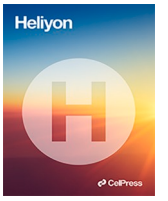
Heliyon 10 (2024) e37598

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Optimisation of quality features of new wheat beers fermented through sequential inoculation of non-*Saccharomyces and Saccharomyces* yeasts

Francesco Grieco [a], Anna Fiore [b], Carmela Gerardi [a], Maria Tufariello [a], Giuseppe Romano [a], Antonietta Baiano [b,*]

[a] Institute of Sciences of Food Production - National Research Council (CNR-ISPA), Via Prov.le, Lecce-Monteroni, 73100, Lecce, Italy
[b] Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria (DAFNE), University of Foggia, Via Napoli 25, 71122, Foggia, Italy

ARTICLE INFO

Keywords:
Beer quality
Design of experiment
Oenological yeasts
Schizosaccharomyces pombe
Sequential fermentation
White craft beer

ABSTRACT

The choice of the starchy ingredients as well as that of the yeasts strongly can represent a useful tool to differentiate the final beers. Our research investigated twelve white beers obtained applying a 2-factor mixed 3-level/4-level experimental design. The first factor was the cereal mixture, with 3 combinations of barley malt (65 %) and unmalted wheat (35 % of common, durum, or emmer). The second factor was the yeast used to carry out the fermentation trials, i.e.: a *S. cerevisiae* starter strain (WB06); an oenological *S. cerevisiae* strain (9502); two mixed starters made of an oenological *Schizosaccharomyces pombe* strain (6956) and, alternatively, one of the two *S. cerevisiae* strains. Most beer attributes were significantly ($p < 0.05$) influenced by the two considered factors with the following exceptions: the wheat species did not affect maltotriose, maltose, pH, total and volatile acidity, floral flavour, and sweetness; the yeast did not exert significant effects on foam colour, turbidity, overall olfactory intensity, yeast flavour, and body. The flavour of fruits and aromatic herbs were not influenced by the factors studied. Alcohol content was maximised using the unmalted durum wheat (~7 %) and *S. cerevisiae* WB06 (~6.8 %). The beer antioxidant content was increased by the use of emmer (566 mg/L) and by the application of the mixed inoculum (478–487 mg/L). The beers made with unmalted common wheat and fermented by the *S. cerevisiae* strains alone obtained the best overall sensory score (3.7). As shown by the Principal Component Analysis, the beers were better classified by the type of unmalted wheat than by the fermenting yeast. A multiple regression analysis was performed by fitting the analytical parameters that highlighted significant differences among the beers to a second-order polynomial model. Data concerning colour, glycerol concentration, FC-TPC, and antioxidant activity were satisfactorily predicted ($R^2 > 0.8$) by the fitted models.

1. Introduction

The addition of unmalted wheat to malted barley is common in brewing to create a specific style known as Belgian wheat beer or Witbier or bière blanche [1]. However, despite the long tradition of the use of raw wheat as a brewing material and the increasing

* Corresponding author.
E-mail addresses: francesco.grieco@ispa.cnr.it (F. Grieco), anna.fiore@unifg.it (A. Fiore), carmela.gerardi@ispa.cnr.it (C. Gerardi), maria.tufariello@ispa.cnr.it (M. Tufariello), giuseppe.romano@ispa.cnr.it (G. Romano), antonietta.baiano@unifg.it (A. Baiano).

https://doi.org/10.1016/j.heliyon.2024.e37598
Received 19 April 2024; Received in revised form 4 September 2024; Accepted 6 September 2024
Available online 8 September 2024

wheat beer production, with few exceptions, little effort has been made to investigate suitable brewing wheat cultivars [2]. For example, winter wheat cultivars 'Gimantis', 'Elixer', 'Rockefeller', and 'Lawina' can be conveniently added to barley malt in unmalted form at a 50 % share to increase alcohol content or in a mix of unmalted and malted forms to maximise sensory attractiveness and total polyphenol content [1]. At the same time, 40 % of unmalted grains ('Risciola' common wheat, 'Dauno III' durum wheat, and 'Padre Pio' emmer) were used to produce white-inspired craft beers, although with a preference for the first two species due to their higher foam amount and fullness and their lower sourness [3].

Wheat beers are usually produced using the top-fermenting (ale) *Saccharomyces cerevisiae* species. However, phylogenetic studies have demonstrated that ale beer strains genetically differ from *S. cerevisiae* strains used in other food sectors, in particular from wine strains, since the peculiar traits of ale beer yeasts have been selected during their domestication [4,5]. Furthermore, many natural top yeast strains are hybrids. Therefore, this is the reason why non-brewing *S. cerevisiae* yeasts have been recently applied in both wort fermentation and beer refermentation as a source of biodiversity and a differentiating factor in the brewing scenery [6]. Rossi et al. [7] performed an in-lab screening of twelve *S. cerevisiae* strains isolated from fermenting grape must, leavened products, wine, and apple stillage, finding a baker's yeast as the most promising in terms of fermentative ability and volatile profile, with quality attributes comparable with those of a standard ale. Other researchers evaluated *S. cerevisiae* strains isolated from wineries. Canonico et al. [8] observed that four of 33 strains isolated as fermenting wine yeasts were suitable for in-bottle refermentation, thanks to their higher production of compounds responsible for fruity and flowery aroma with respect to commercial starter strains. Recently, Baiano et al. [9] tested four oenological *S. cerevisiae* strains for both wort fermentation and beer secondary fermentation. Two of them, isolated from Negroamaro fermenting-must, contributed to maximising the beer's total phenolic content, while a strain recovered from Susumaniello-must contributed to the obtainment of the best sensory properties.

A further challenge is nowadays represented by the use in brewing of non-*Saccharomyces* yeasts due to their ability to produce many secondary metabolites that affect both flavour and taste of beer. Four non-*Saccharomyces* strains (*Schizosaccharomyces pombe, Torulaspora delbrueckii, Saccharomycodes ludwigii, and Lachancea thermotolerans*) have been tested by Callejo et al. [10] in two different trials: in the first one, they were used for both wort fermentation and bottle conditioning; in the second one, they were only used for bottle conditioning of beers fermented by a commercial *S. cerevisiae* strain. *Sc. pombe* gave beers having the highest contents of ethanol and acetaldehyde and the best foam characteristics. Instead, the use of the other three strains was preferable in the producion of low-alcohol beers with a distinctive flavour. Furthermore, species such as *Hanseniaspora guilliermondii*, *Sc. pombe*, and *Lachancea thermotolerans* could be conveniently used to obtain functional beers due to their higher melatonin production [6]. Wine yeast strains of *Hanseniaspora guilliermondii, Zygosaccharomyces bailii*, and *Torulaspora delbrueckii* used in sequential fermentation with a commercially available *S. cerevisiae* strains enhanced the sensory characteristics of the resulting beer [11].

To the best of our knowledge, non-*Saccharomyces* yeasts have been used almost exclusively for the fermentation of barley malt wort and are exceptionally integrated with adjuncts of sorghum, as in Einfalt's research [12]. The inoculation of non-*Saccharomyces* followed by *Saccharomyces* strains is therefore unexplored.

For the first time, the present work was aimed to optimise quality characteristics (in particular those related to concentration of healthy compounds and sensory profile) of novel white craft beers brewed from a mixture of barley malt and unmalted wheat species (alternatively common, durum, emmer). This mixture was submitted to sequential fermentation using a *Sc. pombe* strain isolated from grape-must alternatively combined with two *S. cerevisiae* strains (one commercial and the other of oenological origin).

2. Materials and methods

2.1. Materials

The Pilsner barley malt used in the experimental trials was produced by Agroalimentare Sud (Melfi, Italy). Three unmalted wheat species were considered: common wheat cv. Risciola and durum wheat cv. Dauno III (from now on referred to as C and D, respectively), produced by Soc. Cooperativa Agricola Valleverde (Bovino, Italy) starting from seeds selected by CREA-CI Research Centre (Foggia, Italy); and emmer (E) (*Triticum dicoccum*) produced in the experimental fields of CREA-CI Research Centre.

Three yeast strains were used: the commercial *S. cerevisiae* strain WB06 supplied by Lesaffre Italia (Sissa Trecasali, Italy); *S. cerevisiae* ITEM9502 (from now on referred to as *S. cerevisiae* 9502) isolated from Susumaniello grape (Tristezza et al. [13]); the *oenological Sc. pombe* strain ITEM 6956 (from now on referred to as *Sc. pombe* 6956). The two oenological strains belong to the ITEM Microbial Culture Collection of CNR-ISPA (http://www.ispacnr.it/collezioni-microbiche).

