Attività di ricerca scientifica svolta nell'ambito del Progetto V.I.B.R.I.S.

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Obiettivo Generale del Progetto

L'obiettivo generale della proposta progettuale è: lo sviluppo di protocolli di vinificazione per la produzione di vini bianchi a ridotto contenuto di solfiti, longevi e di elevata qualità sensoriale, avendo come oggetto di studio quattro varietà di uva specifiche del territorio della Costiera Amalfitana: Fenile, Ripoli, Ginestra e Pepella (*Vitis vinifera* L.)

Obiettivi Specifici perseguiti dal gruppo di ricerca della Sezione di Scienze della Vigna e del Vino

Analisi chimico-enologica delle uve raccolte nei campi a maturazione;

Sviluppo di protocolli innovativi a ridotto contenuto di anidride solforosa;

Analisi chimico-enologica dei vini ottenuti secondo i protocolli innovativi a ridotto contenuto di anidride solforosa proposti;

Analisi sensoriale dei vini ottenuti secondo i protocolli innovativi a ridotto contenuto di anidride solforosa proposti.

Caratterizzazione chimico-metabolica delle uve Fenile, Ginestra, Pepella e Ripoli (*Vitis vinifera* L.)

I vitigni autoctoni destano grande interesse da parte di studiosi e produttori, in quanto presentano caratteristiche chimico-metaboliche funzionali alla produzione di vini ad elevata identità territoriale.

L'Italia vanta un'ampia selezione di vitigni autoctoni e, tra questi, si annoverano quattro varietà della costiera amalfitana, caratterizzate da una ricca biodiversità: Fenile, Ginestra, Pepella e Ripoli. Nel 2005 questi quattro vitigni sono stati iscritti nel "Catalogo Nazionale delle varietà di vite".

Nell'ambito del progetto VIBRIS, è stato condotto, per la prima volta, uno studio scientifico finalizzato a delineare il profilo metabolico delle suddette quattro varietà di uva bianca, mediante esperimenti basati su tecniche avanzate quali la Risonanza Magnetica Nucleare (NMR) e la Spettrometria di massa accoppiata alla cromatografia liquida (LC-MS/MS).

Campioni di uva delle quattro cultivar oggetto di indagine sono stati raccolti nel 2021 e sono stati identificati sulla base delle descrizioni riportate in "La risorsa genetica della vite in Campania" di M. Manzoz e A. Monaco (eds. Se.S.I.R.C.A., Regione Campania).

Per ogni campione d'uva sono state separate le bucce dai vinaccioli, per essere estratti, separatamente, con una soluzione idroetanolica e analizzati mediante NMR e LC-MS/MS.

Gli spettri ^{1}H-NMR degli estratti idroetanolici delle bucce hanno permesso l'identificazione di diverse classi di composti chimici come carboidrati (glucosio e fruttosio), acidi organici (acido malico e tartarico), aminoacidi e quantità interessanti di un triterpenoide. Attraverso un'accurata indagine strutturale, il triterpenoide individuato negli estratti di bucce è risultato essere l'acido oleanolico. Si tratta di un composto ampiamente diffuso nel mondo vegetale, che, nelle uve, rappresenta un costituente maggioritario della cuticola delle bucce. I triterpenoidi di recente hanno trovato largo impiego come integratori alimentari nel settore nutraceutico e cosmetico, in virtù delle loro spiccate proprietà antitumorali, antiinfiammatorie, cicatrizzanti delle lesioni cutanee, antibiotiche e, non ultime, antivirali. Di fatto, durante la pandemia da COVID-19, l'acido

oleanolico e alcuni suoi analoghi naturali sono stati proposti come potenziali rimedi farmacologici contro il coronavirus. Inoltre, all'acido oleanolico è stata ultimamente attribuita anche una possibile attività antidiabetica, mediata attraverso il potenziamento, nel paziente diabetico, della risposta insulinica e attraverso la promozione della funzionalità delle cellule beta del pancreas deputate alla sintesi dell'insulina. Questo interessante composto potrebbe essere recuperato dalle vinacce nell'ottica dell'economia circolare.

Gli spettri ^{1}H-NMR degli estratti dei vinaccioli hanno evidenziato la presenza di tipici flavan-3-oli, (+)-catechina e (-)-epicatechina, oltre ad un'elevata concentrazione di polimeri, quali i tannini condensati.

L'analisi qualitativa e quantitativa, mediante LC-MS/MS, di bucce e vinaccioli ha portato, inoltre, alla definizione qualitativa e quantitativa dei profili fenolici dei campioni (Tabelle 1 e 2).

Tabella 1. Risultati qualitativi e quantitativi dei composti identificati (mg/g estratto ± SD) negli estratti idroalcolici di bucce Fenile, Ginestra, Pepella and Ripoli

Flavonoli

	quercetin-3-(6"-rhamnosyl)hexoside	**kaempferol 3-(6”-rhamnosyl)hexoside**	**quercetin-3-hexoside**	**kaempferol-3-hexoside**	**quercetin-3-hexuronide**	**kaempferol-3-hexuronide**
F	0.1910 ± 0.0165b	0.1504 ± 0.0141a	1.2381 ± 0.0764c	1.02642 ± 0.0045c	0.6753 ± 0.0622a	0.0366 ± 0.0098d
G	0.1700 ± 0.0028bc	0.1033 ± 0.0059c	2.5391 ± 0.0416b	1.4212 ± 0.0228b	0.6806 ± 0.1007a	0.0750 ± 0.0148c
P	0.1615 ± 0.0015c	0.0982 ± 0.0001c	2.2187 ± 0.1396b	2.0655 ± 0.0573a	0.4460 ± 0.0527b	0.2425 ± 0.0044a
R	0.3123 ± 0.0101a	0.1234 ± 0.0006b	5.1000± 0.0600a	2.0919 ± 0.1293a	0.3807 ± 0.0482b	0.1708 ± 0.0017b

Acidi cinnammici, acidi idrossibenzoici, stilbeni

	coumaric acid	**ferulic acid-*O*-hexoside**	**caffeic acid**	**caffeic acid-*O*- hexoside**	**resveratrol**	**piceid**	**protocatechuic acid**	**dihydroxybenzoic acid-*O*-hexoside**
F	0.0085 ± 0.0001c	0.0085 ± 0.0001c	0.0669 ± 0.0008a	0.0839 ± 0.0013a	0.007 ± 0.0001a	0.3299 ± 0.1986a	0.0026 ± 0.0001c	0.0085 ± 0.0001c
G	0.0100 ± 0.0001a	0.0100 ± 0.0001a	0.0640 ± 0.0003c	0.0486 ± 0.0016b	0.007 ± 0.0001b	0.2461 ± 0.0005a	0.0036 ± 0.0001a	0.0099 ± 0.0001a
P	0.0089 ± 0.0001b	0.0089 ± 0.0001b	0.0653 ± 0.0002b	0.0177 ± 0.0005d	n.d.	0.3386 ± 0.0089a	0.0032 ± 0.0001b	0.0089 ± 0.0001b
R	0.0078 ± 0.0001d	0.0078 ± 0.0001d	0.0668 ± 0.0002a	0.0224 ± 0.0005c	n.d.	0.2675 ± 0.0036a	0.0031 ± 0.0001b	0.0078 ± 0.0001d

Flavanoli

	(-)-epicatechin	**(+)-catechin**	**procyanidin B type**
F	0.1931± 0.0008d	0.4021 ± 0.0026a	1.3983 ± 0.0006c
G	0.2095 ± 0.0005c	0.3542 ± 0.0002c	1.4867 ± 0.0328c
P	0.2580 ± 0.0010a	0.3919 ± 0.0058b	2.9516 ± 0.0063a
R	0.2281 ± 0.0004b	0.2280 ± 0.0004d	2.0962 ± 0.1665b

(F) Fenile; (G) Ginestra; (P) Pepella; (R) Ripoli.

Tabella 2.

