Food Bioscience 52 (2023) 102487

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Application of microbial cross-over for the production of Italian grape ale (IGA), a fruit beer obtained by grape must addition

Gabriella Siesto [a,b], Rocchina Pietrafesa [a], Maria Tufariello [c], Carmela Gerardi [c], Francesco Grieco [c,*], Angela Capece [a,b]

[a] Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy
[b] Spinoff StarFInn S.r.l.s., Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy
[c] Consiglio Nazionale delle Ricerche - Istituto di Scienze delle Produzioni Alimentari (ISPA), Via Prov. Lecce-Monteroni, 73100, Lecce, Italy

ARTICLE INFO

Keywords:
IGA beer
Autochthonous *Saccharomyces cerevisiae* strains
Starter culture
Microbial cross-over
Aroma compound

ABSTRACT

In the last years, the production of grape ale beer has become a trendy choice among brewers in Italy, resulting in a new beer type known as Italian Grape Ale (IGA), a sort of bridge beverage between beer and wine. In this study, three autochthonous *Saccharomyces cerevisiae* strains (CHE-3, P4 and TA4-10), previously isolated from different food types, were tested in comparison with the commercial strain US-05. Fermentations were conducted at a laboratory scale on malt extracts with 15% and 25% grape must adjuncts, and on malt wort (control). After fermentation, the CHE-3, P4 and TA4-10 strains showed significantly higher CO_2 production than US-05. The main analytical parameters (organic acids, real and apparent attenuation, ethanol content, glycerol level and carbohydrate profile) and the volatile compounds related to the organoleptic quality of the experimental beers were strongly influenced both by the grape must addition to the fermentation medium and the used strains. The addition of grape must enhanced the CO_2 production, especially when 25% of grape must was added to the wort. By comparing the tested strains, the highest aromatic expression was observed with 15% grape must adjunct. The present results confirm the importance of media composition and microbial biodiversity to gain more different beers especially in the craft beers sector. Moreover, the outcome data show the significance of microbial cross-over, a novel approach based on the exploitation of microorganisms traditionally used in other agro-food chains also for brewing novel beer types.

1. Introduction

Beer is one of the most ancient and pleasant consumed beverages worldwide. Different types and styles of beer are available on the market in response to consumer's demand, who are increasingly focused on artisanal products (Postigo et al., 2021). Traditionally, beer is brewed from four basic ingredients: water, malt, hops and yeast and the most popular categories of industrial products are denominated 'Ale' and 'Lager', obtained at different fermentation temperatures (16–24 °C and 6–15 °C, respectively). Worldwide, there is a growing interest towards novel beer styles (Denby et al., 2018; Ducruet et al., 2017; Mayer et al., 2016; Romano et al., 2023). In particular, special beers obtained with fruit adjuncts show a complex sensory profile, denoted by harmonious and balanced fruity flavors (Martínez et al., 2017). The fruit beer is one of the most attractive products of micro- and craft breweries (Nardini & Garaguso, 2020) and, at same time, it presents some interesting properties, such as high amount of polyphenols (mainly phenolic acids), aroma compounds and good antioxidant capacity (Gasínski et al., 2020; Kawa-Rygielska et al., 2019; Salanț;ă et al., 2020). In recent years, the Beer Judge Certification Program (BJCP, 2015) established a new original fruit beer made in Italy, known as Italian Grape Ale (IGA), resulting from the fermentation of a mixture of barley malt and grape must. This new brewing process is currently in use in many small regional breweries and is likely to better express the connection with the territory, conjugating the biodiversity of Italian grape cultivars with the brewer's creativity (Garavaglia, 2020). IGA beers are produced utilizing pilsener/pils or other pale base malts with the addition of grapes or grape must in a range from 5% to 40% of the total wort composition at

* Corresponding author. Consiglio Nazionale delle Ricerche - Istituto di Scienze delle Produzioni Alimentari (ISPA), via Prov. le Lecce-Monteroni, 73100, Lecce, Italy.
E-mail address: francesco.grieco@ispa.cnr.it (F. Grieco).

https://doi.org/10.1016/j.fbio.2023.102487
Received 26 January 2023; Received in revised form 13 February 2023; Accepted 15 February 2023
Available online 16 February 2023

the different fermentation stages (boiling, primary or secondary fermentation or bottling) (De Simone et al., 2021). Other than the addition of fruits, herbs, and spices, the choice of yeast starter culture is a further tool that is able to characterize by distinctive aromatic profiles the beers (Capece et al., 2021; Postigo et al., 2021). In brewing, the use of starter cultures is addressed to increase the efficiency of fermentation process, to realize new beers, and to enhance the overall quality of the produced beer (Aquilani et al., 2015; Canonico et al., 2014, 2021; Capece et al., 2018). The sugars naturally occurring in the raw materials are converted into CO_2 and ethanol by yeasts metabolism which is also responsible for the production of many secondary compounds during fermentation, as higher alcohols and esters (Lodolo et al., 2008; Pires et al., 2014), desirable volatile molecules for a pleasant beer. In particular, an emerging trend in food microbiology is the application of microbial cross-over, a technological approach in which microorganisms typically used in some fermentation process are used to improve the quality of other agri-food production/chain (Dank et al., 2021).

Indeed, several studies reported that *Saccharomyces cerevisiae* strains isolated from different foods (as wine and bread) can produce typical and strain-specific fermentative aroma profiles in beer (Cubillos et al., 2019; Marongiu et al., 2015; Rossi et al., 2018).

Typically, a considerable number of different volatile compounds determines beer flavour, but only several of these are recognized as aroma-active compounds (Olaniran et al., 2011, 2017), belonging to the classes of esters, alcohols (higher or fusel alcohols), aldehydes and acids (Biazon et al., 2009).

With the aim to identity a starter culture potentially useful for production of IGA craft beer, three indigenous *S. cerevisiae* strains, isolated from different food matrices were tested in fermentation trials at laboratory scale, using a commercial *S. cerevisiae* strain as control. The four strains were tested in two fermentation media composed of malt extract added with two different grape must amounts (15 and 25%). To the best of our knowledge, this investigation is the first one concerning the employment of the microbial cross-over for the production of IGA craft beer in Southern Italy.

2. Materials and methods

2.1. Yeast strains

Three indigenous *Saccharomyces cerevisiae* strains, belonging to UNIBAS Yeast Collection (UBYC), University of Basilicata (Potenza, Italy), were used in this study: CHE-3, isolated from cherry (Capece A, unpublished data); P4, isolated from sourdough (Capece et al., 2018); TA4-10, isolated during spontaneous fermentation of Inzolia grape must (Capece et al., 2011). These yeast strains were previously selected on the basis of their phenotypic characteristics, such as ability to ferment glucose, maltose, sucrose and fructose, and high fermentative power in laboratory scale trials performed in 100 mL of commercial malt extract (Table S1). The commercial starter US-05 was used as the control. The strains were grown on YPD medium (1% (w/v) yeast extract, 2% (w/v) peptone; 2% (w/v) glucose; 2% (w/v) agar (Oxoid, Hampshire, UK), and maintained at 4 °C for further analysis. Liquid cultures were grown in YPD broth at 28 °C for 24 h.

2.2. Fermentation trials at laboratory scale

A commercial extract malt for Pale Ale beer (MrMalt®, Udine, Italy) was employed according to the manufacturer's instructions. The malt syrup was dissolved in 12 L of sterilized water and the mixture was subdivided in three batches: the control, without any addition (M); IGA15 and IGA25, with addition of natural grape must up 15 and 25%, (v/v), respectively. The main characteristics of the utilized grape must were the following: pH 3.6; TSS 22.0; Yeast Assimilable Nitrogen 211.9 mg N/L. Before the addition, the grape must was heated up to 80 °C and rapidly cooled to room temperature. Experiments were carried out in flasks containing 400 mL of medium inoculated with the yeast strains at a concentration of 1×10^7 cells/mL. The fermentation trials were performed at 20 °C in duplicate under static conditions. Fermentation kinetics were monitored by measuring the weight loss of flasks and reduction of total soluble solids (°Plato) with a refractometer. Once weight loss remained constant for 3 consecutive days, the secondary fermentation was carried out by disposing samples in 250 mL bottles with addition of 5 g/L of sucrose for 2 weeks at 20 °C and, after storage at 4 °C for one month, the samples were analyzed.

2.3. HPLC analysis

Organic acids (tartaric acid, succinic acid and acetic acid) were identified onto an Agilent Hi-Plex H (300 × 7.7 mm) with internal particles of 8.0 μm (Agilent Technologies, Santa Clara, CA, USA). The temperature of the column compartment was maintained at 70 °C. The flow rate applied was 0.4 mL/min with a run time of 30 min. The phase was 4.0 mM/L H_2SO_4 in ultrapure water (Coelho et al., 2018). Standard solutions were injected to obtain the retention time for each compound. For the determination of tartaric, acetic, and succinic acids detection was conducted in the DAD at 210 nm.

