**Supporting Information**

# Staged Fermentation with a *Saccharomyces cerevisiae* Consortium and *Monascus purpureus* for Sustainable Valorization of Persimmon Waste into High-Quality Wine

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## 1 Optimization of Persimmon Pretreatment Conditions

### 1.1 Single-Factor Optimization of Juice yield

The preliminary single-factor experiments identified critical operating ranges for key parameters influencing persimmon juice yield. Pectinase dosage (0.6-2.2 g $kg^{-1}$): Yield plateaued at 1.4-1.8 g $kg^{-1,}$ suggesting enzyme saturation beyond this range (Figure S1a); Temperature (40-60°C): Maximum yield occurred at 40-45°C, with thermal denaturation observed above 50°C (Figure S1b). Processing duration (1-5 h): Yield stabilized after 3-4 h, indicating diminishing returns for extended incubation (Figure S1c).

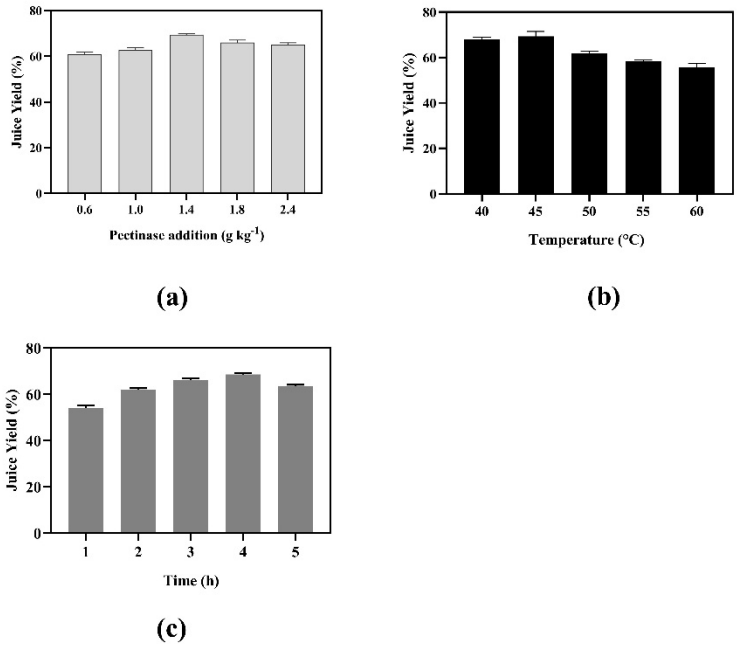


**Figure S1** Single-factor optimization of juice yield: (a) Pectinase addition, (b) Enzymatic temperature, and (c) Enzymatic duration

Based on these results, refined ranges were selected for subsequent orthogonal optimization: Pectinase dosage: 1.4, 1.6, 1.8 g $kg^{-1}$ (covering the plateau region); Temperature: 40, 43, 45°C (spanning the optimal thermal window); Processing duration: 3.0, 3.5, 4.0 h (targeting the stabilization phase). All experiments were conducted in triplicate with juice yield as the primary evaluation metric.

### 1.2 Orthogonal Experimental Design

Range analysis of the orthogonal experiment (Table S1) revealed the following factor hierarchy affecting juice yield: B > A > C (temperature > pectinase dosage > processing duration). This demonstrates that temperature constitutes the primary influencing factor, while pectinase dosage and processing time serve as secondary parameters.

The optimal pretreatment conditions were identified as $A_2B_1C_2$, corresponding to 1.6 g $kg^{-1}$ pectinase dosage, 40°C temperature, 3.5 h processing duration. Under these optimized conditions, the maximum juice yield reached 71.06%. Verification experiments conducted with the established parameters (1.6 g $kg^{-1}$ pectinase, 40°C, 3.5 h) yielded an average juice extraction rate of 70.83%, demonstrating strong consistency with predicted values (relative error: 0.32%).

