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Comparative analysis of physicochemical properties, antioxidant activities, and metabolomic profiles in daylily-supplemented craft beer fermented with different *Saccharomyces* strains

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ABSTRACT

This study investigated the use of daylily as a novel adjunct in craft beer production with four *Saccharomyces* yeast strains. The addition of daylily powder and yeast selection significantly influenced the physicochemical properties, antioxidant activity, and overall metabolome of the beers. Yeast strains exhibited variations in color, alcohol content, phenolic and flavonoid levels, and antioxidant capacity. Metabolomic analysis revealed differences in lipid, amino acid, tannin, and fatty acid synthesis between strains. Volatile profiles also differed markedly in esters, terpenes, higher alcohols, acids, and aldehydes. While 90 % of metabolites were conserved, key differences reflected distinct metabolic regulation among strains. These findings highlight the potential of daylily as a flavorful and bioactive beer ingredient, and emphasize the importance of targeted yeast selection for optimizing beer quality and metabolome. This work provides a practical framework for brewers to develop innovative beers with enhanced functional properties and specialized flavor profiles.

1. Introduction

The craft beer industry has experienced remarkable global growth in recent years, driven by consumer demand for unique flavors and innovative brewing techniques. Unlike traditional industrial beers, craft beers often incorporate non-conventional ingredients and specialized yeast strains to create distinctive flavor profiles (Jaeger et al., 2021). This differentiation strategy has propelled the craft beer market's expansion, catering to consumer demand for premium products with complex sensory attributes.

The use of natural adjuncts in craft brewing has gained attention as a promising strategy to enhance beer flavor and functionality. Several studies have investigated the impact of various adjuncts on beer quality and sensory characteristics. For example, olive leaves, rich in polyphenols such as oleuropein and 3-hydroxytyrosol, were studied for their contribution to bitterness, antioxidant activity, and sensory profile. Results revealed that their addition increased polyphenol content, facilitated the hydrolysis of oleuropein to 3-hydroxytyrosol, and imparted a sour/astringent taste along with a herbal aroma (Guglielmotti et al., 2020). Similarly, the addition of *Sambucus nigra* (elderflower) has been shown to enhance fruity and floral notes in beer, owing to the presence of volatile compounds like monoterpenoids and sesquiterpenoids (Petruţ et al., 2017). Other adjuncts, such as *Parastrephia lucida* leaves, lemon balm, and *Melissae folium* extract, have also been explored, contributing to improved sensory profiles and bioactive properties (Leskosek-Cukalovic et al., 2010; Tirado-Kulieva et al., 2023).

Despite the growing interest in edible flowers as adjuncts in craft beer production, the use of *Hemerocallis* spp. (daylily) flowers remains underexplored. Daylily, a perennial herbaceous plant in the *Liliaceae* family, has a long history of culinary use in Asia and possesses a rich diversity of nutritional and sensory attributes. Analysis shows that dried daylily is rich in carbohydrates (60.1 g/100 g), high-quality proteins (14.1 g/100 g), and minerals like calcium (463 mg/100 g), phosphorus (173 mg/100 g), and iron (16.5 mg/100 g) (Wang et al., 2024). Its floral aroma, attributed to compounds like linalool, geranial, and (*E*)-β-ocimene, suggests that daylily flowers could enhance beer flavor profiles

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and bioactive compound content (Zhou et al., 2023). When compared to other commonly used adjuncts like olive leaves and elderflower, daylily offers unique bioactive properties, with its high content of antioxidant providing additional functional benefits. Its distinctive floral aroma, driven by compounds such as linalool and geranial, can enhance beer aroma, making it an excellent choice for brews that seek complex, floral notes. However, no studies have systematically examined the impact of daylily flowers on the quality and metabolomic profiles of craft beer.

Yeast strain selection is another crucial factor influencing beer fermentation outcomes and final product quality. While industrial *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* strains exhibit consistent fermentation performance, their metabolic profiles often lead to relatively similar flavor characteristics (Gibson et al., 2017). The exploration of diverse yeast strains with distinct metabolic phenotypes has gained traction as a strategy to generate desired flavor attributes in craft beers (Aguiar-Cervera et al., 2024). Saerens et al. (2010) demonstrated significant variations in ester and higher alcohol production among different ale and lager yeast strains, highlighting the potential of strain selection in modulating beer flavor profiles. However, the fermentation performance and metabolic responses of brewing yeast strains in the presence of daylily flower adjuncts have not been systematically investigated.

To address these research gaps, the present study evaluates the effects of four representative *Saccharomyces* yeast strains (*S. pastorianus* W-34/70 and S-189; *S. cerevisiae* WB-06 and S-04) on the physicochemical properties, antioxidant activities, and metabolomic profiles of craft beer produced with 5 % daylily flower powder. We hypothesize that the addition of daylily flowers as an adjunct will significantly enhance the flavor complexity and bioactive compound content of the resulting beers, with these effects varying depending on the yeast strain employed. By conducting a comprehensive analysis of fermentation performance, volatile compound profiles, and metabolomic responses, this study provides insights into the complex interactions between adjunct ingredients and yeast metabolism in craft beer production. The findings aim to contribute to the development of innovative beer formulations with enhanced sensory and functional attributes, expanding the range of options available to craft brewers and consumers alike.

2. Materials and methods

2.1. Materials

Pilsner base malt with 10.5 % protein and 4.5 % moisture was obtained from Euromalt (Baoding) Malt Co., Ltd. (Baoding, China). Vacuum freeze-dried daylily flowers from the Datong Huanghua cultivar, collected in the Datong region of Shanxi Province, China, were processed into an 80-mesh powder and provided by Shanxi Binghua Food Technology Co., Ltd. (Taiyuan, China). Four representative yeast strains were obtained from the Lesaffre Group (Marcq-en-Baroeul, France). They included two *Saccharomyces pastorianus* lager strains W-34/70 (Weihenstephan, Germany) and S-189 (Hürlimann, Switzerland) and two *Saccharomyces cerevisiae* ale strains WB-06 (for wheat beer) and S-04 (for American and British ale styles).

2.2. Craft beer brewing process

Pilsner base malt served as the primary ingredient, and daylily powder was added at 5 % of the malt weight. Wort was prepared using the modified congress method (EBC procedure 4.5.1). The malt was milled to a particle size between 0.2 mm and 2.0 mm and mixed with tap water at a 1:5 ratio. Mashing temperatures were maintained at 48 ± 0.5 °C for 45 min, 63 ± 0.5 °C for 45 min, 72 ± 0.5 °C for 20 min, and 78 ± 0.5 °C for 10 min. The separated wort was boiled at 100 °C for 60 min and then rapidly cooled to the fermentation temperature.

Freeze-dried yeast strains were rehydrated in sterile wort at 25 °C for 24 h, then grown in 100 mL of sterile 12°P wort at 20 °C and 120 rpm for 48 h in a fermentation vessel. Yeast cells in logarithmic growth phase were inoculated at 1×10^6 cells per mL. Primary fermentation proceeded at 20 ± 0.5 °C for 12 days, followed by maturation at 4 ± 0.5 °C for 14 days. The finished beer was filtered through a 0.45 μm polyethersulfone membrane and stored at 4 °C until analysis.

2.3. Physicochemical and antioxidant activity analysis

Beer color parameters were measured using a Konica Minolta CR-400 colorimeter (Konica Minolta, Japan), based on the CIELAB color space (L^*, a^*, b^*). Alcohol content was determined by distillation, original gravity by refractometry, and the degree of fermentation by carbohydrate attenuation ratios, following the EBC Analytica (EBC - European Brewery Convention Analysis committee, 2010). Total soluble solids content was measured with a digital refractometer (PAL-1, Atago, Tokyo, Japan) and quantified in °Brix.

Quality parameters were measured using commercial assay kits (Suzhou Michy Biomedical Technology Co., Ltd., Suzhou, China) in accordance with the manufacturer's instructions. These parameters included reducing sugars (M1506A, with glucose as standard) and total sugars (M1504A, with glucose as standard), ascorbic acid (M0401A), total flavonoids (M0119A, with rutin as standard), total phenolics (M0118A, with gallic acid as standard), and anthocyanins (M0126A). Antioxidant activity was assessed using three different methods: ferric reducing antioxidant power (FRAP, M0111A), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, M0112A), and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, M0113A).

2.4. Non-targeted metabolomics analysis

Non-targeted metabolomics was performed using a TripleTOF 6600+ mass spectrometer (SCIEX, Framingham, MA, USA) connected to an LC-30 A Ultra-High Performance Liquid Chromatography system (Shimadzu Corporation, Kyoto, Japan). Beer samples were freeze-dried and extracted with 70 % methanol containing internal standards. Separation was carried out on a Waters ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA) at 40 °C. The mobile phase consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), with a flow rate of 0.40 mL/min. The gradient program started at 95 % A (0 min), decreased to 35 % A (5 min), lowered to 1 % A (6–7.5 min), and then returned to initial conditions.

Mass spectrometry was performed in both positive and negative ion modes, with electrospray ionization voltages of 5000 V and 4000 V. The ion source temperature was 550 °C, Gas1 and Gas2 pressures were set to 50 psi and 60 psi, and curtain gas was 35 psi. Data were preprocessed using XCMS for peak extraction, K-Nearest Neighbors (KNN) for missing values, and Support Vector Regression (SVR) for peak area correction. Metabolite identification relied on a database match with a comprehensive identification score threshold of 0.5.

2.5. Volatile compound analysis

An Agilent 8890-7000D GC–MS/MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) was used for volatile compound analysis. Solid-Phase Microextraction (SPME) was conducted using a 120 μm Divinylbenzene Carboxen Polydimethylsiloxane Arrow fiber (CTC Analytics AG, Zwingen, Switzerland). Beer samples (0.2 mL) were mixed with NaCl and an internal standard, then extracted at 60 °C for 15 min. The GC temperature program was as follows: initial temperature of 40 °C held for 3.5 min, increased to 100 °C at 10 °C/min, then to 180 °C at 7 °C/min, and finally to 280 °C at 25 °C/min and held for 5 min. Mass spectrometry was performed using Electron Ionization (70 eV) in Selected Ion Monitoring mode.

2.6. Statistical analysis

All experiments were carried out in triplicate, and the results are presented as mean ± standard deviation. Statistical analysis was performed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA) with Duncan's Multiple Range Test at a significance level of $p < 0.05$. Multivariate techniques, including heatmaps, Principal Component Analysis (PCA), and radar charts, were conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Differential metabolites were defined based on $|\text{Log}_2$ Fold Change$| \geq 1.0$ and were mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for enrichment analysis (Kanehisa & Goto, 2000).