Composti	m/z pairs	FENILE				GINESTRA				PEPELLA				RIPOLI			
Catechina	289 (245, 205, 179)	33,57	±	0,10	c	27,60	±	0,03	d	43,00	±	0,08	b	74,30	±	0,07	a
Epicatechina	289 (245, 205, 179)	30,27	±	0,03	c	17,85	±	0,07	d	40,10	±	0,06	b	97,90	±	0,04	a
(Epi)catechina monogallata	441 (289, 169)	2,48	±	0,08	c	0,52	±	0,06	d	9,10	±	0,10	a	8,36	±	0,08	b
(Epi)catechina monoglycoside	451 (289)	2,43	±	0,03	c	3,25	±	0,01	b	0,63	±	0,03	d	4,43	±	0,01	a
Procianidina dimeri (B)	577 (451, 425, 407, 289)	33,49	±	0,07	c	23,52	±	0,07	d	40,78	±	0,11	b	73,16	±	0,10	a
(Epi)catechin diglycoside	613 (577, 289)	1,40	±	0,11	b	2,89	±	0,04	a	0,52	±	0,03	c	3,09	±	0,06	a
Procyanidin dimers monogallate	729 (603, 577, 559, 441, 425, 407)	4,34	±	0,06	c	0,89	±	0,10	d	11,37	±	0,04	a	9,35	±	0,04	b
(Epi)catechin dimers monoglycoside	739 (577, 451, 289, 287)	0,64	±	0,06	a	0,59	±	0,06	a	0,01	±	0,00	c	0,31	±	0,03	b
Procyanidin trimers (B)	865 (739, 695, 577, 575, 543, 449, 407)	22,82	±	0,06	b	15,27	±	0,01	c	8,04	±	0,04	d	47,66	±	0,08	a
Procyanidin dimers digallate (B)	881 (729, 711, 577, 559, 407)	0,47	±	0,06	c	1,13	±	0,03	b	0,35	±	0,04	d	0,40	±	0,04	a
(Epi)catechin dimers diglycoside	901 (407)	0,72	±	0,07	c	1,48	±	0,10	b	0,10	±	0,08	d	1,94	±	0,03	a
Procyanidin trimers monogallate (B)	1017 (729, 865, 847, 677, 891, 695, 575)	2,84	±	0,06	c	0,76	±	0,07	d	10,48	±	0,04	a	8,71	±	0,07	b
Procyanidin tetramers (B)	1153 (1027, 1001, 983, 865, 577, 407)	10,22	±	0,03	b	8,93	±	0,04	c	1,59	±	0,06	d	22,48	±	0,11	a
Procyanidin trimers digallate (B)	1169 (1017, 881, 729, 577)	0,40	±	0,04	b	0,92	±	0,03	a	0,28	±	0,04	b	0,34	±	0,01	b
Procyanidin tetramers monogallate (B)	1305 (1179, 1153, 1017, 1015, 865, 727, 577, 289)	2,08	±	0,07	c	0,56	±	0,06	d	8,91	±	0,03	a	7,93	±	0,03	b
Procyanidin pentamers (B)	1441 (1153, 1151, 865, 863, 577, 289)	4,82	±	0,06	c	6,72	±	0,01	a	0,59	±	0,04	d	6,40	±	0,01	b
Procyanidin tetramers digallate (B)	1457 (1017, 881, 729)	0,36	±	0,06	a	0,46	±	0,07	a	0,24	±	0,03	a	0,32	±	0,07	a
Procyanidin pentamers monogallate (B)	1593 (1441, 1153, 1017, 865, 729, 577, 289)	2,04	±	0,04	c	0,51	±	0,06	d	2,45	±	0,06	b	4,66	±	0,01	a
Procyanidin hexamers (B)	1729* (1153, 865, 577, 289)	2,97	±	0,01	c	4,64	±	0,03	a	0,42	±	0,01	d	3,65	±	0,03	b
Procyanidin pentamers digallate (B)	1745* (1017, 881, 729)	0,24	±	0,06	ab	0,35	±	0,04	a	0,15	±	0,04	b	0,28	±	0,03	ab
Procyanidin hexamers monogallate (B)	1881* (1017, 729, 289)	1,96	±	0,04	a	0,43	±	0,04	d	0,86	±	0,10	c	1,54	±	0,04	b
Procyanidin heptamers (B)	2017* (1441, 1153, 865, 577, 289)	2,66	±	0,06	a	1,90	±	0,07	b	0,29	±	0,04	c	2,84	±	0,03	a
Procyanidin hexamers digallate (B)	2033* (1017, 881, 729)	0,21	±	0,01	a	0,29	±	0,10	a	0,10	±	0,07	a	0,14	±	0,06	a
Procyanidin heptamers monogallate (B)	2169* (1593, 1441, 1305, 1017, 865, 289)	1,88	±	0,01	a	0,29	±	0,06	c	0,25	±	0,04	c	1,06	±	0,04	b
Procyanidin octamers (B)	2305* (1441, 1153, 865, 577, 289)	2,53	±	0,04	a	0,93	±	0,04	c	0,16	±	0,03	d	1,25	±	0,04	b
Procyanidin heptamers digallate (B)	2321* (1017, 881, 729)	0,15	±	0,06	a	0,22	±	0,07	a	0,06	±	0,06	a	0,13	±	0,10	a
Procyanidin octamers monogallate (B)	2457* (1593, 1441, 1305, 1017, 865, 577, 289)	1,56	±	0,04	a	0,18	±	0,06	c	0,03	±	0,03	c	0,87	±	0,01	b
Procyanidin nonamers (B)	2593* (1441, 1153, 865, 577, 289)	0,98	±	0,04	a	0,20	±	0,07	b	0,05	±	0,03	b	1,14	±	0,01	a
Procyanidin octamers digallate (B)	2609* (1017, 881, 729)	0,13	±	0,07	a	0,15	±	0,01	a	0,03	±	0,01	a	0,10	±	0,07	a
Procyanidin nonamers monogallate (B)	2745* (1593, 1441, 1305, 1228, 1084, 1017, 289)	1,22	±	0,04	a	0,08	±	0,03	c	0,01	±	0,01	c	0,35	±	0,03	b
Procyanidin nonamers digallate (B)	2897* (1017, 729)	0,11	±	0,01	a	0,14	±	0,04	a	0,03	±	0,03	a	0,10	±	0,06	a
Procyanidin decamers digallate (B)	3195* (1017, 729)	0,10	±	0,04	a	0,08	±	0,03	a	0,01	±	0,00	a	0,02	±	0,01	a
Total (mg/g)		*172*				*124*				*181*				*385*			
% Galloylation		*13,1*				*6,43*				*24,7*				*11,6*			

Risultati qualitativi e quantitativi dei composti identificati (mg/g estratto ± SD) negli estratti idroalcolici di vinaccioli di Fenile, Ginestra, Pepella and Ripoli

Nello specifico, negli estratti di vinaccioli sono stati individuati principalmente flavanoli monomerici e polimerici (fino ai decameri). I vinaccioli di Ripoli sono risultati molto più ricchi di flavanoli rispetto alle altre tre varietà. Particolarmente elevata è risultata la percentuale di galloilazione dei flavanoli in Pepella e significativamente bassa, in Ginestra. Poiché è noto che una maggiore quantità di polimeri flavanolici e una maggiore percentuale di galloilazione determinano una maggiore astringenza, è chiaro quanto una rapida separazione dei mosti dalle bucce e dai vinaccioli di Pepella e Ripoli sia necessaria per evitare difetti sensoriali nei vini.

Per quanto riguarda i flavanoli presenti nelle bucce, il contenuto di (+)-catechine è risultato superiore a quello delle (-)-epicatechine, con qualche differenza più significativa nel caso di Fenile e Ginestra, rispetto a Pepella e Ripoli. Le procianidine in Pepella e Ripoli erano presenti in concentrazioni maggiori rispetto a Fenile e Ginestra. Dal punto di vista enologico, un'eccessiva presenza di flavanoli e procianidine può essere dannosa, in quanto sono responsabili dell'ossidazione chimica, dell'amarezza e dell'astringenza del vino, motivo per cui è opportuno che, durante la vinificazione di Pepella e Ripoli e, in misura minore, di Fenile e Ginestra, si limiti il più possibile la loro estrazione dalle bucce.

Sempre, nelle bucce, il contenuto di flavonoli, è risultato qualitativamente uguale nelle quattro varietà analizzate, ma sono emerse differenze significative in termini quantitativi: Ripoli è risultata particolarmente ricca di derivati della quercetina, ma con la più bassa quantità di derivati del kaempferolo; Ginestra presentava derivati di quercetina e kaempferolo paragonabili; Fenile e Ripoli avevano un quantitativo di quercetine pari al doppio dei kaempferoli.

Questi dati quantitativi relativi ai flavonoli sono un interessante indice di autenticità e differenziazione varietale e rappresentano, quindi, un importante strumento per la caratterizzazione tassonomica delle uve analizzate.

Sono stati indentificati nelle bucce anche composti appartenenti alla classe dell'acido cinnamico, sostanze cruciali nelle prime fasi di vinificazione. Nel mosto le polifenolossidasi (OPP) ossidano rapidamente questi composti in chinoni altamente reattivi, che sono responsabili delle reazioni di imbrunimento e di una riduzione dell'aroma varietale, a causa della reattività con anidride solforosa e composti aromatici contenenti zolfo. Pertanto, i dati ottenuti in questo studio suggeriscono che, soprattutto per le uve Fenile e Ginestra, durante le prime fasi della vinificazione, è importante limitare l'attività negativa delle OPP, mediante una corretta gestione della temperatura e dell'anidride solforosa.

