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Optimizing malting conditions for the old Sicilian durum wheat landrace Perciasacchi: Effects of steeping time and temperature on malt and beer quality

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ABSTRACT

Recent trends favor using durum wheat malt as a substitute for barley malt, offering craft brewers new opportunities and meeting the demand for unique beer styles. This study investigated the malting process of the Sicilian durum wheat variety Perciasacchi, focusing on the impact of steeping conditions on malt quality. Four malting trials were conducted, varying steeping times (9 and 13 h) and temperatures (15 °C and 25 °C). The physicochemical properties of the resulting malts and worts were analyzed and compared to commercial wheat malt to identify optimal conditions. The highest-performing malt was used in microbrewing, and the beer underwent sensory evaluation. The malt steeped at 25 °C for 13 h showed increased extract yield, enzyme activity, free amino nitrogen, and fermentability, along with reduced β-glucan content and wort viscosity. In contrast, malts steeped at 15 °C demonstrated insufficient modification, evidenced by lower enzyme levels and longer saccharification times. Beer brewed from the optimal malt exhibited improved sensory attributes, including a complex flavor profile, better foam retention, and lower turbidity compared to beer made with commercial malt. Sensory analysis highlighted enhanced wheat aromas, fruity notes, and fewer off-flavors. This study underscored the potential of landraces like Perciasacchi for producing high-quality malts and innovative flavors.

1. Introduction

Innovation in the craft beer industry focuses on ingredients, alcohol content, aging techniques, and packaging strategies to attract a broad consumer base (Baiano, 2021). Ingredient innovation includes novel grain combinations, new hop varieties, yeast cultures, and the use of fruits, vegetables, and flavorings (Alfeo, De Causmaecker, et al., 2018, Alfeo, Jaskula-Goiris, et al., 2018, 2021; Benanti et al., 2023; Francesca et al., 2023; Gugino et al., 2023; Matraxia et al., 2021; Pirrone et al., 2022), aimed at enhancing sensory profiles, personalizing beer styles, or creating new ones. Despite barley's dominant role due to its malting properties, the use of alternative cereals, such as durum wheat (Triticum durum), offers new product characteristics (Alfeo, De Causmaecker, et al., 2018, Alfeo, Jaskula-Goiris, et al., 2018; Mescia et al., 2014). Recent trends involve using durum wheat malt as a partial or full barley malt substitute, providing marketing opportunities for craft brewers and responding to the growing demand for distinctive beer styles. Although some recommend using common wheat varieties for their milling ease and protein content (Mescia et al., 2014), unique properties of durum wheat make it a promising malting option (Alfeo et al., 2021; De Flaviis et al., 2022). Many ancient Italian durum wheat landraces, notable for their rusticity and environmental adaptability, can be cultivated with minimal environmental impact, promoting sustainability in brewing. However, due to its higher protein content, distinct starch, and enzyme profiles, understanding the specific malting process of durum wheat is critical to fully exploit its brewing potential. The malting process is crucial for activating enzymes and modifying the grain's chemical composition to facilitate the subsequent mashing and fermentation steps

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in beer production (Briggs, 1998). Among the different steps in malting, hydration is particularly important for the final quality of the malt. To make the starch stored in the seed available, the endosperm must be hydrated during the steeping stage. Malt quality is determined by the used steeping regime, particularly the hydration time and temperatures used during this stage. It is known that hydration of the kernel promotes enzymatic development and the degradation of proteins and non-starch polysaccharides (Turner et al., 2019). The β-glucans and proteins are among the most important indicators of malt quality (Rani & Bhardwaj, 2021). The high content of these organic compounds is often related to problems of poor filterability, high turbidity in the wort and beer, and loss of efficiency in the brewery (Alfeo et al., 2021; Jin et al., 2012); therefore, their degradation which occurs in malting and begins with steeping is a factor to be achieved to produce a quality malt. The degradation of β-glucans and proteins is linked to the development and activity of β-glucanase and peptidase enzymes which occurs during germination and generally depends on higher hydration and temperature (Bourne & Wheeler, 1984). As demonstrated in several previous studies found in the literature, the rate of degradation of β-glucans during malting follows a 2nd order kinetic (Kuusela et al., 2004); generally, the β-glucan content decreased over time assumes optimal values from the 4th day of germination. The proteins content evolves with a similar pattern; their conversion into soluble proteins by the action of peptidases increases over time giving optimal results around the 4th day of germination (Bourne & Wheeler, 1984; Kuusela et al., 2004; Montanuci et al., 2017; Turner et al., 2019). This study investigated the malting process of the old Sicilian durum wheat landrace Perciasacchi, with a focus on the steeping stage and its impact on malt quality. Four malting trials were conducted, varying steeping times (9 and 13 h) and temperatures (15 °C and 25 °C). The malts and wort produced were analyzed chemically and physically, then compared with commercial wheat malt to identify the optimal malting process for Perciasacchi. The best-performing malt was used in microbrewing, and the resulting beer underwent sensory analysis. This research aimed to enhance understanding of durum wheat malting and explored the potential of Perciasacchi wheat in beer production.

2. Materials and methods

2.1. Wheat grain

The old durum wheat variety Perciasacchi (PE) (*Farro lungo, Melanopus Koern. del Triticum durum DESF*), was cultivated by adopting conventional agronomical management and has been supplied by a commercial cereal farm located in Sicily (Ramacca, CT, Italy). Samples were harvested in June 2023 and stored at 4–6 °C until malting tests carried out in October 2023. After malting the sample were stored under-vacuum at 12 °C for 3 weeks until the analysis.

2.2. Malting conditions

To investigate the influence of steeping temperature and time on the resulting wheat malt, four malting experiments were conducted using two steeping temperatures (15 °C and 25 °C) and two hydration periods (9 h and 13 h) during steeping. Malting tests were performed in triplicate using an automatic malting system (Phoenix Biosystems, Adelaide, Australia) at the Department of Agricultural, Food, and Forest Sciences (SAAF) at the University of Palermo (Italy). Perciasacchi wheat samples were sieved to remove the glumes, husks, and broken and small seeds.

