

Supplementary Information

Molecular hydrogen improves rice storage quality via alleviating lipid deterioration and maintaining nutritional values

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Table S1 Primers of qPCR used in this study

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') |
|------------------------------|--------------------------|----------------------------|
| <i>OsActin</i> (45) | CACGTGAGCAAGGCTCTTTTG | GGCATCCCCCTCCTCATAGA |
| <i>OsUBIQUITIN</i> (46) | GACGGACGCACCCTGGCGAACTAC | TGCTGCCAATTACCATATACCACGAC |
| <i>OsLOX1</i> (XM_015771289) | TTCTTCACCGTCAAGTCGT | TCCGAGAACTGCTCGTC |
| <i>OsLOX2</i> (48) | TACAAGTCCGACGAGGAGGT | CCACATGATGGTGGTCAAGA |
| <i>OsLOX3</i> (49) | TCGCATTGGCTCGCACCT | TCGTCTTCTTCAGCCGCACGAT |

OsActin (reference gene), *beta-actin*; *OsUBIQUITIN* (reference gene), *ubiquitin*; *OsLOX1*, *lipoxygenase 1*; *OsLOX2*, *lipoxygenase 2*; *OsLOX3*, *lipoxygenase 3*.

48. Yang, J.; Wang, Y.; Liu, L.; Liu, L.; Wang, C.; Wang, C.; Li, C. Effects of exogenous salicylic acid and pH on pathogenicity of biotrophy-associated secreted protein 1 (BAS1)-overexpressing strain, *Magnaporthe oryzae*. *Environ. Sci. Pollut. Res.* **2018**, *26*, 13725–13737. <https://doi.org/10.1007/s11356-018-2532-y>.
49. Xu, H.; Wei, Y.; Zhu, Y.; Lian, L.; Xie, H.; Cai, Q.; Chen, Q.; Lin, Z.; Wang, Z.; Xie, H.; et al. Antisense suppression of *LOX3* gene expression in rice endosperm enhances seed longevity. *Plant Biotechnol. J.* **2014**, *13*, 526–539. <https://doi.org/10.1111/pbi.12277>.

Table S2 Profiles of volatiles in one year stored rice (Huruan 1212). Concentrations of volatile compounds were expressed as $\mu\text{g kg}^{-1}$ of rice fresh weight, equivalent to 2,4,6-trimethylpyridine (TMP). Values were mean \pm SD. ND: not detected; Con: Control, irrigated with ditch water; HNW: irrigated with hydrogen nanobubble water.