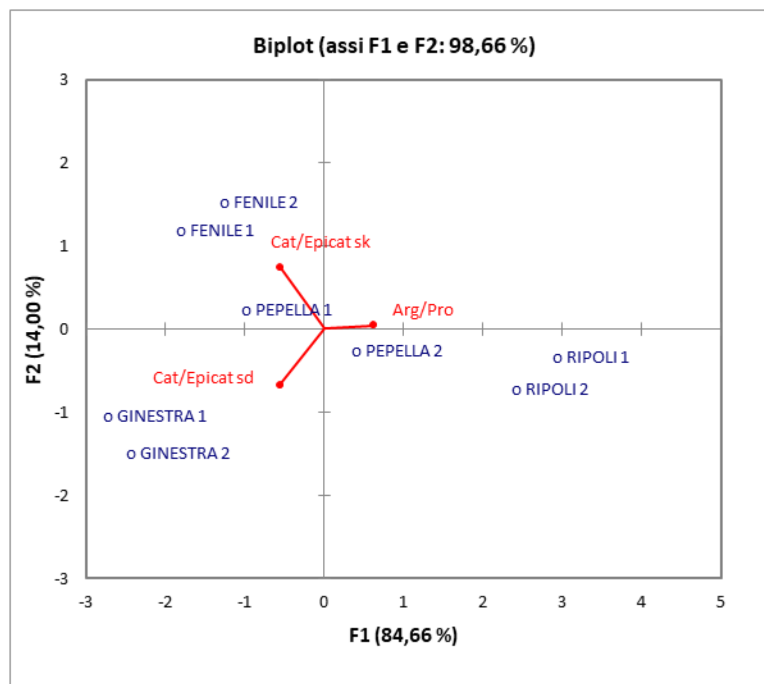
In aggiunta alle analisi descritte, si è definita anche la composizione aminoacidica delle quattro varietà di uve mediante HPLC (Tabella 3).

Tabella 3.

Amino acid	FENILE			GINESTRA			PEPELLA			RIPOLI		
	R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*	
Aspartic acid	21.68	0.005 ± 0.00	a	22.73	0.002 ± 0.00	c	22.63	0.001 ± 0.00	d	23.25	0.004 ± 0.00	b
Glutamic acid	25.86	0.049 ± 0.00	d	27.44	0.158 ± 0.00	c	27.62	0.259 ± 0.00	a	27.43	0.158 ± 0.00	b
Serine	30.44	0.006 ± 0.00	b	31.11	0.002 ± 0.00	c	31.01	0.001 ± 0.00	d	31.17	0.010 ± 0.00	a
Asparagine	31.38	0.011 ± 0.00	b	31.95	0.012 ± 0.00	a	31.87	0.006 ± 0.00	c	32.05	0.003 ± 0.00	d
HO-proline	33.73	0.025 ± 0.00	a	34.12	0.005 ± 0.00	d	34.05	0.006 ± 0.00	c	33.26	0.012 ± 0.00	b
Glutamine	34.36	0.008 ± 0.00	a	34.69	0.002 ± 0.00	b	34.62	0.002 ± 0.00	c	nd	0.000 ± 0.00	d
Histidine	34.95	0.006 ± 0.00	b	35.40	0.003 ± 0.00	c	35.32	0.002 ± 0.00	d	34.32	0.006 ± 0.00	a
Glycine	35.35	0.003 ± 0.00	b	35.70	0.005 ± 0.00	a	35.60	0.002 ± 0.00	d	35.04	0.002 ± 0.00	c
Threonine	36.23	0.019 ± 0.00	a	36.40	0.004 ± 0.00	c	36.36	0.004 ± 0.00	d	35.93	0.009 ± 0.00	b
β-alanine	36.59	0.021 ± 0.00	a	36.82	0.003 ± 0.00	b	36.77	0.002 ± 0.00	c	36.68	0.002 ± 0.00	d
Arginine	37.15	0.185 ± 0.00	a	37.37	0.009 ± 0.00	d	37.33	0.030 ± 0.00	c	37.13	0.110 ± 0.00	b
α-alanine	38.54	0.034 ± 0.00	a	38.79	0.013 ± 0.00	c	38.74	0.008 ± 0.00	d	38.47	0.027 ± 0.00	b
GABA	39.09	0.048 ± 0.00	a	39.35	0.021 ± 0.00	d	39.30	0.024 ± 0.00	c	39.01	0.044 ± 0.00	b
Proline	39.71	0.211 ± 0.00	a	40.04	0.087 ± 0.00	b	40.05	0.023 ± 0.00	d	39.73	0.024 ± 0.00	c
Tyrosine	42.12	0.213 ± 0.00	a	42.53	0.102 ± 0.00	c	42.44	0.097 ± 0.00	d	42.01	0.136 ± 0.00	b
Valine	46.44	0.011 ± 0.00	a	46.64	0.007 ± 0.00	b	46.55	0.006 ± 0.00	d	46.08	0.006 ± 0.00	c
Methionine	48.46	0.015 ± 0.00	a	49.21	0.003 ± 0.00	c	48.61	0.002 ± 0.00	d	48.29	0.008 ± 0.00	b
Cysteine	50.41; 56.80	0.012 ± 0.00	d	50.78; 56.88	0.015 ± 0.00	a	50.66; 56.84	0.015 ± 0.00	b	50.58; 56.85	0.014 ± 0.00	c
Isoleucine	51.83	0.014 ± 0.00	a	52.02	0.005 ± 0.00	b	51.94	0.004 ± 0.00	d	51.80	0.004 ± 0.00	c
Tryptophan	52.34	0.004 ± 0.00	b	52.49	0.001 ± 0.00	d	52.33	0.002 ± 0.00	c	52.51	0.007 ± 0.00	a
Leucine	52.90	0.014 ± 0.00	a	53.16	0.010 ± 0.00	b	53.08	0.008 ± 0.00	c	52.99	0.008 ± 0.00	d
Phenylalanine	53.54	0.007 ± 0.00	a	53.98	0.004 ± 0.00	c	53.87	0.004 ± 0.00	b	53.85	0.002 ± 0.00	d
Ornithine	58.62	0.010 ± 0.00	d	58.80	0.038 ± 0.00	b	58.68	0.043 ± 0.00	a	58.91	0.023 ± 0.00	c
Lysine	59.68	0.004 ± 0.00	a	59.73	0.003 ± 0.00	b	59.70	0.003 ± 0.00	c	59.72	0.003 ± 0.00	bc
Tyramine	69.65	0.001 ± 0.00	d	70.04	0.004 ± 0.00	b	70.06	0.003 ± 0.00	c	70.08	0.020 ± 0.00	a

Fenile è risultata la varietà con più alto contenuto totale di aminoacidi, di cui, tirosina, prolina e arginina, in ordine, sono presenti in maggiore concentrazione. La tirosina è fonte di tirosolo che viene prodotto dai lieviti, durante la fermentazione alcolica, la prolina protegge le piante dallo stress idrico, mentre l'arginina è stata a lungo considerata un indicatore dei livelli di azoto nella vite ed è uno degli aminoacidi più importanti per i lieviti nel mosto, durante la fase stazionaria della fermentazione.

In conclusione, allo scopo di differenziare le varietà analizzate, combinando i dati ottenuti, si è condotta un'analisi PCA, impostata sui rapporti tra aminoacidi più abbondanti (Arg/Pro) e catechina ed epicatechina (Cat/Epicat), rispettivamente in vinaccioli (sd) e bucce (sk). Il rapporto Arginina/Prolina è indice del potenziale valore nutritivo dei mosti per i lieviti, mentre il rapporto Cat/Epitcat è utile al fine di differenziare le varietà d'uva. Dall'analisi si è concluso che Ripoli differisce da Fenile e Ginestra per i rapporti Arg/Pro e Cat/epicat, relativamente ai vinaccioli e che Fenile e Ginestra sono leggermente divergenti, per quanto riguarda i rapporti Cat/Epicat in vinaccioli e bucce, mentre non è stato possibile differenziare chiaramente le uve Pepella, sulla base dell'analisi PCA.