**Table S1** three-factor three-level orthogonal array of persimmon pretreatment

| Run | Factors | | | Juice yield (%) |
|---|---|---|---|---|
| | Pectinase addition (A, g $kg^{-1}$) | Temperature (B, °C) | Time (C, h) | |
| 1 | 1.4 | 40 | 3 | 64.24 |
| 2 | 1.4 | 43 | 3.5 | 56.32 |
| 3 | 1.4 | 45 | 4 | 64.42 |
| 4 | 1.6 | 40 | 3.5 | 71.06 |
| 5 | 1.6 | 43 | 4 | 62.72 |
| 6 | 1.6 | 45 | 3 | 62.74 |
| 7 | 1.8 | 40 | 4 | 67.36 |
| 8 | 1.8 | 43 | 3 | 61.50 |
| 9 | 1.8 | 45 | 3.5 | 64.92 |
| $K_1$ | 92.49 | 100.83 | 94.24 | |
| $K_2$ | 97.76 | 90.27 | 95.65 | |
| $K_3$ | 96.89 | 96.04 | 97.25 | |
| $k_1$ | 30.83 | 33.61 | 31.41 | |
| $k_2$ | 32.58 | 30.09 | 31.88 | |
| $k_3$ | 32.29 | 32.01 | 32.41 | |
| R | 1.75 | 3.52 | 1.00 | |

**2. Multivariate Optimization of Mixed Yeast Fermentation Using Box-Behnken Design**

**Table S2** Box-Behnken experimental design and results for the mixed-yeast fermentation

| Run | Factor A<br>Initial sugar content<br>(°Brix) | Factor B<br>Yeast inoculation ratio<br>(g $kg^{-1}$) | Factor C<br>Temperature<br>(°C) | Response 1<br>Alcohol<br>(%) | Response 2<br>Residual Sugar<br>(g $kg^{-1}$) |
|---|---|---|---|---|---|
| 1 | 22 | 0.25 | 18 | 11.58 | 2.056 |
| 2 | 22 | 0.35 | 18 | 11.46 | 1.592 |
| 3 | 22 | 0.3 | 20 | 11.62 | 1.424 |
| 4 | 23 | 0.25 | 20 | 12.48 | 3.272 |
| 5 | 21 | 0.3 | 18 | 10.58 | 0.968 |
| 6 | 23 | 0.3 | 22 | 12.89 | 4.784 |
| 7 | 22 | 0.25 | 22 | 11.24 | 3.040 |
| 8 | 21 | 0.35 | 20 | 10.9 | 1.296 |
| 9 | 23 | 0.35 | 20 | 10.94 | 2.24 |
| 10 | 22 | 0.3 | 20 | 11.64 | 1.256 |
| 11 | 21 | 0.3 | 22 | 10.74 | 0.880 |
| 12 | 22 | 0.35 | 22 | 11.37 | 2.592 |
| 13 | 22 | 0.3 | 20 | 11.53 | 1.696 |
| 14 | 22 | 0.3 | 20 | 12.1 | 1.888 |
| 15 | 21 | 0.25 | 20 | 12.81 | 1.400 |
| 16 | 22 | 0.3 | 20 | 10.94 | 1.592 |
| 17 | 23 | 0.3 | 18 | 12.75 | 2.000 |