Following the malting conditions for wheat proposed in previous studies (Alfeo et al., 2021; Gugino et al., 2024), for each batch, 500 g of grains were steeped in water at 15 °C (malting program 1, P1) and 25 °C (malting program 2, P2) for 5 h. This was followed by 8 h of air-rest, and then an additional 4 h in water, resulting in a total of 9 h of steeping time and 8 h of air-rest. Germination occurred after 120 h at 15 °C and 95% relative humidity. Subsequently, the samples were dried and kilned for 34.5 h according to the following temperature schedule: 3 h at 55 °C, 12 h at 60 °C, 10 h at 65 °C, 5 h at 70 °C, and 4.5 h at 75 °C.

Similarly, 500 g of grains were steeped in water at 15 °C (malting program 3, P3) and 25 °C (malting program 4, P4) for 5 h, followed by 8 h of air-rest and then an additional 4 h in water. One more cycle of air-rest (4 h), and steeping (4 h) were carried out resulting in a total 13 h of steeping time and 12 h of air-rest. Germination occurred after 120 h at 15 °C and 95% relative humidity. Subsequently, the samples were dried and kilned for 34.5 h using the same temperature schedule mentioned above. The roots and acrospires were removed at the end of the malting processes.

2.3. Malts, hops and yeast strains of micro-brewing trial

The experimental beers were brewed using the Perciasacchi malt (sample P4). The control beer samples were produced using commercial wheat malt (CM) (Wheat Best Maltz ®, Heidelberg, Germany). Rice husks were added to the mash, with 50 g per kilogram of wheat malt included in the recipe. Type 90 pellets of Slovenian Styrian Golding hops, containing 4.6 g 100 g^{-1} alpha acids, were used for bittering and aromatic purposes. For each trial, a commercial strain of *Saccharomyces cerevisiae* (SafAle™ US-05 Fermentis, Lesaffre, France) was inoculated at 0,8 g/L with approximately 2×10^6 CFU/mL. The inoculated strain was incubated at 20 °C, following the procedure described by Holt et al. (2018).

2.4. Micro-brewing conditions

The Klarstein mod. 10031629 “All in one” microbrewery system (Chal-Tec GmbH, Berlin, Germany) was used to brew experimental beers. Seven kilograms of wheat malts were ground using a Mattmill Kompakt double roller mill (Germany) with a roller distance of 1.20 mm. The malt was added to 25 L of water and mashed at 65 °C for 60 min to ensure saccharification, which was verified using an iodine test as suggested by Mayer, H. et al. (2016). The mixture was heated to 78 °C for 10 min to deactivate enzymes. During lautering, wort recirculation over spent grain was performed, followed by rinsing with 12 L of 78 °C water. The resulting 30 L of wort underwent a 60-min boil, with hops added at the start and end (1 g/L). After boiling, the wort was cooled to 20 °C using a stainless-steel plate heat exchanger. Fermentation proceeded for 16 days at 20 °C and was considered complete when specific gravity remained constant for two consecutive days. The beer was stored at 2 °C for 10 days to precipitate yeast, bottled in brown glass, and sucrose (9 g/L) was added for bottle fermentation to produce 6 g of CO_2 per liter.

2.5. Quality parameters of grains and malts

The analyses were performed in triplicate to the Analytica European Brewery Convention (EBC) (2007). Specifically, malt moisture was determined using EBC method 4.2 (2000), while germination energy (GE, %) was calculated using EBC method 3.6.2. The thousand corn weight (TCW, g dry basis) was measured for both wheat and malts according to EBC methods 3.4 and 4.4, respectively. Protein and soluble protein contents were assessed as total nitrogen (TN, g 100 g^{-1} dry basis) and soluble nitrogen (SN, g 100 g^{-1} dry basis) respectively, in accordance with EBC methods 4.3.1 (2004) and 4.9.1 (1997), then multiplied by 5.70. The Kolbach Index (%) was calculated per EBC method 4.9.1 (1997). Malt extract (dry basis %), extract difference, and pH were measured using EBC methods 4.4 (1997), 4.5 (1997), and 4.6 (1997), respectively. Saccharification rate, fermentability (%), free amino nitrogen (FAN, mg 100 g^{-1} dry basis), and wort color were determined using EBC methods 4.4.1 (1997), 4.11.1 (1999), 4.10 (1997), and 8.3 (International Method, 1997), respectively. Filtration speed was measured following EBC method 4.4.3 (1997), while wort viscosity was assessed with an Ostwald viscometer (SI Analytics Typ-Nr.

509 05) in accordance with EBC method 8.2 (1997). Total starch content (g 100 g^{-1} dry basis) was determined using the Megazyme assay kit (AOAC Method 996.11, 2005). β-glucan contents were measured with the Megazyme assay kit (K-BGLU) following EBC Methods 3.10.1 and 8.13.1 for malt and wort samples.

2.6. Enzyme activities

A malt amylase assay kit (K-MALTA, Megazyme International) was used to quantify α- and β-amylases in malt flour. Enzyme activities were measured by absorbance using a Beckman DU650 spectrophotometer (Pasadena, California, US) and reported as units per gram of dry matter (U g^{-1}) in accordance with the methodology provided in the kits. One unit of activity is defined as the amount of enzyme required to release 1 μmol of reducing sugar equivalents per minute under the defined assay conditions. The contents of endo-β-glucanase and endo-1,4-β-Dxylanase were determined using Megazyme assay kits (K-MBGL and Xylazyme tablets, respectively).

2.7. Congress wort sugar profile

The simple sugars were determined in the congress wort samples. The analysis of fructose, glucose, maltose, and sucrose were performed using the analyzer iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China) as reported by Matraxia et al. (2021). All reagents and standards were purchased from R-Biopharm AG (Darmstadt, Germany).