3. Results and discussion

3.1. Color characteristics and fermentation properties

3.1.1. Luminosity

The luminosity (L-value) of daylily-supplemented craft beers varied significantly with the yeast strain (Fig. 1A). The strain with the highest L-value (52.16), indicating a lighter color, was contrasted with the strain with the lowest value (46.23), which resulted in a darker beer appearance ($p < 0.05$). These strain-dependent differences align with previous studies, as several factors influence color variations, such as pigment precursor composition and metabolic pathways (Paszkot et al., 2023). In beers fermented with certain strains, higher β-glucosidase activity may release bound flavonoids, while specific enzymes could promote flavonoid polymerization and precipitation (Harbour, 2023). Moreover, increased acetaldehyde production in some strains may enhance Maillard reactions, forming melanoidins that contribute to a darker color.

3.1.2. Red and yellow values

The a-value (redness) and b-value (yellowness) of the daylily-supplemented craft beers varied significantly among the yeast strains tested (Fig. 1B, C). W-34/70 produced beers with the highest a-value (4.98), while WB-06 yielded the lowest (2.01) ($p < 0.05$). For yellowness, WB-06-fermented beers showed the highest b-value (12.80), and those fermented with S-189 exhibited the lowest (11.02) ($p < 0.05$).

These results suggest that yeast strain selection can significantly affect the color profile of daylily-supplemented craft beers. The observed differences in redness and yellowness are likely due to variations in yeast metabolism and interactions with color-related compounds derived from the daylily and other ingredients. Previous studies have indicated that different yeast strains can influence the color of fermented beverages through their interactions with anthocyanins and other polyphenolic compounds (Postigo et al., 2022). Anthocyanins, which are water-soluble pigments responsible for red, purple, and blue colors in plants, are known to be present in daylily flowers (Guo et al., 2024). The varying ability of yeast strains to absorb, metabolize, or modify these pigments during fermentation could contribute to the observed differences in beer. Additionally, the production of yeast-derived metabolites, such as esters and phenolic compounds, can also influence the color of fermented beverages (Resende Oliveira et al., 2018). The synthesis of these compounds is dependent on the metabolic pathways and enzyme activities of the yeast strains employed.

3.1.3. Soluble solid, total sugar, and reducing sugar content

The soluble solid content in beers fermented by different yeast strains ranged from 5.73 % to 6.13 % (Fig. 1D). Total sugar content ranged from 31.10 to 37.31 mg/mL, while reducing sugar content varied from 9.33 to 11.29 mg/mL, showing significant differences among strains (Fig. 1E, F). The beer fermented by WB-06 had the highest total sugar (37.31 mg/mL) and reducing sugar (11.29 mg/mL) contents. In contrast, the beers fermented by S-189 and S-04 had the lowest total sugar (31.10 mg/mL) and reducing sugar (9.33 mg/mL) contents, respectively ($p < 0.05$).

These results indicate that different yeast strains have distinct sugar utilization patterns and metabolic pathways. The higher total and reducing sugar contents in WB-06 fermented beer may be related to its unique metabolic strategies, including the efficiency of sugar transport systems and the diversity of metabolic pathways. Studies have shown that the hexose transporter in yeast plays a key role in active sugar transport, and its expression level and activity directly affect sugar utilization efficiency (Boles & Oreb, 2018). Moreover, brewing yeasts can utilize sugar substrates through multiple metabolic pathways, such as the pentose phosphate pathway and the Leloir pathway. Variations in these pathways contribute to differences in residual sugar levels (Endalur Gopinarayanan & Nair, 2019).

Although the reducing sugar content is usually positively correlated

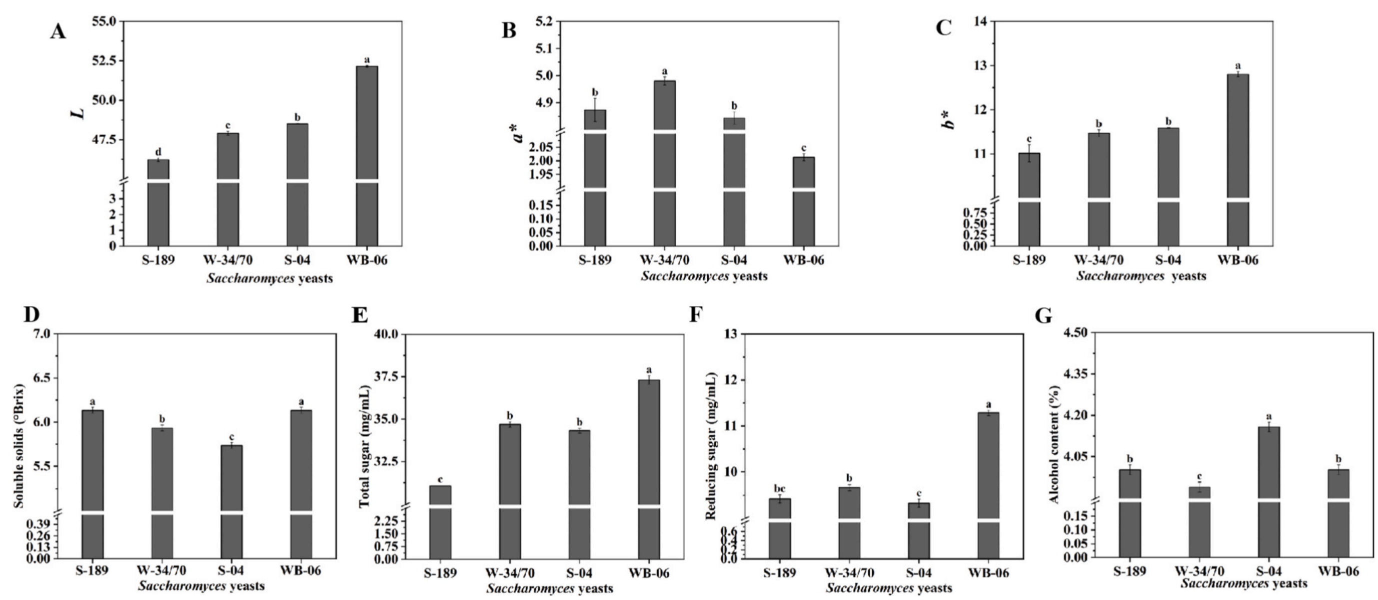

Fig. 1. Color characteristics and fermentation properties of daylily-supplemented craft beers fermented with different *Saccharomyces* yeast strains. (A) Luminosity(L-value), (B) a-value (redness), (C) b-value (yellowness), (D) Soluble solid content, (E) Total sugar content, (F) Reducing sugar content, and (G) Alcohol content. Data represent the mean ± standard deviation of three replicate experiments. Different letters above the bars indicate significant differences among yeast strains ($p < 0.05$).

with the degree of polysaccharide hydrolysis, the residual reducing sugars in WB-06 fermented beer are more likely derived from incompletely consumed maltose rather than incomplete polysaccharide degradation. During beer fermentation, yeasts preferentially utilize low molecular weight sugars such as glucose and maltose, while the utilization of polysaccharides like starch and β-glucan is relatively limited. The presence of residual reducing sugars in WB-06 fermented beer suggests distinctive metabolic regulation patterns of this strain.

3.1.4. Alcohol content

The alcohol content of the beers produced by different yeast strains ranged from 3.94 % to 4.16 % (Fig. 1G). *Saccharomyces cerevisiae* S-04 produced the highest alcohol level (4.16 %), while *Saccharomyces pastorianus* W-34/70 yielded the lowest (3.94 %). These results are consistent with previous studies that have reported higher ethanol yields in *S. cerevisiae* strains compared to other *Saccharomyces* species (Gallone et al., 2016; Langdon et al., 2019).

S-04's superior alcohol production capacity may be attributed to its high attenuation potential (74–82 %), temperature tolerance (15–20 °C), and ability to withstand higher ethanol and CO_2 concentrations during fermentation. Enhanced aldehyde dehydrogenase activity in S-04 may also contribute to the efficient conversion of acetaldehyde to ethanol, although the relationship between this enzyme and ethanol production is complex (Shortall et al., 2021).

3.2. Bioactive components and antioxidant activity

3.2.1. Total phenolics and flavonoids

The selection of yeast strain had a significant impact on the bioactive compound profiles in daylily-supplemented beers (Fig. 2A, B). Strains S-189 and WB-06 exhibited superior phenolic compound production, yielding 0.59 mg/mL and 0.60 mg/mL respectively. These were significantly higher ($p < 0.05$) than those observed in the S-04 strain, which produced 0.46 mg/mL of phenolic compounds.

Notably, the phenolic content achieved with S-189 and WB-06 in daylily-supplemented beers substantially surpassed the contents

