Supplementary Material

Solid-phase extraction followed by gas chromatography-mass spectrometry for revealing the effects of the application of bentonite, tannins and their combination during fermentation in production of white wine

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Table S1. Doses of bentonite applied for protein stabilization of Malvazija istarska wines produced by different bentonite and tannins addition treatments.

Bentonite dose (g/hL)	Protein stability test	Treatment			
		CO	B	T	BT
Initial dose	Heating test	0	95	0	95
Additional dose		193 ± 6	70 ± 0	167 ± 12	70 ± 0
Total dose		193 ± 6 a	165 ± 0 b	167 ± 12 b	165 ± 0 b
Initial dose	Heating with tannin test	0	95	0	95
Additional dose		200 ± 0	83 ± 6	180 ± 17	77 ± 6
Total dose		200 ± 0 a	178 ± 6 b	180 ± 17 b	172 ± 6 b

CO - control wine without added tannins and without bentonite in fermentation, B - bentonite added near the end of fermentation, T - tannins added during fermentation without bentonite in fermentation, BT - bentonite and tannins added during fermentation in the same manner as in T and B treatments. Different lowercase letters in a row represent statistically significant differences between treatments determined by one-way ANOVA and LSD test at $p < 0.05$.

Table S2. Details of identification and quantification of free volatile aroma compounds in Malvazija istarska wines produced by different bentonite and tannins addition treatments, determined after fermentation and after final wine protein stabilization.

Code	Free aroma compounds	Quantification	ID	t_R (min:sec)	LRI_{exp}	LRI_{lit}	LOQ	Conc. range (max.)	Calib. levels (No.)	R^2
	Monoterpenes									
FV-1	Linalool (mg/L)	Quantitative	S, MS	20:22			0.02	1	10	> 0.99
FV-2	α-Terpineol (mg/L)	Quantitative	S, MS	26:30			0.01	0.5	10	> 0.99
FV-3	α-Terpinolene*	Semi-quantitative	S, MS	09:13						
FV-4	Citronellol (mg/L)	Quantitative	S, MS	29:42			0.01	0.5	10	> 0.99
FV-5	Terpendiol I*	Semi-quantitative	RI, MS	37:07	1948	1951				
	C_{13}-norisoprenoid									
FV-6	β-Damascenone	Quantitative	S, MS	31:18			1	10	10	> 0.99
	Alcohols									
FV-7	1-Propanol (mg/L)	Quantitative	S				2	155	3	> 0.99
FV-8	Isobutanol (mg/L)	Quantitative	S				2	154	3	> 0.99
FV-9	Isoamyl alcohol (mg/L)	Quantitative	S				4	313	3	> 0.99
FV-10	1-Hexanol (mg/L)	Quantitative	S, MS	12:00			0.04	2	10	> 0.99
FV-11	*trans*-3-Hexenol*	Semi-quantitative	S, MS	12:16						
FV-12	*cis*-3-Hexenol*	Semi-quantitative	S, MS	13:06						
FV-13	1-Octanol*	Semi-quantitative	S, MS	20:48						
FV-14	Benzyl alcohol*	Semi-quantitative	S, MS	33:36						
FV-15	2-Phenyletanol (mg/L)	Quantitative	S, MS	35:00			0.6	30	10	> 0.99
	Fatty acids									
FV-16	Butyric acid (mg/L)	Quantitative	S, MS	23:42			0.06	3	10	> 0.99
FV-17	Hexanoic acid (mg/L)	Quantitative	S, MS	32:54			0.06	3	10	> 0.99
FV-18	Octanoic acid (mg/L)	Quantitative	S, MS	41:12			0.125	5	10	> 0.99
FV-19	Decanoic acid (mg/L)	Quantitative	S, MS	48:42			0.1	3	10	> 0.99
FV-20	Dodecanoic acid*	Semi-quantitative	RI, MS	56:02	2477	2478				

