the yeast metabolism. Among esters, Ethyl octanoate, which improves the aroma of beers[31] is higher in wheat cultivated at the highest altitudes.

The results observed in this section are in accordance with those of Nešpor et al. (2019) who demonstrated that acetates and fatty acids could be useful to discriminate lager beer produced in the Czech Republic and Spain. Short-chain fatty acids are produced by yeasts during fermentation and maturation and are not released in the wort medium unless an autolysis or cell death occurred due to some physiological stress. During maturation (craft beers are subjected to in-bottle maturation due to secondary fermentation to generate carbonation), Octanoic acid is replaced by Decanoic acid, but a general decrease of these compounds was reported as ageing proceeds, since yeasts used short-chain fatty acids to synthesize glycerides and phospholipids (Briggs et al., 2004). On the other hand, Nonanal is formed through the degradation of malt and cereal derived fatty acids as well as the oxidation of corresponding alcohols, during different steps in beer production (Baert et al., 2012). Wheat cultivated at higher altitudes determined a higher content of both short-chain fatty acid (Decanoic acid, Octanoic acid) and Nonanal content in wheat beers. The fermentation of wheat grown at high altitudes seemed to have caused a greater physiological and oxidative stress to yeasts. Since most stress factors (alcohol content, pH, temperature) could be assumed as constant, this result was ascribed to limited substrate availability. Indeed, wheat grown at high altitude showed a lower total nitrogen content: on average wheat cultivated in F1 showed values of protein on dry basis of 13.95 ± 1.10, which is significantly higher ($p < 0.05$) with respect to those cultivated in F3 (10.37 ± 1.30).

Another PLS regression, using panel attributes as explanatory variables and altitude as a dependent variable, was carried out. The model permitted to calculate one LV that explained only a small part of the overall variance (14% and 10.0% of R^2Y and R^2X respectively). Even though the predictive ability of the model was low, the VIPs value were calculated as shown in Table 4. In particular 6 VIPs had positive β-coefficients (floral, nutty, hop, pear, citrus and wheat) increasing with the log-altitude, whereas 8 VIPs had negative β-coefficients (clarity, malt, sour, phenolic, fermented, sweet, metallic and banana) decreasing with the log-altitude. Pleasant attributes in beers, such as floral and fruity (pear and citrus), increase with increasing altitude, this result is in accordance with several studies that showed that the cultivation at high altitude improves the flavour of different crops (Alessandrini et al., 2017; Bertrand et al., 2012; Špika et al., 2021; De Flaviis et al., 2022d). De Flaviis et al. (2022b), in particular, investigated the VOCs of Vittorio and Solina wheat, showing that altitude promotes the production of VOCs with floral, fruity and green notes.

3.6. Consumer test

The consumer respondents were sexually-balanced 70% of which were aged between 23 and 45 years old; more than 50% were self-assessed connoisseurs of beer and with high involvement. 36% of assessors consumed beer less than once a week, 60% from 2 to 6 times per week, and the 4% every day.