### 3. Multivariate Optimization of *Monascus purpureus* Fermentation Using Box-Behnken Design

**Table S3** Box-Behnken experimental design and results for *Monascus purpureus* fermentation

| Run | Factor A Monascus inoculation (%) | Factor B Temperature (°C) | Factor C pH | Response 1 Sensory score | Response 2 Alcohol (%) | Response 3 Total Ester (mg $mL^{1}$) |
|---|---|---|---|---|---|---|
| 1 | 1.5 | 22 | 4 | 88.9 | 12.6 | 3.36 |
| 2 | 1.5 | 22 | 4 | 89.6 | 12.91 | 3.39 |
| 3 | 1.5 | 20 | 3.8 | 85.7 | 12.39 | 3.08 |
| 4 | 1.5 | 20 | 4.2 | 87.1 | 12.48 | 3.09 |
| 5 | 1.5 | 22 | 4 | 91.5 | 12.69 | 3.72 |
| 6 | 1.5 | 24 | 4.2 | 84.7 | 11.79 | 3.27 |
| 7 | 3 | 22 | 4.2 | 90.1 | 11.32 | 3.01 |
| 8 | 3 | 20 | 4 | 88.6 | 11.24 | 2.84 |
| 9 | 1.5 | 24 | 3.8 | 88.2 | 11.58 | 3.67 |
| 10 | 0 | 22 | 3.8 | 88.3 | 11.97 | 3.43 |
| 11 | 1.5 | 22 | 4 | 92.3 | 11.68 | 4.03 |
| 12 | 3 | 22 | 3.8 | 87.5 | 12.46 | 2.75 |
| 13 | 1.5 | 22 | 4 | 91.7 | 12.02 | 3.46 |
| 14 | 0 | 24 | 4 | 85.1 | 12.14 | 3.32 |
| 15 | 0 | 20 | 4 | 83.2 | 12.35 | 2.57 |
| 16 | 3 | 24 | 4 | 86.7 | 11.77 | 3.62 |
| 17 | 0 | 22 | 4.2 | 84.1 | 12.62 | 3.33 |

**4. Sensory Evaluation Method**

**Table S4** Persimmon wine sensory evaluation criteria

| Category | 9-Point Scale Criteria | Descriptor Anchors | Weight |
|---|---|---|---|
| Color | Pale yellow hue intensity; clarity and brilliance; absence of particles/sediment. | 1: Muddy, cloudy, or discolored; 9: Bright, glossy, particle-free | 15% |
| **Aroma** | Intensity and balance of persimmon fruit notes; absence of off-flavors (e.g., sulfur, acetic) | 1: Overpowering alcohol/off-odors; 9: Elegant, fruity, well-balanced | 25% |
| **Taste** | Sweetness-acidity harmony; mouthfeel richness; absence of bitterness/astringency | 1: Cloying, unbalanced, gritty; 9: Velvety, structured, balanced | 40% |
| Aftertaste | Duration and quality of finish; persistence of pleasant flavors. | 1: Harsh, astringent, or flat; 9: Lingering, harmonious resonance | 20% |