Cascade dried cones (6.7 % α-acid content), peels of bitter orange, and coriander seeds were supplied by Birramia (Querceta, Lucca, Italy).

2.2. Experimental design

A 2-factor mixed 3-level and 4-level experimental design was applied. The first factor was the unmalted wheat species used together with the malted barley as starchy ingredients and the corresponding 3 levels were represented by the mixes of malted barley (65 %) and unmalted C, D, or E (35 %). The second factor was the fermenting yeast, with the following four types of inoculum: *S. cerevisiae* WB06 alone; *S. cerevisiae* 9502 alone,; the sequential inoculation of *Sc. pombe* 6956 (selected for its ability to metabolise maltose and maltotriose) and *S. cerevisiae* WB06; the sequential inoculation of *Sc. pombe* 6956 and *S. cerevisiae* 9502.

2.3. Brewing process

The formulation for 100 L of final beer iwere the following: 135 L water (85 % for mashing and 15 % for sparging); 16.25 kg Pilsner barley malt; 8.75 kg unmalted wheat; 200 g dried hop cones; 100 g peels of bitter orange; 50 g coriander seeds. The brewing trials were performed in a 20 L-Braumeister system (Speidel Tank-und Behälterbau GmbH, Ofterdingen, Germany).

Malted and unmalted cereals were preliminarily coarsely ground with a 2-roller mill (Albrigi Luigi, Stallavena, Italy). The ground cereals were introduced into the mashing water (conductivity, 420 ± 5 μS/cm) when its temperature reached 47 °C. The mashing stages included: protein rest (54 °C; 10 min); β-amylase rest (63 °C; 50 min); α-amylase rest (70 °C; 50 min); mash-off (81 °C; 16 min); sparging with water heated at 81 °C. The obtained wort (5.4 ± 0.2) was then boiled for 65 min. Half the hop was added 5 min after the boil started while the other half was added 45 min later. Coarsely ground coriander and bitter orange peels were added 5 min before the end of boiling.

Each of the worts resulting from the mashing of the three malted barley-unmalted wheat mixtures (C, D, and E) was cooled and whirlpool (original gravity of 1.047 ± 0.001, 1.051 ± 0.001, and 1.049 ± 0.001, respectively), and then divided into 4 aliquots. The first two aliquots were inoculated (~1×10^7 cells/mL) with *S. cerevisiae* WB06 and 9502, respectively. Each of the other two aliquots were firstly inoculated with *Sc. pombe* 6956 (~1×10^7 cells/mL) and, when an intermediate gravity around 1.030 was reached (this happened within 48 h), *S. cerevisiae* WB06 and 9502 (~1×10^7 cells/mL) were alternatively inculated. Fermentations lasted 20 ± 1 days at 23 ± 2 °C (final gravity values, 1.012 ± 0.006). The beers were then stored at 4 ± 1 °C for 2 days. Finally, the racked beers were inoculated with the same *S. cerevisiae* yeast strain used for the previous fermentation (~1×10^5 cells/mL), added with 8 g sucrose per litre, and packaged into 500 mL glass brown bottles. The bottled beer were conditioned at 20 ± 1 °C for 1 month and then stored at 5 ± 1 °C until their opening. The following twelve types of beers were produced combining the worts deriving from the three unmalted wheat species with the four types of fermentation: C-WB06, C-9502; D-WB06, D-9502; E-WB06, E−9502, C-6956/WB06, C-6956/9502, D-6956/WB06, D-6956/9502, E−6956/WB06, E−6956/9502. The production of each type of beer were replicated two times.

Ingredients and brewing procedures were previously characterized and tested in terms of hygiene and safety to ensure that they did not present risks to human health.

2.4. Routine analyses

The analyses of alcohol content (%), soluble solids (as Brix), dry matter (%), CO_2 content (as mg CO_2/L), pH, total acidity (g lactic acid/L), and volatile acidity (g acetic acid/L) were performed according to Baiano et al. [3]. Beer colour was measured at 430 nm according to the Method 9.6 [14] on previously degassed and filtered (0.45 μm) samples and expressed as srm.

Organic acid profile, sugar profile, and glycerol concentrations (g/L) were simultaneously obtained as described by Coelho et al. [15] by an 1100 HPLC system (Agilent, Santa Clara, CA, USA) equipped with a 300 mm × 7.7 mm x 8.0 μm Hi-Plex H column (Agilent Technologies, Santa Clara, CA, USA), a DAD (set at 210 nm for organic acid detection), and a RID (for sugar and glycerol detection). Their quantification was obtained through the ChemStation software (Agilent) on five-point calibration curves of the corresponding standard compounds.

2.5. Analysis of antioxidant compounds and antioxidant activity

The Folin–Ciocalteu method [16] was used to quantify the total antioxidant content (FC-TPC, mg gallic acid equivalents/L) on a calibration curve of gallic acid (20–1000 mg/L range). The beer radical scavenging activity was determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) [17] and expressed as a percentage of DPPH remaining (% DPPH). The phenolic composition was analysed by an 1100 HPLC-DAD system (Agilent, Santa Clara, CA, USA) equipped with a 100 × 4.6 mm × 3 μm RP-C18 Gemini column (Phenomenex, Aschaffenburg, Germany) as described by Aliakbarian et al. [18]. The phenolic compound identification was obtained by comparing their retention times and spectra with those of 18 standard compounds, The identified compounds were quantified on five-point calibration curves of the corresponding standard compounds. The sum of the concentrations of all phenolic compounds (HPLC-TPC) was also calculated.

2.6. Quantitative descriptive analysis

The experimental beers were analysed by a trained panel including twelve panellists whose age was comprised between 25 and 65 years. All panellists have previously obtained a sommelier or technical wine taster certificate. Panellists were asked to sign an informed consent form to take part to the research. They were also made aware that their information would be used solely for the research purposes. A protocol was utilised to protect the rights and privacy of all panellists and data was processed anonymously. The Quantitative Descriptive Analysis (QDA) was performed as described by Baiano et al. [3]. According to the profile sheet (reported in the supplementary material as Fig. S1), the panellists rated five visual (colour, amount, and persistence of foam; colour and turbidity of the liquid fraction), 9 olfactory (overall intensity, finesse, and the following flavours: malt, hop, flower, fruit, spices, yeast, aromatic herbs), 4 gustatory (sweetness, bitterness, saltiness, sourness), and 3 tactile (alcoholic, effervescence, and body/fullness) characteristics. The panellists also assigned an objective score to the overall sensory quality of each beer after its swallowing. The intensity of most descriptors and the overall quality were evaluated on a 5-point scale. Instead, colour was evaluated on the following 4-point scales: 1 (white), 2 (rose), 3 (cream), and 4 (capuchin) for foam; 1 (pale straw yellow), 2 (straw yellow), 3 (golden yellow), and 4 (amber) for the liquid fraction.

2.7. Statistical analysis

Averages and standard deviations were obtained on the data derived from the three analytical replicates erformed for each of the two technological replicates. A two-way ANOVA followed by an LSD test ($p < 0.05$) was useful to highlight the influence of the different unmalted wheat species (C, D, and E) and of the fermentation carried out by the two *S. cerevisiae* yeast strains taken alone or in sequential inoculation after *Schizosaccharomyces pombe* (WB06 and 9502; 6956/WB06 and 6956/9502). A Principal Component Analysis (PCA) was also performed to verify if wheat species, fermenting yeast, or both) could homogeneously group the beer samples according to their analytical characteristics. The R Pearson correlation coefficients calculated at $p < 0.05$ were used to determine relationships among the variables analysed (Table S1). The optimisation of beer formulation was pursued through a multiple regression analysis in which each dependent variable (i.e. the physicochemical and sensory characteristics that showed significant differences among the 12 samples) were fitted to the following second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i}^{k-1} \sum_{j}^{k} \beta_{ij} X_i X_j \quad (1)$$

where: Y is the dependent variable; β_0 is a constant; βi are the linear coefficients; β_{ii} are the quadratic coefficients and β_{ij} are the interactive coefficients. The quality of the fitted model and the significant factors were evaluated through the ANOVA analysis (p-value <0.05). The overall model predictive capability was evaluated by the corresponding coefficient of determination (R^2). The statistically significant correlations are discussed in the text. The statistical analyses were carried out through Statistica for Windows V. 7.0. (Statsoft, Tulsa, OK, USA).