2.8. Microbiological monitoring of alcoholic fermentation

Samples were collected at various stages of Congress worts fermentation, including after yeast strain inoculation on day 0 and every three days during the alcoholic fermentation process (on days 3, 6, 9, and 12). Each analysis of the samples was conducted in triplicate within 24 h of their collection. Yeast loads were monitored by incubating them for 48 h at 25 °C in aerobic conditions on Wallerstein Laboratory (WL) nutrient agar medium (Oxoid, Basingstoke, UK). All samples underwent serial dilution in Ringer's solution (Sigma-Aldrich, Milan, Italy) at 1:10 ratio, followed by spreading onto plates.

2.9. Sensory analysis

A quantitative descriptive analysis was conducted to differentiate beer samples based on sensory descriptors for color, odor, taste, and overall quality. Ten trained panelists (5 males, 5 females, aged 23–30) participated, following the 13.4 EBC methods (1997). Assessments were conducted under controlled conditions at the Sensory Analysis Laboratory of the University of Palermo (Italy), in compliance with ISO 8589:2007 standards. The evaluation covered 32 attributes from the Beer Flavor Wheel by Meilgaard et al. (1979), as reported in EBC methods 10.12 (1979), including visual characteristics (color, turbidity, head color, foam structure, and foam retention), odors (odor intensity, estery, fruity, grainy, malty, hoppy, caramel, phenolic, diacetyl, sulfury, DMS, yeasty, and oxidized), and taste traits (taste intensity, sweetness, acidity, saltiness, bitterness, estery, fruity, grainy, malty, hoppy, oxidized, and body). Sensory attributes were rated on an unstructured nine-point scale from 'absent' to 'high,' and acceptability was also assessed. Water was used to cleanse the palate between samples. The analysis was conducted following the procedures reported by Gugino et al., 2024.

Panelists provided written consent after receiving comprehensive information about the sensory test, ensuring they understood there were no risks associated with odor or taste of the sample. Informed consent forms, signed by both the panelists and the panel leader, were collected prior to the evaluation. All procedures complied with relevant laws, institutional guidelines, and ethical principles.

2.10. Statistical analysis

One-way Analysis of Variance (ANOVA) with Tukey's post hoc test and a principal component analysis (PCA) were performed using Matlab software (MathWorks Inc., Nutick, Massachusetts, United States). Sensory data were analyzed using Smart Sensory Box. All experiments were conducted in triplicate and results were reported as mean ± standard deviation.

3. Result and discussion

3.1. Quality parameter of wheat and malts

The main quality parameters of wheat and malts are summarized in Table 1. The moisture content of both wheat and malts is consistent with existing literature (Alfeo, Jaskula-Goiris, et al., 2018; Faltermaier et al., 2014). Key indicators for high-quality malt include thousand corn weight (TCW), germination energy, and wheat protein content, measured before malting in Perciasacchi wheat. TCW is valuable for maltsters, correlating with larger kernel size, uniform milling, and higher endosperm content (Armstrong et al., 2002). The TCW of Perciasacchi wheat was 48.02 g db, while the malts ranged from 44.50 to 44.74 g db, slightly higher than the control malt (Table 1). Germination energy, indicative of seed health and water sensitivity (Briggs, 1998; Domin et al., 2019; Rani & Bhardwaj, 2021), showed that Perciasacchi had a high germinability of 97.83%, aligning with previous studies (Alfeo, De Causmaecker, et al., 2018, Alfeo, Jaskula-Goiris, et al., 2018). Perciasacchi wheat exhibited a protein content of 12.89 g 100 g^{-1} db, consistent with literature values for wheat malt (Faltermaier et al., 2014). In comparison to unmalted wheat, the proteins in the obtained malts were lower, with protein contents ranging from 11.98 g 100 g^{-1} db to 12.81 g 100 g^{-1} db across different malting regimes. The CM commercial sample had the lowest protein content, while P2 malt had the highest, with no statistically significant differences from the control malt. Protein content is critical for selecting malts for beer production, as it significantly influences the quality of wort and beer. The quantity, type, and size of malt proteins impact wort filtration, fermentability, foam stability, and haze (Faltermaier et al., 2014). High-protein grains can hinder the enzymatic degradation of starch, resulting in suboptimal malt extract production (Brennan et al., 1996). Proteolytic characteristics, including the Kolbach Index (KI), soluble protein, and total protein, are essential for evaluating malt quality and modification. The malts exhibited varying modification degrees, with soluble protein values between 4.21 and 5.06 g 100 g^{-1} db. The CM and P1 malts had the lowest soluble protein values (4.21 and 4.38 g 100 g^{-1} db, respectively), while P3 and P4 malts showed intermediate values (4.58 and 4.99 g 100 g^{-1} db, respectively). P2 malt had the highest soluble protein content at 5.06 g 100 g^{-1} db. These differences correlated with Kolbach Index values, which ranged from 34.84% in P1 to 39.74% in P4, similar to P2 malt (39.51% KI). This trend aligns with previous findings that show a positive correlation between the Kolbach Index and steeping degree (Hassani et al., 2014; Rostovskaya et al., 2020). The starchy endosperm is the primary source of energy in the of germinating grains, and its content is significantly influenced by the embryo's development during germination. The starch content of the studied samples ranged from 57.17 g 100 g^{-1} db for P4 to 60.33 g 100 g^{-1} db for P3. No significant differences were observed in the starch content of the malts obtained with different steeping regimes and the control malt.

The β glucan content varied among the samples studied with the lowest values in P2 and P4 malts (0.31 g 100 g^{-1} db), intermediate levels in P3 and CM malts (0.54 and 0.37 g 100 g^{-1} db, respectively), and the highest in P1 malt (0.60 g 100 g 100 g^{-1} db). The β glucan content is typically lower when steeping and germination processes involve high temperatures and longer hydration times (Montanuci et al., 2017).