The maltodextrin, sucrose, maltose, maltotriose, glucose, fructose, glycerol and ethanol concentration were quantified using an Agilent Hi-Plex Ca column (300 mm × 7.7 mm) with internal particles of 8.0 μm (Agilent Technologies, Santa Clara, CA, USA). The mobile phase used was deionized water and a constant flow rate of 0.6 mL/min for a run time of 30 min. Sugars detection was carried out by refractive index detection (RID). Quantification of individual organic acids and sugar were performed directly by Chem-Station software (Agilent) using a five-point regression curve ($R^2 \geq 0.99$) on the basis of authentic standards.

2.4. Volatilomic assay

The contents of main secondary compounds, such as acetaldehyde, ethyl acetate, acetoin, n-propanol, isobutanol, n-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol, were quantified by direct injection into a gas-chromatographic system (GC Agilent 7890). The sample was prepared and analyzed following the procedure described by Capece et al. (2021).

The analysis of volatilomic profile was carried out by a solid phase micro extraction in combination with a gas chromatography coupled to mass spectrometry (HS-SPME-GC/MS). According to the methods of Tufariello et al. (2019), 100 μL of internal standard solution (ISTD, 4-methyl-2-pentanol, 300 mg/L) was added to a volume of 5 mL of beer in a 20 mL headspace vial (Alltech Corp., Deerfield, IL, USA). A 50/30 DVB-CAR-PDMS solid phase microextraction (SPME) fiber (Supelco, Bellofonte, PA) was inserted into the vial and let to adsorb volatiles for 30 min at 40 °C and then transferred to the injector port (250 °C) where desorption occurred in 2 min. Splitless mode was selected as injection mode. GC-MS analyses were performed on a GC 6890 (Agilent Technologies, Palo Alto, CA) coupled to an Agilent MSD 5973 Network detector using a HP-INNOWAX capillary column (60 m × 0.25 mm, 0.25 μm, J&W Scientific Inc., Folsom, CA, USA) as reported by Tufariello et al. (2019). The annotation of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, P > 90%), with the retention data of commercially available standards and MS data reported in the literature. Concentration of each volatile compound was assessed by the internal standard method following this equation:

VOCs concentration = (VOC GC peak area/IS GC peak area) × IS concentration as reported in previous scientific studies (Kang et al., 2010; Perestrelo et al., 2018).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the differences among the four tested strains in the fermentative performance for each medium used: malt wort, malt wort added with 15% and 25% of grape must. Two-way ANOVA was carried out to evaluate the influence of the fermenting yeast strain (first independent variable) and the fermenting media (second independent variable) on each of the analytical parameters (dependent variable).

Data of volatile organic compounds were submitted to classical two-way clustering analysis, by using Ward's algorithm and Euclidean similarity index. The Z-scores were used to reduce the dimensionality of the data and to find the best differentiation between yeast strains and type of the media used to produce beers (Capece et al., 2021). The data obtained were converted to Z-scores, calculated as follows:

$$\text{Z-score} = (X - \mu)/\sigma$$

X = concentration of the aroma compound, μ = mean value of all strains per measured compound and σ = standard deviation of values per tested compound.

All the analytical parameters detected in the experimental beers, both by HPLC analysis and volatilomic assay, were submitted to Principal Component Analysis (PCA).

All statistical analyses were carried out using the Past free software ver. 4.2 (Natural History Museum-University of Oslo, Oslo, Norway) (Hammer et al., 2001).

3. Results and discussion

3.1. Fermentative aptitude of Saccharomyces cerevisiae strains

The three *S. cerevisiae* strains CHE-3, P4 and TA4-10, isolated from cherry, sourdough and grape must, respectively, were for the first time tested as starter for the production of IGA craft beer.

The first step of our procedure was addressed to evaluate the fermentative performance (measured as CO_2 grams produced during the process) of the four strains on brewing worts with or without the addition of grape must up 15 and 25% (Fig. 1A–C). The CO_2 production increases with the addition of grape must, with the highest level in beers obtained in wort added with 25% of grape must (Fig. 1C). Similar results were reported by Cioch-Skoneczny et al. (2022); these authors found the highest mass loss, due to CO_2 evolution, in the fermentation of samples added with the higher content of grape must in comparison to the wort without any addition. The grape must addition influences also the fermentative kinetics; in fact, the fermentation rate, calculated as CO_2 g/day over the first 3 days of fermentation, showed the highest value for IGA 25 samples (Fig. S1). For example, P4 strain exhibited a fermentation rate of 4.08, 5.92 and 7.46 CO_2 g/day for wort without grape must, IGA 15 and IGA 25 samples, respectively. These data are in line with a recent research by Castro Marin et al. (2021), in which fermentations performed in samples supplied with 10 and 20% of Cv. Lambrusco grapes must showed kinetics faster than the one showed by the control malt wort. The higher the amount of grape must present in the samples increased the number of carbon sources, the key factor in the yeast metabolism; this is strictly correlated with the rate of carbohydrate conversion, which directly influences the speed of the entire fermentative process and its course (Cioch-Skoneczny et al. (2022)). By analyzing the different strain behaviour in each condition, the P4 strain showed the highest release of CO_2 in wort without grape must addition (sample M, Fig. 1A) during the first fermentation days, with an increase of CO_2 production from 7.77 to 14.07 g CO_2/400 mL, whereas the lowest values were observed for CHE-3 strain (from 4.98 to 12.60 g CO_2/400 mL). After the sixth fermentation day and up to the end of the monitored period (13 days), the highest fermentative activity was exhibited by the commercial starter US-05. At the end of the process, no statistical differences were found among the three selected strains.

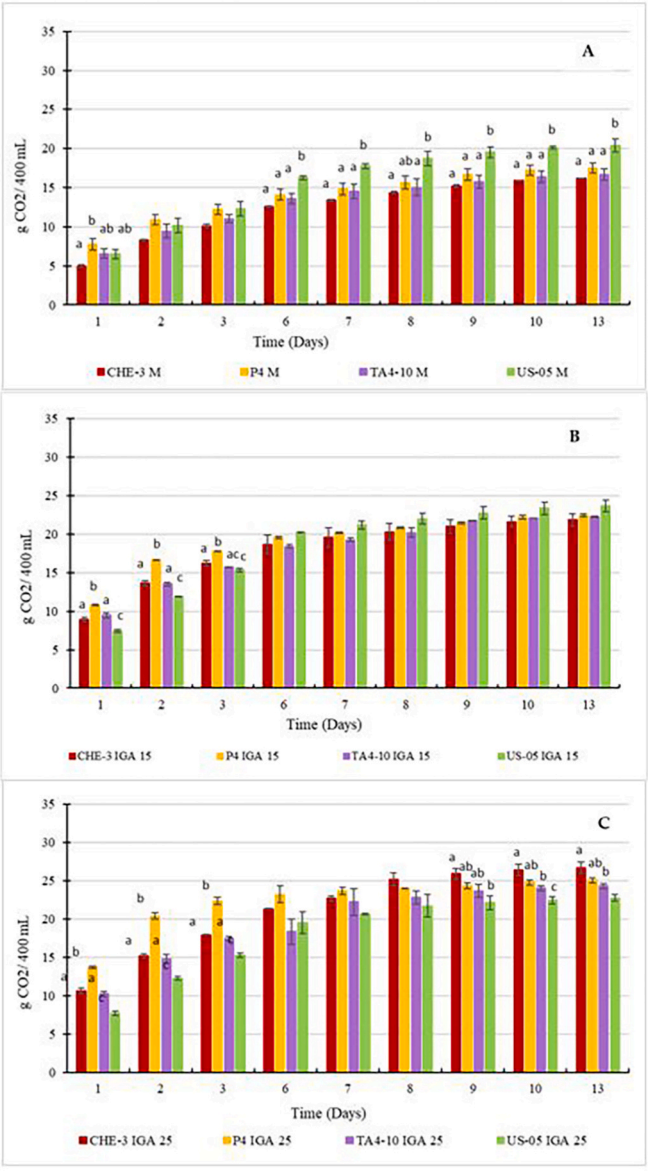

Fig. 1. Fermentative performance of 4 *S. cerevisiae* strains, reported as g of CO_2 produced during the fermentation of conventional wort (A) and wort added with 15 and 25% of grape must (B and C, respectively). Letters on plot bars indicate significant differences ($p < 0.05$) among the four strains for each time. Data are expressed as the means of duplicate experiments ±standard deviation.

Concerning the fermentative performance in wort added with 15% of grape must (IGA15, Fig. 1B), in the first three days of fermentation, P4 yielded the highest CO_2 production (from 10.82 to 17.78 g CO_2/400 mL), whereas US-05 produced the lowest CO_2 amount (ranged from 7.43 to 15.32 g CO_2/400 mL). After the sixth fermentation day, the commercial starter showed CO_2 production levels very similar to the three indigenous strains. Indeed, after sixth fermentation day no significant differences were registered among all the strains.