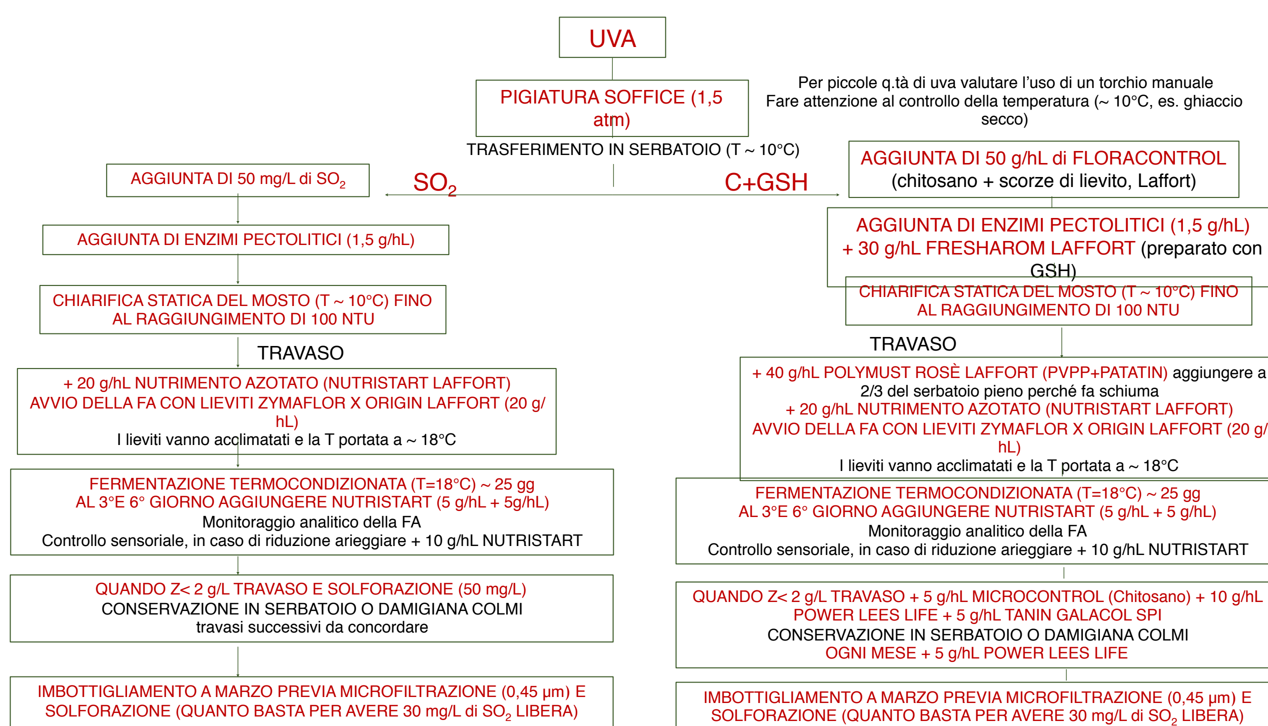
Classificazione delle varietà d'uva in base al rapporto aminoacidico (arginina/prolina) e dei flavanoli (catechina/epicatechina) estratti dai vinaccioli (sd) e dalle bucce (sk).

I risultati dell'analisi chimico-metabolica sono fondamentali per i produttori, per sviluppare pratiche viticole ed enologiche funzionali alla produzione di vini di alta qualità e con un'elevata identità territoriale, con indubbie ricadute sulla reputazione delle cantine coinvolte come pure dell'intero territorio della costiera amalfitana.

Inoltre, come anticipato, gli specifici profili metabolici delle quattro varietà d'uva senz'altro custodiscono il segreto della loro sopravvivenza negli aspri ambienti pedoclimatici della Costiera Amalfitana e potrebbero fornire interessanti informazioni al fine di selezionare i cloni di vite adatti a specifici ambienti pedoclimatici soggetti a rapidi mutamenti, come conseguenza dei cambiamenti climatici in atto.

Protocolli di vinificazione 2021 e 2022 e analisi sensoriale dei vini prodotti in base al protocollo 2021 e 2022

Alla luce dei dati chimico-analitici ottenuti sulle uve, per ciascuna varietà di uva, sono stati elaborati due protocolli di vinificazione: uno convenzionale con impiego di SO_2 ed uno innovativo, sviluppato in base alle più recenti conoscenze in tema di alternative all'impiego di SO_2 in enologia. Nello specifico, nel protocollo convenzionale di vinificazione in bianco la protezione dalle ossidazioni e dalle alterazioni microbiche è stata gestita mediante l'addizione di anidride solforosa (SO_2) in diverse fasi del processo di vinificazione. Nel secondo protocollo di vinificazione (C+GSH), invece, non è stata prevista alcuna addizione di SO_2, ma la protezione antiossidante, sia in fase pre- che post-fermentativa, e il controllo microbiologico sono stati gestiti mediante l'impiego di additivi e coadiuvanti enologici innovativi, in particolare chitosano (C) e derivati del lievito ricchi in glutatione (GSH). Di seguito sono riportati i protocolli elaborati nel 2021:



Un punto cruciale del progetto è stato la valutazione della qualità sensoriale dei vini sperimentali ottenuti secondo i due protocolli. Al fine di valutare se l'applicazione dei due protocolli di vinificazione avesse determinato differenze sensoriali tra i vini ottenuti dalla stessa varietà di uva, sono stati condotti dei test di analisi sensoriale discriminante. Il panel era composto da 20 giudici (8 femmine e 12 maschi) di età compresa tra i 24 ed i 51 anni (età media 31). Ai giudici sono state servite le coppie di campioni costituite dai due vini della stessa varietà ottenuti secondo i due protocolli di vinificazione sperimentali (convenzionale con aggiunta di SO_2) e alternativo con chitosano e derivati del lievito ricchi in glutatione (C+GSH)). Il test ha previsto due fasi: una prima fase in cui è stato chiesto ai giudici di valutare l'esistenza di differenze nell'odore dei vini e una seconda fase in cui ai giudici è stato chiesto di assaggiare i vini e valutare quindi l'esistenza di eventuali differenze nel profilo sensoriale globale.

I vini delle varietà Ripoli, Ginestra, Pepella e Fenile ottenuti con i due protocolli di vinificazione sono risultati significativamente diversi all'odore. Nel caso del campione Ripoli, il 75% della giuria ha percepito i vini come diversi all'odore, ma la differenza non è risultata essere statisticamente significativa ($p<0,05$). Per i vini Pepella

e Fenile, l'esistenza di differenze sensoriali è stata confermata anche per il profilo sensoriale globale, mentre nel caso di Ginestra e Ripoli la differenza non è risultata essere statisticamente significativa ($p<0,05$).

Al fine di indagare più finemente le differenze sensoriali emerse nell'analisi sensoriale discriminante, è stata condotta un'analisi sensoriale quantitativo-descrittiva dei vini sperimentali. Il panel era composto da 8 giudici (6 femmine e 2 maschi) di età compresa tra i 24 e 51 anni. I giudici selezionati sono stati addestrati per il riconoscimento degli odori dei vini bianchi e la quantificazione della loro intensità usando scale di misura.

Per la valutazione dei descrittori olfattivi è stata utilizzata una scheda messa a punto dal gruppo di ricerca per l'analisi sensoriale dei vini bianchi ed utilizzata dai giudici in fase di addestramento. L'intensità dei descrittori olfattivi è stata valutata utilizzando una scala di misura a 9 punti (1= molto debole, 2 = debole, 3 = medio, 4 = forte, 5 = molto forte; con la possibilità di assegnare un punteggio intermedio tra due punti consecutivi).

I risultati riportati in Figura 1 mostrano come la varietà Fenile vinificata con l'aggiunta di SO_2 ha note odorose di Fruttato, Agrumato, Frutta Esotica, Floreale e Vegetale/Erbaceo più intense rispetto a quelle del vino vinificato senza aggiunte di SO_2; in quest'ultimo, risulta molto più evidente la presenza di difetti olfattivi, in particolare, diversi giudici hanno riportato odori di vernice/solvente ed ossidato.

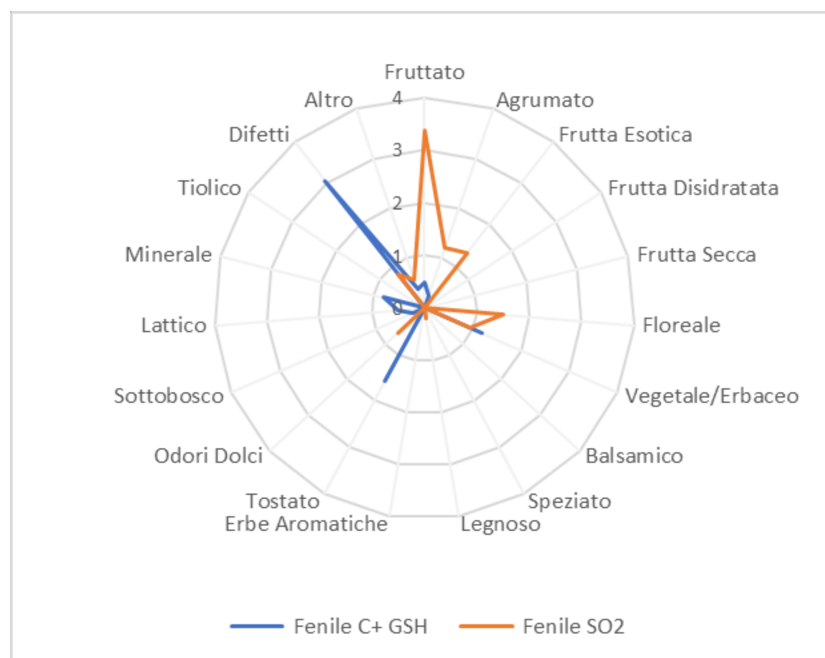


Figura 1. Profili olfattivi dei vini sperimentali della varietà Fenile

Per quanto riguarda i vini ottenuti dalla vinificazione della varietà Ginestra (Figura 2), innanzi tutto si può notare che le note odorose percepite per i due vini sono di debole intensità. Il vino ottenuto secondo il protocollo convenzionale con aggiunta di SO_2 presentava note odorose floreali e di odori dolci più intense e dominanti, mentre il vino ottenuto senza aggiunte di SO_2 presentava una maggiore intensità per i descrittori "Lattico" e "Difetti" (Ossidato e Acescenza).