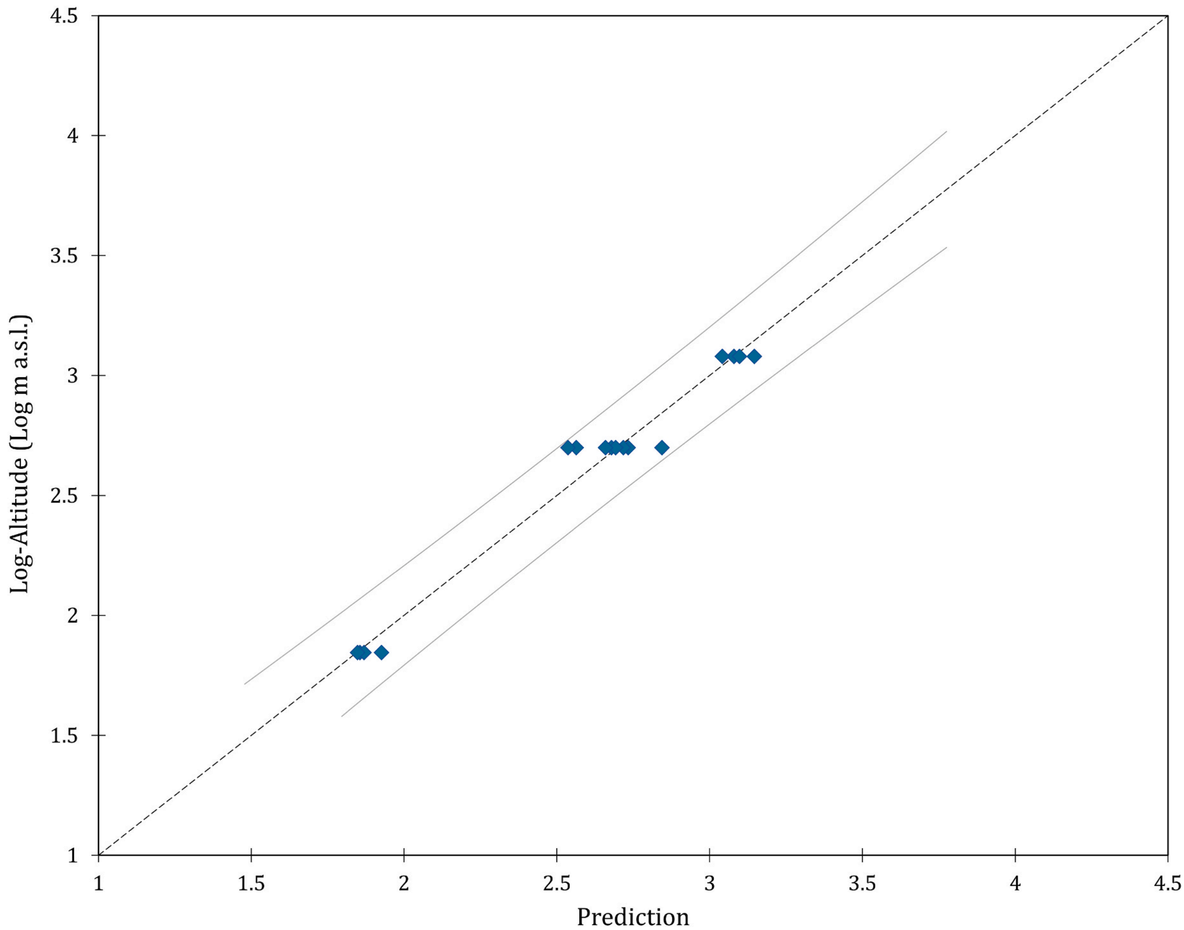

Fig. 6. PLS regression plot between predicted and observed altitude values with confidence intervals.

Table 4
PLS-R results of different craft beers with wheat cultivated at different altitudes: variables importance in projection (VIP >1) and standardized coefficient (β).

	PLS-R (Variables)	VIP	β
VOCs	Ethyl 9-decenoate	1.752	0.201
	2-Ethylhexanol	1.640	0.394
	Decanoic acid	1.440	0.264
	Ethyl decanoate	1.347	0.034
	Ethyl nonanoate	1.338	−0.112
	Octanoic acid	1.325	0.178
	Ethyl dodecanoate	1.233	0.123
	Ethyl octanoate	1.199	0.083
	Heptyl acetate	1.071	−0.079
	Nonanal	1.030	0.123
Panel attributes	Floral	2.386	0.096
	Clarity	1.560	−0.063
	Nutty	1.448	0.059
	Hop	1.383	0.056
	Pear	1.334	0.054
	Citrus	1.334	0.054
	Malt	1.246	−0.050
	Sour	1.245	−0.050
	Phenolic	1.145	−0.046
	Fermented	1.134	−0.046
	Wheat	1.115	0.045
	Sweet	1.081	−0.044
	Metallic	1.068	−0.043
	Banana	1.007	−0.041

Friedman robust test permitted to evaluate significant differences of centred assessors scores between samples (Table S2). The R beer was not significantly different from other beers, meaning that the assessors preferred it in the same way of craft beers despite their different styles. In general, 40VF1 was the most appreciated craft wheat beer while 40VF3 was the worst. The main significant differences were observed among the samples 40VF1, 40VF2 and 40VF3. Noteworthy, in Vittorio wheat, the cultivation at different altitudes considerably influenced the liking scores of assessors. On the contrary, Solina wheat beers did not show significant differences based on altitude, suggesting that this old landrace may be used to obtain wheat beers with constant quality traits regardless of the geographical origin of wheat.

To better visualize the relations among psychographic variables, samples and consumers, a PCA was performed. Two PCs that explain 46.5% of the total variance permitted to discriminate beers depending on origin as shown in Fig. 7. Considering only the beers with the same wheat origin (F2), the wheat amount is confirmed as the main factor also in the consumer test. Moreover, samples 40F2 were preferred by aged assessors; whereas samples 40F3 were appreciated by habitual consumers with higher involvement, and self-assessed knowledge, who likely prefer flavour-balanced beers (see Section 3.2.1) rather than beers with characterizing attributes. Based on a further PLS-R computed by using psychographic variables as factors, self-assessed knowledge did not result as a VIP (Supplementary Fig. S3), on the contrary, involvement, frequency of use, and age were important factors for consumer's liking.