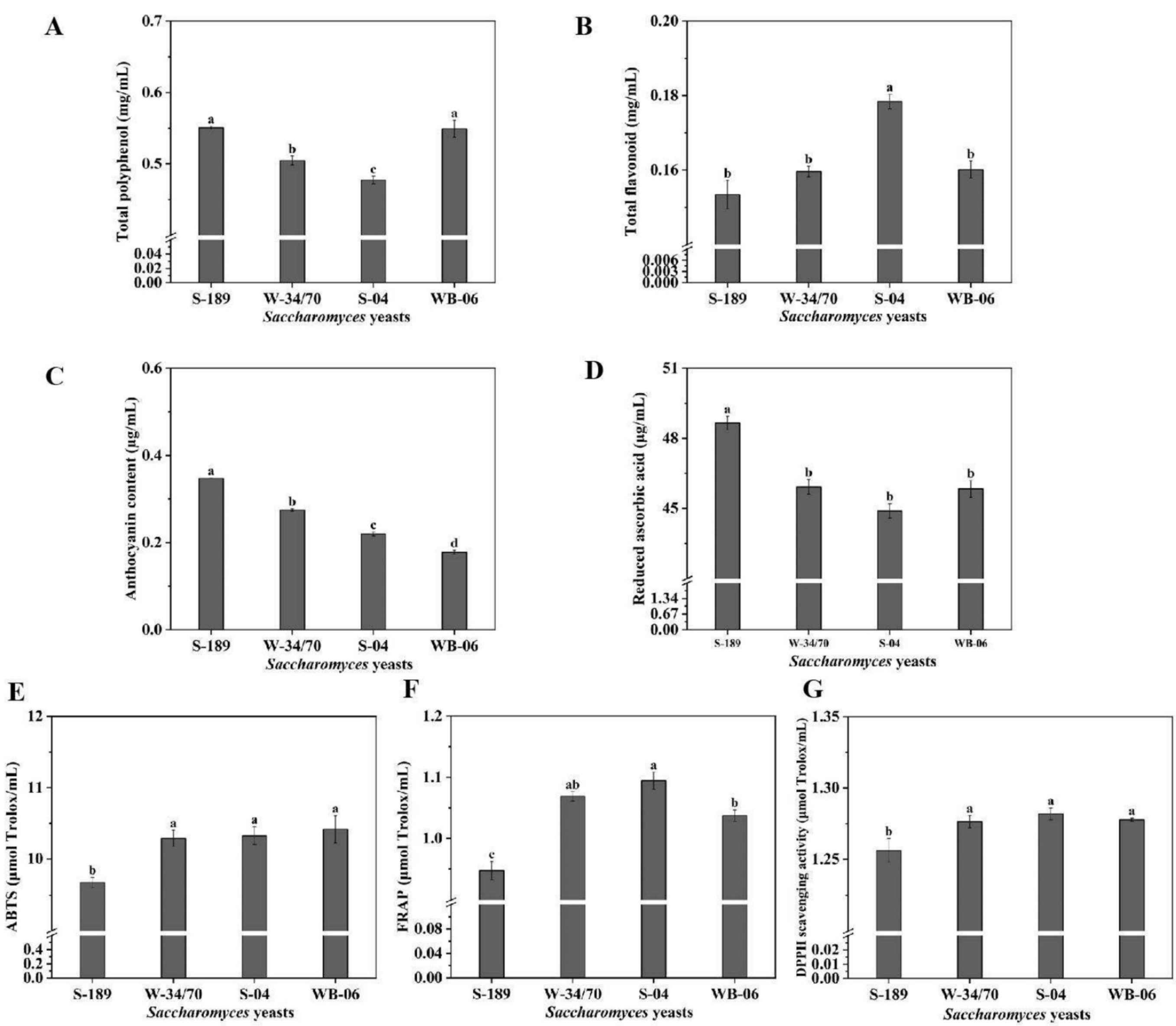

Fig. 2. Bioactive components and antioxidant activity of daylily-supplemented craft beers fermented with different *Saccharomyces* yeast strains. (A) Total phenolics, (B) Flavonoids, (C) Anthocyanins, (D) Ascorbic acid, (E) ABTS antioxidant activity, (F) FRAP antioxidant activity, and (G) DPPH radical scavenging activity. Data represent means ± standard deviation from three replicate experiments. Different letters above the bars indicate significant differences among yeast strains ($p < 0.05$).

typically reported in commercial beers, which range from 0.15 to 0.34 mg/mL (Zhao et al., 2010). This comparison emphasizes the remarkable effect of daylily supplementation in enhancing the phenolic profile of the final product when fermented with appropriate yeast strains. The elevated phenolic content in beers produced with S-189 and WB-06 suggests these strains have superior polyphenol hydrolysis capabilities, likely mediated by heightened β-glucosidase activity (Muradova et al., 2023). This feature could potentially contribute to improved antioxidant properties and distinctive flavor characteristics in the resulting beers.

In contrast, the S-04 strain demonstrated the highest flavonoid concentration at 0.18 mg/mL, slightly exceeding the levels achieved by S-189 (0.16 mg/mL), W-34/70, and WB-06 (both 0.14 mg/mL). While the flavonoid content in daylily-supplemented beers fermented with these strains was not as markedly elevated compared to commercial beers as the phenolic content, the results still indicate the potential for daylily to enhance the flavonoid profile of the final product.

3.2.2. Anthocyanins and ascorbic acid

S-189 fermented beers demonstrated superior retention of bioactive compounds, achieving the highest concentrations of both anthocyanins (0.35 μg/mL) and ascorbic acid (48.65 μg/mL) (Fig. 2C, D). These values were significantly higher ($p < 0.05$) than those obtained with other strains. The enhanced anthocyanin preservation in S-189 fermentations likely results from an optimal balance between glycosidase activity and oxidative enzyme expression(Lin et al., 2023). Furthermore, the elevated levels of ascorbic acid may be attributed to strain-specific retention mechanisms or limited degradation by ascorbate peroxidase (Zandi & Schnug, 2022).

3.2.3. Antioxidant activity

The evaluation of antioxidant capacity through ABTS, FRAP, and DPPH assays revealed distinct patterns among different yeast strains in daylily-supplemented beers (Fig. 2E, F, G). W-34/70, S-04, and WB-06 fermentations demonstrated significantly higher ABTS radical scavenging capacity (10.29–10.42 μmol Trolox/mL) compared to S-189 (9.67 μmol Trolox/mL, $p < 0.05$). In the FRAP analysis, S-04 exhibited the highest reducing power (1.09 μmol Trolox/mL), followed by W-34/70 (1.07 μmol Trolox/mL) and WB-06 (1.04 μmol Trolox/mL), with S-189 showing significantly lower activity (0.95 μmol Trolox/mL, $p < 0.05$). These strain-specific variations align with findings from previous studies in American Pale Ale (APA) craft beer production, where yeast strain selection was shown to significantly influence antioxidant properties (Viana et al., 2021).

The DPPH assay results showed relatively minor differences among strains, with W-34/70, S-04, and WB-06 fermentations (approximately 1.28 μmol Trolox/mL) slightly exceeding S-189 (1.26 μmol Trolox/mL). This pattern of varying responses across different antioxidant assays is consistent with research in APA craft beer, where each antioxidant methodology assessed specific chemical actions and produced results on different scales. The contrasting performance of S-189 across different assays suggests that strain-specific metabolic characteristics differently affect various antioxidant mechanisms, similar to how M15 yeast exhibited lower antioxidant capacity in ABTS and FRAP assays compared to US-05 in APA craft beer production (Viana et al., 2021).

In the present study, differences in antioxidant capacity among yeast strains can likely be attributed to their varying abilities to preserve and transform phenolic compounds during fermentation. This finding is consistent with research on APA craft beer, where enhanced antioxidant capacity was associated with higher concentrations of specific compounds such as epicatechin gallate and procyanidins in beers fermented with US-05 strain compared to M15 strain (Viana et al., 2021). The results demonstrate that yeast strain selection significantly influences the functional properties of specialty beers, particularly their antioxidant characteristics, which is crucial for developing beers with enhanced health-beneficial properties.

3.3. Non-targeted metabolomic analysis

3.3.1. Global heatmap analysis and OPLS-DA analysis

The non-targeted metabolomic analysis was conducted to assess the metabolic profiles of four brewing yeast strains (S-189, W-34/70, S-04, and WB-06) used in the production of daylily-supplemented beer. The global heatmap clearly reveals substantial differences in the metabolic patterns across the yeast strains, indicating distinct quantitative variations in key metabolite groups, such as amino acids and derivatives, benzene derivatives, organic acids, and phenolic acids (Fig. 3A). The heatmap highlights the unique metabolic traits of each yeast strain, emphasizing how these differences may influence the flavor and aroma profiles of daylily-supplemented beers. By selecting yeast strains with distinct metabolic profiles, brewers can tailor the flavor complexity of their beers, enhance specific sensory attributes, and even optimize stability and mouthfeel.

S-189 is notably enriched in amino acids and peptides, which are compounds well known for contributing to savory umami flavors, thereby enhancing the complexity and depth of the beer's taste. This suggests that beers brewed with S-189 may have a more umami-rich profile. Such flavor enhancement could make S-189 an excellent choice for brewers aiming to develop beers with richer, more savory taste profiles, such as wheat beers or other styles where complexity and depth are essential. Conversely, WB-06 is characterized by a significant production of phenolic acids, which are linked to astringency, bitterness, and mouthfeel. These attributes are critical in beer styles like IPAs or stouts, where pronounced bitterness or a robust mouthfeel is desired (Lentz, 2018). Therefore, WB-06 may impart a more astringent and bitter character to the beer, making it an ideal strain for brewers looking to create beers with a stronger, more intense finish. W-34/70 and S-04 display relatively balanced metabolic profiles, providing a broader spectrum of flavor production. These strains are versatile, enabling brewers to create beers with a variety of flavor profiles, depending on the fermentation conditions and adjuncts used. For example, these strains may be ideal for brewers who want flexibility in flavor modulation or for creating balanced beers that do not overly emphasize bitterness or sweetness. Selecting these yeast strains allows brewers to craft beers that appeal to a wide range of consumers by offering complexity without overwhelming any single flavor note.

OPLS-DA S-plot analysis was performed to identify potential biomarkers distinguishing the metabolic profiles of the yeast strains (Figs. 3B–G). The S-04 vs S-189 plot (Fig. 3B) shows clear separation, with S-189 exhibiting higher levels of amino acids and peptides that may contribute to enhanced savory flavors, while S-04 has a more balanced metabolite composition. The W-34/70 vs S-189 plot (Fig. 3C) reveals subtle differences, with S-189 showing slightly higher amino acid abundance. In contrast, the W-34/70 vs S-04 plot (Fig. 3D) indicates a reciprocal relationship, with S-04 favoring organic acids (associated with tartness and acidity) and W-34/70 enriched in phenolic compounds (contributing to bitterness and astringency). WB-06 exhibits marked differences compared to S-189 and S-04 (Fig. 3E and F), with elevated levels of phenolic acids that can impart bitterness and astringency. The WB-06 vs W-34/70 plot (Fig. 3G) further highlights WB-06's unique profile, characterized by higher levels of phenolic and benzene derivatives, contributing to its distinctive bitterness.

These findings demonstrate how strain-specific metabolic differences can be leveraged to influence the sensory properties of daylily-supplemented beers. By selecting specific yeast strains based on their metabolic traits, brewers can create beers with desired flavor profiles, such as emphasizing savory umami notes (S-189), increasing bitterness and astringency (WB-06), or producing a balanced flavor spectrum (S-04 and W-34/70). This targeted approach enables brewers to optimize their recipes for specific beer styles, enhancing both sensory appeal and functional properties.