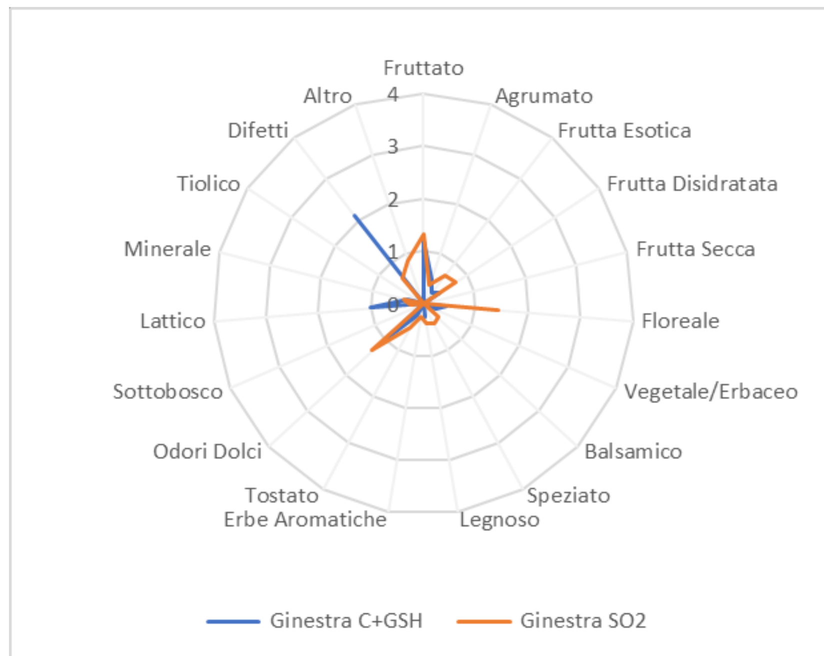


Figura 2. Profili olfattivi dei vini sperimentali della varietà Ginestra

In Figura 3 sono riportati i profili olfattivi dei vini ottenuti dalla varietà Pepella. Come si può notare dal grafico, in entrambi i vini il profilo olfattivo è dominato dalla presenza di difetti d'odore. La natura e l'origine di tali difetti è però diversa nei due vini. In particolare, i giudici hanno riportato la presenza di odore di riduzione nel "Pepella SO_2", mentre il campione "Pepella C+GSH" presentava difetti di Ossidato e Acescenza.

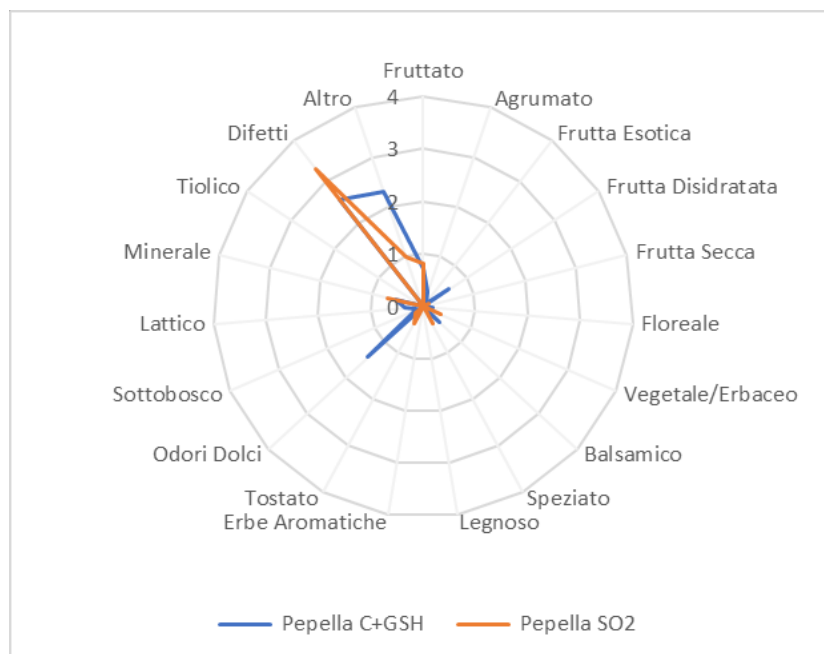


Figura 3. Profili olfattivi dei vini sperimentali della varietà Pepella

Come si evince dalla Figura 4, i due vini della varietà Ripoli hanno ottenuto punteggi simili per quanto riguarda le note odorose piacevoli associate ai vini (Fruttato, Agrumato, Floreale); le differenze si riscontrano nella

presenza di una nota "Lattico" molto più intensa nel campione ottenuto senza l'aggiunta di SO_2 e di un odore di "frutta secca", assente nel vino con SO_2. In quest'ultimo sono state anche riportate note di "erbe aromatiche", "minerale" e "tiolico" e "odori dolci", assenti nell'altro campione.

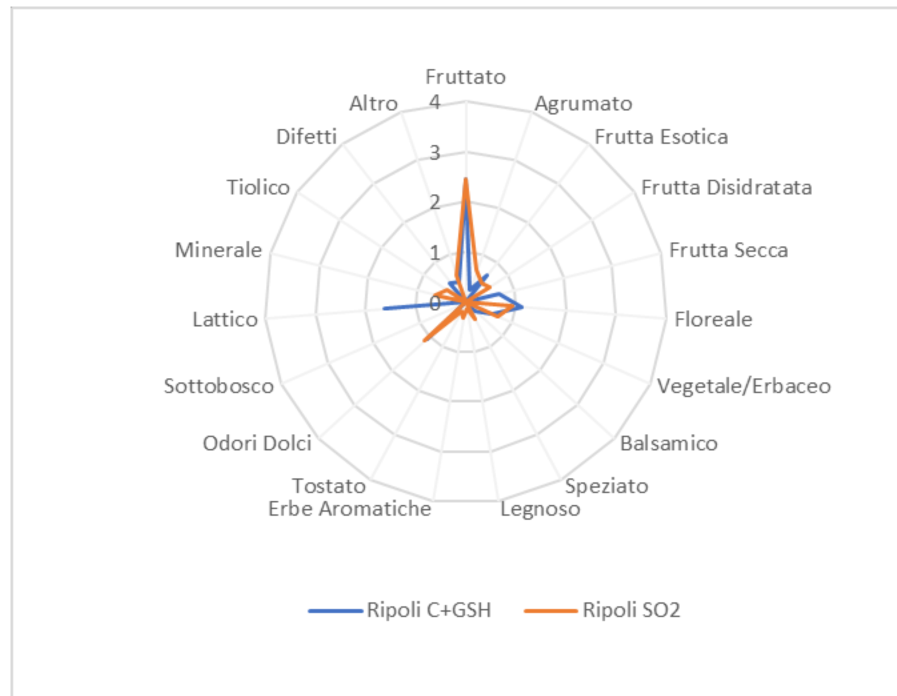


Figura 4. Profili olfattivi dei vini sperimentali della varietà Ripoli

Al fine di indagare a livello molecolare le differenze riscontrate a livello sensoriale, è stata analizzata la frazione volatile dei vini sperimentali.