Table 1
Wheat and malts quality parameters.

Sample	Moisture (g 100 g^{-1})	TCW (g db)	Proteins (g 100 g^{-1} db)	Sol. Proteins (g 100 g^{-1} db)	Kolbach Index (%)	Starch (g 100 g^{-1} db)	β-glucan (g 100 g^{-1} db)	GE (%)
Wheat grain								
PE	10.00 ± 0.21	48.02 ± 0.11	12.89 ± 0.11	–	–	65.73 ± 0.43	–	97.83 ± 0.29
Wheat malt								
P1	5.31 ± 0.04^{b}	44.55 ± 0.13^{b}	12.56 ± 0.39^{a}	4.38 ± 0.18^{a}	34.84 ± 0.67^{a}	59.67 ± 0.34^{a}	0.60 ± 0.15^{b}	–
P2	5.85 ± 0.04^{d}	44.74 ± 0.09^{b}	12.81 ± 0.64^{a}	5.06 ± 0.18^{c}	39.51 ± 0.72^{c}	59.49 ± 1.30^{a}	0.31 ± 0.08^{a}	–
P3	5.18 ± 0.04^{a}	44.50 ± 0.12^{b}	12.57 ± 0.19^{a}	4.58 ± 0.14^{ab}	36.46 ± 0.57^{b}	60.33 ± 1.69^{a}	0.54 ± 0.13^{ab}	–
P4	5.60 ± 0.00^{c}	44.64 ± 0.22^{b}	12.54 ± 0.44^{a}	4.99 ± 0.23^{bc}	39.74 ± 0.44^{c}	57.17 ± 3.46^{a}	0.31 ± 0.03^{a}	–
CM	5.53 ± 0.06^{c}	37.03 ± 0.06^{a}	11.98 ± 0.05^{a}	4.21 ± 0.06^{a}	35.13 ± 0.50^{ab}	58.22 ± 0.25^{a}	0.37 ± 0.01^{ab}	–

db = dry basis; GE = germination energy; TCW = thousand corn weight.
Values in the same column followed by different letter are statistically different ($p < 0.05$).

3.2. Congress wort characteristics

Table 2 summarizes the characteristics of Congress worts. The extract value, a key parameter for malt selection, was evaluated to determine the impact of steeping conditions on malt and wort characteristics. In the samples, the extract contents ranged from 69.78% db (P1) to 79.24% db (CM), with malts steeped at 25 °C (P3 and P4) showing higher extract levels than those steeped at 15 °C (P1 and P2). The fine-coarse difference, an indicator of malt homogeneity, was highest in P1 (1.75% db) and lowest in CM (1.60% db), with the other samples showing similar, lower values. Wort pH ranged from 6.09 (CM) to 6.43 (P1), with higher pH in malts steeped for 9 h (P1 and P2) than in P3 and P4. There were no significant differences in the color of Perciasacchi worts, ranging from 2.83 to 3.44 EBC units (P1 and P4, respectively), while CM showed a darker color than other samples (4.56 EBC). Viscosity of wort remained within acceptable levels for all samples; the viscosity impacts filtration and turbity of wort and beer, with higher viscosity indicating suboptimal cell wall hydrolysis (Deme et al., 2020; Rani et al., 2021). For wort at 8 °P (Plato degree), the recommended viscosity should ideally be in the region of 1.6 cP (Briggs, 2004). The malt samples viscosity values ranged from 1.07 to 1.44 mPa s, in sample P1 and sample CM, respectively. Our study found significant differences in β-glucan values among the samples, ranging from 1.92 mg L^{-1} to 31.91 mg L^{-1} for P1 and CM, respectively. The P4 malt exhibited a higher value than the P1, P2 and P3 malts. While statistically different, the β-glucan content of the P4 malt (27.68 mg L^{-1}) was close to that of the CM malt. An important aspect to consider is the Free Amino Nitrogen (FAN) content. FAN refers to nitrogen compounds that are metabolized or assimilated by the yeast during fermentation. These compounds encompass α-amino acids (excluding proline, which does not fall under this category), ammonia, and small peptides (Hill & Stewart, 2019). In the fermentation process, the ideal FAN content required for optimal yeast growth is approximately 130 mg L^{-1}, as highlighted by Stewart et al. (2017). FAN values in the samples varied from 73.51 mg L^{-1} (P1) to 90.14 mg L^{-1} (CM), with P4 showing higher levels (89.49 mg L^{-1}) than the other experimental malts, indicating that extended hydration (13 h) and higher steeping temperature (25 °C) promote FAN development (Hassani et al., 2014; Rostovskaya et al., 2020). Fermentability ranged from 73.04% (P1) to 79.51% (P4), with P2, P3, and CM showing intermediate values (76.35%–78.08%). Saccharification time during congress mash was evaluated, representing the duration required for starch to convert into sugars (Kunze, 2004). High-quality malt completes saccharification within 10 min, while extended times indicate poor starch disintegration (Kumar et al., 2013). Among the samples, P4 and CM saccharified within 10–15 min, while others exceeded 15 min, with P1 taking 25–30 min. Saccharification time for Perciasacchi malts showed an inverse relationship with α-amylase content (refer to Table 3).

3.3. Enzyme activities

Enzyme activity of malt is an important aspect to consider in the brewing industry because of its influence on the beer characteristic and quality. The enzymatic activity is not only fundamental for the successful fermentation of wort by yeast but also plays a critical role in shaping the sensory attributes and mouthfeel of the final products

Table 3
Enzymes activity of malts.