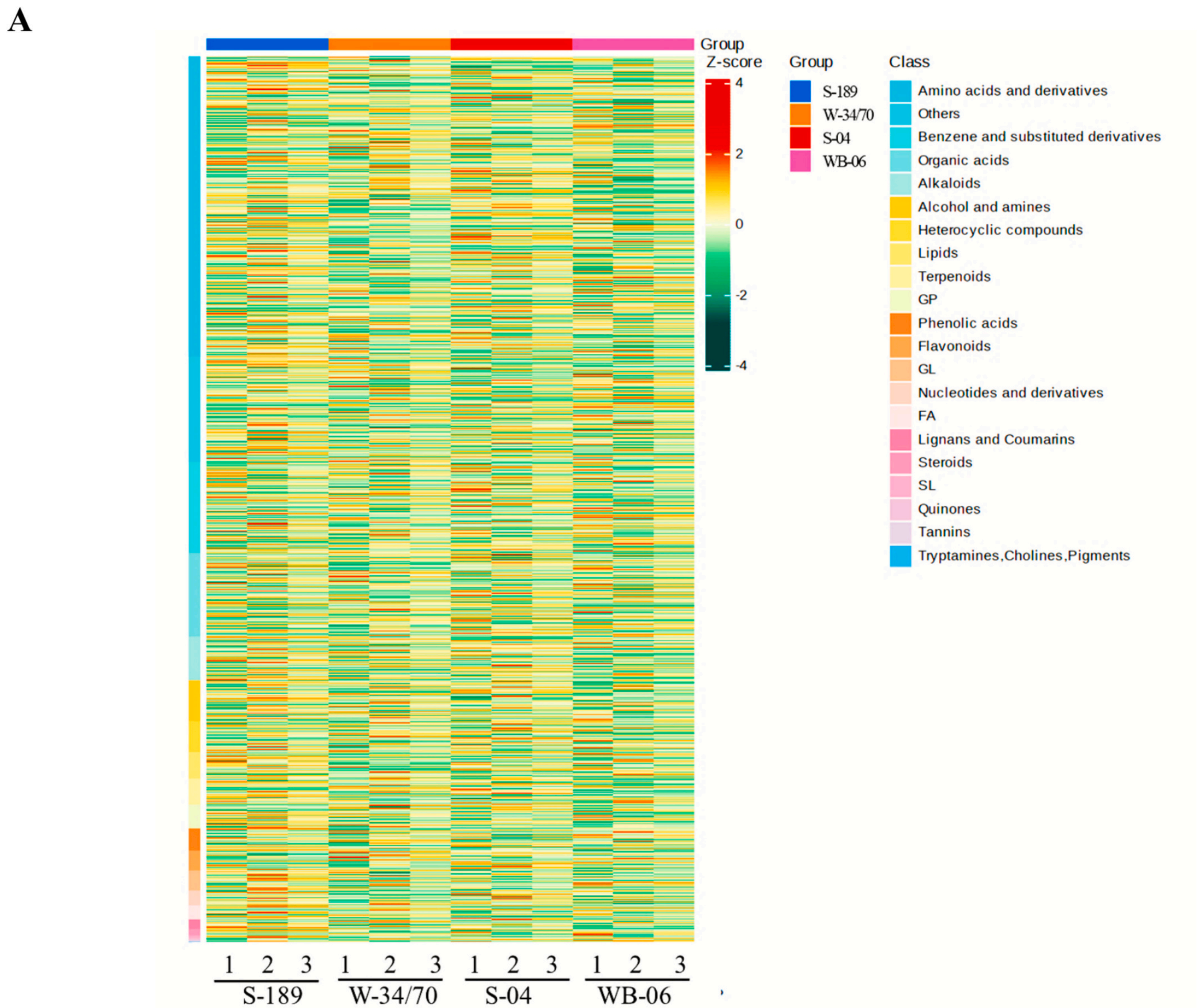

Fig. 3. Non-targeted metabolomic analysis of daylily-supplemented beers fermented with different *Saccharomyces* yeast strains. (A) Global heatmap analysis of metabolite abundance across strains and replicates. OPLS-DA S-plot analysis comparing (B) S-04 vs S-189, (C) W-34/70 vs S-189, (D) W-34/70 vs S-04, (E) WB-06 vs S-189, (F) WB-06 vs S-04, and (G) WB-06 vs W-34/70.

3.3.2. Differential metabolite abundance and KEGG pathway enrichment analysis

Metabolic differences between yeast strains were analyzed, revealing that over 90 % of metabolites did not show significant variations, indicating a high degree of conservation in the core metabolic networks of yeast. However, several specific metabolic pathways exhibited notable strain-dependent differences. Compared to the S-04 strain, S-189 showed a downregulation of 753 metabolites (Fig. 4A), suggesting that S-189 may possess more efficient substrate utilization or stricter regulation of biosynthetic pathways (Pires et al., 2014). WB-06 exhibited numerous upregulated metabolites, particularly in comparison with S-189 (363 metabolites) and W-34/70 (489 metabolites) (Fig. 4E, F), suggesting that WB-06 may have an advantage in specific synthetic pathways or stress response mechanisms. These metabolic differences in WB-06 could make it a suitable strain for producing beers with complex and robust flavor profiles, especially under stressful fermentation conditions that require resilience, such as high alcohol concentrations or fluctuating temperatures.

KEGG pathway enrichment analysis further highlighted significant differences between strains in central carbon metabolism, amino acid biosynthesis, lipid metabolism, and secondary metabolism (Figs. 4G–L). S-04 was enriched in histidine metabolism (Rich factor 0.5, $p < 0.05$) and β-alanine metabolism (Rich factor 0.6, $p < 0.05$) compared to S-189 (Fig. 4G). This suggests that S-04 may have higher nitrogen assimilation efficiency and amino acid synthesis capacity, which could lead to enhanced fermentation performance and more distinct flavor characteristics, such as umami or savory notes in the final beer. Enhanced histidine and β-alanine metabolism could also result in desirable sensory and functional properties in the final product, such as a more rounded flavor profile and improved mouthfeel (Zhou et al., 2025). Brewers could therefore select S-04 for beer styles where a balanced and savory profile is preferred, such as in wheat beers or lighter ales.

The improved lipid composition may benefit the mouthfeel and shelf stability of the beer, particularly in beers where a smooth and full-bodied mouthfeel is desired, such as stouts or porters. The enhanced lipid composition may also play a role in preserving the beer's stability,

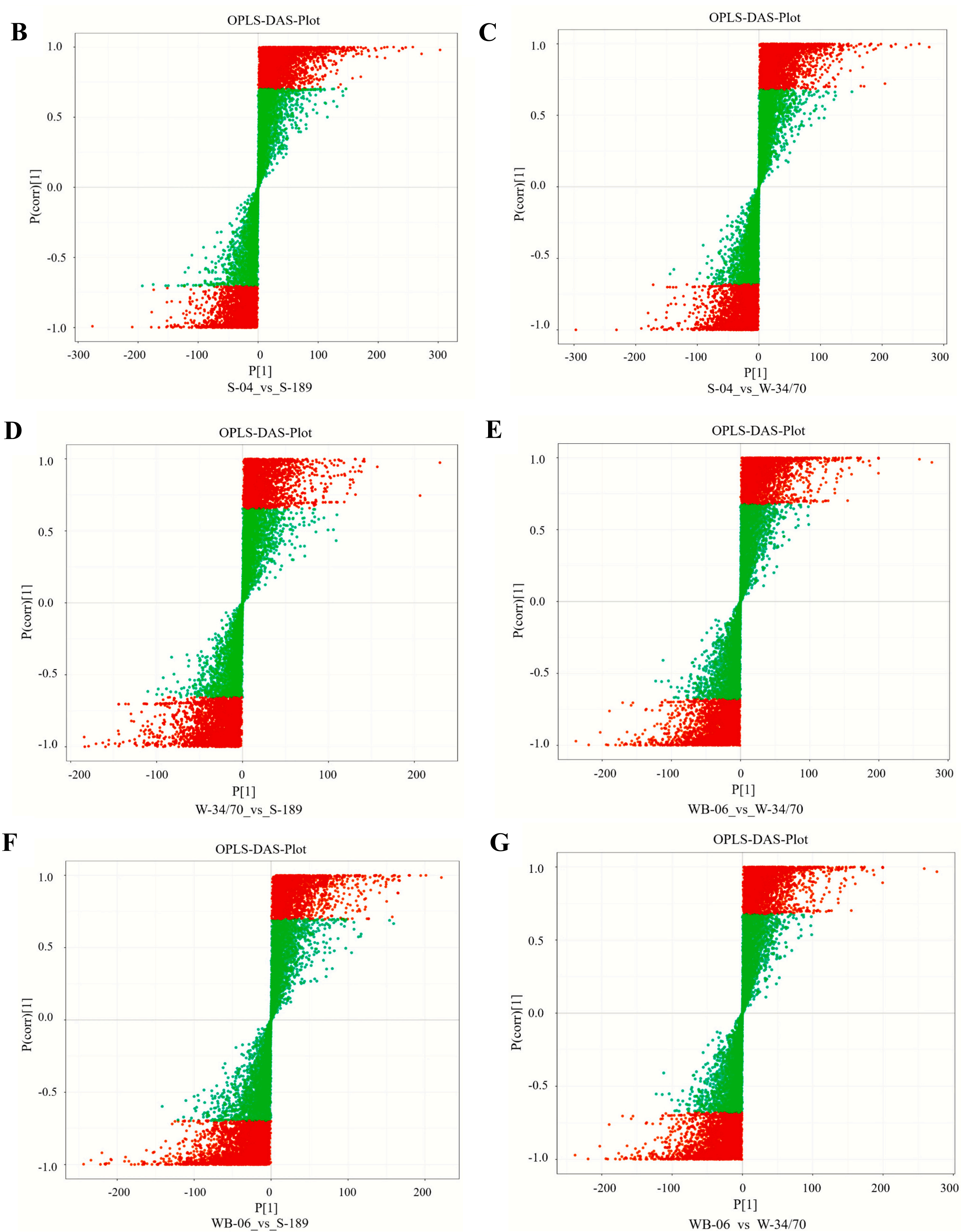

Fig. 3. (*continued*).

contributing to a more consistent product during storage (de Lima et al., 2022). In contrast, WB-06 showed significant enrichment in β-lactam antibiotic biosynthesis (Rich factor 0.9, $p < 0.01$) and pentose phosphate pathway (Rich factor 0.7, $p < 0.05$) compared to S-189 (Fig. 4K). Active pentose phosphate pathway metabolism supports NADPH production, which is crucial for biosynthesis and antioxidant processes, potentially