3. Results and discussion

According to the Beer Judge Certification Program [19], the typical characteristics of a white beer include an original gravity within 1.044–1.052, a final gravity comprised in the 1.008–1.012 range, a colour of 2–4 srm, and an alcohol content from 4.5 % to 5.5 %. Our experimental beers had comparable gravity values but slightly more intense colours, from straw to golden, when durum wheat and emmer were used, and slightly higher alcohol content. Table 1 reports the results of the analysis of variance concerning the obtained data and paragraphs 3.1 and 3.2 describe the effects of wheat species and fermenting yeasts, respectively.

3.1. Effects of wheat species

As highlighted by Table 1, almost all physical, chemical, and sensory parameters were influenced by wheat species. The exceptions to this rule concern maltodextrins and maltotriose contents, pH, total and volatile acidity, floral, fruty, and aromatic herb flavours, which did not have significant differences among the beers. The colour become significantly darker ranging from C to E beers, consistently with the colourimetric indices of the corresponding cereal mixtures used as starchy materials (L*: 42.7, 39.7, and 36.2; a*: 9.2, 9.7, and 6.2; b*: 33.0, 31.2, and 27.0, for C, D, and E, respectively), and their coloured compound contents [20]. The lowest and the highest values for alcohol, residual soluble solid, dry matter, and maltodextrin contents were detected in C- and D-beers, respectively, despite no significant differences among the initial starch amounts of the corresponding raw materials (66.91 and 67.17 % for mixtures containing common and durum wheat, respectively). The different behaviour of durum and common wheat may be attributed to the higher endo-β-glucanase/endo-1,4-β-D-xylanase activities of the first that, by degrading the non-starch polysaccharides of the cell walls, increase starch accessibility to amylolytic enzymes [21]. The emmer had higher starch content (67.88 %) than durum wheat, but the presence of hulls may have interfered with the action of amylases during mashing. Maltotriose and maltose contents were notinfuenced by the wheat species. Regarding monosaccharides, the concentration of residual fructose was far greater than that of glucose (double in D- and E-beers; triple in C-beers), which is in agreement with the sugar composition of wheat grains [22]. E− and C-beers showed, respectively, the highest and the lowest glucose concentrations, while the opposite behaviour was observed for fructose. D-beers had intermediate concentrations of both monosaccharides. These results are consistent with the influence of genotypes on cereal composition [22,23]. Glycerol and ethanol contents showed an opposite behaviour, since the first is produced as a consequence of the diversion of carbon flux from ethanol [24].

The carbon dioxide contents of our beers significantly varied with the wheat species used according to the following decreasing order: C > E > D, showing a trend opposite to that of the residual soluble solid content (the greater the quantity of sugar fermented by the yeasts, the greater the carbon dioxide produced). Our experimental CO_2 contents were significantly lower than those (4.3–4.7 g/L) detected by Belcar et al. [1] in witbier-style beers produced from mixtures of barley malt (50 %) and unmalted winter wheats (50 %) because carbon dioxide increases as the amount of unmalted wheat increases. While pH, total and volatile acidity were independent of the type of unmalted wheat, the organic acid composition was affected by wheat species. The C-beers had the highest content of citric acid and the lowest contents of all the other organic acids. This is in agreement with the data reported by Lovegrove et al. [22], who detected significantly lower concentrations of total organic acids in bread wheat.

According to the FC-TPC results (Table 1), C- and E-beers showed the lowest and the highest total antioxidant content, respectively. This data highlighted the significant effects of genotype and was in agreement with the findings of Zrcková et al. [25] who found our same order of concentration for common wheat and emmer. The opposite situation was observed for HPLC-TPC ($r = -0.5720$), with C- and E-beers showed the highest and the lowest sum of the concentrations of individual phenolic molecules, respectively, thus highlighting that, in addition to polyphenols, other reducing compounds contributed to the beer antioxidant activity. Indeed, the negative

Table 1
Results of the two-way ANOVA applied to the experimental data to highlight the influence of wheat species and fermenting yeast strains on physical, chemical, and sensory parameters of beers.

Variables		Effect of wheat species				Effect of yeast strains				
		C	D	E	Significance	WB06	9502	6956/ WB06	6956/ 9502	Significance
Colour (srm)		3.329a	4.320b	5.980c	*	4.253a	4.371b	4.787c	4.762c	*
Alcohol content (%)		5.81a	6.99c	6.27b	*	6.79c	6.25b	6.20a	6.19a	*
Soluble solids (°Bx)		3.61a	4.72c	4.31b	*	3.65a	5.56c	3.64a	4.03b	*
Dry Matter (%)		3.96a	6.51c	4.17b	*	3.77a	5.84c	5.22bc	4.69 ab	*
Sugars (g/L)	Maltodextrins	15.19a	17.60b	15.94 ab	*	15.30 ab	21.49b	13.92a	14.24a	*
	Maltotriose	3.34a	3.52a	2.87a	ns	1.78a	9.94b	0.62a	0.64a	*
	Maltose	1.62a	1.90a	1.87a	ns	1.75b	3.47c	1.05a	0.91a	*
	Glucose	0.26a	0.29 ab	0.31b	*	0.18a	0.13a	0.34b	0.47c	*
	Fructose	0.90b	0.66 ab	0.60a	*	0.65a	0.71a	0.80b	0.72 ab	*
Glycerol (g/L)		3.70b	3.57 ab	3.49a	*	3.11b	2.06a	4.67c	4.50c	*
CO_2 (g/L)		3.76c	2.83a	3.01b	*	2.92a	3.40c	3.15b	3.33c	*
pH		4.11a	4.11a	4.10a	ns	4.11b	4.07a	4.13c	4.21d	*
Total acidity (g lactic acid/L)		2.06a	2.08a	2.04a	ns	2.10b	2.03a	2.09b	2.02a	*
Volatile acidity (g acetic acid/L)		0.58s	0.58s	0.54a	ns	0.58c	0.56 ab	0.55a	0.57c	*
Organic acids (g/L)	Citric	1.07b	1.00 ab	0.98a	*	0.97a	0.98 ab	1.07b	1.06b	*
	Malic	1.12a	1.69b	1.74b	*	1.45a	1.47a	1.70b	1.45a	*
	Succinic	1.36a	1.52b	1.39a	*	1.50 ab	1.11a	1.54b	1.54b	*
	Lactic	0.61a	0.73c	0.66b	*	0.67b	0.58a	0.74c	0.69b	*
	Fumaric	0.01a	0.02b	0.02b	*	0.02b	0.02b	0.01a	0.01a	*
	Acetic	1.47a	1.70b	1.86b	*	1.60 ab	1.77bc	1.89c	1.44a	*
FC-TPC (mg gallic acid/L)		337a	468b	566c	*	417a	446b	478c	487c	*
Phenolics (mg/L)	Gallic acid	21.8c	20.0b	13.4a	*	36.6d	25.4c	9.8b	5.9a	*
	4-Hydroxybenzoic acid	2.0b	2.2c	1.6a	*	2.2c	2.1c	1.9b	1.5a	*
	Catechin	4.8b	3.5a	6.8c	*	6.9d	5.6c	3.2a	4.4b	*
	Vanillic acid	1.9b	2.0c	0.9a	*	1.5a	1.7b	1.8c	1.6b	*
	Caffeic acid	1.5b	3.8c	1.3a	*	1.9a	1.9a	2.8c	2.2b	*
	Syringic acid	4.1c	2.8a	3.6b	*	3.7c	3.6b	3.6b	3.1a	*
	Epicatechin	13.9b	13.8b	12.1a	*	13.1b	15.5d	10.9a	13.6c	*
	Chlorogenic acid	21.3c	19,1b	7.7a	*	16.3c	21.2d	14.2b	12.5a	*
	Epigallocatechin	16.2c	15.2b	12.1a	*	15.7b	13.5a	13.2a	15.6b	*
	Ferulic acid	1.9a	2.1c	2.0b	*	2.0a	2.1b	2.0a	2.0a	*
	p-Coumaric acid	1.4b	1.3a	1.4b	*	1.3a	1.6b	1.3a	1.3a	*
	Sinapic acid	7.0c	6.5b	4.8a	*	5.5a	6.0b	6.8c	6.0b	*
	Epicatechingallate	25.4b	26.3c	10.4a	*	25.1c	25.9c	17.3b	14.2a	*
	Rutin	8.1c	6.5b	5.3a	*	10.5d	6.9c	3.9a	5.3b	*
	Resveratrol	1.7c	1.4b	1.2a	*	1.5c	1.6d	1.4b	1.3a	*
	Rosmarinic acid	4.7a	14.6b	15.9c	*	12.5c	12.7c	10.8a	11.1b	*
	Quercetin	1.4b	2.1c	1.3a	*	1.6b	1.6b	1.4a	1.6b	*
	Kaempferol	13.1c	2.0a	5.0b	*	6.0a	7.5c	6.3b	7.0c	*
HPLC-TPC (mg/L)		152c	145b	107a	*	160d	156c	113b	110a	*
Antioxidant Activity (% DPPH)		62.0c	52.1b	38.1a	*	51.9b	51.6b	50.0a	49.4a	*
Colour	Foam	1.0a	1.1 ab	1.2b	*	1.1a	1.1a	1.2a	1.1a	ns
	Liquid	2.6a	2.9b	3.0c	*	2.7 ab	2.6a	2.9bc	3.1c	*
Foam	Amount	3.6b	3.5b	3.2a	*	3.9c	3.3b	3.4b	3.0a	*
	Persistence	3.1b	3.2b	2.7a	*	3.6c	2.9 ab	3.0b	2.7a	*
Turbidity		3.6c	3.3b	2.6a	*	3.2a	3.1a	3.1a	3.3a	ns
Flavour characteristics	Overall Olfactory Intensity	3.3b	3.3b	2.9a	*	3.2a	3.3a	3.1a	3.0a	ns
	Olfactory Finesse	3.8b	3.7b	3.3a	*	3.9c	3.8bc	3.1a	3.6b	*
	Malty	2.7c	2.6 ab	2.4a	*	2.6 ab	2.7b	2.4a	2.6 ab	*
	Hoppy	3.1b	2.8 ab	2.6a	*	3.0b	2.9 ab	2.6a	2.8 ab	*
	Floral	2.4a	2.5a	2.3a	ns	2.4 ab	2.6b	2.3 ab	2.2a	*
	Fruity	2.6a	2.5a	2.3a	ns	2.6a	2.5a	2.4a	2.4a	ns
	Spicy	2.6b	2.4 ab	2.3a	*	2.4 ab	2.4 ab	2.2a	2.5b	*
	Yeast	2.7b	2.5 ab	2.3a	*	2.5a	2.4a	2.6a	2.5a	ns
	Aromatic herbs	2.1a	2.1a	2.1a	ns	2.2a	2.1a	2.1a	2.1a	ns
Gustatory characteristics	Sweetness	2.4a	2.3a	2.3a	ns	2.5b	2.3 ab	2.2a	2.3 ab	*
	Bitterness	3.0b	2.9a	2.9a	*	2.9 ab	2.8a	3.0 ab	3.1b	*