Finally, also in wort added with 25% of grape must the P4 strain produced the highest amount of CO_2 during the first three days (IGA25, Fig. 1C), as already observed both in M and IGA15 fermentations. After the eighth fermentation day, the CHE-3strain showed the highest CO_2 production, (26.71 ± 0.74 g CO_2/400 mL). However, at the end of the process the three indigenous strains showed significantly higher CO_2 production than the commercial one, thus indicating that these strains are better able to utilize the fermentable sugars provided by the added

grape must. These results confirmed that the exploitation of the brewing potential of microorganisms of different origin, such as *Saccharomyces* strains isolated from other fermented food and beverage, is a powerful tool for special beer production (De Simone et al., 2021). In fact, the brewing potential of yeasts isolated from other traditional fermented beverages, such as cachaça (Araújo et al., 2018), tequila (Cubillos et al., 2019), or African alcoholic spirits (Johansen et al., 2019) has been recently assessed. In addition to alcoholic beverages, other fermented foods could also serve as a source for novel yeast isolation (Tamang et al., 2020; Pasqualone et al., 2018). Moreover, numerous *S. cerevisiae* strains isolated from Brazilian distilleries have been employed in high gravity beer production, undoubtedly displaying to be novel starter cultures for the brewing industry (Christofoleti-Furlan et al., 2020).

3.2. Beer analytical profiles

At the end of secondary fermentation, the experimental beers produced by the four *S. cerevisiae* strains in the three different media (denoted as M, IGA15 and IGA25) were analyzed for analytical parameters and by-products related to organoleptic quality.

The carbohydrate profile of wort samples and experimental beers are illustrated in Table 1. As regards the different fermentation media, maltose, maltotriose and maltodextrine were found to be the most abundant saccharides in malt wort, whereas the highest content of glucose and fructose was found in wort added with grape must, in direct proportion with the amount of grape must added. No residual maltose was found in the samples fermented with CHE-3, P4 and TA4-10 strains (with exception of the low content detected in beer from malt wort inoculated with TA4-10 strain), whereas all the samples inoculated with commercial strain US-05 contained low maltose amounts, without difference statistically significant among the three experimental beers. Fructose was detected in none of the samples, while a glucose content ranging from 1.67 to 3.47 mg/mL were detected in all the IGA samples, with exception of CHE-3 IGA15. As already reported for the fermentation media, the beers obtained by malt worth contained the highest content of maltotriose and maltodextrine. The results of the two-way ANOVA showed that: i) the strain influenced mainly maltotriose, maltose content, ii) the fermentation media significantly influenced the content of all fermentable sugars, iii) the interaction between media and strain affected mainly maltose and glucose content (Table 1).

Table 1
Carbohydrate content of fermentation media and experimental beers (g/L).

Samples	Maltodextrin	Maltotriose	Maltose	Glucose	Fructose
Malt wort	19.12	26.38	50.22	10.8	3.27
IGA15	17.14	20.17	34.56	17.14	13.16
IGA25	17.37	18.61	38.18	28.63	25.41
CHE-3 M	16.99 ± 0.88^{a}	25.91 ± 2.12^{a}	ND	ND	ND
CHE-3 IGA15	14.07 ± 0.20^{a}	19.03 ± 0.01^{a}	ND	ND	ND
CHE-3 IGA25	12.71 ± 0.07^{b}	16.21 ± 0.78^{b}	ND	3.47 ± 0.02	ND
P4 M	18.30 ± 0.12^{a}	18.82 ± 0.76^{a}	ND	ND	ND
P4 IGA15	16.12 ± 0.13^{b}	15.58 ± 0.11^{b}	ND	1.67 ± 0.05^{a}	ND
P4 IGA25	14.37 ± 0.14^{c}	15.88 ± 0.09^{b}	ND	1.78 ± 0.12^{a}	ND
TA4-10 M	17.96 ± 0.06^{a}	23.05 ± 0.01^{a}	2.08 ± 0.03	ND	ND
TA4-10 IGA15	15.96 ± 0.10^{a}	20.00 ± 0.09^{b}	ND	2.47 ± 0.10^{a}	ND
TA4-10 IGA25	16.01 ± 1,66^{a}	18.01 ± 0.05^{c}	ND	2.82 ± 0.12^{a}	ND
US-05 M	17.00±0-17^{a}	10.65 ± 0.14^{a}	0.88 ± 0.01^{a}	ND	ND
US-05 IGA15	15.70 ± 0.25^{a}	7.32 ± 0.91^{b}	0.67 ± 0.01^{a}	2.80 ± 0.01^{a}	ND
US-05 IGA25	14.31 ± 0.79^{a}	7.62 ± 0.16^{b}	1.08 ± 0.29^{a}	2.06 ± 0.58^{a}	ND
p Strains (A)	6.89E-3	4.30E-10	1.80E-08	1.72E-3	/
p Type of beers (B)	2.38E-05	9.62E-07	1.16E-06	4.22E-10	/
p Interaction A x B	4.48E-1	9.83E-3	6.65E-08	1.68E-06	/

Data are reported as mean ± standard deviation of two independent replicates, ND = not detected. Superscript letters indicate significant differences ($p < 0.05$) among each strain in three different types of beers (M, IGA15, IGA25) for each parameter. *P* Strains (A), type of beers (B) and their Interaction (A x B) = result of Two-ways factorial ANOVA performed on the experimental beers using as factors the yeast strains and the type of wort used for the production of beers.

The sugar profile of the beers reflected the tested yeast strain. In fact, the monosaccharides were virtually completely fermented, whereas the catabolism of the oligosaccharides, in particular the maltotriose, varied for the four yeast starter. The ability to metabolize maltose and maltotriose is not common, thus restricting the brewing potential only to a few species (Cubillos et al., 2019). Maltotriose is fermented slowly and sometimes incompletely, so traces may remain in beer (Li et al., 2020). Our results confirmed that the utilization degree as well as the ability to utilize these sugars is strain dependent (Meier-Dörnberg et al., 2017). The CHE-3 and P4 strains (isolated from fruit and sourdough, respectively) were more efficient in maltose fermentation than the commercial strain US-05. Rossi et al. (2018) selected a baking yeast as the most promising strain by comparing *S. cerevisiae* strains isolated from different agri-food chains, showing that sourdough could represent an important source of biodiversity for selection of autochthonous starter cultures for craft beer production.

The data regarding to the main analytical parameters detected in the experimental beers were reported in Table 2. As shown by the real (RA) and apparent attenuation (AA), fermentation progressed to a greater extent in the IGA25 samples, in particular in beer obtained by US-05, with RA and AA values of 72.19 and 88.08%, respectively, whereas the lowest values were detected in the M beers for all the tested strains. The highest amount of ethanol content was found in IGA25 beers, with exception of sample fermented with commercial strain US-05. This beer contained the lowest ethanol amount among the three samples fermented with this strain (3.76% v/v).

Glycerol is the by-product of ethanol fermentation carried out by *S. cerevisiae* that contributes to body and mouthfeel, with influence on beer flavor (Langstaff et al., 1991; Zhao et al., 2015). The lowest amounts of glycerol were found in beers from malt wort, ranging between 2.05 and 3.75 g/L, whereas the highest amounts were detected in samples obtained by wort added with 25% of grape must (between 7.14 and 3.70 g/L). The increase in glycerol level seen in IGA25 beers, which were the samples with the highest ethanol content (with the exception of US-05 beers) could be related to the defensive mechanism adopted by *S. cerevisiae* yeast, which produces glycerol to mitigate the toxic effects of high ethanol concentrations in the environment (Udom et al., 2019).

The addition of grape must enhanced the content of acetic acid for CH3 and US-05 samples, while for the experimental beers obtained by P4 and TA4-10 fermentations the level of acetic acid was higher in beers from malt wort than IGA beers. However, the differences in acetic acid content were not statistically significant in beers fermented with P4 strain. Otherwise, the beer produce by the TA4-10 strain fermenting malt wort (M) contained a level of acetic acid significantly higher than acetic acid detected in TA4-10 IGA25 (594.89 and 399.78 mg/L, respectively).

The presence of tartaric acid in IGA beers was not surprising, since this acid is naturally abundant in grapes (dos Santos Lima et al., 2015). The same trend was not observed for succinic acid content. In fact, although grapes contain a small amount of succinic acid, the content of this acid was higher in beer from malt wort than IGA beers, with the exception of sample obtained by P4 fermentation. Similar result was

Table 2
Main analytical parameters of the laboratory scale beers.