| Family | Number | Compound | MW | MF | Compound CID | Content ($\mu\text{g kg}^{-1}$) | |
|-----------|--------|------------------------|--------|---|-----------------|-----------------------------------|-------------------|
| | | | | | | Con | HNW |
| Aldehydes | 1 | Pentanal | 86.13 | $\text{C}_5\text{H}_{10}\text{O}$ | 8063 | 19.77 \pm 2.68 | 6.62 \pm 0.77 |
| | 2 | Hexanal | 100.16 | $\text{C}_6\text{H}_{12}\text{O}$ | 6184 | 270.10 \pm 19.03 | 183.71 \pm 1.40 |
| | 3 | Heptanal | 114.19 | $\text{C}_7\text{H}_{14}\text{O}$ | 8130 | 23.68 \pm 1.86 | 12.73 \pm 0.68 |
| | 4 | Benzaldehyde | 106.12 | $\text{C}_7\text{H}_6\text{O}$ | 240 | 17.62 \pm 1.18 | 32.25 \pm 1.55 |
| | 5 | Octanal | 128.21 | $\text{C}_8\text{H}_{16}\text{O}$ | 454 | 17.30 \pm 2.49 | 11.56 \pm 0.57 |
| | 6 | (E)-2-Octenal | 126.20 | $\text{C}_8\text{H}_{14}\text{O}$ | 5283324 | 20.02 \pm 0.79 | 18.99 \pm 1.48. |
| | 7 | Nonanal | 142.24 | $\text{C}_9\text{H}_{18}\text{O}$ | 31289 | 74.98 \pm 2.97 | 106.8 \pm 6.05 |
| | 8 | (E)-2-Nonenal | 140.22 | $\text{C}_9\text{H}_{16}\text{O}$ | 5283335 | 12.84 \pm 0.39 | 9.47 \pm 2.08 |
| | 9 | Decanal | 156.26 | $\text{C}_{10}\text{H}_{20}\text{O}$ | 8175 | 22.36 \pm 1.05 | 28.89 \pm 2.74 |
| | 10 | Undecanal | 170.29 | $\text{C}_{11}\text{H}_{22}\text{O}$ | 8186 | 3.51 \pm 0.13 | 5.53 \pm 0.12 |
| | 11 | 2-Butyl-2-octenal | 182.30 | $\text{C}_{12}\text{H}_{22}\text{O}$ | 5362697 | 5.43 \pm 0.50 | 3.01 \pm 0.25 |
| Alcohols | 1 | Ethanol | 46.07 | $\text{C}_2\text{H}_6\text{O}$ | 702 | ND | 3.84 \pm 0.38 |
| | 2 | 1-Pentanol | 88.15 | $\text{C}_5\text{H}_{12}\text{O}$ | 6276 | 22.05 \pm 2.48 | 24.68 \pm 1.57 |
| | 3 | 1-Hexanol | 102.17 | $\text{C}_6\text{H}_{14}\text{O}$ | 8103 | 82.58 \pm 11.22 | 98.49 \pm 4.08 |
| | 4 | 1-Heptanol | 116.20 | $\text{C}_7\text{H}_{16}\text{O}$ | 8129 | 7.37 \pm 0.57 | 10.09 \pm 0.82 |
| | 5 | 1-Octen-3-ol | 128.21 | $\text{C}_8\text{H}_{16}\text{O}$ | 18827 | 35.92 \pm 1.34 | 21.88 \pm 0.88 |
| | 6 | Cis-linalool oxide | 170.25 | $\text{C}_{10}\text{H}_{18}\text{O}_{22}$ | 6428573 | 6.21 \pm 0.27 | 8.03 \pm 0.09 |
| | 7 | Terpinen-4-ol | 154.25 | $\text{C}_{10}\text{H}_{18}\text{O}$ | 11230 | 3.68 \pm 0.06 | 2.28 \pm 0.05 |
| | 8 | <i>p</i> -Menthan-1-ol | 156.26 | $\text{C}_{10}\text{H}_{20}\text{O}$ | 89437 | 13.94 \pm 1.73 | 9.16 \pm 0.12 |
| Ketones | | Acetone | 58.08 | $\text{C}_3\text{H}_6\text{O}$ | 180 | 10.34 \pm 1.14 | 15.22 \pm 0.54 |
| | 2 | 2-Heptanone | 114.19 | $\text{C}_7\text{H}_{14}\text{O}$ | 8051 | 46.60 \pm 1.46 | 34.98 \pm 1.06 |
| | 3 | 4-Methyl-cyclohexanone | 112.17 | $\text{C}_7\text{H}_{12}\text{O}$ | 11525 | 9.65 \pm 0.9 | 6.96 \pm 0.60 |