(*continued on next page*)

Table 1 (*continued*)

Variables		Effect of wheat species				Effect of yeast strains				
		C	D	E	Significance	WB06	9502	6956/WB06	6956/9502	Significance
	Saltiness	2.4a	2.6 ab	2.7b	*	2.4a	2.6 ab	2.7b	2.5 ab	*
	Sourness	2.6a	2.7b	2.6a	*	2.7b	2.6a	2.6a	2.7b	*
Tactile	Alcoholic	2.9 ab	3.0b	2.8a	*	3.0b	2.8a	2.9 ab	2.9 ab	*
characteristics	Effervescence	3.3b	3.1a	3.1a	*	3.3b	3.2 ab	3.2 ab	3.0a	*
	Body/Fullness	3.1b	3.0 ab	2.8a	*	2.9a	3.1a	3.0a	2.9a	ns
Overall Sensory Quality		3.7b	3.3 ab	3.0a	*	3.7b	3.7b	3.0a	3.1a	*

In column, different letters and the asterisk indicate significant differences at $p < 0.05$ by LSD multiple range test; ns: not significant.
C: malted barley 65%-unmalted common wheat 35 %; D: malted barley 65%-unmalted durum wheat 35 %; E: malted barley 65%-unmalted emmer 35 %; 9502: *Saccharomyces cerevisiae* isolated from grape must; WB06: commercial *Saccharomyces cerevisiae*; 6956: *Schizosaccharomyces pombe*; FC-TPC: Total Antioxidant Content by Folin-Ciocalteau assay; HPLC-TPC: Sum of the concentrations of the individual phenolic compounds determined by HPLC-DAD.

relationships between FC-TPC and antioxidant activity (r = −0.945) and the positive Pearson correlation coefficient between HPLC-TPC and antioxidant activity (r = 0.631) would indicate that compounds other than phenols (probably melanoidins) [26] exerted their antioxidant activity in our beers and that, instead, the phenolic compounds as a whole even exerted pro-oxidant actions. The beer phenolic profile reinforces this supposition since C-beers showed the highest % of remaining DPPH regardless of their highest concentrations of most phenolics (i.e. gallic acid, ECG, kaempferol, rutin, chlorogenic acid, sinapic acid, syringic acid, and resveratrol) while E-beers had the lowest % of remaining DPPH regardless of their lowest concentrations of most phenolic acids and catechine-derivatives. The beers produced with durum wheat contained intermediate concentrations of most phenolic compounds.

In brewing, the use of different cereals is a way to achieve beer differentiation [27]. The wheat species significantly affected most sensory attributes of the experimental beers with the exception of: floral and fruity flavours, to which the trained panel assigned intermediate scores; the flavour of aromatic herbs and sweetness, whose intensity received medium-low scores. This behaviour was already detected by De Flaviis et al. [28], who observed that a greater influence of the place of cultivation (altitude in particular) with respect to genotype for herbal, floral, and fruity flavours. The score attributed to sweetness was consistent with the typical mouthfeel of witbiers that must finish dry [19].

Consistently with the instrumental measurement of colour, significant differences attributable to the wheat species were also observed for the sensory evaluation of this parameter. The foam colour varied from ice-white (C-beers) to milky-white (E-beers), while that of the liquid portion varied from yellow (C-beers) to golden yellow (E-beers) as a consequence of the solubilisation of pigments derived from cereals during mashing. The differences between the beers were minimal but sufficient to be evaluated by the eye of expert panellists. Evidence of the role of phenolic compounds in haze production is the high correlation between turbidity and HPLC-TPC (0.586), both higher in C-beers and lower in E-beers.

The beers produced with emmer received the lowest ratings for most sensory attributes: amount and persistence of foam; turbidity; overall olfactory intensity and finesse; intensity of malty, hoppy, spicy, yeast flavours; perception of alcohol content and fulness. As a result, their overall sensory quality was judged inferior to that of the other types of beer, which is consistent with the results of previous research [3]. The beers produced from the common wheat obtained the highest ratings for intensity of malty, hoppy, spicy, and yeast flavours, bitterness, effervescence, fullness, and overall sensory quality. The beers produced from durum wheat showed intermediate scores for most sensory parameters (beer colour; malty, hoppy, spicy, and yeast flavours; saltiness and fullness), and the highest alcoholic sensory perception, in agreement with its highest alcoholic content.

Based on the Pearson correlation coefficients, the overall sensory quality was negatively correlated with the colour of foam (−0.508), the colour of the liquid portion (−0.648), and saltiness (−0.599), while it was positively correlated with foam features (amount, 0.559; persistence, 0.500); turbidity (0.535); overall flavour intensity and finesse (0.656 and 0.810); intensity of malty, hoppy, and floral flavour (0.726, 0.818, and 0.605); fullness (0.552).

3.2. *Effects of yeast strains*

The yeast strain used in fermentation affected all physical-chemical parameters and most sensory attributes (Table 1).

First of all, the sample colours were in a very narrow range, but the differences were significant, ranging from the lowest value (~4.3 srm) detected on WB06 and the highest values (around 4.7 srm) measured on the beers submitted to the sequential fermentation trials (6956/WB06 and 6956/9502). Regarding these colour differences, some studies stated that high fermentation yeasts could be responsible for browning and melanoidin oxidation [29,30].