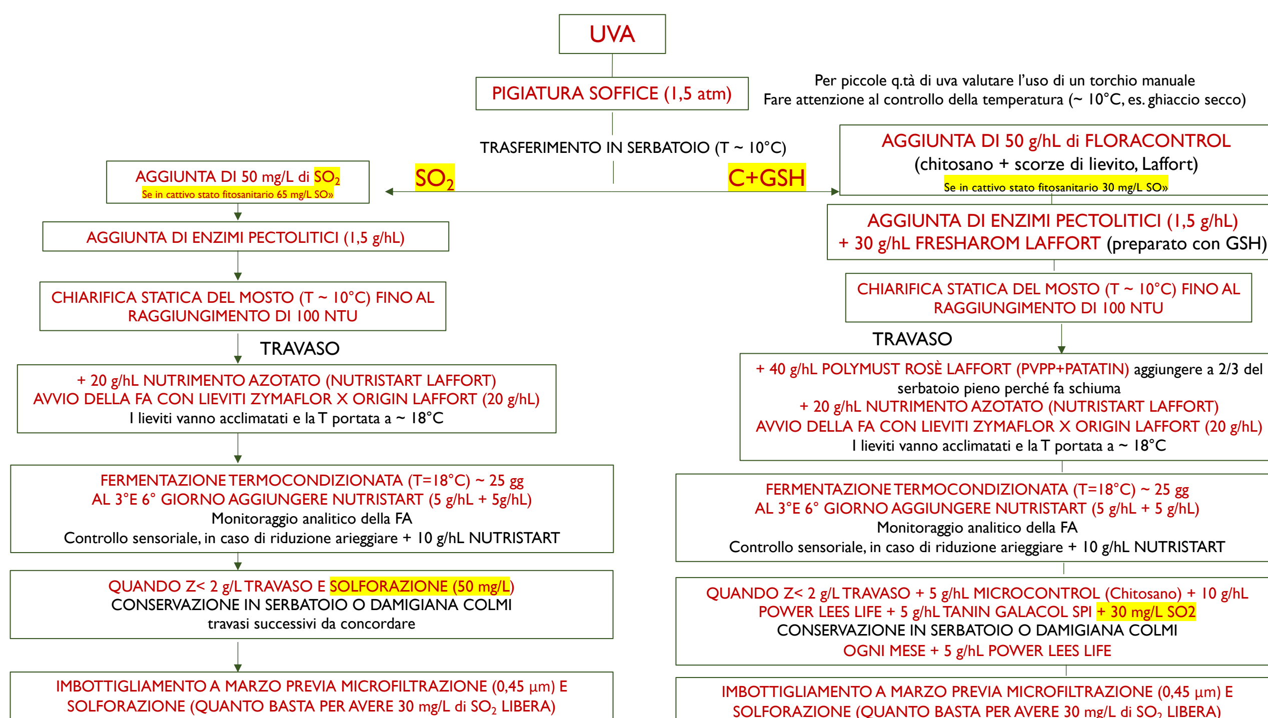
Sono stati determinati 34 composti volatili, appartenenti a diverse classi chimiche: esteri, alcoli, acidi, terpeni, norisoprenoidi e composti solforati.

I risultati dell'analisi chimica delle molecole volatili dei vini sperimentali ottenuti dalla varietà Fenile, evidenziano che le maggiori differenze tra i due protocolli di vinificazione riguardano le classi chimiche degli esteri acetici e degli alcoli. Gli esteri acetici appartengono agli aromi di fermentazione e sono responsabili principalmente di odori fruttati. La loro maggiore concentrazione nel vino FSO_2 potrebbe spiegare la maggiore intensità di odori fruttati rilevata in questo vino all'analisi sensoriale. Al contrario, gli alcoli superiori, responsabili di note odorose correlate negativamente alla qualità nei vini, sono più abbondanti nel campione FC+GSH.

In conclusione, dall'analisi sensoriale e dall'analisi della frazione volatile dei vini sperimentali è emerso che l'applicazione dei due protocolli di vinificazione ha determinato risultati diversi a seconda della varietà di uva di partenza. Le maggiori differenze a carico della frazione volatile sono state riscontrate per le classi chimiche degli esteri e degli alcoli superiori. Essendo tali classi rappresentate principalmente da aromi di fermentazione, questi risultati suggeriscono che la presenza o assenza di SO_2 durante i processi fermentativi abbia determinato differenze nel metabolismo fermentativo e/o nelle specie microbiche presenti. Una prima analisi della frazione volatile per classi chimiche di composti, suggerisce che le differenze sensoriali significative riscontrate per i vini della varietà Fenile (FSO_2 più fruttato di FC+GSH) potrebbero essere dovute alla maggiore concentrazione di esteri acetici in FSO_2.

Sulla scorta dei risultati ottenuti, per la vendemmia del 2022 è stato proposto un protocollo di vinificazione in parte modificato per correggere i “difetti” evidenziati nei vini prodotti secondo il protocollo del 2021.

Di seguito si riporta lo schema del protocollo 2022 con in evidenza le modifiche rispetto al 2021.



La modifica al protocollo consisteva nell'aggiunta di 30 mg/L di SO_2 alla sfecciatura, effettuata quando la concentrazione in solidi solubili solubili risultava essere inferiore a 2 g/L, oltre agli additivi alternativi già previsti nel protocollo iniziale; inoltre, solo nel caso in cui le uve si presentassero in cattivo stato fitosanitario, era prevista un'aggiunta di 20 mg/L di SO_2 all'ammostatura.

Anche nel secondo anno di sperimentazione, al fine di valutare se l'applicazione dei due protocolli di vinificazione avesse determinato differenze sensoriali tra i vini ottenuti dalla stessa varietà di uva, sono stati condotti dei test di analisi sensoriale discriminante. Il panel era composto da 21 giudici (8 femmine e 13 maschi) di età compresa tra 21 e 72 anni (età media 34 anni). Ai giudici sono state servite le coppie di campioni costituite dai due vini della stessa varietà ottenuti secondo i due protocolli di vinificazione sperimentali: convenzionale con aggiunta di SO_2 (codice: SO_2) e alternativo con chitosano e derivati del lievito ricchi in glutatione (codice: C+GSH). La domanda posta ai giudici in questo tipo di test è: i campioni all'interno di una coppia sono uguali o diversi? Il test è stato condotto secondo due modalità in sessioni separate: sia considerando solo l'odore dei vini che in seguito a degustazione, quindi considerando il profilo sensoriale globale. Per monitorare la capacità discriminante del panel, tra i campioni sperimentali è stato introdotto un test di controllo che consisteva in una coppia costituita da uno stesso vino bianco, diverso da quelli della sperimentazione. A differenza dei risultati ottenuti nel primo anno di sperimentazione (protocollo di vinificazione 2021), per tutte le varietà, eccetto Ripoli, non sono state riscontrate differenze statisticamente significative tra i vini ottenuti con i due protocolli di vinificazione (SO_2 vs C+GSH) ($\alpha=0.05$). Questo risultato

è molto interessante considerando che per questo tipo di test la giuria è composta da un elevato numero di giudici non specificatamente addestrati all'analisi sensoriale. Alla luce di ciò, il risultato del test è rappresentativo di quello che potrebbe essere percepito da un normale consumatore dei vini in oggetto. L'assenza di differenze significative rilevate mostra che per queste varietà l'uso del protocollo alternativo con aggiunta ridotta di SO_2 ha prodotto vini non significativamente diversi rispetto al protocollo convenzionale, quantomeno per un normale consumatore di vino.

Successivamente, una giuria di analisi sensoriale selezionata ed addestrata è stata utilizzata per ottenere i profili olfattivi dei vini oggetto di studio. La giuria era composta da 10 giudici, 4 maschi e 6 femmine, di età compresa tra 24 e 54 anni ed età media di 34,5 anni. I giudici sono stati addestrati all'analisi sensoriale del vino, ma considerando la tematica del progetto, una buona parte dell'addestramento è stata finalizzata al riconoscimento e alla quantificazione di difetti ossidativi sia di natura chimica che microbiologica (acescenza). In particolare, questa seconda parte dell'attività del WP dedicato all'analisi sensoriale dei vini sperimentali è stata finalizzata a valutare se la criticità relativa alla comparsa di difetti d'odore d'ossidazione, soprattutto nei vini prodotti con protocollo alternativo, fosse stata superata grazie al protocollo di vinificazione modificato.

La figura 5 riporta l'intensità media del descrittore d'odore "ossidato", per i vini sperimentali prodotti nei due anni di sperimentazione. Secondo i focus group che hanno preceduto le sedute di misura e finalizzati a raggiungere il consenso della giuria sull'interpretazione dei descrittori, questo termine si riferisce ai caratteristici odori che compaiono nei vini bianchi in seguito ad ossidazione chimica, come "marsala", "liquoroso", "curry", "frutta essiccata". Si ricordi che il protocollo convenzionale (SO_2) è rimasto invariato nei due anni, mentre il protocollo alternativo senza SO_2 (C+GSH) è stato modificato nel 2022 prevedendo un ridotto apporto di SO_2 alla sfecciatura al fine di superare le criticità qualitative riscontrate nel 2021.