	AA#	RA##	Ethanol (%v/v)	Glycerol (g/L)	Acetic acid (g/L)	Tartaric acid (g/L)	Succinic acid (g/L)
CHE-3 M	65.55 ± 0.71[a]	53.69 ± 0.58[a]	3.72 ± 0.02[a]	2.95 ± 0.31[a]	0.45 ± 0.02[a]	ND	7.53 ± 0.05[a]
CHE-3 IGA15	74.58 ± 0.59[b]	61.13 ± 0.48[b]	5.44 ± 0.11[b]	4.61 ± 0.03[b]	0.49 ± 0.001[a]	2.13 ± 0.01[a]	6.88 ± 0.02[b]
CHE-3 IGA25	84.23 ± 0.54[c]	69.04 ± 0.45[c]	6.40 ± 0.03[b]	5.30 ± 0.01[b]	0.58 ± 0.006[b]	2.46 ± 0.01[b]	6.49 ± 0.03[c]
P4 M	70.50 ± 0.71[a]	57.79 ± 0.68[a]	4.25 ± 0.12[a]	3.75 ± 0.15[a]	0.49 ± 0.01[a]	ND	7.43 ± 0.12[a]
P4 IGA15	78.96 ± 0.29[b]	64.72 ± 0.24[b]	5.78 ± 0.10[b]	6.04 ± 0.13[b]	0.45 ± 0.05[a]	2.13 ± 0.05[a]	7.28 ± 0.04[a]
P4 IGA25	84.81 ± 0.37[c]	69.51 ± 0.22[c]	6.60 ± 0.33[b]	7.14 ± 0.02[c]	0.46 ± 0.007[a]	2.43 ± 0.22[a]	7.91 ± 0.31[a]
TA4-10 M	69.50 ± 0.71[a]	56.97 ± 0.58[a]	3.85 ± 0.02[a]	3.09 ± 0.16[a]	0.40 ± 0.01[a]	ND	7.07 ± 0.08[a]
TA4-10 IGA15	79.58 ± 0.59[b]	65.23 ± 0.48[b]	4.80 ± 0.10[b]	5.02 ± 0.09[b]	0.55 ± 0.03[b]	2.09 ± 0.05[a]	5.92 ± 0.03[b]
TA4-10 IGA25	85.00 ± 0.54[c]	69.67 ± 0.45[c]	5.67 ± 0.03[c]	5.86 ± 0.09[c]	0.59 ± 0.01[b]	2.60 ± 0.16[b]	5.65 ± 0.03[b]
US-05 M	79.50 ± 0.81[a]	65.16 ± 0.58[a]	4.70 ± 0.01[a]	2.05 ± 0.00[a]	0.35 ± 0.015[a]	ND	7.53 ± 0.14[a]
US-05 IGA15	87.92 ± 0.59[b]	72.06 ± 0.48[b]	4.60 ± 0.59[a]	3.63 ± 0.38[ab]	0.47 ± 0.02[b]	2.01 ± 0.11[a]	6.12 ± 0.53[a]
US-05 IGA25	88.08 ± 0.64[b]	72.19 ± 0.45[b]	3.76 ± 0.07[a]	3.70 ± 0.30[b]	0.55 ± 0.03[b]	1.92 ± 0.07[a]	6.56 ± 0.18[a]
p Strains (A)	7.30E-12	7.45E-12	1.19E-02	1.19E-08	7.99E-04	1.76E-03	2.28E-05
p Type of beers (B)	1.32E-14	1.34E-14	1.03E-04	5.32E-10	1.27E-01	2.45E-15	8.83E-05
p Interaction A x B	8.58E-07	8.81E-07	3.07E-03	1.78E-02	6.48E-07	3.59E-03	5.66E-03

Data are means ± sd of two independent replicates. # = apparent attenuation, ## = real attenuation. ND = not detected. Superscript letters indicate significant differences ($p < 0.05$) among each strain in three different types of beers (M, IGA15, IGA25) for each parameter. *P* Strains (A), type of beers (B) and their Interaction (A x B) = result of Two-ways factorial ANOVA performed on the experimental beers using as factors the yeast strains and the type of wort used for the production of beers.

observed for experimental beers obtained by inoculating the commercial strain US-05. The two-way ANOVA analysis showed that the strains influenced mainly AA and RA values, the type of beer wort affected mainly tartaric acid content, AA and RA values, whereas the interaction affected mainly AA, RA and acetic acid content.

3.3. Beer volatilomic profile

Thirty-nine volatile compounds were detected by HS-SPME-GC/MS of the experimental beers produced by the four different strains in three different fermentation media, including 6 alcohols, 4 volatile acids, 19 esters, 3 aldehydes and ketones, 4 terpenes, 1 phenol, 1 hydrocarbon, and 1 lactone (Table S2). In Table 3, we have reported principal higher alcohols and their esters identified and quantified by GC-FID and GC-MS. It is worth noting the significantly ($p < 0.05$) positive interaction Strains (A) x Type of beers (B) on the volatile concentrations among different samples.

Fig. 2 shows the volatile organic compounds that were detected in the beers samples using four different starters and fermentation media. The greatest aromatic expression across the four used strains was observed when 15% grape must was added to the malt wort.

The highest level of aroma compounds was found in IGA15 beer, brewed with the indigenous strain CHE-3, with levels around 580 mg/L, a level very similar to the amount found in beer fermented with the commercial strain US-05. However, also in the other two types of produced beers, M and IGA25, these strains showed similar levels of total content of volatile compounds. The beers brewed with P4 strain contained the lowest levels of volatiles in all the fermentation media used (280 mg/L in M, 450 mg/L in IGA25 and 400 mg/L in IGA25, respectively).

Among the different compounds, esters and alcohols play a very important role in the organoleptic characteristics of alcoholic beverages (Tufariello et al., 2021). In particular, esters, such as isoamyl acetate, ethyl hexanoate and ethyl octanoate (associated with banana, sweet and sour apple notes, respectively; Table 3), positively influence the aroma with fruity notes, thanks to their low perception threshold (Ocvirk et al., 2018; Pires et al., 2014; Thompson-Witrick et al., 2015).

The addition of grape must at 15% increased ester concentration. In fact, the highest values were found in all the IGA15 beers, ranging from 77.57 mg/L (TA4-10) to 141.71 mg/L (CHE-3). The addition of 25% of grape must increased the ester concentration in comparison to beers from malt wort for CHE-3 and P4, whereas when the TA4-10 and US-05 starters were used, the highest esters level were found in the beers from malt wort without additions. The lowest esters concentrations were found in the M and IGA25 beers brewed with the strain P4 (44.10 and 55.28 mg/L, respectively).

Different studies (Piddocke et al., 2009; Younis & Stewart, 2000) reported that the sugar composition of the fermented wort may influence the changes in the aromatic profile of the final beer. Indeed, the authors indicated that the wort rich in easily assimilable glucose and fructose usually produce beers characterized by higher contents of esters than those rich in maltose. The mechanisms explaining the effect of individual assimilable sugar on ester production are not yet fully understood and controversial results are reported. Younis and Stewart (1998) suggested that higher levels of glucose increase acetyl-CoA, which is the main substrate for acetate ester synthesis, whereas maltose-rich wort may weakly induce acetyl-CoA formation for acetate ester production (Shindo et al., 1992). Conversely, other authors found that an increase of maltose levels as a sole carbon source in a synthetic medium determined an increasing tendency to accumulate acetate esters (Saerens et al., 2008).

Higher alcohols, also known as fusel alcohols, are the most abundant organoleptic compounds present in beer (Baiano et al., 2023). In fact, previous studies reported that the beer may contain more than forty different higher alcohols, including n-propanol, isobutanol, benzyl alcohol, 2-phenylethanol, amyl alcohol and isoamyl alcohol (Loviso & Libkind, 2019; Thompson-Witrick et al., 2015). Higher alcohols amounts below 300 mg/L confer flower, pleasant and refreshing notes, and impart desirable warming character, which gives complexity to the beers, whereas higher concentrations cause a burning sensation and bring an alcohol or solvent aroma to the nose (Olaniran et al., 2017).

The most abundant alcohols detected in our beer were the isobutanol in beers brewed from malt wort without addition (M) and the isoamyl alcohol in the IGA25. The total amount of alcohols was highest in beers with 25% grape must addition, with values ranging from 230.69 mg/L (strain P4) to 331.40 mg/L (US-05). The increase of isoamyl alcohol concentration in the obtained IGA beers is due to the microbial action occurring throughout the fermentation process (Hazelwood et al., 2008). In fact, yeast synthesize this alcohol by the Ehrlich pathway using isoleucine as precursors, whose microbial synthesis is enhanced in yeast strains characterized by a high fermentative activity (Castro Marin et al., 2021.

The terpenes present in IGA beers may originate from both hops and grape must and they can play an important role on the aroma profile of

Table 3
Concentration (mg/L) of most important aroma compounds in laboratory-scale beers produced with 4 different yeast strains (CHE-3, P4, TA4-10, and US-05) using malt wort and malt wort supplemented with 15 and 25% of grape must (M, IGA15 and IGA25, respectively).