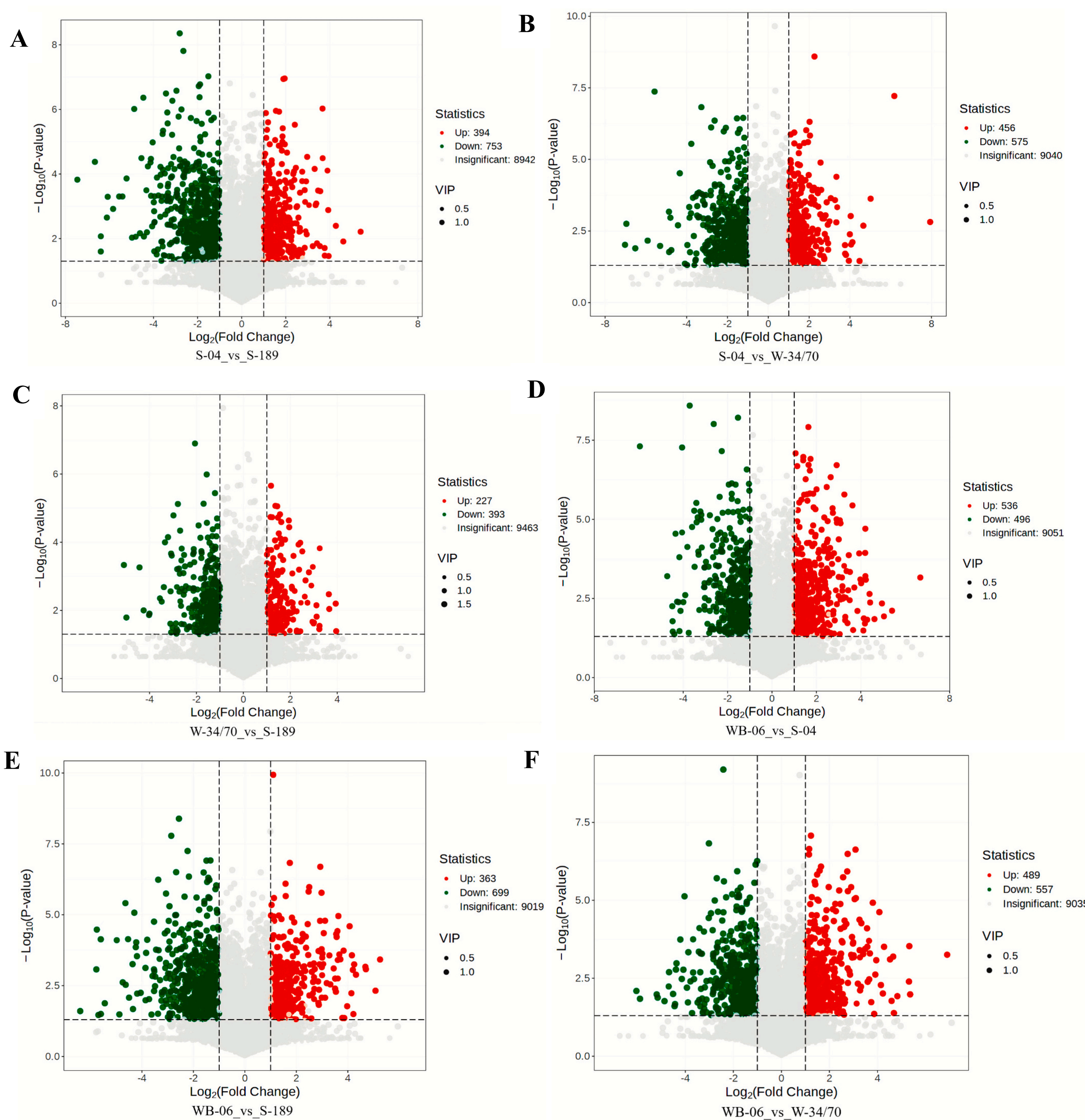

Fig. 4. Volcano plot analysis and KEGG pathway enrichment of metabolomic data comparing yeast strains used in daylily-supplemented beer fermentation. Volcano plots (A–F) compare S-04 vs S-189 (A), S-04 vs W-34/70 (B), W-34/70 vs S-189 (C), WB-06 vs S-04 (D), WB-06 vs S-189 (E), and WB-06 vs W-34/70 (F). KEGG pathway enrichment (G–L) compares S-04 vs S-189 (G), S-04 vs W-34/70 (H), W-34/70 vs S-189 (I), W-34/70 vs S-04 (J), WB-06 vs S-189 (K), and WB-06 vs W-34/70 (L). Metabolites and pathways with p-value < 0.05 are regarded as significantly enriched.

benefiting the beer's flavor stability and oxidation resistance (Bertels et al., 2021). This could be especially advantageous for brewers looking to create beers with a longer shelf life without sacrificing freshness or flavor integrity.

Furthermore, WB-06 also demonstrated enrichment in several pathways compared to W-34/70, including histidine metabolism, β-alanine metabolism, and the pentose phosphate pathway (Fig. 4K, L). These findings suggest that WB-06 may possess unique capabilities in amino acid biosynthesis and oxidative stress responses, which could further contribute to the development of complex flavor profiles and improved fermentation performance. Brewers might consider WB-06 for producing specialty beers where complex flavors, such as bitter, astringent, and umami notes, are desired, as well as beers that require strong fermentation resilience and a prolonged shelf life.

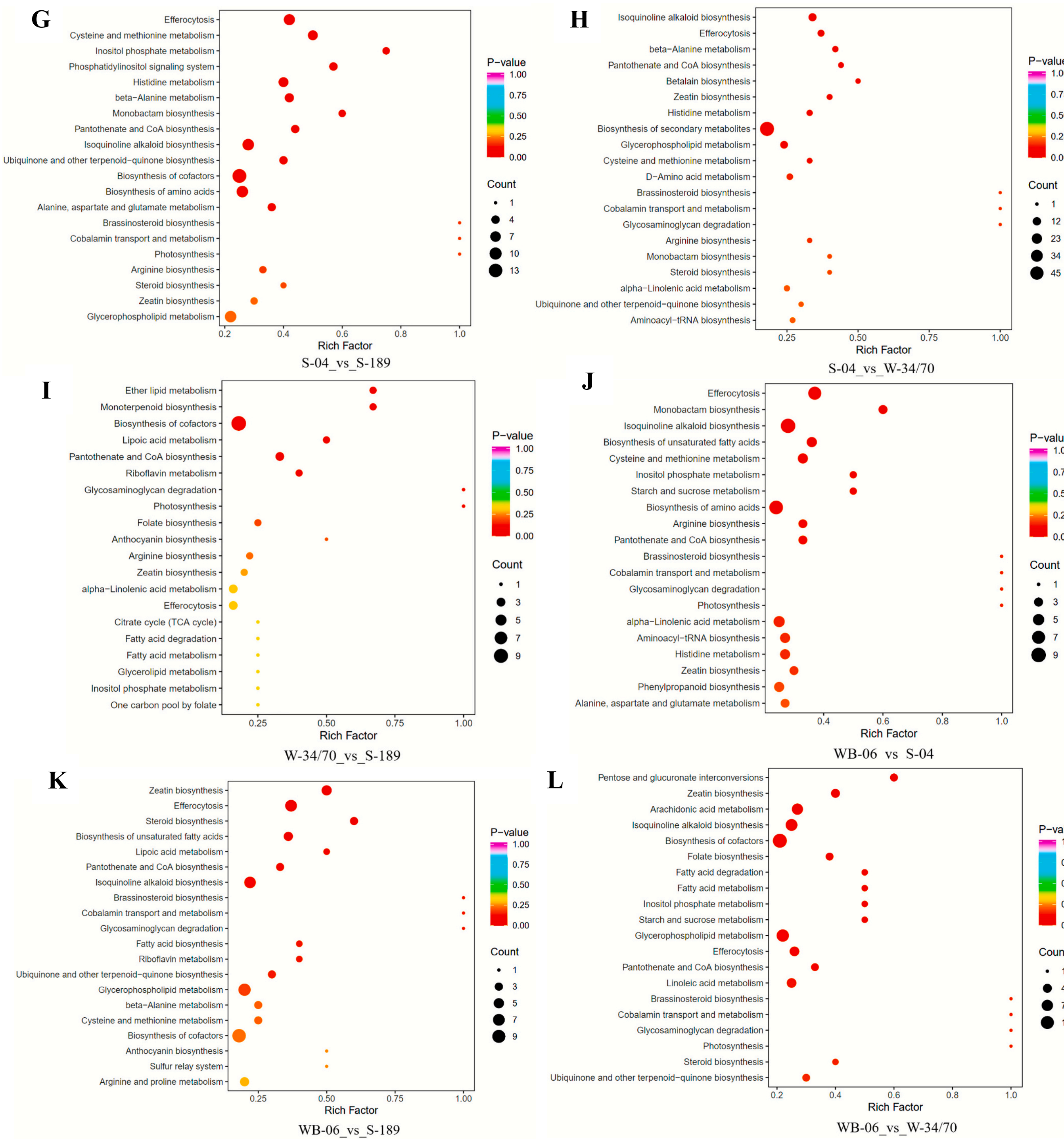

Fig. 4. (*continued*).

3.4. Volatile metabolomic analysis

3.4.1. Clustering heatmap analysis and principal component analysis

The hierarchical clustering heatmap (Fig. 5A) reveals clear variations in the levels of different classes of volatile metabolites produced by the four strains during the fermentation of daylily-supplemented beer. S-189 exhibited the highest relative abundance of flavor-active compounds, particularly esters and terpenes, which are crucial for imparting fruity, floral, and spicy notes to the beer (Holt et al., 2019). These compounds are particularly important in beers where complex aromas are desired, such as fruit-forward or aromatic styles like IPAs. The higher ester and terpene concentrations in S-189 suggest that it may be an ideal choice for brewers aiming to create beers with bold, aromatic profiles. In contrast, WB-06 showed elevated concentrations of heterocyclic compounds and higher alcohols, which are typically associated with malty, solvent-like, and spicy flavors. These metabolic differences suggest that S-189 may be more suitable for producing ester- and terpene-rich beers with complex aromatic profiles, while WB-06 could be more appropriate for brewing beers that benefit from higher alcohol or heterocyclic compound contributions.