	Ethyl esters									
FV-21	Ethyl butyrate (mg/L)	Quantitative	S, MS	03:18			0.025	0.5	10	> 0.99
FV-22	Ethyl hexanoate (mg/L)	Quantitative	S, MS	07:42			0.02	1	10	> 0.99
FV-23	Ethyl octanoate (mg/L)	Quantitative	S, MS	15:18			0.02	1	10	> 0.99
FV-24	Ethyl decanoate (mg/L)	Quantitative	S, MS	24:18			0.02	1	10	> 0.99
	Acetate esters									
FV-25	Ethyl acetate (mg/L)	Quantitative	S, MS				2	173	3	> 0.99
FV-26	Isoamyl acetate (mg/L)	Quantitative	S, MS	04:46			0.06	3	10	> 0.99
FV-27	Hexyl acetate	Quantitative	S, MS	08:57			10	200	10	> 0.99
FV-28	2-Phenethyl acetate (mg/L)	Quantitative	S, MS	31:12			0.06	3	10	> 0.99
	Other esters									
FV-29	Ethyl lactate (mg/L)	Quantitative	S, MS	11:30			0.4	20	10	> 0.99
FV-30	Ethyl 3-hydroxybutanoate* (mg/L)	Semi-quantitative	RI, MS	18:38	1512	1507				
FV-31	Diethyl succinate* (mg/L)	Semi-quantitative	RI, MS	25:48	1674	1678				
FV-32	Methyl 4-hydroxybutanoate*	Semi-quantitative	MS	29:30						
FV-33	Ethyl 4-hydroxybutanoate* (mg/L)	Semi-quantitative	RI, MS	31:16	1802	1819				
FV-34	Diethyl malate* (mg/L)	Semi-quantitative	RI, MS	40:18	2034	2039				
FV-35	Monomethyl succinate*	Semi-quantitative	RI, MS	51:08	2317	2350				
FV-36	Monoethyl succinate* (mg/L)	Semi-quantitative	RI, MS	52:24	2373	2367				
FV-37	Ethyl *p*-coumarate* (mg/L)	Semi-quantitative	MS	82:30						
	Other									
FV-38	Acetaldehyde (mg/L)	Quantitative	S				2	144	3	> 0.99
FV-39	Benzaldehyde*	Semi-quantitative	S, MS	18:36						
FV-40	Methanol (mg/L)	Quantitative	S				2	151	3	> 0.99
FV-41	Guaiacol*	Semi-quantitative	S, MS	33:00						
FV-42	4-Vinylguaiacol*	Semi-quantitative	S, MS	45:42						
FV-43	Acetoin*	Semi-quantitative	S, MS	9:10						
FV-44	3-Hydroxy-2-pentanone*	Semi-quantitative	RI, MS	11:12	1326	1327				
FV-45	γ-Butyrolactone*	Semi-quantitative	RI, MS	22:58	1612	1607				
FV-46	Pantolactone*	Semi-quantitative	RI, MS	39:34	2016	2028				

FV-47	3-Methylthiopropanol* (mg/L)	Semi-quantitative	RI, MS	27:12	1708	1710				
FV-48	Tryptophol* (mg/L)	Semi-quantitative	MS	81:18						

Abbreviations: ID – identification of compounds: S – retention time consistent with that of a pure standard; LRI – linear retention index consistent with that found in literature; MS – mass spectra consistent with that from NIST 2.0 mass spectra electronic library or literature; t_R retention time in minutes:seconds; LRI_{exp} - linear retention index obtained experimentally; LRI_{lit} - linear retention index from the literature; LOQ – limit of quantification; R^2 – coefficients of determination of calibration curves used for quantification.

Table S3. Details of identification and quantification of bound aroma compounds in Malvazija istarska wines produced by different bentonite and tannins addition treatments, determined after fermentation and after final wine protein stabilization.