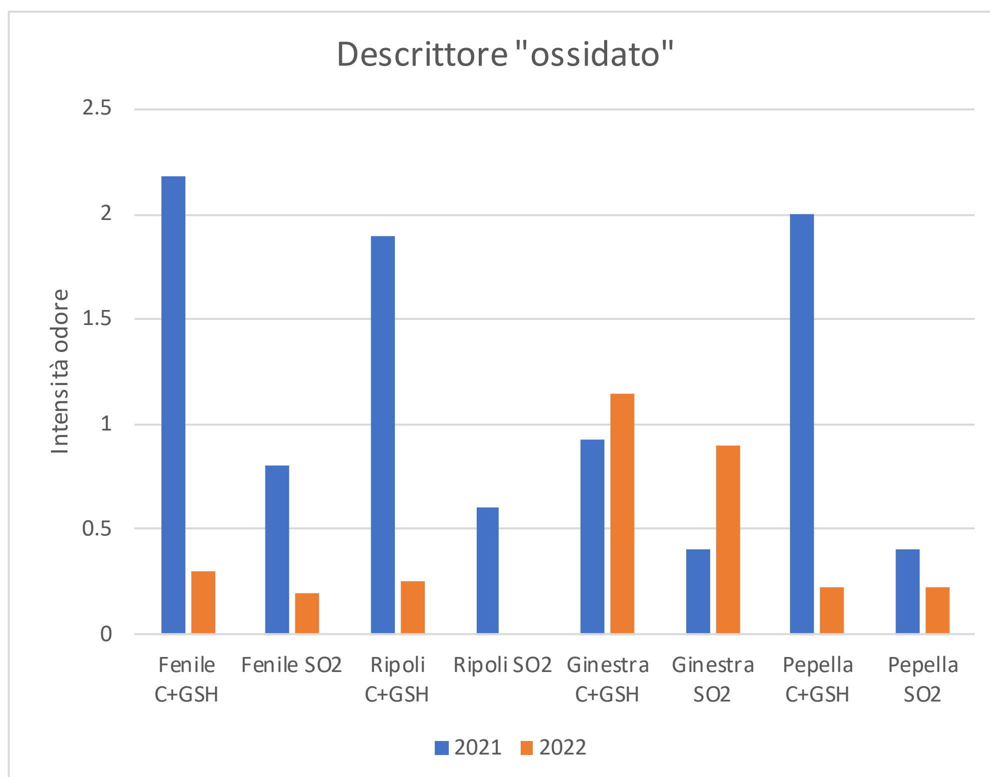


Figura 5. Intensità media del descrittore d'odore "ossidato" rilevato durante l'analisi quantitativo-descrittiva nei vini sperimentali prodotti nei due anni di sperimentazione.

Mentre nel 2021 i vini prodotti con il protocollo C+GSH presentavano un'intensità del descrittore "ossidato" significativamente più elevata rispetto al corrispondente vino "SO2", sebbene debole, nel 2022 l'intensità del descrittore si è drasticamente ridotta ed è interessante notare che è paragonabile a quella del corrispondente vino convenzionale. Questo dimostra che l'applicazione del protocollo modificato 2022 ha consentito di ridurre le dosi di SO_2 e di contenere la comparsa di difetti d'ossidazione chimica, grazie all'impiego concomitante di chitosano, derivati di lievito ricchi in glutatione e tannini enologici. Molto interessante anche il risultato ottenuti per il descrittore "acescenza" nei vini Ginestra e Pepella, riportati nella figura 6.

Nel primo anno di sperimentazione, i vini delle varietà Ginestra e Pepella prodotti senza l'aggiunta di SO_2 (C+GSH) mostravano un debole odore di spunto acetico, che invece non era presente nei vini analoghi prodotti con l'aggiunta di SO_2. Questa criticità è stata efficacemente superata nel secondo anno, quando nei vini a ridotto apporto di SO_2 (C+GSH) non presentavano il difetto di spunto acetico. La modifica del protocollo ha evidentemente consentito un migliore controllo sullo sviluppo batterico e una maggiore stabilità microbiologica.

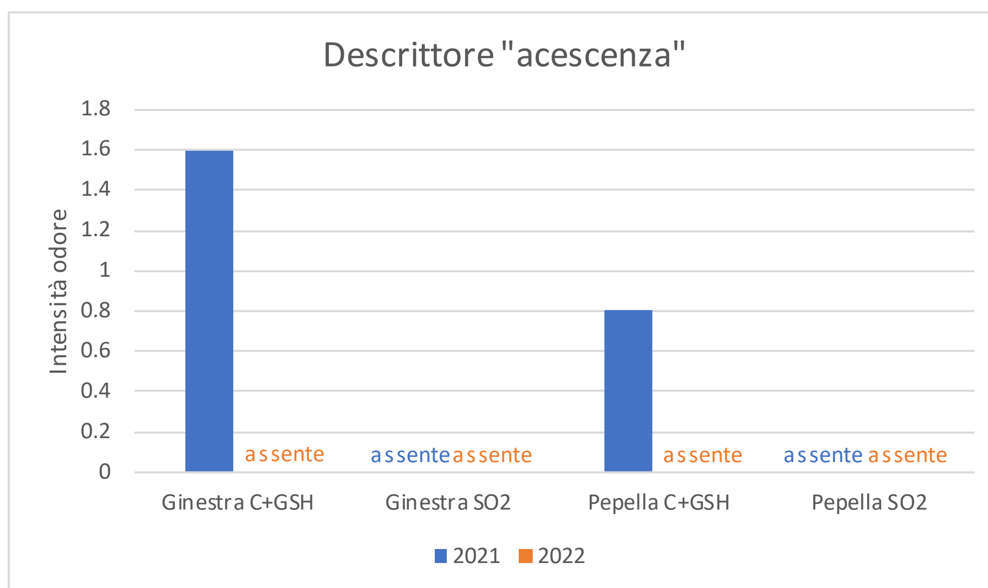


Figura 6. Intensità media del descrittore d'odore "acescenza" rilevato durante l'analisi quantitativo-descrittiva dei vini sperimentali Ginestra e Pepella, prodotti nei due anni di sperimentazione.

Conclusione

La riduzione delle dosi di SO_2 in vinificazione è una tematica estremamente sentita dall'industria enologica. Tuttavia, considerando le azioni protettive svolte dall'SO_2, antimicrobica e antiossidante, l'effetto della sua eliminazione dal processo di vinificazione o della riduzione delle dosi può incidere negativamente sulla qualità del vino. Pertanto, protocolli di vinificazione alternativi "no-sulphite" o "low-sulphite" vanno sviluppati e valutati attentamente mediante un approccio scientifico. Nel presente progetto, inizialmente è stata effettuata un'approfondita analisi delle uve provenienti dalle quattro varietà oggetto di studio: Fenile, Ripoli, Pepella e Ginestra.

Successivamente, nel primo anno di progetto un protocollo di vinificazione convenzionale con aggiunta di SO_2 è stato confrontato con un protocollo alternativo che non prevedeva alcuna addizione di SO_2, ma in cui sono stati utilizzati additivi e coadiuvanti di origine naturale ad azione antimicrobica e antiossidante (chitosano di origine fungina, lieviti inattivi ricchi in glutatione, tannini enologici). I vini prodotti sono stati analizzati da un punto di vista chimico e sensoriale. Le analisi chimiche hanno mostrato che l'azione dei coadiuvanti alternativi utilizzati ha sortito l'effetto desiderato, ma i livelli di protezione dal rischio ossidativo a carico della materia

colorante dei vini era inferiore a quella dell'SO_2. L'analisi sensoriale ha messo in evidenza la comparsa di difetti d'ossidazione nei vini prodotti con protocollo alternativo. Sebbene tali difetti fossero di debole intensità, considerando la loro azione mascherante sull'identità sensoriale del vino e le potenzialità qualitative delle varietà considerate nello studio, si è ritenuto opportuno modificare il protocollo alternativo senza solfiti aggiunti, prevedendo l'aggiunta di una piccola dose di SO_2 alla sfecciatura, in modo da aumentare la protezione antiossidante. I risultati delle analisi chimiche e sensoriali dei vini sperimentali hanno mostrato che il protocollo alternativo a ridotta dose di solfiti aggiunti è stato in grado di superare le criticità rilevate nel primo anno e di produrre vini di buona qualità sensoriale.

I risultati ottenuti nell'ambito del progetto e relativi all'analisi delle varietà d'uva sono stati pubblicati su una rivista scientifica a diffusione internazionale:

D'Amato, M., Cerulli, A., Errichiello, F., Gambuti, A., Moio, L., Forino, M., & Piacente, S. (2023).

Chemical characterization of four ancient white wine grapes (Vitis vinifera L.) from the Amalfi coast.

Food Chemistry Advances, 2, 100201.

Food Chemistry Advances 2 (2023) 100201

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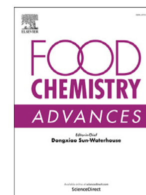

Chemical characterization of four ancient white wine grapes (*Vitis vinifera* L.) from the Amalfi coast

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ARTICLE INFO

Keywords:
Fenile grapevine
Ginestra grapevine
Pepella grapevine
Ripoli grapevine
Amalfi coast grapevines analysis

ABSTRACT

Minor grape varieties often possess key traits to produce wines with specific territorial identity. Italy is home to a wide selection of autochthonous grapevines. In 2005, four white grape varieties from the Amalfi coast, Fenile, Ginestra, Pepella and Ripoli were registered in the National Catalogue grapevines. They are all complementary varieties with a rich biodiversity and linked to an ancient wine heritage. Studies on such varieties have not been conducted yet. Here, the metabolite content of the above four grape varieties is described. Ripoli was the richest in quercetin derivatives while very low was the content of kaempferol derivatives; Pepella possessed an equal amount of quercetins and kaempferols; Ginestra and Fenile featured a quercetins abundance twice that of kaempferols. Also, the (+)-catechin content was higher than (–)-epicatechin's; B-type procyanidins occurred in higher concentrations in Pepella and Ripoli than in Fenile and Ginestra. Hydroxycinnamic acids were also identified and quantified. Seeds from each variety were carefully analyzed and flavanols were identified up to decamers along with their galloylation percentage. The obtained data are a tool of variety differentiation and useful to develop appropriate vinification procedures to ameliorate the quality of wines.