| | | | | | | | |
|----------------|---|--|--------|--|-----------|------------|------------|
| Ketones | 4 | 6-Methyl-5-hepten-2-one | 126.20 | C ₈ H ₁₄ O | 9862 | 12.93±1.16 | 24.80±1.68 |
| | 5 | 3-Octen-2-one | 126.20 | C ₈ H ₁₄ O | 5363229 | 3.38±0.19 | 3.38±0.16 |
| | 6 | (R,S)-5-Ethyl-6-methyl-3E-hepten-2-one | 154.25 | C ₁₀ H ₁₈ O | 146160920 | 12.42±1.33 | 7.42±0.34 |
| | 7 | 6,10-Dimethyl-2-undecanone | 198.34 | C ₁₃ H ₂₆ O | 95495 | 3.52±0.12 | 3.05±0.02 |
| | 8 | Geranylacetone | 194.31 | C ₁₃ H ₂₂ O | 1549778 | 11.86±0.80 | 17.06±0.41 |
| | 9 | 6,10,14-trimethylpentadecan-2-one | 268.50 | C ₁₈ H ₃₆ O | 10408 | 4.23±0.58 | 2.47±0.17 |
| Esters | 1 | Formic acid, octylester | 158.24 | C ₉ H ₁₈ O ₂ | 8176 | 19.10±1.66 | 20.47±0.18 |
| | 2 | Dodecyl acrylate | 240.38 | C ₁₅ H ₂₈ O ₂ | 75084 | 17.17±1.56 | 12.98±0.61 |
| | 3 | Sulfurous acid, octadecyl 2-propyl ester | 376.60 | C ₂₁ H ₄₄ O ₃ S | 6420358 | 7.74±0.66 | 7.61±0.17 |
| | 4 | Octadecanoic acid, 2-oxo-, methyl ester | 312.50 | C ₁₉ H ₃₆ O ₃ | 620002 | 5.71±0.18 | 5.65±0.14 |
| | 5 | Dimethyl phthalate | 194.18 | C ₁₀ H ₁₀ O ₄ | 8554 | 5.81±0.41 | 8.43±0.48 |
| Others | 1 | Acetic acid | 60.05 | C ₂ H ₄ O ₂ | 176 | 16.20±5.19 | 9.59±0.52 |
| | 2 | Ethylbenzene | 106.16 | C ₈ H ₁₀ | 7500 | 3.32±0.58 | 9.61±1.09 |
| | 3 | Undecane | 156.31 | C ₁₁ H ₂₄ | 14257 | 3.80±0.26 | 4.25±0.06 |
| | 4 | 2-Aminomethyl-5-methylamino-1,3,4-oxadiazole | 128.13 | C ₄ H ₈ N ₄ O | 581786 | 4.29±0.33 | 5.22±0.18 |
| | 5 | Tridecane | 184.36 | C ₁₃ H ₂₈ | 12388 | 4.61±0.17 | 6.54±0.48 |
| | 6 | 5-pentyloxolan-2-one | 156.22 | C ₉ H ₁₆ O ₂ | 7710 | 8.21±0.76 | 10.34±0.47 |
| | 7 | Hexadecane | 226.44 | C ₁₆ H ₃₄ | 11006 | 3.48±0.18 | 3.05±0.57 |

Table S3 Profiles of hydrolyzed amino acids in stored rice (Huruan 1212). Contents were expressed as mg g⁻¹ of rice dry weight. Values were mean \pm SD. Con: Control, irrigated with ditch water; HNW: irrigated with hydrogen nanobubble water.

| Type | Content (mg g ⁻¹) | |
|---------------|------------------------------------|------------------------------------|
| | Con | HNW |
| Threonine | 2.11 \pm 0.10 | 2.65 \pm 0.06 |
| Valine | 2.25 \pm 0.70 | 3.51 \pm 0.05 |
| Methionine | 0.29 \pm 0.13 | 0.27 \pm 0.06 |
| Isoleucine | 1.88 \pm 0.31 | 2.36 \pm 0.11 |
| Leucine | 4.18 \pm 0.15 | 4.95 \pm 0.10 |
| Phenylalanine | 2.76 \pm 0.09 | 3.40 \pm 0.17 |
| Histidine | 1.54 \pm 0.02 | 1.84 \pm 0.02 |
| Lysine | 2.41 \pm 0.05 | 2.80 \pm 0.09 |
| Total | 17.44 \pm 1.21 | 21.80 \pm 0.28 |
| Aspartic acid | 6.07 \pm 0.06 | 6.89 \pm 0.15 |
| Serine | 2.67 \pm 0.20 | 3.39 \pm 0.09 |
| Glutamate | 11.46 \pm 1.14 | 14.12 \pm 0.11 |
| Glycine | 3.30 \pm 0.14 | 3.68 \pm 0.02 |
| Alanin | 3.70 \pm 0.35 | 4.29 \pm 0.07 |
| Cystine | 0.99 \pm 0.28 | 0.68 \pm 0.03 |
| Tyrosine | 2.08 \pm 0.02 | 2.60 \pm 0.06 |
| Arginine | 5.15 \pm 0.21 | 6.24 \pm 0.34 |
| Proline | 4.43 \pm 1.47 | 3.94 \pm 0.05 |
| Total | 39.89 \pm 0.80 | 45.84 \pm 0.59 |