Code	Bound aroma compounds	Quantification	ID	t_R (min:sec)	LRI_{exp}	LRI_{lit}	LOQ	Conc. range (max.)	Calib. Levels (No.)	R^2
	Monoterpenes									
BV-1	*trans*-furan Linalool oxide*	Semi-quantitative	S, MS	15:10						
BV-2	*cis*-furan Linalool oxide*	Semi-quantitative	S, MS	16:24						
BV-3	Linalool (mg/L)	Quantitative	S, MS	20:06			0.02	1	10	> 0.99
BV-4	Hotrienol*	Semi-quantitative	RI, MS	22:46	1608	1613				
BV-5	α-Terpineol (mg/L)	Quantitative	S, MS	26:34			0.01	0.25	10	> 0.99
BV-6	*trans*-pyran Linalool oxide*	Semi-quantitative	RI, MS	27:58	1730	1740				
BV-7	Citronellol (mg/L)	Quantitative	S, MS	29:42			0.01	0.5	10	> 0.99
BV-8	Nerol (mg/L)	Quantitative	S, MS	30:40			0.01	0.5	10	> 0.99
BV-9	Geraniol (mg/L)	Quantitative	S, MS	32:48			0.01	0.5	10	> 0.99
BV-10	Terpendiol I*	Semi-quantitative	RI, MS	36:54	1948	1951				
BV-11	*trans*-8-Hydroxy-linalool*	Semi-quantitative	RI, MS	48:38	2266	2246				
BV-12	7-Hydroxy-geraniol*	Semi-quantitative	MS	49:54						
BV-13	*cis*-8-Hydroxy-linalool*	Semi-quantitative	RI, MS	49:57	2305	2292				
BV-14	*trans*-Geranic acid*	Semi-quantitative	S, MS	50:46						
	C_{13}-norisoprenoids									
BV-15	β-Damascenone	Quantitative	S, MS	31:18			0.25	2	10	> 0.99
BV-16	3-Hydroxy-β-damascone*	Semi-quantitative	RI, MS	56:42	2510	2504				
BV-17	3-Oxo-α-ionol*	Semi-quantitative	RI, MS	59:41	2597	2615				
BV-18	3-Hydroxy-7,8-dihydro-β-ionol*	Semi-quantitative	RI, MS	63:41	2738	2770				
BV-19	Vomifoliol*	Semi-quantitative	MS	74:05						
	Alcohols		RI, MS							
BV-20	1-Hexanol (mg/L)	Quantitative	S, MS	11:37			0.04	1	10	> 0.99
BV-21	*trans*-3-Hexenol*	Semi-quantitative	RI, MS	12:07	1346	1356				
BV-22	*cis*-3-Hexenol*	Semi-quantitative	S, MS	12:48						

BV-23	*trans*-2-Hexenol*	Semi-quantitative	S, MS	13:48						
BV-24	1-Octen-3-ol*	Semi-quantitative	S, MS	15:46						
BV-25	1-Octanol*	Semi-quantitative	S, MS	20:27						
BV-26	2-Phenylethanol*	Semi-quantitative	S, MS	34:42						
	Other									
BV-27	Benzaldehyde*	Semi-quantitative	S, MS	18:40						
BV-28	4-Vinylguaiacol*	Semi-quantitative	S, MS	45:26						
BV-29	Tryptophol*	Semi-quantitative	MS	81:00						

Abbreviations: ID – identification of compounds: S – retention time consistent with that of a pure standard; LRI – linear retention index consistent with that found in literature; MS – mass spectra consistent with that from NIST 2.0 mass spectra electronic library or literature; t_R retention time in minutes:seconds; LRI_{exp} - linear retention index obtained experimentally; LRI_{lit} - linear retention index from the literature; LOQ – limit of quantification; R^2 – coefficients of determination of calibration curves used for quantification.

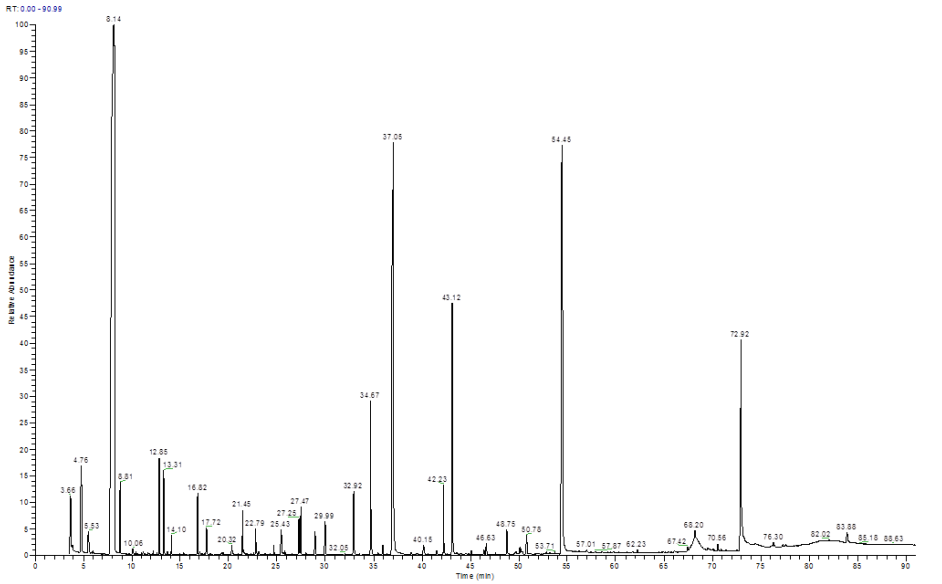

Figure S1. (a) Representative GC-MS chromatograms of a SPE extract of free volatile aroma compounds from Malvazija istarska white wine; **(b)** Representative GC-MS chromatograms of a SPE extract of bound aroma compounds from Malvazija istarska white wine.