As expected, *S. cerevisiae* WB06 was able to produce the highest alcohol volume and leave the lowest amounts of residual sugars, while the oenological *S. cerevisiae* 9502 produced an intermediate alcohol volume but left the highest amounts of unfermented maltodextrins, maltotriose, and maltose. The sequential fermentation led to only slightly lower alcohol production, since *Sc. pombe* was left to ferment on its own (therefore with low efficiency) for just two days before the inoculation of *S. cerevisiae* [31]. However, the sequential fermentation significantly contributed to the consumption of maltodextrins, maltotriose, and maltose (compounds remained in higher concentrations in beers fermented by *S. cerevisiae* strains alone), enriching beers with the following key compounds for the beer sensory characteristics: glycerol, citric and succinic acids, whose higher concentrations were detected in all beers obtained

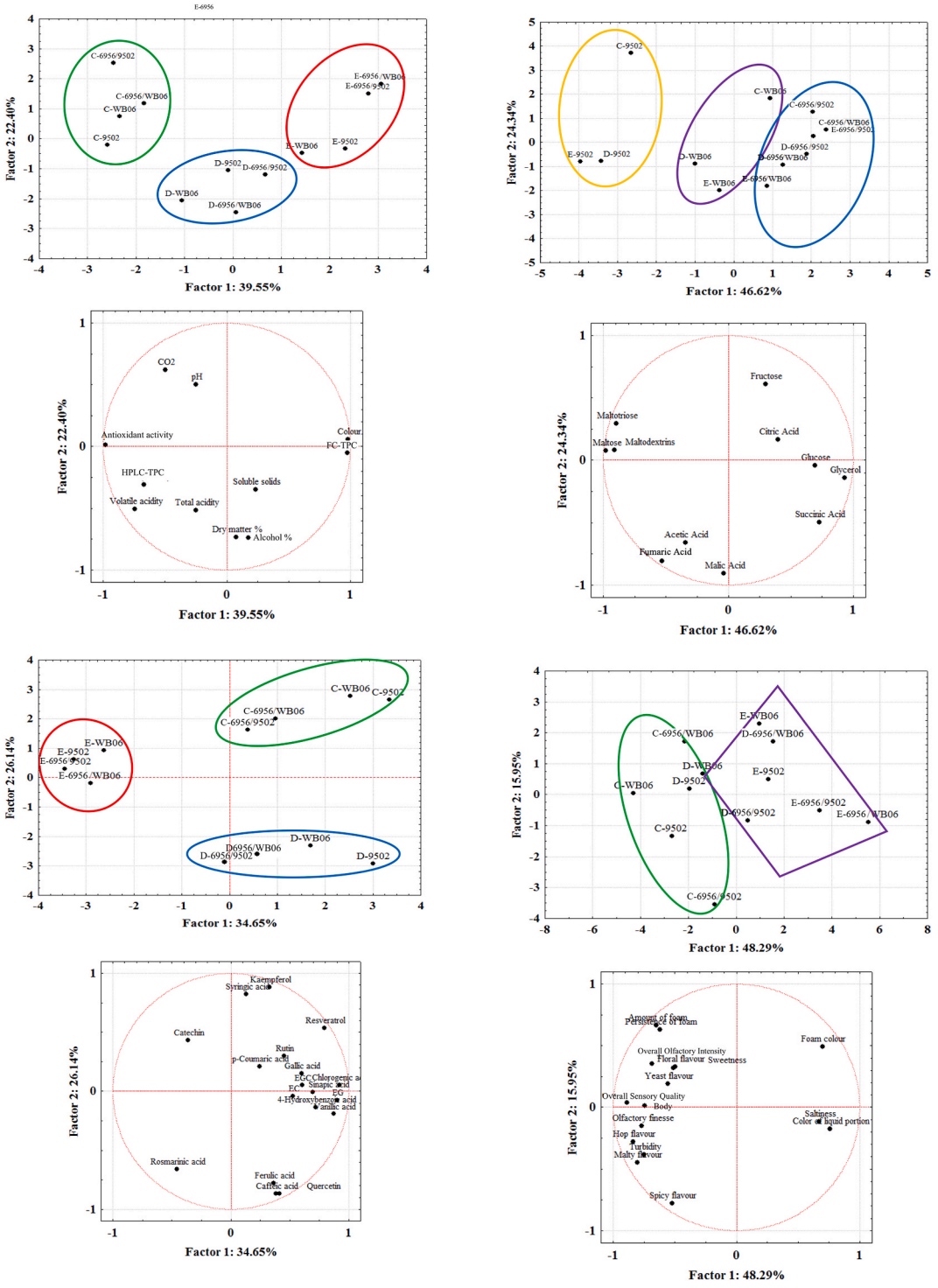


Fig. 1. Biplot of scores (on the left) and loadings (on the right) obtained with the application of Principal Component Analysis to (a) physical-chemical, (b) sugars and organic acids, (c) polyphenol, (d) sensory data sets along the first and second component. FC-TPC: Total Antioxidant Content obtained by Folin-Ciocalteau assay; HPLC-TPC: Total Phenolic Content as the sum of the concentrations of the individual phenolic compounds determined by HPLC-DAD; EC: Epicatechin; EGC: Epigallocatechin; EG: Epicatechingallate.

through sequential fermentation; malic, lactic, and acetic acids, whose higher concentrations were detected in beers inoculated with *Sc. pombe* and *S. cerevisiae* WB06; compounds responsible for volatile acidity, mostly present in beers fermented by *Sc. pombe* and *S. cerevisiae* 9502. *S. cerevisiae* 9502 was able to produce the highest amount of carbon dioxide both when inoculated alone and in conjunction with *Sc. pombe*. The beers fermented by *S. cerevisiae* WB06 alone or in sequential fermentation with *Sc. pombe* showed the highest total acidity.

The beers produced through sequential fermentation showed the highest overall antioxidant content (FC-TPC) and the highest radical scavenging activity; once again, HPLC-TPC showed a trend opposite to that of FC-TPC, being higher in beers inoculated solely with *S. cerevisiae* strains. Regarding the specific phenolic profiles, the beers inoculated only with the *S. cerevisiae* strains alone also had the highest concentrations of most phenolic compounds, namely gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, rosmarinic acid, catechin, epicatechingallate, rutin, and resveratrol.The beers inoculated with *S. cerevisiae* WB06 also had the highest concentrations of syringic acid while epicatechin, ferulic acid, *p*-coumaric acids, and kaempferol were detected in greater amounts in the samples fermented by *S. cerevisiae* 9502. The phenolic acids vanillic, caffeic, and sinapic were contained in higher concentrations in beers fermented by *Sc. pombe* and *S. cerevisiae* WB06. The active role of fermenting yeasts in modifying the beer phenolic profiles is known. due to the absorption of these compounds on their cell walls that are negatively charged. However, recently studies [32] have demonstrated that the extent of this phenomenon depends on the content of mannan in the cell walls and that such content changes during the yeast growth phases, being higher during the exponential phase and dcreasing when the yeast entered the stationary phase and varies with yeast strain. Moreover, the phenolic adsorption ability also depends on yeast strains [33] as well as different strains can show a certain specificity in adsorbing some phenolic compounds over others [34]. In light of the obtained results, the yeast strains used in our study showed different behaviours with the various classes of phenolic compounds: *S. cerevisiae* WB06 had a high affinity for hydroxycinnamic acids and kaempferol, while *S. cerevisiae* 9502 showed a higher specificity for caffeic acid and epigallocatechin; *Sc. pombe* highlighted high affinity for hydroxybenzoic acids, rutin, resveratrol, and rosmarinic acid.

Concerning the sensory properties, any significant difference was highlighted among the beers fermented by the various yeasts for the following attributes: foam colour, turbidity, overall olfactory intensity, fruity, yeast and aromatic herb flavours, and body. The differences in colour among the beer liquid portions were minimal but significant, with the following hierarchy from the lightest to the most pigmented: 9502, WBB06, GP18-WB06, GP18-9502. In agreement with the instrumental measurement of colour, the darker beers whre those obtained through sequential fermentation. To reinforce the discussion concerning the effects of yeasts on colour, the latter was negatively correlated with HPLC-TPC (−0.692), indicating a slight oxidation of beers having lower phenolic concentrations. Yeasts also played an important role in the development of foam, which was increased by *S. cerevisiae* WB06 and depressed by sequential fermentation involving 6956 and 9502 strains. This result was not consistent with the findings of Callejo et al. [10]. However, they used the non-*Saccharomyces* strains in beer conditioning instead of at the beginning of primary fermentation. Yeast strains also affected the flavour profile by: enhancing the hoppy flavour and olfactory finesse (significantly maximised by WB06) or malty and floral flavour (significantly maximised by 9502); significantly depressing the intensity of malty, hoppy, spicy flavours and the overall olfactory finesse (6956/WB06); significantly minimising the intensity of floral flavour and maximising the intensity of spicy flavour (6956/9502). Regarding gustatory and tactile characteristics, the beers inoculated with *S. cerevisiae* WB06 received the significantly highest ratings for sweetness, sourness, alcoholic perception, and effervescence and the significantly lowest scores for saltiness, while the beers fermented by the oenological *S. cerevisiae* obtained the significantly lowest ratings due to a reduced alcohol perception. The sequential fermentation resulted in the following bad sensory combinations: the lowest sweetness and highest saltiness for the samples fermented by 6956/WB06, and the lowest effervescence and highest bitterness and sourness for the beers fermented by 6956/9502. Consequently, the trained panel assigned the highest overall sensory ratings to the sampless obtained with single fermentations of *S. cerevisiae* strains. Our findings are in substantial agreement with Loira et al. [35], who listed the production of undesirable sensory characteristics among the disadvantages of using *Sc. pombe*.