Aroma	Ethyl octanoate	Ethyl hexanoate	Ethyl acetate	Isoamyl acetate	Phenyl acetate	Phenyl-ethanol	*n*-Propanol	Isobutanol	Amyl alcohol	Isoamyl alcohol	Acetaldehyde	Acetoin
	Rose, honey	Apple, fruity	Fruity, solvent	Banana	Apple, aniseed	Rose	Alcohol, sweet	Solvent	Alcoholic, banana	Alcoholic, banana, sweet	Green apple, sweet	Butter
CHE-3 M	19.47 ± 4.21^{a}	ND	20.19 ± 1.34^{a}	0.72 ± 0.14^{a}	1.95 ± 0.41^{a}	12.82 ± 3.54^{a}	14.62 ± 1.16^{a}	77.50 ± 1.49^{a}	31.76 ± 0.49^{a}	66.26 ± 5.23^{a}	57.63 ± 0.11^{a}	13.62 ± 0.35^{a}
CHE-3 IGA15	49.63 ± 5.14^{b}	3.61 ± 0.45	16.16 ± 0.25^{b}	2.33 ± 0.12^{b}	8.12 ± 1.17^{b}	16.31 ± 4.17^{a}	14.27 ± 0.39^{a}	62.90 ± 2.72^{b}	29.80 ± 2.34^{a}	89.17 ± 1.88^{b}	97.97 ± 3.97^{b}	8.35 ± 0.55^{b}
CHE-3 IGA25	27.92 ± 4.22^{a}	ND	15.97 ± 0.76^{b}	2.79 ± 0.21^{b}	5.92 ± 0.85^{b}	10.12 ± 2.94^{a}	11.54 ± 0.11^{b}	59.08 ± 0.81^{b}	33.34 ± 0.53^{a}	82.90 ± 2.54^{b}	68.37 ± 2.79^{a}	8.53 ± 0.20^{b}
P4 M	12.45 ± 3.55^{a}	ND	20.56 ± 1.40^{a}	1.28 ± 0.28^{a}	5.32 ± 0.36^{a}	8.90 ± 2.55^{a}	14.91 ± 0.07^{a}	65.43 ± 2.25^{a}	29.41 ± 3.86^{a}	64.88 ± 4.97^{a}	32.30 ± 3.48^{a}	12.95 ± 1.15^{a}
P4 IGA15	39.18 ± 6.11^{b}	ND	14.65 ± 3.07^{ab}	2.63 ± 0.64^{b}	13.88 ± 5.24^{b}	12.57 ± 3.18^{a}	13.83 ± 0.68^{a}	58.84 ± 2.90^{ab}	36.08 ± 4.41^{ab}	100.42 ± 7.12^{b}	54.12 ± 3.63^{b}	11.93 ± 1.37^{a}
P4 IGA25	14.86 ± 4.25^{a}	ND	11.39 ± 1.71^{b}	2.24 ± 0.33^{b}	10.39 ± 2.41^{b}	11.50 ± 3.25^{a}	14.58 ± 1.19^{a}	43.98 ± 0.22^{b}	46.85 ± 2.98^{b}	102.79 ± 4.46^{b}	93.09 ± 7.01^{c}	6.87 ± 0.25^{b}
TA4-10 M	31.91 ± 5.22^{a}	3.97 ± 0.15^{a}	20.20 ± 1.84^{a}	3.21 ± 0.52^{a}	1.89 ± 0.35^{a}	4.14 ± 0.24^{a}	14.95 ± 1.17^{a}	67.24 ± 2.40^{a}	31.73 ± 1.01^{a}	56.82 ± 5.73^{a}	33.37 ± 2.91^{a}	9.68 ± 1.37^{a}
TA4-10 IGA15	23.87 ± 4.15^{a}	4.24 ± 0.22^{a}	18.13 ± 2.05^{a}	2.47 ± 0.42^{a}	4.51 ± 0.24^{b}	11.38 ± 2.57^{b}	16.85 ± 0.55^{a}	65.01 ± 4.07^{a}	49.33 ± 1.32^{b}	93.67 ± 1.05^{b}	38.39 ± 1.02^{ab}	18.15 ± 0.47^{b}
TA4-10 IGA25	34.79 ± 4.88a	8.90 ± 0.28 b	9.02 ± 0.11 b	4.39 ± 0.64^{a}	7.86 ± 2.18^{b}	25.85 ± 4.18^{c}	14.13 ± 2.15^{a}	40.93 ± 3.42^{b}	46.17 ± 2.06^{b}	110.84 ± 2.26^{c}	44.56 ± 3.54^{b}	8.39 ± 1.89^{a}
US-05 M	49.23 ± 5.31a	1.52 ± 0.01a	11.33 ± 1.76a	1.17 ± 0.14^{a}	3.19 ± 0.27^{a}	22.01 ± 5.01^{a}	12.67 ± 1.89^{a}	38.70 ± 6.58^{a}	20.43 ± 3.21^{a}	41.45 ± 6.69^{a}	31.99 ± 1.98^{a}	7.03 ± 1.74^{a}
US-05 IGA15	74.76 ± 5.84 b	7.87 ± 1.25 b	10.66 ± 0.87a	2.46 ± 0.18^{b}	ND	23.75 ± 3.58^{a}	16.91 ± 3.65^{a}	46.45 ± 5.75^{a}	27.33 ± 2.82^{a}	67.36 ± 3.25^{ab}	71.49 ± 2.51^{b}	7.77 ± 1.11^{ab}
US-05 IGA25	29.33 ± 6.32c	4.11 ± 0.11c	12.99 ± 2.15a	1.30 ± 0.31^{a}	1.68 ± 0.44^{b}	7.97 ± 2.15^{b}	18.95 ± 0.63^{b}	41.76 ± 2.32^{a}	28.98 ± 0.68^{a}	72.57 ± 5.04^{b}	72.46 ± 7.06^{b}	13.72 ± 1.58^{b}
p Strains (A)	2.82E-06	6.06E-11	1.92E-3	2.03E-05	4.12E-10	2.35E-2	9.70E-2	1.30E-06	5.16E-07	2.14E-06	1.02E-07	1.48E-2
p Type of beers (B)	4.83E-06	1.45E-07	3.22E-4	1.92E-4	1.72E-1	9.02E-2	4.27E-1	7.05E-06	8.69E-06	2.44E-08	1.65E-08	8.58E-3
p Interaction A x B	1.00E-4	4.73E-08	6.69E-3	9.45E-4	1.72E-07	2.06E-4	4.71E-2	1.24E-3	1.59E-3	1.13E-2	2.18E-06	2.26E-06

Data are means ± sd of two independent replicates. # = apparent attenuation, ## = real attenuation. ND = not detected. Superscript letters indicate significant differences ($p < 0.05$) among each strain in three different types of beers (M, IGA15, IGA25) for each parameter. *P* Strains (A), type of beers (B) and their Interaction (A x B) = result of Two-ways factorial ANOVA performed on the experimental beers using as factors the yeast strains and the type of wort used for the production of beers.

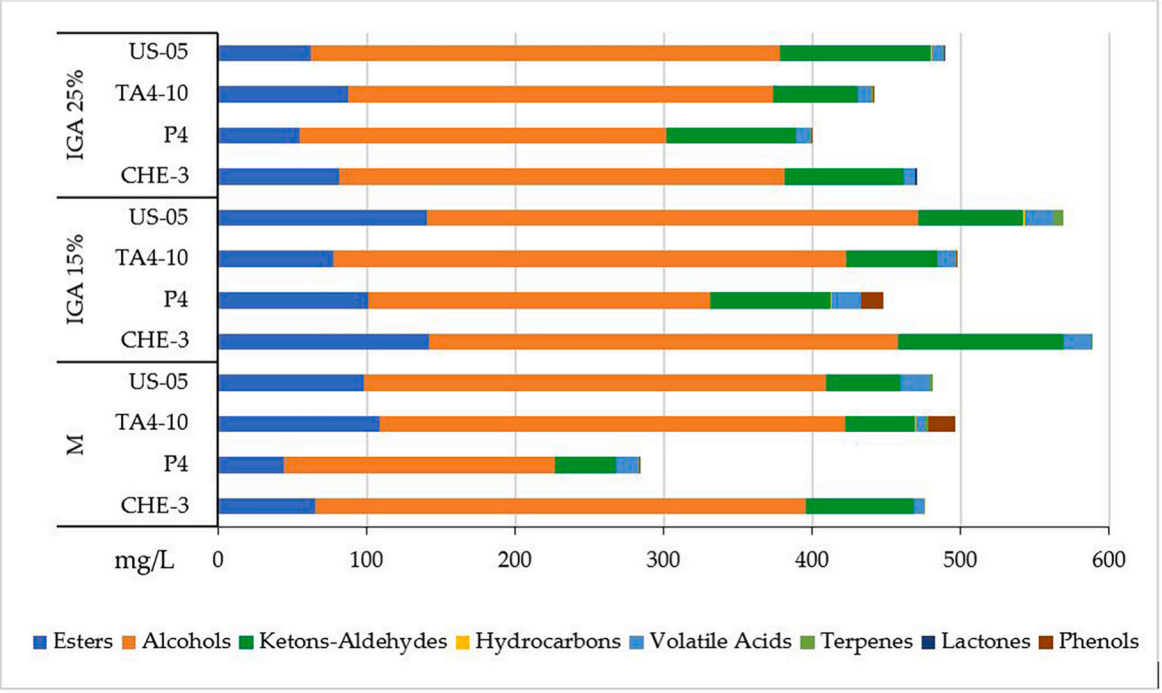

Fig. 2. Classes of principal volatile compounds of the experimental beers produced with 4 different yeast strains (CHE-3, P4, TA4-10, and US-05) using malt wort and malt wort supplemented with 15 and 25% of grape must (M, IGA 15 and IGA 25, respectively).

final product (Dietz et al., 2021). In IGA15 e IGA25 samples, only four terpenes were identified, they being linalool, α-terpineol, citronellol, and geraniol (Table S2). The highest concentration of terpenes was found in beer added with 15% of grape must fermented with the indigenous strain CHE-3.