Sample	α-amylase (CU g^{-1} db)	β-amylase (BU g^{-1} db)	Endo-β-glucanases (U kg^{-1} db)	Endo-1,4-β-Dxylanase (U g^{-1} db)
P1	70.47 ± 0.92^{a}	31,09 ± 0,56^{a}	10,78 ± 0,44^{a}	0.60 ± 0.02^{a}
P2	88.14 ± 0.89^{c}	33.88 ± 1.35^{b}	17.57 ± 1.06^{b}	0.75 ± 0.03^{b}
P3	82.45 ± 0.53^{b}	32.45 ± 1.21^{ab}	11.5 ± 0.54^{a}	0.68 ± 0.02^{ab}
P4	115.23 ± 1.98^{d}	34.41 ± 0.54^{bc}	32.41 ± 1.74^{c}	0.99 ± 0.05^{c}
CM	71.44 ± 1.79^{a}	36.86 ± 0.74^{c}	17.53 ± 1.08^{b}	0.95 ± 0.03^{c}

db = dry basis; BU = Betamyl Units; CU = Ceralpha Units; U=Units of enzyme. Enzymes activity calculated on Perciasacchi wheat malt by different malting regimes (P1, P2, P3, P4) and Commercial wheat malt (CM).
Values in the same column followed by different letter are statistically different ($p < 0.05$).

Table 2
Quality attributes of Congress worts.

Sample	Extract (% db)	Extract fine-course (% db)	pH	Colour (EBC unit)	Viscosity (mPa.s)	β-glucan (mg L^{-1})	FAN (mg L^{-1})	Fermentability (%)	Saccharification (min)
P1	69.78 ± 2.86^{a}	1.75 ± 0.04^{c}	6.43 ± 0.04^{c}	2.83 ± 0.58^{a}	1.14 ± 0.06^{a}	3.57 ± 0.21^{a}	73.51 ± 2.88^{a}	73.04 ± 2.57^{a}	20 < s < 30^{c}
P2	73.46 ± 0.58^{ab}	1.64 ± 0.02^{ab}	6.40 ± 0.02^{c}	3.25 ± 0.22^{a}	1.11 ± 0.06^{a}	1.92 ± 0.10^{a}	82.22 ± 1.42^{ab}	76.35 ± 0.92^{ab}	15 < s < 20^{b}
P3	74.4 ± 1.53^{b}	1.67 ± 0.01^{b}	6.25 ± 0.08^{b}	2.78 ± 0.61^{a}	1.10 ± 0.11^{a}	6.7 ± 0.40^{b}	81.68 ± 6.21^{ab}	75.33 ± 2.65^{ab}	15 < s < 20^{b}
P4	77.45 ± 1.16^{bc}	1.62 ± 0.03^{ab}	6.23 ± 0.03^{b}	3.44 ± 0.27^{a}	1.07 ± 0.10^{a}	27.68 ± 1.57^{c}	89.49 ± 6.27^{c}	79.51 ± 1.21^{b}	10 < s < 15^{a}
CM	79.24 ± 0.52^{c}	1.60 ± 0.01^{a}	6.09 ± 0.02^{a}	4.56 ± 0.14^{b}	1.44 ± 0.02^{b}	31.91 ± 0.89^{d}	90.14 ± 0.06^{c}	78.08 ± 1.37^{ab}	10 < s < 15^{a}

db = dry basis; s = saccarification; FAN = free amino nitrogen; Congress worts of Perciasacchi wheat malt by different malting regimes (P1, P2, P3, P4) and Commercial wheat malt (CM).
Values in the same column followed by different letter are statistically different ($p < 0.05$).

(Kunze, 2004). One of the key enzymes involved is α-amylase, responsible of the starch hydrolysis to maltose and dextrins (Bamforth, 2009; Farias et al., 2021). In tandem, β-amylase complements this process by converting dextrins into maltose. The enzymatic characteristics of the studied malts have been presented in Table 3, revealing notable differences for both for α- and β -amylases among the samples. The α-amylase activity, for instance, exhibited a substantially higher level in P4 malt. Specifically, the α-amylase content ranged from 70.47 to 115.23 CU g^{-1} db, with the lowest value observed in sample P1, followed by CM, P3 and P2, while the highest value was recorded in P4 malt. The β-amylase activity ranged between 31.09 BU g^{-1} db in P1 and 36.86 BU g^{-1} db in CM. Sample P3 (32.45 BU g^{-1} db) exhibited an activity similar to that founded in P1 and P2, while sample P4 had a value of 34.41 BU g^{-1} db, which was between P2 and CM. The duration and temperature of steeping were found to influence the amylolytic activity of the malts. In particular, the results are in accordance with those of other studies, indicating that the highest activity for α- and β-amylases occurred following an increase in temperature (Usansa et al., 2009). The study compared the level of endo-β-glucanases and endo-1,4-β-Dxylanase of the durum wheat malts with that of commercial malt. These two enzymes are crucial for the degradation of non-starch polysaccharides, which affect the wort viscosity and contribute to filtration difficulties during the brewing process. Inadequate hydrolysis of these polysaccharides during the malting process raises their concentration in wort and beer (Shaluk et al., 2019). The samples showed variable endo-β-glucanases activity, ranging from 10.78 to 32.41 U kg^{-1} db for P1 and P4, respectively. The highest values were recorded in the samples tested under a higher steeping temperature, P2 and P4. The endo-1, 4-β-Dxylanase levels ranged between 0.60 U g^{-1} db and 0.99 U g^{-1} db, with the lowest value for P1 and the highest for P4. The commercial malt value (0.95 U g^{-1} db) was similar to that of sample P4. The higher activity of the enzymes endo-β-glucanases and endo-1,4-β-Dxylanase in this malt sample in comparison to the other malt samples was the primary factor contributing to the reduction in viscosity observed in the congress wort of sample P4 (Table 2). Among the malting tests studied, those with a steeping temperature of 25 °C showed a higher development of endo-β-glucanases and endo-1,4-β-Dxylanase. These findings underscore the substantial variations in enzymatic activity within the malts.

3.4. Congress wort sugar profile

To comprehensively evaluate the impact of malting procedures on Perciasacchi wheat quality, our study focused on sugar profiles of the congress worts. The sugar profiles are normally influenced by various factors, including starch content, non-starch polysaccharides, and enzymatic activity resulting from the mashing conditions (Fox G., 2016).