3.3. Principal Component Analysis (PCA)

PCA was applied to verify the relative strength of wheat species and the type of fermentation that affects beer quality. Analyses were performed on the data sets of physical-chemical parameters (Fig. 1a), sugars and organic acids concentrations (Fig. 1b), phenolic concentrations (Fig. 1c), and sensory attributes (Fig. 1d). The first two factors accounted for 61.95 %, 70.96 %, 60.79 %, and 64.24 % of the total variance of the above mentioned data sets.

Based on the data corresponding to the physical-chemical parameters, Fig. 1a shows that the beers were homogeneously clustered into three groups corresponding to the three unmalted wheat species used in brewing. C-beers appear mainly located in the portion of the factorial plan distinguished by negative values of Factor 1 and positive values of Factor 2, corresponding to the highest CO_2 and HPLC-TPC contents and the lowest antioxidant activity. The opposite part of the factorial plan hosts the emmer-based beers, having the highest FC-TPC concentrations and the darkest colour. The beers obtained from unmalted durum wheat are located in the half of the plan corresponding to negative values of Factor 2 and are comprised between −1 and +1 values of Factor 1. They are mainly characterized by the highest contents of soluble solid, dry matter, and alcohol.

Sugar and organic acid profiles are among beer compounds those that are mainly affected by the yeast fermentative metabolism, allowing the individuation of three homogeneous groups corresponding to (Fig. 1b): beers fermented by *S. cerevisiae* 9502, located in the portion of the factorial plan corresponding to negative values of Factor 1 and characterized by the highest concentrations of unfermented maltose, maltotriose, and maltodextrins; the cluster that included all beers obtained through sequential fermentation, located in the portion of the factorial plan corresponding to positive values of Factor 1 and characterized by the highest concentrations of fructose, glucose, glycerol, and citric acid; the beers fermented by *S. cerevisiae* WB06, distributed around the factorial plane centre

and therefore devoid of distinctive characteristics. Sugar profiles often compared in metabolomic studies, generally together with α-acid and amino acid contents was previously found able to discriminate between nonalcoholic and alcoholic beers [36] while organic acid composition has never appeared in beer classification studies.

Based on the concentrations of phenolic compounds (Fig. 1c), i.e. compounds deriving from the starchy materials and hops, the beers were homogeneously grouped in clusters corresponding to the three unmalted wheat species. In the factorial plan, the great distance between emmer-based beers and those produced with durum and soft wheat can be precisely attributed to the strongly different phenolic composition of the first, characterized by the highest contents of catechin and rosmarinic acid and by generally

Table 2
Estimated regression coefficients and coefficients of determination of the quadratic models that describe the weight of wheat species and fermenting yeast strains on the considered beer variables.

Variables	Intercept	Linear, Quadratic, and Interactive Effects of wheat species (W) and yeast strains (Y)					R^2 of the Quadratic Models
		W	Y	W^2	Y^2	W[a]Y	
Colour (srm)	2.813 *(0.000)*			0.279 *(0.000)*		0.086 *(0.010)*	0.916 *(0.000)*
Alcohol content (%)	2.725 *(0.019)*	4.041 *(0.003)*		0.952 *(0.005)*			0.357 *(0.001)*
Soluble solids (°Bx)	5.563 *(0.000)*		0.538 *(0.011)*				0.257 *(0.011)*
Dry Matter (%)		9.883 *(0.017)*		−2.446 *(0.017)*			0.242 *(0.050)*
Maltodextrins	22.188 *(0.000)*		−2.380 *(0.011)*				0.257 *(0.011)*
Maltotriose (g/L)	20.700 *(0.000)*		−13.090 *(0.007)*		2.036 *(0.029)*		0.482 *(0.001)*
Maltose (g/L)	6.146 *(0.000)*		−3.134 *(0.001)*		0.465 *(0.011)*		0.636 *(0.000)*
Glucose (g/L)						0.034 *(0.011)*	0.260 *(0.011)*
Fructose (g/L)	1.027 *(0.000)*	−0.152 *(0.005)*					0.304 *(0.005)*
Glycerol (g/L)			2.015 *(0.001)*		−0.219 *(0.04)*		0.842 *(0.000)*
CO_2 (g/L)	3.947 *(0.000)*	0.373 *(0.027)*					0.202 *(0.030)*
Malic acid (g/L)	0.892 *(0.000)*	0.312 *(0.002)*					0.357 *(0.002)*
Succinic acid (g/L)	1.089 *(0.000)*		0.134 *(0.009)*				0.272 *(0.009)*
Lactic acid (g/L)		0.422 *(0.025)*	0.052 *(0.010)*	−0.099 *(0.033)*			0.421 *(0.011)*
Fumaric acid (g/L)		0.019 *(0.010)*		−0.004 *(0.032)*			0.500 *(0.001)*
FC-TPC (mg gallic acid/L)	202.811 *(0.000)*	114.417 *(0.000)*			3.383 *(0.020)*		0.881 *(0.000)*
HPLC-TPC (mg/L)	207.640 *(0.000)*		−18.072 *(0.003)*	−5.891 *(0.004)*			0.510 *(0.001)*
Antioxidant Activity (% DPPH)	64.614 *(0.000)*			−2.971 *(0.000)*			0.928 *(0.000)*
Colour of Foam	0.889 *(0.000)*	0.115 *(0.001)*					0.399 *(0.001)*
Colour Liquid portion	2.406 *(0.000)*					0.083 *(0.001)*	0.374 *(0.001)*
Turbidity	3.798 *(0.000)*			−0.134 *(0.000)*			0.621 *(0.000)*
Olfactory Finesse	4.323 *(0.000)*			−0.071 *(0.010)*	−0.051 *(0.002)*		0.485 *(0.001)*
Malty flavour	2.847 *(0.000)*					−0.049 *(0.001)*	0.395 *(0.001)*
Hoppy flavour	3.306 *(0.000)*	−0.240 *(0.007)*					0.288 *(0.007)*
Spicy flavour	2.973 *(0.000)*	−0.338 *(0.001)*			−0.038 *(0.009)*	0.077 *(0.022)*	0.539 *(0.001)*
Yeast flavour	2.562 *(0.000)*				0.039 *(0.001)*	−0.069 *(0.001)*	0.472 *(0.001)*
Alcoholic	2.306 *(0.000)*	0.427 *(0.007)*	0.236 *(0.011)*	−0.114 *(0.004)*	−0.042 *(0.020)*		0.551 *(0.003)*
Body/Fullness	3.281 *(0.000)*					−0.053 *(0.033)*	0.190 *(0.03)*
Overall Sensory Quality	4.674 *(0.000)*	−0.344 *(0.024)*	−0.247 *(0.026)*				0.357 *(0.010)*

FC-TPC: Total Phenolic Content by Folin-Ciocalteau assay; HPLC-TPC: Sum of the concentrations of the individual phenolic compounds determined by HPLC-DAD.

[a] *p*-values are reported in brackets.

lower concentrations of other phenols. C- and D-beers share similar values of Factor 1 and can be discriminated by the different values of Factor 2, positive for common wheat based beers and negative for those produced from durum wheat, substantially due to the high kaempferol content of the first and the high rosmarinic acid content of latter. Our results could be considered as an evolution of the findings of Gouvinhas et al. [37] who were able to classify commercial Portuguese beers belonging to different brewing styles based on their contents in ortho-diphenols, total phenols, flavonoids and their antioxidant activity (ABTS and DPPH).

PCA applied to the sensory data (Fig. 1d) allowed the separation of samples in only two groups, one clustering D- and E-beers together, which were characterized by more intense colour of both foam and liquid portion and by a higher saltiness, and the other one including beers made from common wheat, which showed the better sensory characteristics (higher malty, hop, yeast, and spicy flavour; higher effervescence and body). These results are slightly different from those of De Flaviis et al. [28], who found that the effects of genotype on wheat beer sensory properties are less important than those of the wheat percentage, place of wheat cultivation, and altitude.