Among the aldehydes, the most abundant was acetaldehyde, whereas among the ketones, the most abundant one was acetoin. Acetaldehyde was detected in IGA15 beer brewed with CHE-3 strain (97.97 mg/L) and in IGA25 brewed with the commercial strain US-05 (93.09 mg/L), whereas lower concentrations of this compound were identified in all three types of beer produced with the strain TA4-10 (values ranging from 33.37 mg/L in M to 44.56 mg/L in IGA25) (Table 1). The addition of grape must to malt wort increased acetaldehyde content in all the samples, thus confirming the evidence that its synthesis during fermentation is enhanced by the presence of high glucose concentration (Briggs et al., 2004; Piddocke et al., 2009.

Among the volatile acids analyzed, the most abundant was octanoic acid followed by decanoic acid (Table S2). Total volatile acids were found in higher concentrations in all four IGA15 beers, with values ranging from 11.72 mg/L (TA4-10) to 19.34 mg/L (US-05).

The only identified lactone (i.e. y-nonalattone) was absent in control beer (M), but it was detected in the IGA15 beer produced with P4 and TA4-10 strains (0.13 mg/L), whereas it was present in all the IGA25 beers (0.05 mg/L for P4; 0.15 mg/L for TA4-10). The highest concentration of 4-vinyl guaiacol (18.22 mg/L) was detected in M beers brewed with TA4-10 strain and in IGA 15 produced with P4 strain (14.85 mg/L).

The differences in the volatilomic profiles among the beer samples based on the used yeast strains and fermentation media were further evaluated by performing a clustering analysis (Fig. 3). The major volatile compounds (i.e., higher alcohols, acetate esters, ethyl esters, volatile acids, aldehydes, ketones, pyrazines, terpenes, and volatile phenols), were normalized based on the Z-score. Red colours indicate that the amount of the volatile compounds was less than the average value, whereas the blue colours indicate that the volatile compound levels were higher than average level. The aroma compound levels in the beers produced with the strain CHE-3 in the 3 different media were similar; in fact, these beers were clustered in the same group. The strains P4 and US-05 produced beers characterized by similar aroma levels using malt wort and malt supplemented with 15% of grape must, which clustered together but separated by the IGA25 beers. As regards TA4-10 strains, all the beers produced by this starter were located in different groups, indicating that the metabolic behaviour of this strain is strongly influenced by the composition of fermentation medium.

In order to compare overall the characteristics of the twelve experimental beers, both for chemical parameters and organoleptic characteristics, all the analytical parameters detected in the samples were submitted to PCA. The plot of all the experimental beers on the plane defined by the first two components is shown in Fig. 4. The two principal components, PC1 and PC2, accounted for 55.58% of the total variance (36.38 and 19.20%, respectively). This analysis separated the beers obtained by malt wort (located in the left side of the graphs) by IGA15 and IGA25 beers, located in the right side of the scatterplot. Furthermore, among the IGA beers, both the samples fermented with the commercial starter US-05 were separated by all the IGA beers fermented with autochthonous strains.

4. Conclusions

The results obtained in this study showed that the contribution of both the fermentation substrate and yeast strain significantly influenced the process kinetics and chemical characteristics of IGA beers. The three selected *S. cerevisiae* strains showed higher fermentative activity and higher ability to utilize the sugars present in the different fermentation media compared to the commercial brewing strain.

Concerning the analytical composition of the experimental beers, the addition of grape must significantly enhanced the content of ethanol, and as expected, of glycerol and volatile compounds, such as esters and some higher alcohols. However, independently of the tested strains, the highest aromatic expression was observed in the beers obtained by adding 15% grape must.

This study showed that yeast starter cultures deriving from other traditional food-chains can produce quality-enhanced IGA beer, thus

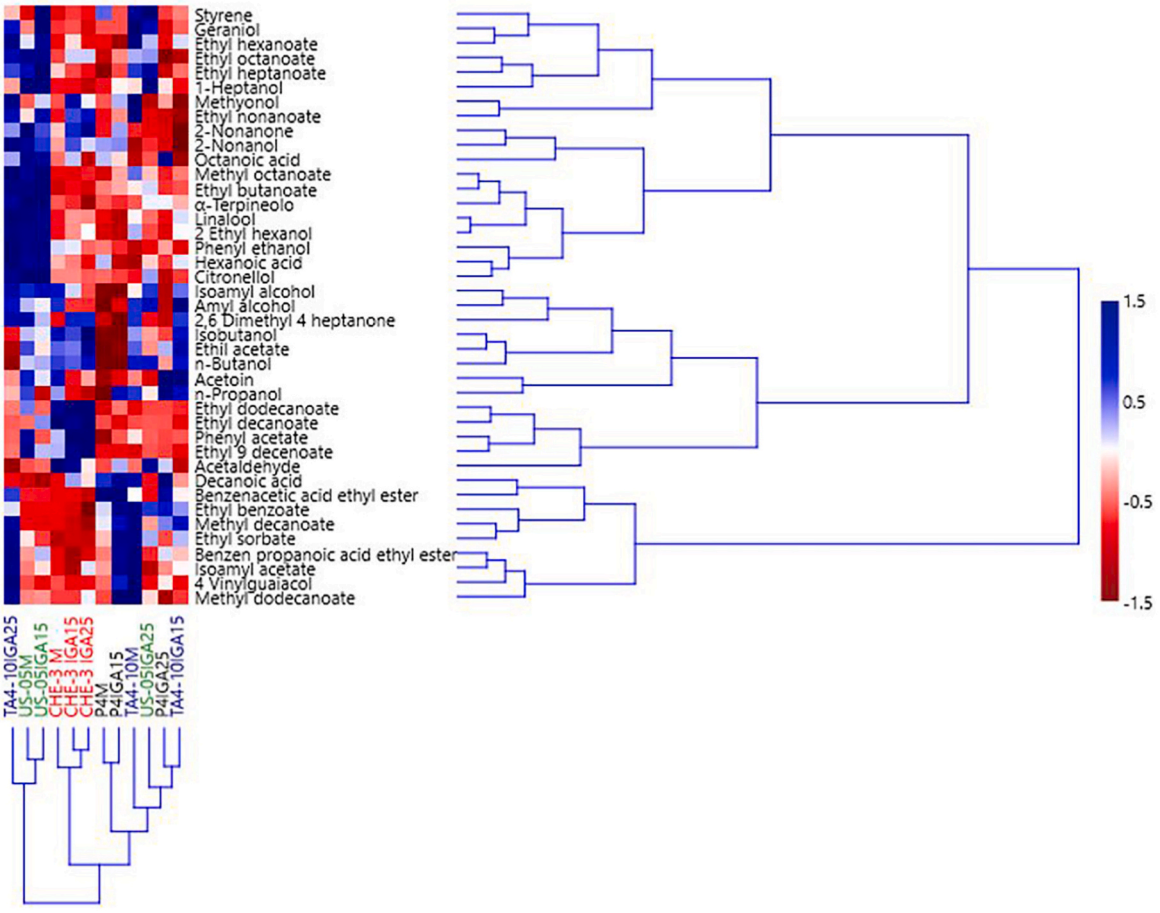

Fig. 3. Heat map visualization and clustering results of volatile compounds detected in the experimental beers produced with 4 different yeast strains (CHE-3, P4, TA4-10, and US-05) using malt wort and malt wort supplemented with 15 and 25% of grape must (M, IGA15 and IGA25, respectively). The data were converted to Z-scores to easily visualize which yeast strains are relevant aroma producers in relation to average production. For each fermentation substrate, Z-Scores were calculated as follows: Z-score = (X – μ)/σ, where X is the concentration of the aroma compound, μ is the mean value of all strains per measured aroma and σ is the standard deviation of values per tested aroma.