The PCA score plot (Fig. 5B) offers further confirmation of the strain-specific metabolic differences observed in the heatmap. The first two

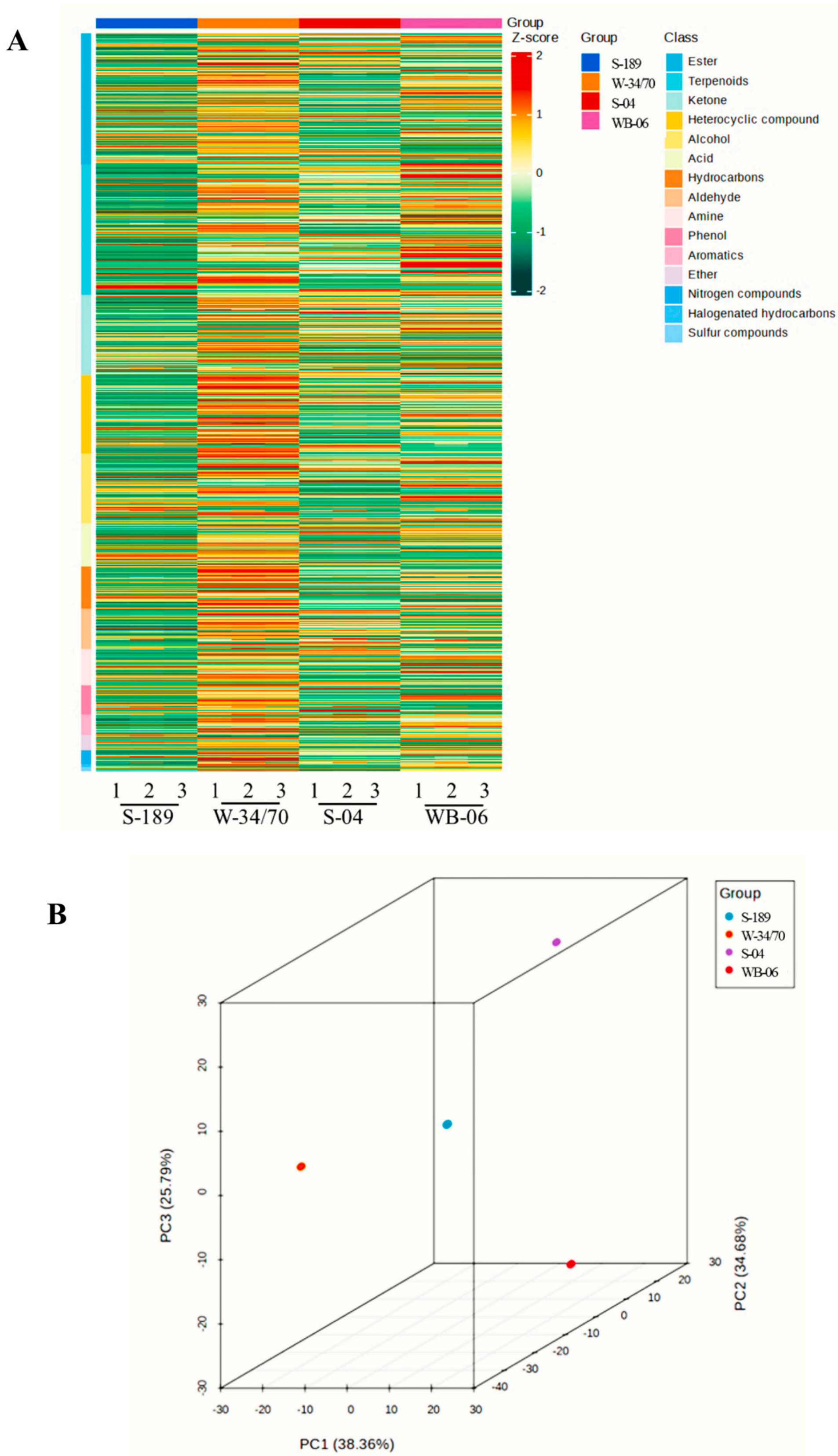

Fig. 5. Volatile metabolomic analysis of daylily-supplemented beers fermented with different yeast strains. (A) Hierarchical clustering heatmap of volatile metabolite. (B) PCA score plot of volatile metabolomic profiles.

principal components, PC1 and PC2, together account for 73.04 % of the total variance, which indicates that these two components capture most of the metabolomic differences among the samples. This highlights how strain-specific metabolic differences can directly influence the volatile composition and, ultimately, the flavor profile of daylily-supplemented beers. The clear clustering of samples fermented with the same yeast strain suggests that the metabolic profiles are strongly correlated with the genotypic traits of each strain (Monnin et al., 2024; Pires et al., 2014). This finding underscores the importance of strain selection in influencing the volatile composition and sensory attributes of daylily-supplemented beer. By choosing specific yeast strains, brewers can achieve particular flavor targets, whether enhancing aromatic complexity (S-189) or increasing robustness and maltiness (WB-06).

The distinct groupings observed in the PCA plot align with the patterns seen in the hierarchical clustering heatmap. S-189 and WB-06 are positioned at opposite ends of the plot, indicating their contrasting metabolite profiles. In contrast, W-34/70 and S-04 occupy intermediate positions, suggesting that these strains may offer more balanced or versatile flavor outcomes compared to the more specialized profiles of S-189 and WB-06.

3.4.2. Volcano plot and differential metabolite change analysis

The volcano plot analysis and differential metabolite fold change bar charts were employed to elucidate the strain-specific metabolic characteristics of four *Saccharomyces* strains used in the fermentation of daylily-supplemented beer. The volcano plots (Fig. 6A–F) reveal distinct

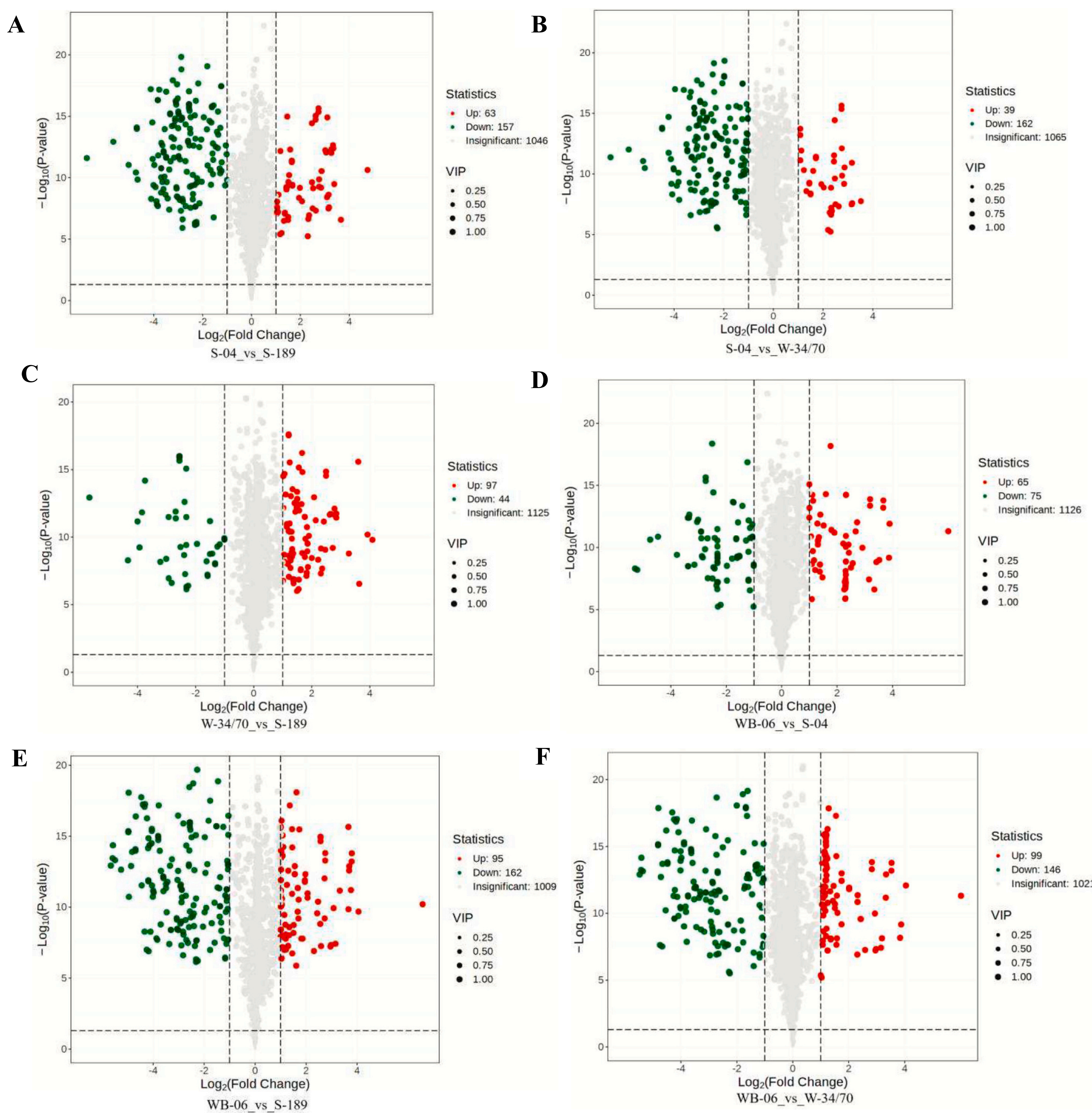

Fig. 6. Volcano Plots (A–F) and Differential Metabolite Fold Change Bar Charts (G–L) of the Yeast Strains Used in Daylily-Supplemented Beer Fermentations.