Based on these findings, PCA applied to physical-chemical parameters or to profiles of organic acids, sugars, and phenolics proves to be a powerful tool in classifying beers by wheat species or yeast. Moreover, this method is simpler to apply when compared to more refined multivariate analysis techniques such as the integrated multi-omics approach proposed by De Flaviis et al. [38] to categorize beers upon style, wheat concentration, and yeast type. The potential of our approach depends on its ability to discriminate among beers belonging to the same brewing style.

3.4. Multiple regression analysis

The ability to predict key beer parameters is a challenge that fascinates researchers. The indicators that mainly interest researchers are those related to safety and quality aspects. As an example, Rodríguez-Saavedra et al. [39] applied a binary logistic regression model based on alcohol content, iso-α-acid content, and pH to predict the microbial spoilage susceptibility of craft beer.

In this work, the multiple regression analysis was applied with the aim of build the equations able to describe the relationships among each physical, chemical, and sensory parameter of the beers and the considered factors (wheat species and fermenting yeast strains); evaluate the extent of these effects on each dependent variables through the quantification of their regression coefficients; evaluate the models fitting capability.

Table 2 lists the analytical parameters for which models with a significant ($p < 0.05$) predictive ability have been found, and for each of them, only the significant ($p < 0.05$) linear, quadratic, and interactive terms are reported. The statistical analysis stated that the experimental results concerning colour, glycerol concentration, FC-TPC, and antioxidant activity were well described by the quadratic model ($R^2 > 0.8$). On the other hand, the statistical analysis fitted the experimental data slightly less well ($0.5 \geq R^2 \leq 0.8$) for dependent variables such as maltose, and fumaric acid concentration; HPLC-TPC; and the sensory attributes turbidity, spicy flavour, and alcohol perception. Instead, R^2 values were lower than 0.5 for the equations that describe the behaviour of all the other parameters. Therefore, the implications of these results concern the possibility of predicting with a certain accuracy mainly the parameters correlated with the health aspects of the product (antioxidant activity and content) and the visual characteristics of beers while other physochemical and sensory indices are influenced by the type of starchy raw material and yeast but also depend to a variable extent on other factors related to the additional raw materials and to the production process and therefore require a more complex predictive approach.

As can be inferred from these data, the parameters can be ideally divided into the following 4 groups, based on the factor(s) capable of predicting them.

1) parameters depending on wheat species - this group include compounds whose concentrations in beer is strongly related to their concentrations in the starchy ingredients, products whose biosynthesis depends on the content of precursors in the starchy ingredients, and sensory characteristics whose intensity is influenced by the starchy ingredients: alcohol content, dry matter, fructose, CO_2, malic acid, fumaric acid; antioxidant activity, foam colour, turbidity, hoppy flavour;
2) parameters depending on yeast strain, i.e. compounds influenced by the yeast metabolism such as soluble solids, maltodextrins, maltotriose, maltose, glycerol, succinic acid;
3) parameters depending on the interactive effects of wheat species and yeast strain - this group includes parameters whose intensity is influenced by the interactions of the considered factors and their single contributions cannot be separated: glucose concentration, colour of liquid portion, malty flavour, and body fullness;
4) parameters depending on the contribution of both factors - this cluster comprises parameters for which the single effects of the two factors is significant, i.e. spectrophotometric colour, lactic acid, FC-TPC, HPLC-TPC, olfactory finesse, spicy flavour, alcohol perception, overall sensory quality.

4. Conclusions

Sequential fermentation of worts produced from a mixture of barley malt and unmalted wheat grains (alternatively common, durum, or emmer wheat) was performed using a *Sc. pombe* of oenological origin followed by inoculation of an *S. cerevisiae* strain (commercial or isolated from oenological material). Two single fermentations by the *S. cerevisiae* strains were also performed. The highest overall sensory scores were attributed to the beers produced from common wheat and fermented by the two *S. cerevisiae* strains alone. The use of *Sc. pombe* at the beginning of the primary fermentation before *S. cerevisiae* inoculation enhanced the antioxidant content of the corresponding beers with the disadvantage of compromising their sensory quality. Physical-chemical parameters and the

phenolic distribution were mainly influenced by the 'wheat species' factor, as suggested by the homogeneous groupings of the beers depending on the cereal mixtures used. The effect of the wheat species factor was weaker for sensory characteristics, with beers produced from durum wheat or emmer resulting indistinguishable from each other. Sugar and organic acid profiles allowed the clustering of beers by fermenting yeast. The multiple regression analysis produced models with high predictive capacity for colour, alcohol %, carbon dioxide, soluble solids, dry matter; total antioxidant content, total phenolic concentration, antioxidant activity, and sensory most parameters (foam colour, turbidity, olfactory finesse, flavours of malt, hop, spicy and yeast, body, overall sensory quality).

These results represent a starting point for even more in-depth studies. The future prospects of using unconventional ingredients and yeasts as a tool for diversification of craft beers are encouraging although many cause-effect relationships are still to be understood. Future trends in brewing as in other food sectors also include the increasing use of predictive models of the product characteristics as a function of both formulations and process parameters as well as the development of softwares for the automated management of manufacturing processes.

Funding

The results presented in this manuscript were obtained within the project entitled "Dal Campo al Boccale" (Acr. BEˆ2R) funded by PSR PUGLIA 2014/2020-Misura 16-Cooperazione-Sottomisura 16.2 - "Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie".

Ethical statement

Approval by an ethics committee was not needed for this study because the craft beers object of the research are made with procedures/ingredients (yeasts included) characterised in terms of hygiene and safety (they are not novel foods), and, thus, they do not present risks to the health of users. The sensory study was performed using human volunteers who were previously asked to sign an informed consent form. An appropriate protocol for protecting the rights and privacy of all participants was utilised.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Francesco Grieco: Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition. **Anna Fiore:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Carmela Gerardi:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Maria Tufariello:** Writing – review & editing. **Giuseppe Romano:** Methodology, Investigation. **Antonietta Baiano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgement

The authors thank Dr. Marco Taurino and Mrs. Annamaria Pascali for their technical and administrative assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37598.

References

[1] J. Belcar, J. Buczek, I. Kapusta, J. Gorzelany, Quality and Pro-Healthy Properties of Belgian Witbier-style beers relative to the cultivar of winter wheat and raw materials used, Foods 11 (2022) 1150, https://doi.org/10.3390/foods11081150.

[2] A. Faltermaier, D. Waters, T. Becker, E. Arendt, M. Gastl, Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal–a review, J. Inst. Brew. 120 (2014) 1–15.

[3] A. Baiano, A. Fiore, B. la Gatta, M. Tufariello, C. Gerardi, F. Grieco, Single and interactive effects of unmalted cereals, hops, and yeasts on quality of white-inspired craft beers, Beverages 9 (1) (2023) 9, https://doi.org/10.3390/beverages9010009.

[4] S.S. González, E. Barrio, A. Querol, Molecular characterisation of new natural hybrids of *Saccharomyces cerevisiae* and *S. kudriavzevii* in brewing, Appl. Environ. Microbiol. 74 (2008) 2314–2320.

[5] J.-L. Legras, D. Merdinoglu, J.-M. Cornuet, F. Karst, Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history, Mol. Ecol. 16 (2007) 2091–2102.

[6] V. Postigo, A. Sánchez, J.M. Cabellos, T. Arroyo, New approaches for the fermentation of beer: non-*Saccharomyces* yeasts from wine, Fermentation 8 (2022) 280, https://doi.org/10.3390/fermentation8060280.

[7] S. Rossi, B. Turchetti, V. Sileoni, O. Marconi, G. Perretti, Evaluation of *Saccharomyces cerevisiae* strains isolated from non-brewing environments in beer production, J. Inst. Brew. 124 (2018) 381–388.

[8] L. Canonico, F. Comitini, M. Ciani, Dominance and influence of selected *Saccharomyces cerevisiae* strains on the analytical profile of craft beer refermentation, J. Inst. Brew. 120 (2014) 262–267.

[9] A. Baiano, A. Fiore, B. la Gatta, M. Tufariello, V. Capozzi, N. De Simone, C. Gerardi, F. Grieco, Unmalted cereals, oenological yeasts, and in-bottle sugar addition as synergic strategies to enhance the quality of craft beers 10, 8, https://doi.org/10.3390/beverages10010008, 2024.