The findings related to the sugar profiles of the examined samples are showed in Table 4. The content of simple sugars was found to be similar to the values reported in the literature for wheat malts (Alfeo et al., 2021; Gonu & Withayagiat, 2023). Notably, statistically significant variations were observed across all sugars, signifying that the sugar profiles were influenced by the different steeping conditions. Whit the exception of sucrose, P4 malt exhibited the highest levels for all sugars. In detail, the D-fructose content ranged between 0.60 and 1.02 g L^{-1}, with the highest concentration observed in sample P4 and the lowest in P1. A similar trend was observed in the case of glucose, and total simple sugars. D-glucose content ranged from 3.16 g L^{-1} (P1) to 5.15 g L^{-1} (P4), while sucrose exhibited values between 4.52 (P4) and 7.29 g L^{-1} (P3). Simple sugars were found to be in the range of 40.9–47.17 g L^{-1}, respectively for P1 e CM, it is noteworthy that the total content of simple sugars in P4 malt (47.15 g L^{-1}) was similar to that of the CM sample. With regard to maltose content, we observed a range between 30.55 and 36.47 g L^{-1}, respectively in P3 and P4. Samples P1 and P2 exhibited statistically similar values to sample P1, while the commercial sample CM (35.48 g L^{-1}) was similar to sample P4. In consideration of the sugar profile after fermentation of the congress worts, illustrated in Table 4, the residual simple sugars showed a markedly low concentration and exhibited slight variability between the samples. In particular, the total simple sugars ranged from 0.14 g L^{-1} (samples P2 and P4) to 0.23 g L^{-1} (sample P3). Concerning the composition of the sugar profiles, maltose and sucrose represent the most to the residual sugars in all the samples. Maltose was the most abundant, with a range of 0.04–0.11 g L^{-1}, followed by sucrose, with a range of 0.04–0.07 g L^{-1}. Both fructose and glucose were totally metabolized by the yeast during fermentation and were present in the fermented wort in trace amounts, at concentrations of 0.03–0.05 g L^{-1} for glucose and 0.01–0.02 g L^{-1} for fructose, respectively.

3.5. Microbiological monitoring of congress wort

Yeast levels detected during diverse phases of alcoholic fermentation are presented in Fig. 1. The microbiological levels in the wort were below the detection limit before yeast inoculation. The inoculation rate performed was as planned, with a slight difference between trials, with values between 6.1 and 6.6 log (CFU/mL). The microbial load values in the different trials increased until day 6 and then declined, attributed to various factors such as a lack of required nutrients from the strains (Francesca et al., 2023; Lodolo et al., 2008). Furthermore, the diverse trials demonstrated a comparable trend of fermentation, consistent with the typical fermentation process of beer wort (Matraxia et al., 2023; Tan et al., 2021). The beer wort made of Perciasacchi malt resulted in a regularly fermentation process as the yeast was provided with the necessary nutrients required.

3.6. Sensory descriptive analysis

Following the analysis of malts from the four malting trials and the

Table 4
Sugars profile of congress worts.

Sample	D-fructose (g L^{-1})	D-glucose (g L^{-1})	Sucrose (g L^{-1})	Maltose (g L^{-1})	Tot. Simple sugar (g L^{-1})
Initial sugar					
P1	0.60 ± 0.03^{a}	3.16 ± 0.12^{a}	5.06 ± 0.25^{a}	$32.07 \pm 1,79^{a}$	40.9 ± 2.18^{a}
P2	0.84 ± 0.05^{b}	3.97 ± 0.10^{b}	6.17 ± 0.34^{b}	31.45 ± 1.00^{a}	42.43 ± 1.48^{a}
P3	0.97 ± 0.05^{c}	4.33 ± 0.16^{c}	7.29 ± 0.36^{c}	30.55 ± 1.23^{a}	43.14 ± 1.79^{ab}
P4	1.02 ± 0.06^{c}	5.15 ± 0.18^{d}	4.52 ± 0.23^{a}	36.47 ± 1.16^{b}	47.15 ± 1.61^{b}
CM	0.51 ± 0.02^{a}	5.12 ± 0.04^{d}	6.07 ± 0.18^{b}	35.48 ± 0.01^{b}	47.17 ± 0.23^{b}
Final sugar					
P1	0.01 ± 0.00^{a}	0.03 ± 0.00^{a}	0.07 ± 0.00^{c}	0.11 ± 0.00^{f}	0.22 ± 0.00^{c}
P2	0.01 ± 0.00^{a}	0.05 ± 0.00^{c}	0.04 ± 0.00^{b}	0.04 ± 0.00^{a}	0.14 ± 0.00^{a}
P3	0.02 ± 0.00^{b}	0.04 ± 0.00^{b}	0.07 ± 0.00^{d}	0.09 ± 0.00^{d}	0.23 ± 0.01^{c}
P4	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.03 ± 0.00^{a}	0.08 ± 0.00^{c}	0.16 ± 0.00^{b}
CM	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.04 ± 0.00^{b}	0.05 ± 0.00^{b}	0.14 ± 0.00^{a}

Sugars profile on congress worts of Perciasacchi wheat malt by different malting regimes (P1, P2, P3, P4) and Commercial wheat malt (CM).
Values in the same column followed by different letter are statistically different ($p < 0.05$).