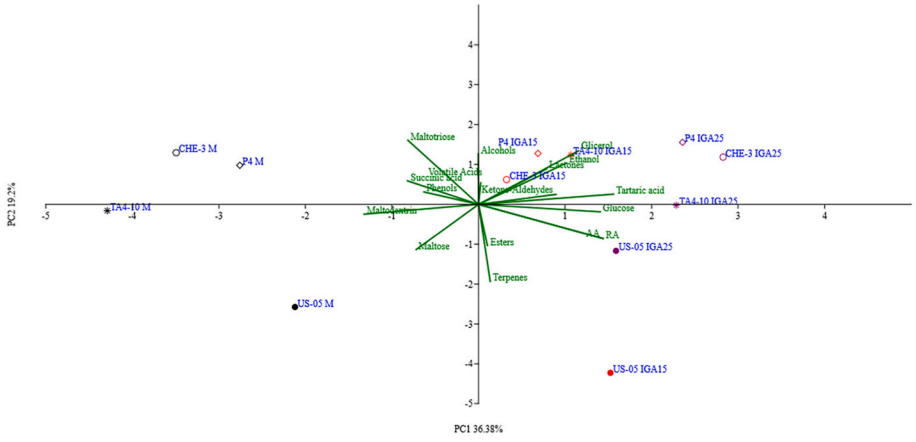

Fig. 4. Principal component analysis (PCA) biplot of volatile aroma compounds, carbohydrate content, organic acids and analytical parameters (real and apparent attenuation, ethanol, glycerol, acetic acid) determined in beers obtained using the four different yeast strains (CHE-3, P4, TA4-10, and US-05) to ferment malt wort (M) or malt wort added with 15% (IGA15) and 25% (IGA25) of grape must.

confirming the potential of cross-over fermentation for novel beverages. Moreover, these findings confirm that wild microorganisms, isolated from different food sources and maintained in the microbial collections, represent a very interesting reservoir of novel starter cultures which contribute to product differentiation, particularly desired in the craft beers sector. Further investigations at brewery scale are at the present underway to validate these strains as novel starters for IGA production.

CRediT authorship contribution statement

Gabriella Siesto: Investigation, Methodology, Writing – original draft. **Rocchina Pietrafesa:** Investigation, Methodology, Writing – original draft. **Maria Tufariello:** Investigation, Validation. **Carmela Gerardi:** Investigation, Validation. **Francesco Grieco:** Supervision, Writing – review & editing. **Angela Capece:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Funding information

This research did not receive any specific grant from funding agencies in the public, commercial, or

Not-for-profit sectors.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

No data was used for the research described in the article.

Acknowledgements

The JRU MIRRI-IT (https://www.mirri-it.it/) is greatly acknowledged for scientific support. The investigation was partially supported by the project "Birra: dal campo al boccale - BEˆ2R" (P.S.R. Puglia 2014/2020 -Misura 16). The Authors are grateful to Domenico Genchi and Giuseppe Romano (CNR-ISPA) for their skilled technical support and Pasquale Del Vecchio (CNR-ISPA) for the data stewardship supplied.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102487.

References

Aquilani, B., Laureti, T., Poponi, S., & Secondi, L. (2015). Beer choice and consumption determinants when craft beers are tasted: An exploratory study of consumer preferences. *Food Quality and Preference, 41*, 214–224.

Araújo, T. M., Souza, M. T., Diniz, R. H. S., Yamakawa, C. K., Soares, L. B., Lenczak, J. L., de Castro Oliveira, J. V., Goldman, G. H., Barbosa, E. A., Campos, A. C. S., Castro, I. M., & Brandão, R. L. (2018). Cachaça yeast strains: Alternative starters to produce beer and bioethanol. *Antonie van Leeuwenhoek, 111*, 1749–1766.

Baiano, A., Fiore, A., la Gatta, B., Tufariello, M., Gerardi, C., Savino, M., & Grieco, F. (2023). Single and interactive effects of unmalted cereals, hops, and yeasts on quality of white-inspired craft beers. *Beverages, 9*, 9.

Biazon, C. L., Brambilla, R., Rigacci, A., Pizzolato, T. M., & dos Santos, J. H. Z. (2009). Combining silica-based adsorbents and SPME fibers in the extraction of the volatiles of beer: An exploratory study. *Analytical and Bioanalytical Chemistry, 394*, 549–556.

BJCP. (2015). *Beer style guidlines, beer Judge certification Program*. Internet: https://www.bjcp.org/docs/2015_Guidelines_Beer.pdf. (Accessed 15 December 2022).

Briggs, D., Boulton, C., Brookes, P., & Stevens, R. (2004). *Brewing science and practice*. Cambridge: Woodhead Publishing Limited, ISBN 18-5-573490-7.

Canonico, L., Comitini, F., & Ciani, M. (2014). Dominance and influence of selected *Saccharomyces cerevisiae* strains on the analytical profile of craft beer refermentation. *Journal of the Institute of Brewing, 120*, 262–267.

Canonico, L., Zannini, E., Ciani, M., & Comitini, F. (2021). Assessment of non-conventional yeasts with potential probiotic for protein-fortified craft beer production. *Lebensmittel-Wissenschaft und -Technologie, 145*, Article 111361.

Capece, A., De Fusco, D., Pietrafesa, R., Siesto, G., & Romano, P. (2021). Performance of wild non-conventional yeasts in fermentation of wort based on different malt extracts to select novel starters for low-alcohol beers. *Applied Sciences, 11*, 801.

Capece, A., Pietrafesa, R., & Romano, P. (2011). Experimental approach for target selection of wild wine yeasts from spontaneous fermentation of "Inzolia" grapes. *World Journal of Microbiology and Biotechnology, 27*, 2775–2783.

Capece, A., Romaniello, R., Pietrafesa, A., Siesto, G., Pietrafesa, R., Zambuto, M., & Romano, P. (2018). Use of *Saccharomyces cerevisiae* var. *boulardii* in co-fermentations with *S. cerevisiae* for the production of craft beers with potential healthy value added. *International Journal of Food Microbiology, 284*, 22–30.

Castro Marin, A., Baris, F., Romanini, E., Lambri, M., Montevecchi, G., & Chinnici, F. (2021). Physico-chemical and sensory characterization of a fruit beer obtained with the addition of cv. Lambrusco grapes must. *Beverages, 7*, 34.

Christofoleti-Furlan, R. M., Portugal, C. B., Varize, C. S., Muynarsk, E. S. M., Alcarde, A. R., & Basso, L. C. (2020). Unraveling Brazilian bioethanol yeasts as novel starters for high-gravity brewing. *Food Research International, 135*, Article 109282.

Cioch-Skoneczny, M., Królak, K., Tworzydło, Z., Satora, P., & Skoneczny, S. (2022). In *Characteristics of beer brewed with unconventional yeasts and addition of grape must, pulp and marc*. European Food Research and Technology. https://doi.org/10.1007/s00217-022-04166-w.

Coelho, E. M., da Silva Padilha, C. V., Miskinis, G. A., de Sá, A. G. B., Pereira, G. E., de Azevêdo, L. C., & dos Santos Lima, M. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167.

Cubillos, F. A., Gibson, B., Grijalva-Vallejos, N., Krogerus, K., & Nikulin, J. (2019). Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. *Yeast, 36*, 383–398.

Dank, A., van Mastrigt, O., Yang, Z., Dinesh, V. M., Lillevang, S. K., Weij, C., & Smid, E. J. (2021). The cross-over fermentation concept and its application in a novel food product: The dairy miso case study. *LWT - Food Science and Technology, 142*, Article 111041.

De Simone, N., Russo, P., Tufariello, M., Fragasso, M., Solimando, M., Capozzi, V., Grieco, F., & Spano, G. (2021). Autochthonous biological resources for the production of regional craft beers: Exploring possible contributions of cereals, hops, microbes, and other ingredients. *Foods, 10*, 1831.

Denby, C. M., Li, R. A., Vu, V. T., Costello, Z., Lin, W., Chan, L. J. G., Williams, J., Donaldson, B., Bamforth, C. W., Petzold, C. J., Scheller, H. V., Garcia Martin, H., & Keasling, J. D. (2018). Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nature Communications, 9*, 965.

Dietz, C., Cook, D., Wilson, C., Oliveira, P., & Ford, R. (2021). Exploring the multisensory perception of terpene alcohol and sesquiterpene rich hop extracts in lager style beer. *Food Research International, 148*, Article 110598.

Ducruet, J., Rébénaque, P., Diserens, S., Kosińska-Cagnazzo, A., Héritier, I., & Andlauer, W. (2017). Amber ale beer enriched with goji berries -The effect on bioactive compound content and sensorial properties. *Food Chemistry, 226*, 109–118.