3.7. Internal preference mapping

In order to identify consumer clusters and their driven attributes, an internal preference map was computed as shown in Fig. 8. Following the panel driven approach (see section 3.2.1), only the 40% beers were used

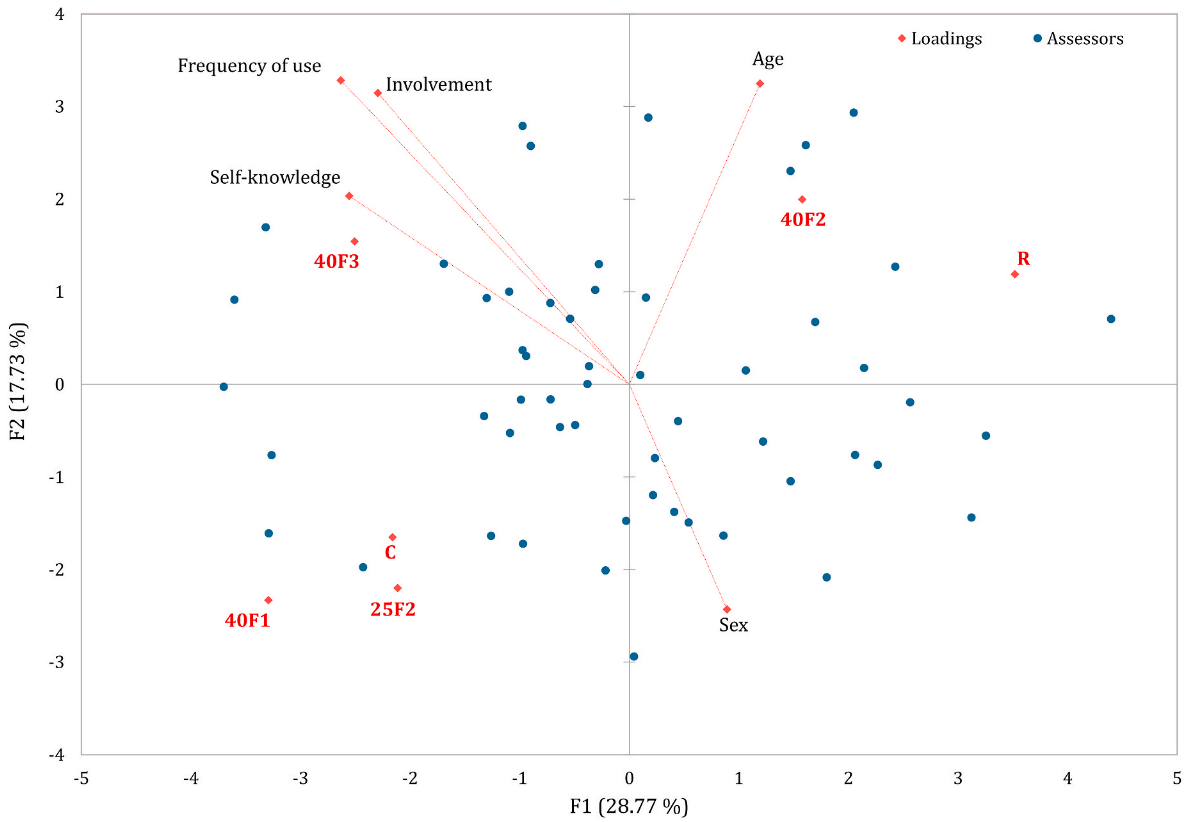

Fig. 7. PCA bi-plots obtained by using the consumer test dataset, including psychographic variables. All the craft wheat beers were used as sample scores. Score nomenclature: (25) 25% wheat amount; (40) 40% wheat amount; (C) Control beer sample; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua (F3; 1200 m a.s.l.); Rocca Pia.