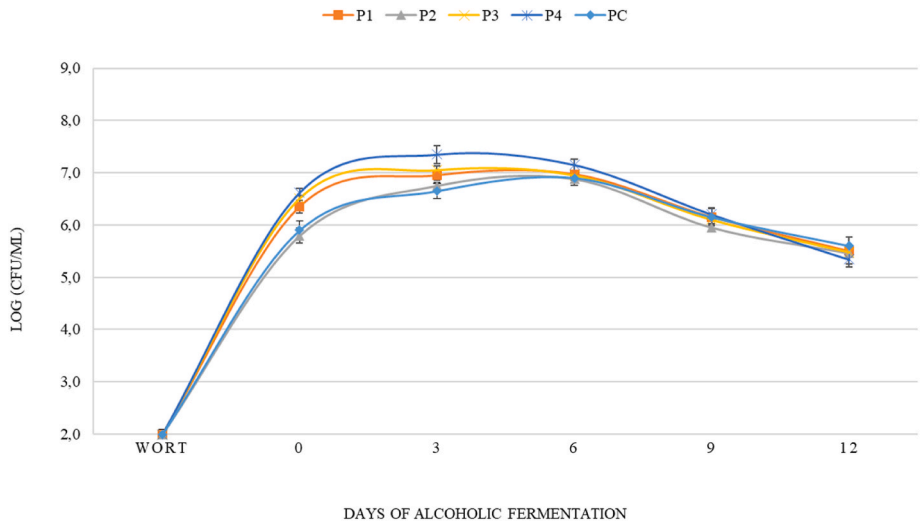

Fig. 1. Results of microbiological analyses during congress wort fermentation under real conditions. Beer fermented by *S. cerevisiae* US05.

subsequent fermentation of Congress worts, a micro-brewing trial was conducted using the P4 malt sample. The characteristics of the experimental beer produced with P4 malt were then compared to those of beer brewed with the commercial wheat malt (CM) (Fig. 2a, 2b, 2c). With regard to the visual characteristics (Fig. 2a), the beer brewed with Perciasacchi malt (P4) had a lighter color compared to the reference beer (CM) as already noted in the characteristics of the congress wort proposed in Table 2. The beers were significantly different in terms of turbidity ($p < 0.001$), P4 beer was much clearer than the control beer. The observed increase in turbidity may be attributed to a reduction in the activity of the enzymes endo-β-glucanases and endo-1,4-β-D-xylanase, which could result in a higher residual amount of compounds such as β-glucans and arabinoxylans in the control beer, contributing to an elevated turbidity in the wort and beer (Kahle et al., 2021; Speers et al., 2003). The visual analysis revealed an enhanced foam structure, consisting in small bubble size, which contributed to superior foam retention in the P4 beer. The stability of foam is dependent upon the equilibrium between those components that facilitate foam formation, including polypeptides, hop bitter acids, metal ions and melanoidins, and those that inhibit it, such as ethanol, lipids and detergents. The principal proteins that contribute to foam stability, derived from malt, are Protein Z4 and Lipid Transfer Protein (LTP1) (Bamforth, 2023). With regard to the aroma attributes (Fig. 2b), the beers exhibited notable differences in the attributes "grainy" ($p < 0.01$), "sulfury" ($p < 0.001$) and "oxidized" ($p < 0.05$). These last two attributes are typically regarded as undesirable characteristics. In particular, the "sulfury" attribute, which was the most intense in CM beer, is associated with compounds produced through yeast metabolism. Some of these compounds, such as hydrogen sulphide, have a very low perception threshold and, even in small traces, can significantly alter the organoleptic characteristics of beer. A reduction of free nitrogen compound as FAN can reduce yeast metabolism, resulting in slower fermentation and the production of beers with elevated hydrogen sulfide levels (Ferreira & Guido, 2018). The taste characteristics have been reported in the radar graph in Fig. 2c. The panel of tasters identified notable differences in the intensity of the taste ($p < 0.05$), acid ($p < 0.05$), fruitiness ($p < 0.05$) and oxidized attributes ($p < 0.05$). The P4 beer exhibited greater acidity and a more pronounced fruity attribute compared to the CM commercial beer. Additionally, the taste indicated a more pronounced oxidized note in the CM beer compared to the beer produced with P4 malt.

The panel rated the P4 beer as being more pleasant overall than the CM beer.

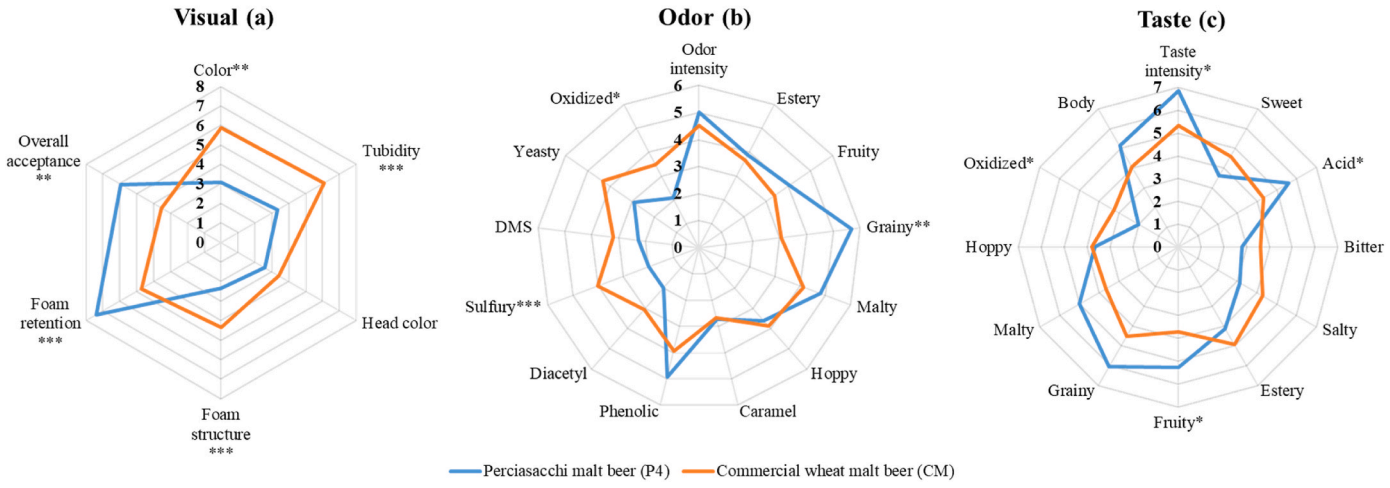

Fig. 2. Sensory analysis performed on visual (a), odor (b) and taste (c) of wheat malted beers: spider plot of average scores for aroma determined by judges during tasting sessions. Beers produced by micro-brewing trials with Perciasacchi malt obtained by malting at steeping conditions of 25 °C for 13 h (P4) and Commercial wheat malt (CM). Symbols: ****, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.*

3.7. Multivariate data analysis

Fig. 3 presents the results of the principal component analysis on the malt samples obtained from the different malting trials. Principal component one (F1) can explain about 60.07% of the variance, while principal component two (F2) accounted for 31.69% of the totale variance (92.39%).