Garavaglia, C. (2020). The emergence of Italian craft breweries and the development of their local identity. In N. Hoalst-Pullen, & M. W. Patterson (Eds.), *The geography of beer: Culture and economics* (pp. 135–147). Cham, Switzerland: Springer International Publishing, ISBN 978-3-030-41654-6.

Gasínski, A., Kawa-Rygielska, J., Szumny, A., Czubaszek, A., Gąsior, J., & Pietrzak, W. (2020). Volatile compounds content, physicochemical parameters, and antioxidant activity of beers with addition of mango fruit (Mangifera Indica). *Molecules, 25*, 3033.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica, 4*, 1–9.

Hazelwood, L. A., Daran, J.-M., van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology, 74*, 2259–2266.

Johansen, P. G., Owusu-Kwarteng, J., Parkouda, C., Padonou, S. W., & Jespersen, L. (2019). Occurrence and importance of yeasts in indigenous fermented food and beverages produced in sub-Saharan Africa. *Frontiers in Microbiology, 10*, 1789.

Kang, W., Xu, Y., Qin, L., & Wang, Y. (2010). Effects of different β-d-glycosidases on bound aroma compounds in Muscat grape determined by HS-SPME and GC-MS. *Journal of the Institute of Brewing, 116*, 70–77.

Kawa-Rygielska, J., Adamenko, K., Kucharska, A. Z., Prorok, P., & Píorecki, N. (2019). Physicochemical and antioxidative properties of Cornelian cherry beer. *Food Chemistry, 281*, 147–153.

Langstaff, S. A., Guinard, J. X., & Lewis, M. J. (1991). Instrumental evaluation of the mouthfeel of beer and correlation with sensory evaluation. *Journal of the Institute of Brewing, 97*, 427–433.

Li, M., Du, J., & Zhang, K. (2020). Profiling of carbohydrates in commercial beers and their influence on beer quality. *Journal of the Science of Food and Agriculture, 100*, 3062–3070.

Lodolo, E. J., Kock, J. L., Axcell, B. C., & Brooks, M. (2008). The yeast *Saccharomyces cerevisiae* - the main character in beer brewing. *FEMS Yeast Research, 8*, 1018–1036.

Loviso, C. L., & Libkind, D. (2019). Synthesis and regulation of flavor compounds derived from brewing yeast: Esters. *Revista Argentina de Microbiología, 50*, 436–446.

Marongiu, A., Zara, G., Legras, J.-L., Del Caro, A., Mascia, I., Fadda, C., & Budroni, M. (2015). Novel starters for old processes: Use of *Saccharomyces cerevisiae* strains isolated from artisanal sourdough for craft beer production at a brewery scale. *Journal of Industrial Microbiology and Biotechnology, 42*, 85–92.

Martínez, A., Vegara, S., Martí, N., Valero, M., & Saura, D. (2017). Physicochemical characterization of special persimmon fruit beers using bohemian pilsner malt as a base. *Journal of the Institute of Brewing, 123*, 319–327.

Mayer, H., Ceccaroni, D., Marconi, O., Sileoni, V., Perretti, G., & Fantozzi, P. (2016). Development of an all rice malt beer: A gluten free alternative. *LWT - Food Science and Technology, 67*, 67–73.

Meier-Dörnberg, T., Hutzler, M., Michel, M., Methner, F.-J., & Jacob, F. (2017). The importance of a comparative characterization of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* strains for brewing. *Fermentation, 3*, 41.

Nardini, M., & Garaguso, I. (2020). Characterization of bioactive compounds and antioxidant activity of fruit beers. *Food Chemistry, 305*, Article 125437.

Ocvirk, M., Mlinarič, N. K., & Košir, I. J. (2018). Comparison of sensory and chemical evaluation of lager beer aroma by gas chromatography and gas chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 98*, 3627–3635.

Olaniran, A. O., Hiralal, L., Mokoena, M. P., & Pillay, B. (2017). Flavour-active volatile compounds in beer: Production, regulation and control. *Journal of the Institute of Brewing, 123*, 13–23.

Olaniran, A. O., Maharaj, Y. R., & Pillay, B. (2011). Effects of fermentation temperature on the composition of beer volatile compounds, organoleptic quality and spent yeast density. *Electronic Journal of Biotechnology, 14*(2), 5-5.

Pasqualone, A., Summo, C., Laddomada, B., Mudura, E., & Coldea, T. E. (2018). Effect of processing variables on the physico-chemical characteristics and aroma of borş, a traditional beverage derived from wheat bran. *Food Chemistry, 265*, 242–252.

Perestrelo, R., Silva, C., Silva, P., & Câmara, J. S. (2018). Unraveling vitis vinifera L. grape maturity markers based on integration of terpenic pattern and chemometric methods. *Microchemical Journal, 142*, 367–376.

Piddocke, M. P., Kreisz, S., Heldt-Hansen, H. P., Nielsen, K. F., & Olsson, L. (2009). Physiological characterization of brewer's yeast in high-gravity beer fermentations with glucose or maltose syrups as adjuncts. *Applied Microbiology and Biotechnology, 84*, 453–464.

Pires, E. J., Teixeira, J., Brányik, T., & Vicente, A. (2014). Yeast: The soul of beer's aroma - a review of flavour-active esters and higher alcohols produced by the brewing yeast. *Applied Microbiology and Biotechnology, 98*, 1937–1949.

Postigo, V., García, M., Cabellos, J. M., & Arroyo, T. (2021). Wine *Saccharomyces* yeasts for beer fermentation. *Fermentation, 7*, 290.

Romano, G., Tufariello, M., Calabriso, N., Del Coco, L., Fanizzi, F. P., Blanco, A., Carluccio, M. A., Grieco, F., & Laddomada, B. (2023). Pigmented cereals and legume grains as healthier alternatives for brewing beers. *Food Bioscience, 52*, Article 102463.

Rossi, S., Turchetti, B., Sileoni, V., Marconi, O., & Perretti, G. I. F. (2018). Evaluation of *Saccharomyces cerevisiae* strains isolated from non-brewing environments in beer production. *Journal of the Institute of Brewing, 124*, 381–388.

Saerens, S. M., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R. (2008). Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Applied and Environmental Microbiology, 74*, 454–461.

Salanță, L. C., Coldea, T. E., Ignat, M. V., Pop, C. R., Tofană, M., Mudura, E., Borșa, A., Pasqualone, A., Anjos, O., & Zhao, H. (2020). Functionality of special beer processes and potential health benefits. *Processes, 8*, 1613.

dos Santos Lima, M., Dutra, M. D. C. P., Toaldo, I. M., Corrêa, L. C., Pereira, G. E., de Oliveira, D., Bordignon-Luiz, M. T., & Ninow, J. L. (2015). Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration. *Food Chemistry, 188*, 384–392.

Shindo, S., Murakani, J., & Koshino, S. (1992). Control of acetate ester formation during alcohol fermentation with immobilized yeast. *Journal of Fermentation and Bioengineering, 73*, 370–374.

Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., Mayo, B., Westerik, N., & Hutkins, R. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety, 19*, 184–217.

Thompson-Witrick, K. A., Rouseff, R. L., Cadawallader, K. R., Duncan, S. E., Eigel, W. N., Tanko, J. M., & O'Keefe, S. F. (2015). Comparison of two extraction techniques, solid-phase microextraction versus continuous liquid-liquid extraction/solvent-assisted flavor evaporation, for the analysis of flavor compounds in Gueuze lambic beer. *Journal of Food Science, 80*, C571–C576.

Tufariello, M., Fragasso, M., Pico, J., Panighel, A., Castellarin, S. D., Flamini, R., & Grieco, F. (2021). Influence of non-*Saccharomyces* on wine chemistry: A focus on aroma-related compounds. *Molecules, 26*(3), 644.

Tufariello, M., Pati, S., D'Amico, L., Bleve, G., Losito, I., & Grieco, F. (2019). Quantitative issues related to the headspace-SPME-GC/MS analysis of volatile compounds in wines: The case of Maresco sparkling wine. *LWT - Food Science and Technology, 108*, 268–276.

Udom, N., Chansongkrow, P., Charoensawan, V., & Auesukaree, C. (2019). Coordination of the cell wall integrity and high-osmolarity glycerol pathways in response to ethanol stress in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology, 85*, 00551-19.

Younis, O. S., & Stewart, G. G. (1998). Sugar uptake and subsequent ester and higher alcohol production by *Saccharomyces cerevisiae*. *Journal of the Institute of Brewing, 104*, 255–264.

Younis, O. S., & Stewart, G. G. (2000). The effect of wort maltose content on volatile production and fermentation performance in brewing yeast. In K. Smart (Ed.), *Brewing yeast fermentation performance* (pp. 170–176). Oxford: Blackwell.

Zhao, X., Procopio, S., & Becker, T. (2015). Flavor impacts of glycerol in the processing of yeast fermented beverages: A review. *Journal of Food Science & Technology, 52*(12), 7588–7598.