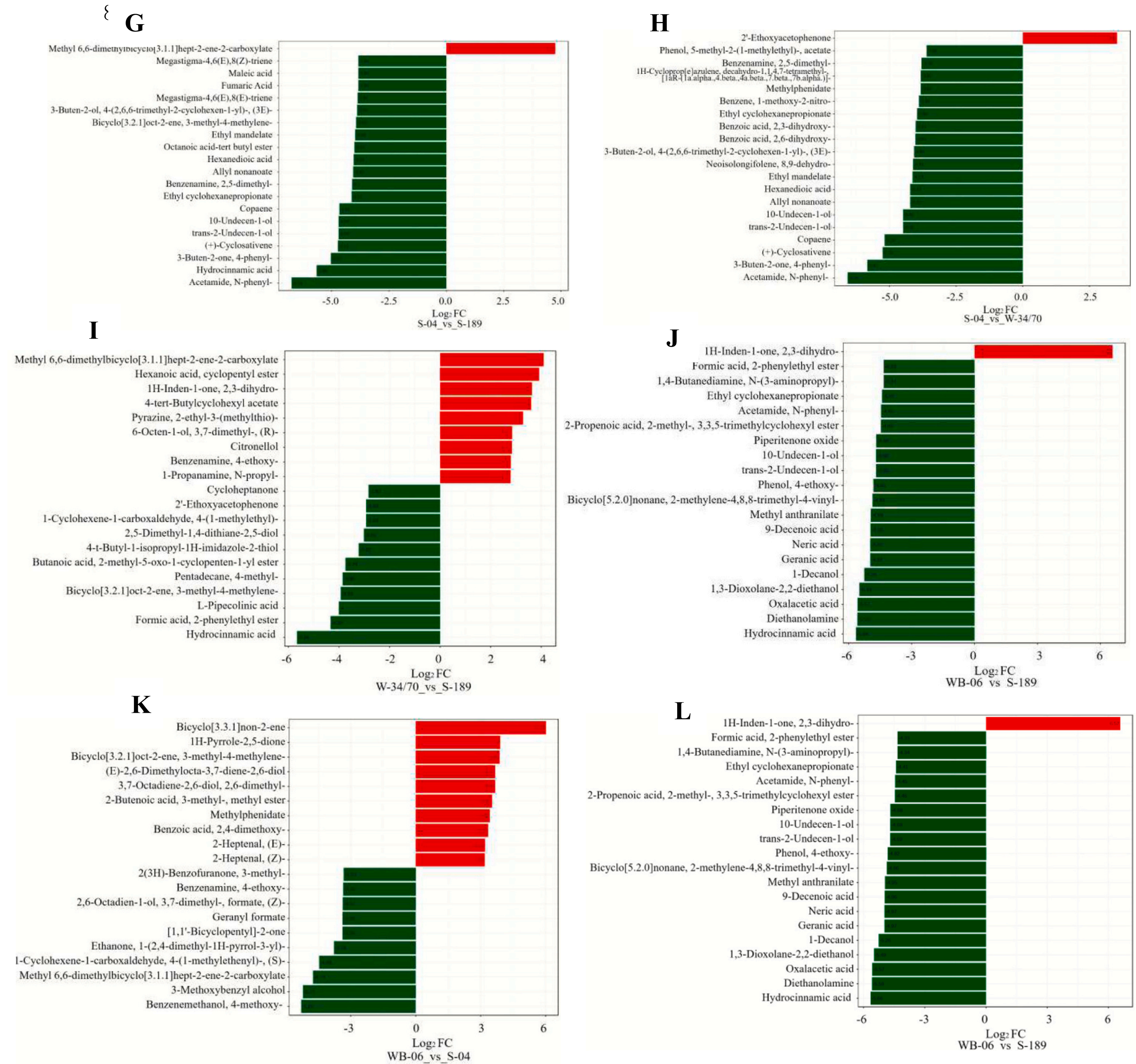

Fig. 6. (*continued*).

metabolic profiles among the strains. S-189 exhibited 157 significantly downregulated ($Log_2FC < -1$) and 63 upregulated ($Log_2FC > 1$) metabolites compared to S-04 (Fig. 6A). The downregulated metabolites showed high statistical significance ($-Log_{10}$ *P*-values up to 20), suggesting that S-189 may utilize different regulatory pathways than S-04, potentially impacting flavor and aroma compounds. This indicates that S-189 may produce beers with a more refined or subtle flavor profile, making it suitable for crafting beers with lighter, more aromatic characteristics. In the S-04 vs. W-34/70 comparison (Fig. 6B), 162 downregulated and 39 upregulated metabolites were observed in W-34/70, with most downregulated metabolites having high VIP values (>0.75). This suggests contrasting metabolic routes between the two strains, which could contribute to variations in fermentation performance and flavor profiles (Tian, Xiong, Yu, Chen, & Lou, 2023). W-34/70's ability to modulate fermentation metabolites could benefit brewers looking for a yeast strain that can create balanced, nuanced beers with subtle flavors and aromas. W-34/70 showed enhanced metabolic activity compared to S-189 (Fig. 6C), with 97 upregulated and 44 downregulated metabolites, many exhibiting Log_2FC values from 2 to 4. This suggests increased production of flavor compounds or stress tolerance in W-34/70. WB-06 consistently demonstrated unique metabolic characteristics across all comparisons (Fig. 6D–F), suggesting potential differences in pathway regulation compared to the other strains (Gibson et al., 2017). These unique characteristics may contribute to WB-06's potential for producing distinctive flavor profiles, especially those that require enhanced alcohol and heterocyclic compound contributions, which are valuable for crafting complex, full-bodied beers.

The differential metabolite fold change bar charts (Fig. 6G–L) provide quantitative insights into strain-specific metabolic patterns, highlighting changes in esters, alcohols, acids, and aldehydes that can impact the flavor balance of the final beer (Li et al., 2022). In the S-04 vs. S-189 comparison (Fig. 6G), Methyl 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-carboxylate was upregulated ($Log_2FC = 1.87$), while Hydrocinnamic acid was downregulated ($Log_2FC = -6.74$). This suggests that S-189 may favor the production of fruity and floral flavors, while S-04's metabolic pathway could contribute to a more balanced profile with subtle acidity. In the W-34/70 vs. S-189 comparison (Fig. 6I), 1H-Inden-1-one, 2,3-dihydro-Formic acid, 2-phenylethyl ester ($Log_2FC = 3.57$) and 1,4-Butanediamine, N-(3-aminopropyl)-Ethyl cyclohexanepropionate ($Log_2FC = 2.73$) were upregulated, which can contribute fruity, floral, or spicy aromas (Tian, Xiong, Yu, Chen, & Lou, 2023; Yuan et al., 2024). This underscores W-34/70's potential for creating daylily beers with more complex flavor profiles. The WB-06 vs. S-189 comparison (Fig. 6K) revealed increased 1H-Inden-1-one, 2,3-dihydro-Formic acid, 2-phenylethyl ester ($Log_2FC = 3.38$) and reduced Hydrocinnamic acid ($Log_2FC = -3.57$) in WB-06, highlighting its ability to enhance flavor-active esters while minimizing off-flavor precursors, potentially leading to improved taste stability in daylily beers. Brewers

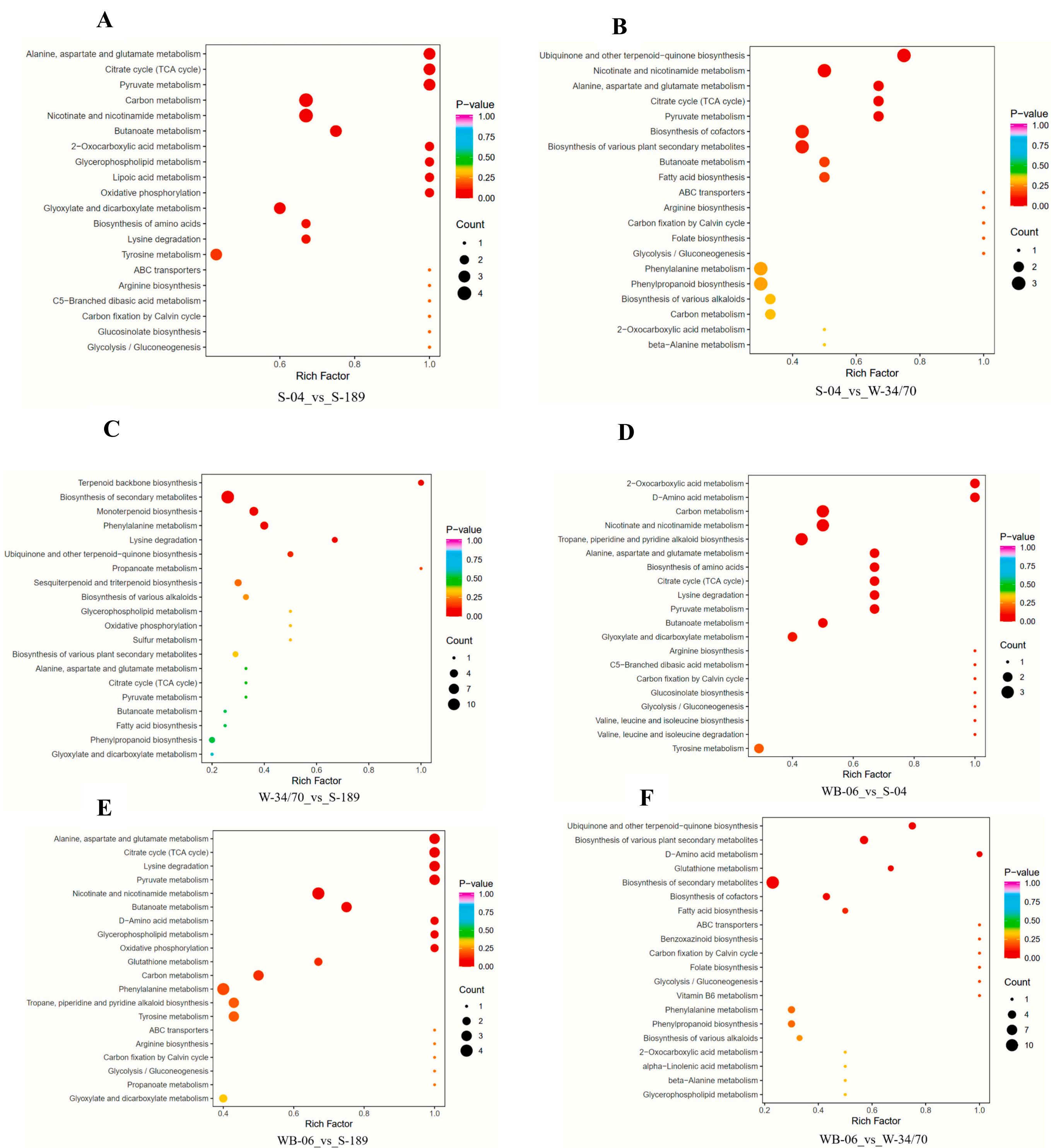

Fig. 7. KEGG pathway enrichment analysis and sensory characteristic analysis of differential metabolites among the yeast strains used in the daylily-supplemented beer fermentations. (A–F) Rich factor plots displaying the enrichment of metabolic pathways based on the differential metabolites identified in the pairwise comparisons of the yeast strains. (G–L) Radar plots displaying the number of metabolites associated with different flavor categories in the pairwise comparisons of the yeast strains.