The primary component, that can explain most part of the variance, was positively influenced by endo-1,4-β-Dxylanase, FAN, extract, and β-amylase. The parameters on the negative side of the principal component one included starch, and β-glucan content.

The second pricipal component (F2) was primarily positively affected by Kolbach Index, α-amylase and protein. On the other side, the viscosity and the color of the congress wort negatively affected the second principal component. How observed, the first principal component spread the samples according to endosperm starch degradation, samples plotted on the positive side (P4 and CM) were the samples with the higher endosperm degradation, while the samples on the negative side showed the lower endosperm degradation. The second principal component spread the samples according to the protein degradation and overall modification degree, samples plotted on the positive side (P4 and P2) were the samples showing optimal modification degree while the samples on the negative side (CM and P1) were the lower modified samples. Samples P3 and P2 that showed intermediate characteristics were plotted on the middle of the biplot.

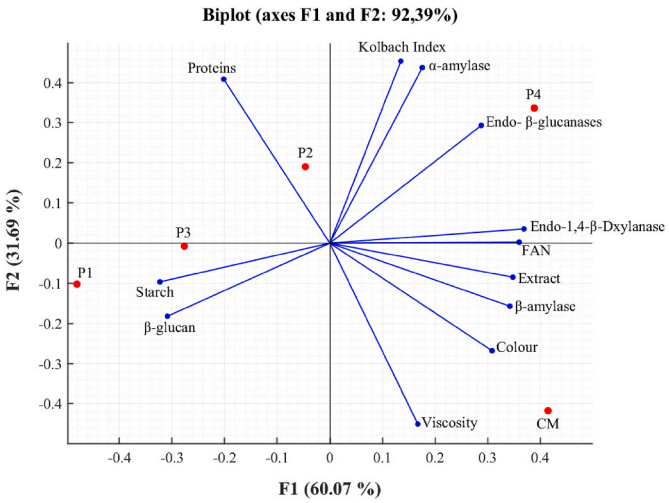

Fig. 3. Score and loading plots of PCA of the malt samples of Perciasacchi wheat malt obtained by different malting regimes (P1, P2, P3, P4), and Commercial wheat malt (CM).

4. Conclusion

The investigations conducted in this study revealed that the steeping process exerts a significant influence on malt quality. In particular, the P4 malting profile, characterized by the steeping of grains at 25 °C for 13 h, was found to be optimal to produce Perciasacchi wheat malt, outperforming the other profiles tested (P1, P2 and P3). The P4 malt displayed notable enhancements in several quality parameters, including extract yield, free amino nitrogen content (FAN), enzyme activity (α-amylase, β-amylase, endo-β-glucanases, endo-1,4-β-Dxylanase), fermentability and sugar content. Furthermore, P4 malt demonstrated comparable or superior quality to commercial wheat malt (CM), with a reduction in undesirable parameters such as β-glucans and wort viscosity. The results showed that P1 and P3 malts, steeped at 15 °C for 9 and 13 h, respectively, exhibited similar characteristics but differed from P2, P4, and CM malts. P1 and P3 demonstrated inadequate modification, as indicated by low enzyme levels, Kolbach Index values, and extended saccharification times. In contrast, P2 and P4 malts, steeped at 25 °C for 9 and 13 h, respectively, showed significant improvements in key quality parameters, including higher Kolbach Index, increased α-amylase content, and reduced β-glucans and wort viscosity. Microbrewing trials utilizing P4 malt, which exhibited the most favorable technological characteristics among the malts tested, demonstrated that beer production using 100% Perciasacchi wheat malt was not constrained. The sensory evaluation indicated that beer produced with P4 malt exhibited advantages in several attributes when compared to beer produced with commercial CM malt. These advantages included a lighter colour, less turbidity, and a more foam retention. Furthermore, the sensory analysis revealed a more complex and nuanced aroma and taste profile for the P4 beer, with a more pronounced wheat odor and fewer negative attributes such as a sulphury and oxidized flavour. Furthermore, the P4 beer exhibited a greater intensity of flavour, characterized by more pronounced notes of acidity and fruitiness than the commercial CM beer.

The investigation of the malting process for durum wheat varieties represents an innovative approach that promises to produce high-quality malts derived from ancient wheat varieties. These distinctive malts have the potential to impart distinctive organoleptic characteristics to the final products, thereby offering a novel and engaging sensory experience.

CRediT authorship contribution statement

Ignazio Maria Gugino: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Data curation, Conceptualization. **Antonino Pirrone:** Writing – review & editing, Visualization, Validation, Software, Methodology, Data curation. **Luca Gaetano Lo Porto:** Visualization, Validation, Methodology, Data curation. **Noemi Giammusso:** Visualization, Validation, Data curation. **Vincenzo Alfeo:** Writing – review & editing, Visualization, Validation, Software, Methodology, Data curation. **Gert De Rouck:** Writing – review & editing, Validation, Supervision, Formal analysis. **Nicola Francesca:** Validation, Methodology, Investigation, Conceptualization. **Aldo Todaro:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, 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.

Data availability

Data will be made available on request.

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