**5. Aroma Profile of Persimmon Wine**

**Table S5** Total volatile components in persimmon wine fermented by different strains

| No | Compounds | Fermented methods | | | | | Odor Description |
|---|---|---|---|---|---|---|---|
| | | MSF | EC | Aq | F33 | Mixed Yeast | |
| 1 | Pentanol | 0.51 | 6.97 | 2.58 | 3.8 | 12.54 | Alcohol-like odor |
| 2 | Active pentanol | - | 2.52 | 1.33 | 1.41 | 5.63 | Alcohol-like aroma |
| 3 | 2,3-Butanediol | - | 0.11 | - | 0.16 | 0.07 | Fruity, creamy notes |
| 4 | Phenylethyl alcohol | 73.08 | 28.64 | 33.62 | 42.31 | 47.06 | Rose-like fragrance |
| 5 | 4-Hydroxyphenethyl alcohol | 0.23 | 0.04 | - | 0.06 | 0.19 | Sweet floral-fruity aroma |
| 6 | 1-Decanol | 0.16 | - | - | - | - | Waxy, sweet, floral, fruity aroma |
| 7 | Decyl methanol | 0.12 | 0.05 | 0.02 | 0.02 | 0.06 | Mild sweet rose-like scent |
| 8 | Farnesol | - | 0.12 | 0.03 | - | 0.17 | Lily-of-the-valley floral character |
| 9 | Citronellol | - | 0.29 | 0.10 | 0.19 | - | Fresh green rose-like aroma |
| 10 | 1-Decanol | 0.16 | - | - | - | - | Waxy, sweet, floral, fruity aroma |
| 11 | Terpinen-4-ol | 0.43 | - | - | - | - | Spicy note |
| 12 | Nonanediol | 0.68 | - | - | - | - | Rosy, waxy, fruity lipid aroma |
| 13 | Lauryl alcohol | 0.12 | - | - | - | - | Tuberose, violet-like scent |
| 14 | Geranylgeraniol | 0.28 | - | - | - | - | Blooming rose aroma |
| 15 | Ethyl hexanoate | - | 0.15 | 0.31 | 0.23 | 0.09 | Fruity aroma |
| 16 | Ethyl octanoate | - | - | 0.71 | 1.08 | 0.94 | Brandy-like aroma |
| 17 | Ethyl phenylacetate | - | 0.09 | 0.03 | 0.10 | 0.03 | Alcoholic, sweet, floral notes |
| 18 | Phenethyl acetate | 1.72 | 1.36 | 6.96 | 2.37 | 4.73 | Rose, honey-like fragrance |
| 19 | Ethyl 9-decenoate | - | 0.09 | - | 0.27 | 1.24 | Mild fruity, milky aroma |
| 20 | Ethyl decanoate | - | 0.54 | 0.42 | 0.75 | 1.08 | Alcoholic, sweet, floral notes |
| 21 | Ethyl 3-methylbutyl succinate | - | - | - | - | 0.03 | Apple, banana-like aroma |
| 22 | Diethyl succinate | 0.09 | - | - | - | - | Pleasant odor |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 23 | Tributyl phosphate | - | - | - | - | 0.19 | - |
| 24 | Ethyl 3-hydroxytetradecanoate | - | - | - | 0.07 | 0.16 | - |
| 25 | Methyl laurate | 0.20 | 0.37 | 0.45 | 0.73 | 0.20 | Coconut, mushroom notes |
| 26 | Methyl myristate | - | - | - | - | 0.02 | - |
| 27 | Ethyl 2-methylacetoacetate | 0.12 | - | - | - | - | Fruity aroma |
| 28 | Ethyl undecanoate | 0.03 | - | - | - | - | Wine-like, nutty, coconut aroma |
| 29 | Isobutyl cis-3-hexenyl ester | 0.02 | - | - | - | - | Banana or pear-like aroma |
| 30 | Ethyl 9-hexadecenoate | - | 0.29 | 0.18 | - | 0.09 | - |
| 31 | Dioctyl terephthalate | 0.44 | - | - | - | 0.53 | - |
| 32 | Ethyl palmitate | 0.16 | 0.43 | 0.27 | 0.24 | 0.30 | Nutty, cocoa-like aroma |
| 33 | Ethyl (E)-9-octadecenoate | - | - | - | - | 0.08 | Floral, fruity aroma |
| 34 | Isoamyl acetate | - | 0.06 | 0.18 | - | - | Banana-like aroma |
| 35 | 2-Phenylethyl decanoate | - | - | 0.10 | 0.04 | - | Fruity, wine-like aroma |
| 36 | Isoamyl propionate | 0.14 | - | - | - | - | Fresh sweet fruity aroma |
| 37 | Trimethyl orthobutyrate | 0.09 | - | - | - | - | - |
| 38 | 2,3-Dihydrobenzofuran | - | 0.13 | 0.17 | 0.18 | 0.06 | Nutty aroma |
| 39 | Phenethylamine | 0.06 | - | - | - | - | - |
| 40 | Chloroxylenol | - | - | - | - | 0.05 | - |
| 41 | 2,4-Di-tert-butylphenol | 3.17 | - | 6.07 | - | 5.38 | - |
| 42 | Germacrane | 0.02 | - | - | - | - | Herbal plant-like aroma |
| 43 | 3,4-Dimethylbenzaldehyde | 2.99 | 0.48 | 0.59 | 0.54 | 0.12 | - |
| 44 | Farnesal | - | 0.04 | - | - | - | Spicy note |
| 45 | Pentadecanal | 0.47 | - | - | - | - | Spicy note |
| 46 | Decanal | 0.05 | - | - | - | - | Citrus peel-like odor |
| 47 | 2-Nonanone | 0.08 | - | - | - | - | Fruity aroma |
| 48 | Isolongifolene ketone | 0.06 | - | - | - | - | Dry woody aroma |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 49 | Hexadecanal | 0.11 | - | - | - | - | Weak floral, waxy aroma |
| 50 | 8-Nonen-2-one | 0.21 | - | - | - | - | - |
| 51 | Phenylpropanol hydrate | 0.10 | - | - | - | - | Unique aromatic character |
| 52 | Ketones (Total) | 0.39 | 0.17 | 0.15 | | 0.08 | |
| | Count | 32 | 21 | 20 | 19 | 27 | |