[10] M.J. Callejo, J.J. García Navas, R. Alba, C. Escott, I. Loira, M.C. González, A. Morata, Wort fermentation and beer conditioning with selected non-*Saccharomyces* yeasts in craft beers, Eur. Food Res. Technol. 245 (2019) 1229–1238, https://doi.org/10.1007/s00217-019-03244-w.

[11] V. Postigo, T. O'Sullivan, T. Elink Schuurman, T. Arroyo, Non-conventional yeast: behavior under pure culture, sequential and aeration conditions in beer fermentation, Foods 11 (2022) 3717, https://doi.org/10.3390/foods11223717.

[12] D. Einfalt, Barley-sorghum craft beer production with *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Metschnikowia pulcherrima* yeast strains, Eur. Food Res. Technol. 247 (2021) 385–393, https://doi.org/10.1007/s00217-020-03632-7.

[13] M. Tristezza, L. Fantastico, C. Vetrano, G. Bleve, D. Corallo, G. Mita, F. Grieco, Molecular and technological characterisation of *Saccharomyces cerevisiae* strains isolated from natural fermentation of Susumaniello grape must in Apulia, Southern Italy, Int. J. Microbiol. (2014) 897428, https://doi.org/10.1155/2014/897428.

[14] European Brewery Convention, Method 9.6—colour of beer: spectrophotometric method, in: Analytica-EBC, Schweizer Brauerei-Rundschau: Zürich, Switzerland, 1975.

[15] E.M. Coelho, C.V. da Silva Padilha, G.A. Miskinis, A.G.B. de Sá, G.E. Pereira, L.C. de Azevêdo, M. dos Santos Lima, Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: method validation and characterisation of products from northeast Brazil, J. Food Compos. Anal. 66 (2018) 160–167, https://doi.org/10.1016/j.jfca.2017.12.017.

[16] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[17] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.

[18] B. Aliakbarian, A.A. Casazza, P. Perego, Valorization of olive oil solid waste using high pressure–high temperature reactor, Food Chem. 128 (2011) 704–710.

[19] Beer Judge Certification Program (BJCP), Style guidelines: beer style guidelines, Available online at: https://www.bjcp.org/docs/2015_Guidelines_Beer.pdf, 2015. (Accessed 11 March 2024).

[20] J. Lachman, P. Martinek, Z. Kotíková, M. Orsák, M. Šulc, Genetics and chemistry of pigments in wheat grain – a review, J. Cereal. Sci. 74 (2017) 145–154, https://doi.org/10.1016/j.jcs.2017.02.007.

[21] V. Alfeo, G. De Francesco, V. Sileoni, S. Blangiforti, R. Palmeri, G. Aerts, G. Perretti, A. Todaro, Physicochemical properties, sugar profile, and non-starch polysaccharides characterisation of old wheat malt landraces, J. Food Compos. Anal. 102 (2021) 103997, https://doi.org/10.1016/j.jfca.2021.103997.

[22] A. Lovegrove, J. Dunn, T.K. Pellny, J. Hood, A.J. Burridge, A.H.P. America, L. Gilissen, R. Timmer, Z.A.M. Proos-Huijsmans, J.P. van Straaten, D. Jonkers, J. L. Ward, F. Brouns, P.R. Shewry, Comparative compositions of grain of bread wheat, emmer and spelt grown with different levels of nitrogen fertilisation, Foods 12 (4) (2023) 843, https://doi.org/10.3390/foods12040843.

[23] P.R. Shewry, F. Brouns, J. Dunn, J. Hood, A.J. Burridge, A.H.P. America, L. Gilissen, Z.A.M. Proos-Huijsmans, J.P. van Straaten, D. Jonkers, P.A. Lazzeri, J. L. Ward, A. Lovegrove, Comparative compositions of grain of tritordeum, durum wheat and bread wheat grown in multi-environment trials, Food Chem. 423 (2023) 136312, https://doi.org/10.1016/j.foodchem.2023.136312.

[24] S. Michnick, J.L. Roustan, F. Remize, P. Barre, S. Dequin, Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for GPD1 encodingglycerol 3-phosphate dehydrogenase, Yeast 13 (1997) 783–793, https://doi.org/10.1002/(SICI)1097-0061(199707)13:9%3C783::AID-YEA128%3E3.0.CO;2-W.

[25] M. Zrcková, I. Capouchová, L. Paznocht, M. Eliášová, P. Dvořák, P. Konvalina, D. Janovská, M. Orsák, L. Bečková, Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year, Plant Soil Environ. 65 (2019) 260–266, https://doi.org/10.17221/134/2019-PSE.

[26] A. Martinez-Gomez, I. Caballero, C.A. Blanco, Phenols and melanoidins as natural antioxidants in beer. structure, reactivity and antioxidant activity, Biomolecules 10 (2020) 400, https://doi.org/10.3390/biom10030400.

[27] R. Cadenas, I. Caballero, D. Nimubona, C.A. Blanco, Brewing with starchy adjuncts: its influence on the sensory and nutritional properties of beer, Foods 10 (2021) 1726, https://doi.org/10.3390/foods10081726.

[28] R. De Flaviis, V. Santarelli, D. Mutarutwa, S. Grilli, G. Sacchetti, A unifying approach to wheat beer flavour by chemometric analyses, Could we speak of 'terroir', Curr. Res. Food Sci. 6 (2023) 100429 https://doi.org/10.1016/j.crfs.2022.100429.

[29] V. Postigo, M. García, J.M. Cabellos, T. Arroyo, Wine *Saccharomyces* yeasts for beer fermentation, Fermentation 7 (2021) 290, https://doi.org/10.3390/fermentation7040290.

[30] M. Matukas, V. Starkute, E. Zokaityte, G. Zokaityte, D. Klupsaite, E. Mockus, J.M. Rocha, R. Ruibys, E. Bartkiene, Effect of Different yeast strains on biogenic amines, volatile compounds and sensory profile of beer, Foods 11 (2022) 2317, https://doi.org/10.3390/foods11152317.

[31] Á. Benito, F. Calderón, S. Benito, Mixed alcoholic fermentation of *Schizosaccharomyces pombe* and *Lachancea thermotolerans* and its influence on mannose-containing polysaccharides wine composition, AMB Expr 9 (17) (2019), https://doi.org/10.1186/s13568-019-0738-0.

[32] R.A. Harbah, T.V. Meledina, A.A. Morozov, The effect of yeast growth stages on the absorption of polyphenols, Agron. Res. 18 (2020) 1673–1679, https://doi.org/10.15159/AR.20.053.

[33] A. Pulvirenti, L. De Vero, G. Blaiotta, R. Sidari, G. Iosca, M. Gullo, A. Caridi, Selection of wine *Saccharomyces cerevisiae* strains and their screening for the adsorption activity of pigments, phenolics and ochratoxin A, Fermentation 6 (2020) 80, https://doi.org/10.3390/fermentation6030080.

[34] A. Rinaldi, G. Blaiotta, M. Aponte, L. Moio, Effect of yeast strain and some nutritional factors on tannin composition and potential astringency of model wines, Food Microbiol. 53 (2016) 128–134, https://doi.org/10.1016/j.fm.2015.09.013.

[35] I. Loira, A. Morata, F. Palomero, C. González, J.A. Suárez-Lepe, *Schizosaccharomyces pombe*: a promising biotechnology for modulating wine composition, Fermentation 4 (3) (2018) 70, https://doi.org/10.3390/fermentation4030070.

[36] C. Andrés-Iglesias, C.A. Blanco, O. Montero, Sugar, hop α-acid, and amino acid contents contribute to the differential profile between nonalcoholic and alcoholic beers Food Anal, Methods 12 (2019) 59–68, https://doi.org/10.1007/s12161-018-1338-3.

[37] I. Gouvinhas, C. Breda, A.I. Barros, Characterization and discrimination of commercial Portuguese beers based on phenolic composition and antioxidant capacity, Foods 10 (2021) 1144, https://doi.org/10.3390/foods10051144.

[38] R. De Flaviis, V. Santarelli, S. Grilli, G. Sacchetti, An integrative multi-omics approach aimed to gain insight on the effect of composition, style, yeast, and wheat species on wheat craft beer flavour, Food Chem. 441 (2024) 138387, https://doi.org/10.1016/j.foodchem.2024.138387.

[39] M. Rodríguez-Saavedra, K. Pérez-Revelo, A. Valero, M.V. Moreno-Arribas, D. González de Llano, A binary logistic regression model as a tool to predict craft beer susceptibility to microbial spoilage, Foods 10 (2021) 1926, https://doi.org/10.3390/foods10081926.