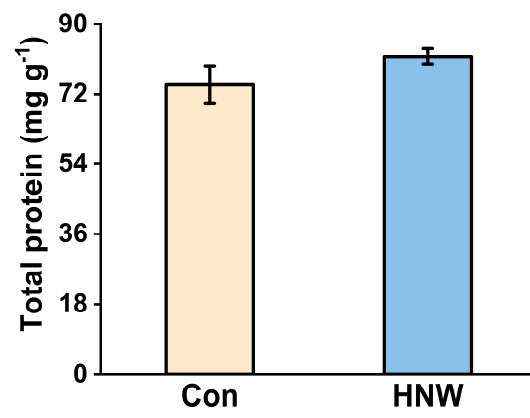


Figure S1 Profiles of total protein contents from different treatments in stored rice.

Values are means \pm standard deviation (SD) of three biological repetitions.

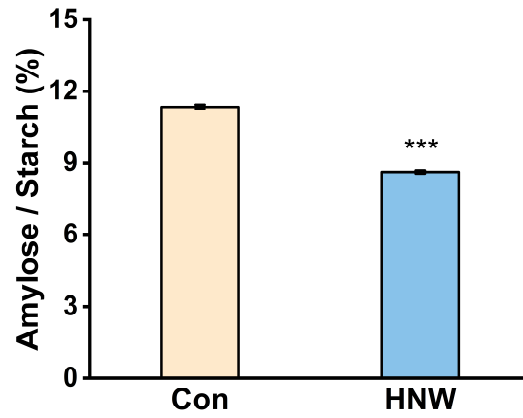


Figure S2 Ratio of amylose in starch from different treatments in stored rice. Values are means \pm standard deviation (SD) of three biological repetitions. *** indicate significant difference (t -test, $P < 0.001$).

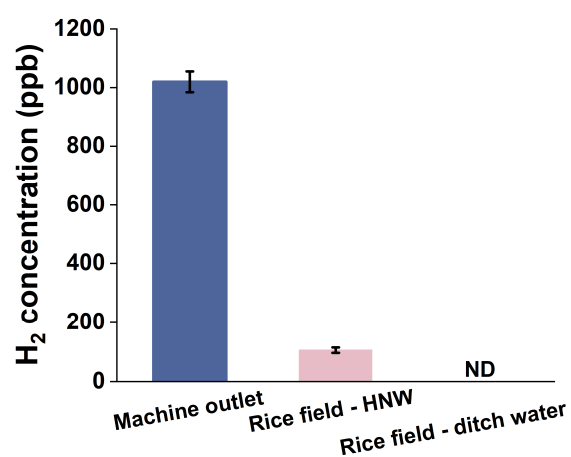


Figure S3 Concentration of H₂ in the machine outlet and fields with different treatments (measured on 27 June 2020). Values are means \pm standard deviation (SD) of six repetitions in each field randomly. ND: not detected.

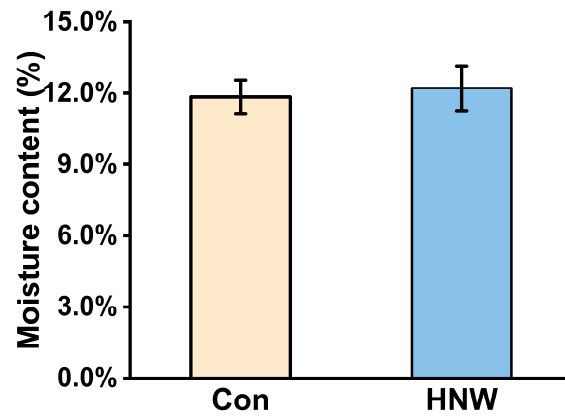


Figure S4 Profiles of moisture content from different treatments in stored rice. Values are means \pm standard deviation (SD) of three biological repetitions.