

Impact of oenological processing aids and additives on the genetic traceability of 'Nebbiolo' wine produced with withered grapes

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Supplementary material

Table S1. Primers and probes used for the SNP genotyping.

| ID marker | SNP position | Allele Nebbiolo | Allele non-Nebbiolo | ID Oligo | Primer and Probe sequences 5'-3' | Length of the fragment (bp) |
|-----------|---------------|-----------------|---------------------|----------------|----------------------------------|-----------------------------|
| SNP_14783 | chr8_13053532 | G | A | For | GAGCACAATCAACAATTATCCATTT | 83 |
| | | | | Rev | TGGTTGTGTTAATAGCAGGCAA | |
| | | | | Probe Allele A | FAM-TAAAAAAGTGTTAAGGTGATAAT-NFQ | |
| | | | | Probe Allele G | VIC-TAAAAAAGTGTTAAGGTGATGAT-NFQ | |
| SNP_15082 | chr8_19402046 | T | C | For | TCTCTTCTGGCATGGAAATCAAT | 89 |
| | | | | Rev | TAGATTACGGGCCAAGCTGA | |
| | | | | Probe Allele T | FAM-TCTCATTTTCCTCATTAT-NFQ | |
| | | | | Probe Allele C | VIC-TCTCATTTTCCTCATCATG-NFQ | |

Table S2. Characterization of 'Nebbiolo' wine before the treatments. Data are mean values \pm SDs of two replicates.

| Wine parameter | |
|--|------------------|
| Ethanol (% v/v) | 13.61 \pm 0.00 |
| Glycerol (g/L) | 11.08 \pm 0.02 |
| pH | 3.46 \pm 0.00 |
| Total acidity (g/L tartaric acid) | 6.32 \pm 0.08 |
| Malic acid (g/L) | 0.04 \pm 0.02 |
| Lactic acid (g/L) | 1.95 \pm 0.00 |
| Tartaric acid (g/L) | 1.60 \pm 0.06 |
| Citric acid (g/L) | 0.13 \pm 0.01 |
| Acetic acid (g/L) | 0.30 \pm 0.00 |
| Free SO₂ (mg/L) | 10.50 \pm 0.71 |
| Total SO₂ (mg/L) | 76.48 \pm 0.91 |
| Turbidity (NTU) | 15.05 \pm 0.01 |
| Color intensity (AU) | 8.29 \pm 0.01 |
| Hue | 0.75 \pm 0.00 |
| L* | 16.2 \pm 0.0 |
| a* | 46.99 \pm 0.04 |
| b* | 27.12 \pm 0.02 |
| Total phenolics (mg/L (-)-epicatechin) | 3163 \pm 70 |
| Total anthocyanins (mg/L malvidin-3-O-glucoside chloride) | 148.4 \pm 1 |
| Total flavonoids (mg/L (+)-catechin) | 1063 \pm 6 |

1 **Table S3.** DNA quantity and quality extracted from 'Nebbiolo' wines treated with different additives and processing aids using Plant/Fungi DNA
2 Isolation Kit (Norgen). For each treatment repetition, one sample was extracted (R1, R2, and R3). Purity and yield measured using NanoDrop.
3 Allelic profiles of genotyping assays SNP_15082 and SNP_14783. '-' in the allelic profile denotes an incorrect allelic call; '+' indicates samples
4 that correctly amplified, and 'nd' stands for 'not detected'. Data are means of 3 replicates \pm standard deviation. Values followed by different letters
5 within a column are significantly different ($p < 0.05$, Kruskal-Wallis test with Conover's Comparison test). CONTR20: untreated control sampled
6 one year before the application of additives; CONTR: untreated control; BEN: bentonite; GEL: gelatine; VEG: vegetable protein; PVPP:
7 polyvinylpyrrolidone; YST: yeast hulls; CHT: chitosan; MAN: mannoprotein; ARG: arabic gum; POL: potassium polyaspartate; TAN: grape
8 skin tannin.

| Sample | Treatment | NanoDrop Quantification | | | SNP_14783 | | | SNP_15082 | | |
|---------|-------------------------|------------------------------|------------------------------------|------------------------------------|-----------|----|----|-----------|----|----|
| | | DNA yield [ng/mL of wine] | A ₂₆₀ /A ₂₈₀ | A ₂₆₀ /A ₂₃₀ | Alleles | | | Alleles | | |
| | | | | | R1 | R2 | R3 | R1 | R2 | R3 |
| CONTR20 | - | 33.7 \pm 6.5 a | 1.35 \pm 0.15 a | 0.51 \pm 0.15 a | + | + | + | + | + | + |
| CONTR | - | 5.2 \pm 1.1 b | 1.13 \pm 0.11 a | 0.26 \pm 0.04 a | nd | - | nd | - | - | - |
| BEN | Bentonite | 3.4 \pm 1.8 b | 1.13 \pm 0.08 a | 0.23 \pm 0.14 a | - | - | - | - | - | - |
| GEL | Gelatine | 6.8 \pm 3.1 b | 1.19 \pm 0.07 a | 0.31 \pm 0.01 a | - | - | - | - | - | - |
| VEG | Vegetables protein | 7.6 \pm 1.8 b | 1.27 \pm 0.05 a | 0.31 \pm 0.03 a | - | - | - | - | - | - |
| PVPP | Polyvinylpyrrolidone | 5.8 \pm 0.9 b | 1.05 \pm 0.19 a | 0.24 \pm 0.09 a | - | - | - | - | - | - |
| YST | Yeasts hulls | 7.5 \pm 3.1 b | 1.18 \pm 0.06 a | 0.27 \pm 0.02 a | nd | nd | nd | nd | nd | nd |
| CHT | Chitosan | 7.4 \pm 2.4 b | 0.98 \pm 0.06 a | 0.21 \pm 0.04 a | - | nd | nd | nd | nd | - |
| MAN | Yeast mannoprotein | 11.9 \pm 5.1 b | 1.17 \pm 0.09 a | 0.25 \pm 0.03 a | - | - | - | - | - | - |
| ARG | Arabic gum | 16.6 \pm 19.9 b | 1.11 \pm 0.06 a | 0.29 \pm 0.06 a | nd | nd | nd | nd | nd | nd |
| POL | Potassium polyaspartate | 6.7 \pm 0.8 b | 1.08 \pm 0.09 a | 0.26 \pm 0.06 a | + | nd | nd | nd | + | nd |
| TAN | Grape skin tannin | 5.5 \pm 1.2 b | 1.03 \pm 0.14 a | 0.31 \pm 0.12 a | - | nd | - | nd | nd | - |