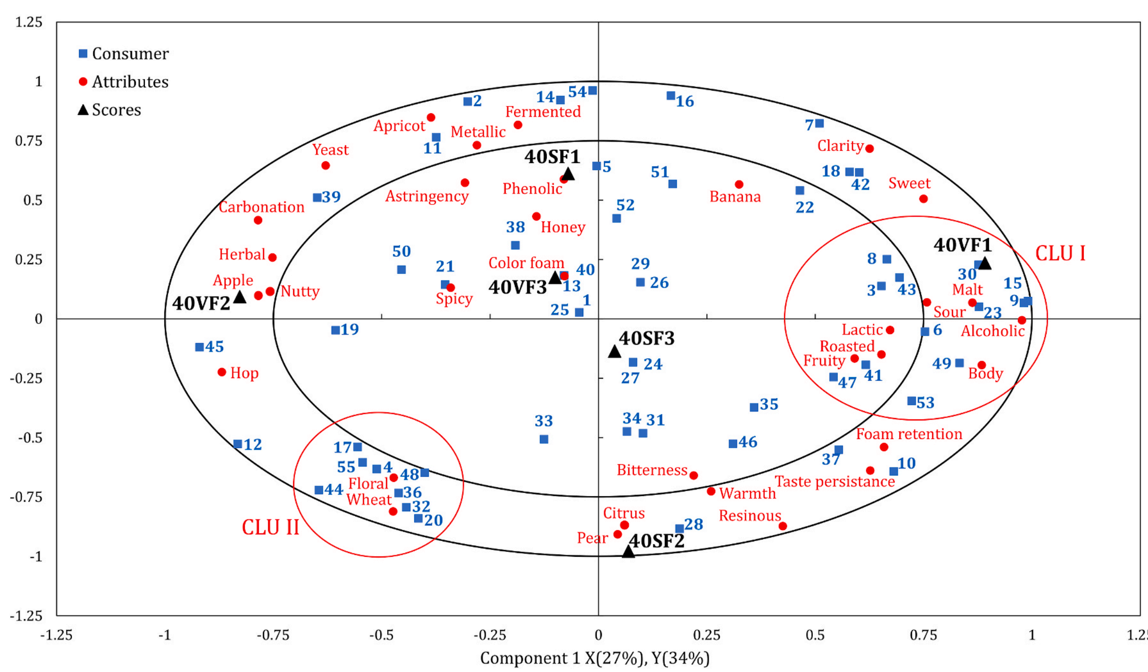

Fig. 8. Internal preference map obtained by PLS regression between consumer test (blue squares), beer sample scores (black triangles) and panel attributes (red circles). Red ellipses highlight two major consumer clusters named CLU I and CLU II respectively. Score nomenclature: (40) 40% wheat amount; (V) Vittorio wheat; (S) Solina wheat (F1; 70 m a.s.l.); Corropoli (F2; 500 m a.s.l.); Introdacqua (F3; 1200 m a.s.l.); Rocca Pia. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to compute t-scores (Fig. 8). The method used was a PLS regression between consumer test (X) and panel attributes (Y). The first two LVs explained the 65% and 54% of R^2Y and R^2X respectively. Despite the limited number of participants in consumer test, two main different clusters (CLU I, CLU II) can be observed and were circled in Fig. 8. CLU I was composed of the 22% of the assessors that preferred the 40VF1 sample and were driven by sour, malt, alcoholic, body, roasted and fruity attributes, instead CLU II was composed by the 15% of the assessors that preferred 40VF2 and 40SF2 samples and were driven by wheat and floral attributes.

4. Conclusion

The unifying approach used in this study permitted to demonstrate that the origin of wheat (variety and place of cultivation) is of utmost importance in defining the sensory profile of wheat beers, with the place of cultivation (altitude in particular) being even more important than genotype. Selected odours (e.g. herbal and fruity) increased with the increasing of the altitude of wheat cultivation and were appreciated by consumers who were familiar with beer, whilst others (malt, fermented, and banana) decreased with the increasing of altitude.

The variations in ingredients quality dictated by a specific geographical combination could produce, a 'taste of the place' that one can term the 'terroir' of beer. Climate change could, however, modify beer terroir since global warming is affecting the altitude of wheat cultivation by promoting the use of rustic local wheat variety. Thus, the findings of this work can help to valorise both local primary productions with a unique and distinctive VOCs pattern as well as their end-product whose sensory profile is linked to a specific 'terroir'.

A possible limit of this study is that the samples were not representative of commercial beers, thus further research is needed in order to validate these results, especially in beer with more complex formulation (e.g. higher amount of hops and characteristic ingredients) according to different brewery styles. Moreover, expanding the beer characterization to other chemical compounds, responsible for taste and mouthfeel, would help to further support and eventually valorise the 'terroir' identity of craft beer.

CRediT authorship contribution statement

Riccardo De Flaviis: Methodology, Investigation, Formal analysis, Writing – original draft, Visualization. **Veronica Santarelli:** Methodology, Investigation, Formal analysis, Writing – original draft, Visualization. **Delvana Mutarutwa:** Investigation. **Sergio Grilli:** Investigation. **Giampiero Sacchetti:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.100429.

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