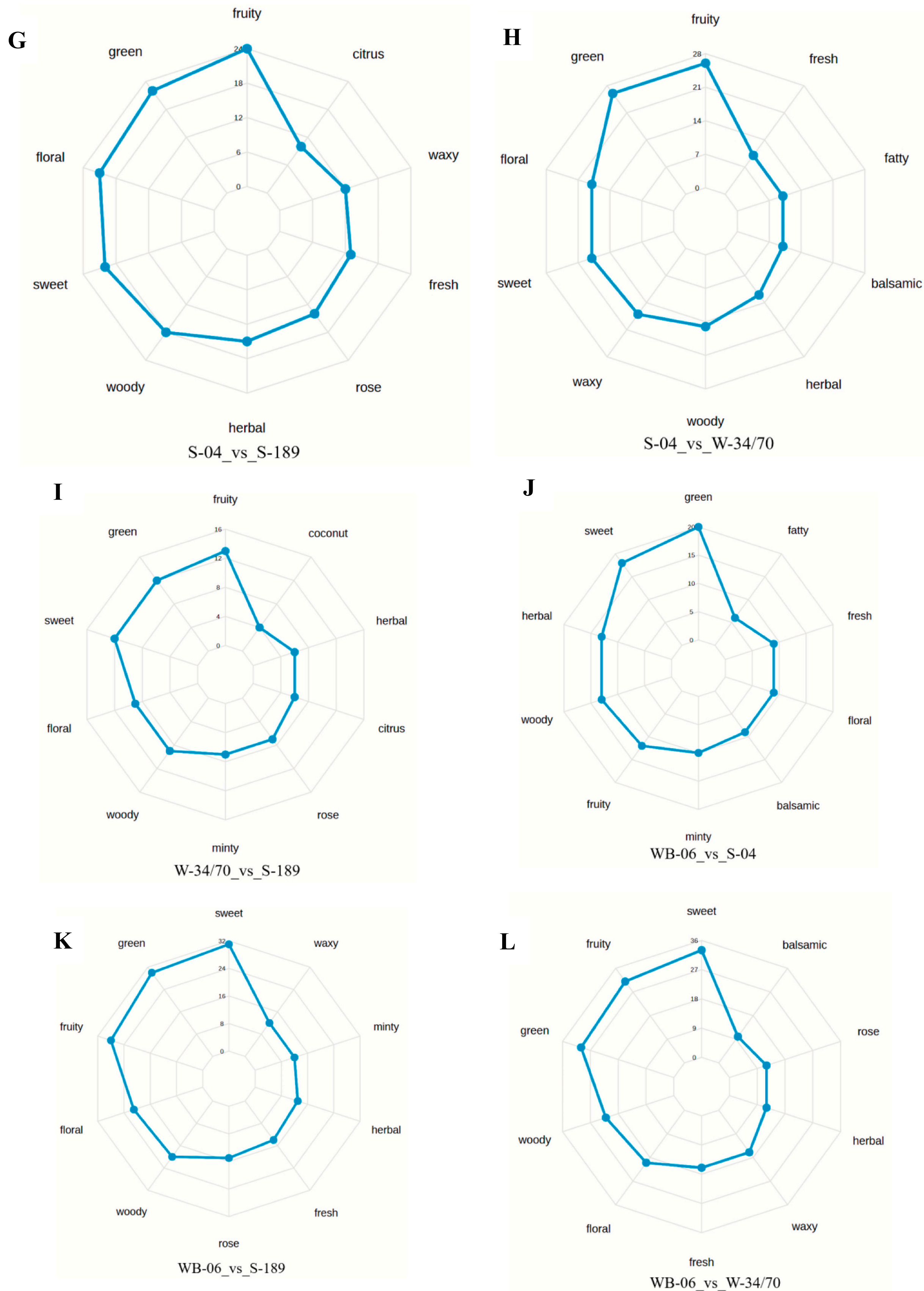

Fig. 7. (*continued*).

can thus leverage WB-06's metabolic profile for beers that combine complex aromas with improved stability, especially in long-aging beers.

3.4.3. KEGG pathway enrichment and sensory characteristic analysis of differential metabolites

The KEGG pathway enrichment analysis highlights significant metabolic differences among the four strains in both central and secondary metabolic pathways (Fig. 7A–F). Notably, S-04 and S-189 exhibited the lowest enrichment *P*-values in energy metabolism pathways, such as the citric acid cycle and pyruvate metabolism (Fig. 7A), suggesting that these strains prioritize efficient energy production for fermentation. This aligns with the known roles of the citric acid cycle and pyruvate metabolism in providing the necessary ATP and intermediates for growth and fermentation (Pires et al., 2014). In contrast, W-34/70 showed substantial enrichment in terpene and monoterpene biosynthesis relative to S-189 (Fig. 7C), indicating a potential advantage in the synthesis of flavor-active compounds. This pathway is particularly significant for producing terpenes, which are responsible for imparting citrus, floral, and herbal notes to fermented beverages. Breweries seeking to enhance the aromatic complexity of daylily-supplemented beer should consider using W-34/70 to boost the floral and citrus profiles, which are key to the beer's sensory appeal (Keșa et al., 2021).

Further analysis of the enrichment factors revealed that WB-06 and S-189 had the highest Rich factors in ubiquinone and nicotinamide metabolism (Fig. 7F), which are critical for the biosynthesis of cofactors involved in various metabolic reactions. The ubiquinone pathway is crucial for the mitochondrial electron transport chain and oxidative phosphorylation, while nicotinamide metabolism is involved in NAD+ biosynthesis, which is essential for redox reactions during fermentation (Duncan et al., 2024). This enrichment suggests that both strains may have enhanced cofactor biosynthesis, potentially contributing to their metabolic efficiency and fermentation performance.

On the other hand, S-04 and W-34/70 displayed elevated enrichment in multiple amino acid biosynthesis pathways (Fig. 7B, E). This suggests that these strains have robust nitrogen assimilation capacities, which may be beneficial for yeast growth and the production of various amino acids, some of which contribute to the formation of flavor-active compounds such as esters and higher alcohols (Roberts et al., 2023). Such metabolic traits may allow S-04 and W-34/70 to produce beers with a broader range of flavor profiles, especially in terms of esters and higher alcohols, which are key contributors to the fruity, floral, and spicy aromas typical of many beer styles. These pathways are vital for yeast metabolism, influencing the final aromatic profile of the beer. Additionally, specialized secondary pathways, such as caffeoylamine metabolism enrichment in WB-06 and diaminobutyric acid degradation in W-34/70, were observed (Fig. 7D, E). These pathways are involved in the degradation of specific amino acids, which may influence flavor precursors, especially those related to bitterness and astringency. This suggests that W-34/70 and WB-06 can be valuable for brewers looking to manipulate specific bitter or astringent notes, important in certain beer styles like IPAs or stouts.

The sensory characteristic analysis confirmed strain-specific flavor contributions, emphasizing the metabolic pathways that shape the aroma profile of daylily-supplemented beer (Fig. 7G–L). The comparison of S-04 vs S-189 (Fig. 7G) showed that S-04 produced prominent fruity (24), green (24), citrus (16), and waxy (16) descriptors. This aligns with its higher energy metabolism efficiency, which supports fermentation processes that favor the production of these specific volatile compounds. The fruity and citrus characteristics suggest that S-04 could be well-suited for beers that emphasize freshness and liveliness, like wheat ales or light lagers. In contrast, S-04 vs W-34/70 (Fig. 7H) highlighted W-34/70's ability to generate stronger fruity (28), green (28), fresh (14), and fatty (14) notes. These results are consistent with the terpene and monoterpene biosynthesis enrichment observed in W-34/70, underscoring its capacity for enhancing floral and citrus notes that are key to the sensory appeal of daylily beers.

In the W-34/70 vs S-189 comparison (Fig. 7I), W-34/70 demonstrated a unique ability to produce coconut (12) and herbal (8) flavors. This could be attributed to its enriched metabolic pathways related to sesquiterpene and triterpene biosynthesis, which are known to contribute to such notes. For brewers, this could mean that W-34/70 is particularly suited for producing beers with a more complex, tropical flavor profile. WB-06 showed advantageous sensory profiles across multiple comparisons. WB-06 vs S-04 (Fig. 7J) indicated strengths in green (20), fatty (15), sweet (32), and balsamic (27) descriptors, highlighting its ability to create more complex and nuanced flavor profiles. This suggests that WB-06 can contribute to creating well-rounded beers with a mix of sweet, green, and herbal flavors, ideal for producing balanced, aromatic daylily beers. WB-06 vs S-189 (Fig. 7K) further emphasized WB-06's strong sweet (32) and green (28) flavors, with prominent fruity (20) and floral (16) notes, suggesting its potential for producing balanced, aromatic daylily beers. The comparison of WB-06 vs W-34/70 (Fig. 7L) showed WB-06's dominant fruity (36) and sweet (36) descriptors, with moderate herbal and balsamic contributions. This pattern supports the idea that WB-06 can produce daylily beers with a rich, multifaceted flavor profile that combines sweetness with refreshing, herbal qualities. This pattern supports the idea that WB-06 can produce daylily beers with a rich, multifaceted flavor profile that combines sweetness with refreshing, herbal qualities.

4. Conclusion

This study demonstrates that different *Saccharomyces* yeast strains significantly influence the physicochemical characteristics, antioxidant activity, and metabolomic profiles of daylily-supplemented craft beer. *Saccharomyces pastorianus* strains W-34/70 and S-189 reinforce color stability and phenolic content, with W-34/70 promoting floral and herbal aromas and S-189 supporting lighter beer styles featuring higher antioxidant capacity. In contrast, *Saccharomyces cerevisiae* strains S-04 and WB-06 excel in alcohol production and bioactive compound enrichment. S-04 achieves the highest alcohol content and antioxidant potential, while WB-06 imparts astringent and polyphenolic flavor attributes.

Metabolomic analyses confirm the unique metabolic pathways and flavor compound formation of each strain, providing a robust scientific basis for targeted yeast selection in craft beer production. Non-targeted metabolomic and volatile compound examinations highlight substantial metabolic diversity among the four strains, each exhibiting distinct advantages in central carbon metabolism, amino acid synthesis, lipid transformation, and secondary metabolism. These comprehensive profiles, together with observed physicochemical and bioactive component variations, emphasize the potential for tailored yeast choice to achieve specific beer quality and flavor objectives.

However, potential variability due to different brewing conditions and daylily cultivars should be considered. Variations in fermentation temperature, oxygen levels, and other environmental factors could influence yeast performance and, consequently, the beer's final quality. Additionally, the selection of daylily cultivars with unique bioactive profiles may further impact the beer's flavor and aroma characteristics. Future research should focus on optimizing brewing conditions, selecting specific daylily cultivars, and investigating the effects of aging on the sensory attributes and stability of daylily-supplemented beers. These directions will help refine brewing practices and maximize the potential of daylily as a brewing adjunct.

CRediT authorship contribution statement

Yuwen Mu: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Chaozhen Zeng:** Software, Formal analysis, Data curation, Conceptualization. **Yulong Ni:** Visualization, Validation, Formal analysis. **Shiyu Zhang:** Methodology, Formal analysis,

Conceptualization. **Jianbin Yang:** Validation, Supervision, Software. **Yuqin Feng:** Writing – review & editing, Validation, Supervision.

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.

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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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