1. Introduction

The Italian Peninsula is home to a wide selection of autochthonous grapevine varieties (*Vitis vinifera* L.), many of which are quite rare and might disappear over the next future due to the lack of conservation programs. Nonetheless, there is a growing interest in minor grape varieties as they often feature successful survival rates even in harsh environments thanks to their prolonged vegetative cycle, and possess key traits to produce wines endowed with specific territorial identity (Fraga et al., 2012). Among others, possible advantages deriving from the preservation and characterization of local cultivars may be the identification of parasite-free planting material and the obtainment of desirable agronomic properties as well as cultivar qualities (Nicolas et al., 2016).

Across the Italian Campania Region, historical, cultural and topographical factors might have contributed to the preservation of ancient grape varieties, especially in remote or inaccessible areas with limited connections or communications, thus retaining a high viticultural biodiversity (Costantini et al., 2005; Muccillo et al., 2014). Additionally, the volcanic origin of several soils might have averted the spread of phylloxera, thus preventing the extinction of autochthonous vineyards and cultivars (Farabollini & Lugeri, 2021). One such area is the Amalfi coast that is certainly renowned all over the world for its breathtaking views, emerald sea and lush vegetation, but also for its spectacular vineyards cultivated on steep terraces (Tarolli et al., 2014). The extreme conditions, under which vines are grown along the Amalfi coast banks, have prompted the Italian Government to officially classify such vineyards as heroic (Italian Ministerial Decree VVQ n. 6/2019), since they comply with the decree requirements including cultivation above 500 m and on slopes over 30%.

In 2005, four white grape varieties from the Amalfi Coast were registered in the National Catalogue of Italian grapevines for wine production: Fenile, Ginestra, Pepella, and Ripoli (Costantini et al., 2005). They are all complementary varieties characterized by a rich biodiversity and linked to an ancient wine heritage.

Fenile is a variety mainly found in the territories of Furore, Positano and Amalfi. It has conical grape bunches of medium compactness, small (about 2 gs) elliptical berries with thin, yellow and pruinose skin, colorless and soft pulp, and 2–3 dark brown and cylindrical seeds per berry. There are no documents in literature regarding this variety, but the name might be due to its typical white-gold color reminiscent of hay. Fenile yields a modest production because of its low-weight bunches. Also, its thin skin requires a prompt harvest in order to prevent rot-

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https://doi.org/10.1016/j.focha.2023.100201
Received 4 October 2022; Received in revised form 2 January 2023; Accepted 31 January 2023
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ting (Ministero Italiano delle Politiche Agricole, Alimentari e Forestali, 2013).

Ginestra is a grape variety cultivated in the areas of Furore, Tramonti, Corbaia and Positano. The name derives from the scent of broom that in Italian is called 'ginestra'. The earliest references to this variety are dated back to the 19th century and it has long been mistakenly confused with the Falanghina variety. From recent DNA-based molecular characterization of grape genotypes, it was found that Ginestra is exclusively cultivated along the Amalfi coast. It has conical, medium long and compact grape bunches, medium (about 2,5 gs) elliptical berries with yellow-green and slightly waxy skin, colorless and firm pulp, and 2–3 medium and globose seeds per berry (Ministero Italiano delle Politiche Agricole, Alimentari e Forestali, 2013).

Pepella variety, spread over a few limited areas of the Amalfi coast including Tramonti and Ravello, has conical pyramidal grape bunches, small or very small (less than 2 gs) and not uniform elliptical berries with yellow-green and slightly waxy skin, colorless and firm pulp, and 2–3 light brown and globose seeds per berry. Typical of this variety is the sporadic occurrence of large grapes on each bunch, with the rest of them quite small featuring a size of peppercorns, from which the name 'Pepella' is derived (Ministero Italiano delle Politiche Agricole, Alimentari e Forestali, 2013).

Ripoli (or Ripolo), once rife across the slopes of Mons Lactarius, is now present only in small areas in the municipalities of Furore and Positano. Ripoli has cylindrical pyramidal grape bunches, small (less than 2 gs) rounded berries with thin, yellow and moderately pruinose skin, colorless and soft pulp, and 2–3 dark brown and globose seeds per berry. Cultivated on pergolas, Ripoli bunches feature weights quite varied and usually below average. Thus, its production is not always constant (Ministero Italiano delle Politiche Agricole, Alimentari e Forestali, 2013).

Scientific reports on the metabolic profile of these four historical white berried grapes from the Amalfi Coast are quite limited or missing in literature. This is particularly disappointing if we consider that they are quite marginal productions grown in a few limited vineyards and worth to be preserved. In order to define, and eventually promote, the varietal typicity of ancient grapes genotypes, a deep knowledge of their chemical composition is a prerequisite. To this aim, an untargeted chemical qualitative and quantitative analysis based on three different yet complementary techniques, including Diode Array Detector-HPLC (DAD-HPLC), Mass Spectrometry and NMR, was undertaken. The secondary metabolites profiles of both seeds and skins along with the aminoacidic content of the whole berry from each analyzed grape variety were defined.

The obtained data were further used for a comparative study of the four varieties by multivariate data analysis using Principal Component Analysis (PCA).

Our chemical characterization with a particular focus on compounds of enological interest is critical to develop specific vinification processes of the above mentioned ancient grape varieties.

Additionally, the understanding of the molecular basis of the adaptation of these varieties to harsh soil and often unfavorable climatic conditions may suggest possible genetic strategies to ameliorate other grape varieties adaption to climate changes.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade (> 99%), water purity was Milli-Q (Merck-Millipore, Darmstadt, Germany). The solvents for LC-MS and NMR analyses were purchased from Sigma Aldrich (Milan, Italy) and Deltek (Napoli, Italy).

All of the amino acid standards and diethylethoxymethylenemalonate (DEEMM) were purchased from Sigma-Aldrich (Milan, Italy).

2.2. Grape samples

Samples of four cultivars of *Vitis vinifera* L., namely Fenile, Ginestra, Pepella, and Ripoli, were harvested in 2021. For each variety 2 kg of grape clusters were harvested and the sampling was performed to ensure that analyses will be representative of all the rare vineyards across the Amalfi coast where such varieties could be unambiguously identified. Identification of each variety was based on descriptions reported in 'La risorsa genetica della vite in Campania' by Manzo and Monaco (2001). Soon after harvesting, grapes samples were transported to the lab and their enological parameters were determined as follows:

Fenile (sugar content: 14.2 ± 0.10 °Brix; total acidity: 6.24 ± 0.03; pH 3.68 ± 0.03)

Ginestra (sugar content: 22.5 ± 0.10 °Brix; total acidity: 6.15 ± 0.11; pH 3.11 ± 0.01)

Pepella (sugar content: 15.8 ± 0.10 °Brix; total acidity: 7.26 ± 0.02; pH 3.15 ± 0.00)

Ripoli (sugar content: 17.2 ± 0.06 °Brix; total acidity: 4.84 ± 0.05; pH 3.33 ± 0.01)

For each grape variety grape clusters were quickly frozen at –40 °C. For secondary metabolites analyses, grape berries were picked from frozen grape clusters coming from the same harvest as to obtain 500 g of grape berries. The organic material was processed freshly thawed. For the analysis of amino acids, 25 g of destemmed berries were processed freshly thawed.

2.3. Grape skins and seeds extractions

500 g (fresh weight) of berries from Fenile, Ginestra, Pepella and Ripoli, respectively, were manually fractioned in skins and seeds, softly dried and shredded.

Skins (60.4 g from Fenile; 110.0 g from Ginestra; 62.7 g from Pepella; 68.1 g from Ripoli) from each variety were extracted with 300 mL of a Ethanol:Water (8:2 v/v) mixture at room temperature, for 24 h under stirring. Extracts from each variety were filtered through filter paper, concentrated under vacuum, and then partitioned twice against dichloromethane (300 mL x 2). The aqueous layer was then partitioned twice against ethyl acetate (200 mL x 2); finally, the residual aqueous layer was partitioned twice against butanol (200 mL x 2). Dry weights of extracted skins were the following: Fenile 5.8 g; Ginestra 11.7 g; Pepella 6.5 g; Ripoli 7.1 g.

Likewise, seeds (9.3 g from Fenile; 13.9 g from Ginestra; 9.2 g from Pepella, 9.8 g from Ripoli) were extracted as reported above for skins. Seeds after shredding were extracted by using in the order 100 mL of dichloromethane, ethyl acetate, and butanol, respectively. Each of the above extractions was performed twice. 2.0 mL of each extract from either skins or seeds were lyophilized and subjected to NMR and LC-MS/MS analyses.

2.4. NMR analyses

NMR analyses were carried out on a Bruker Ascend-600 MHz spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm. Methanol-d_4 (99.95%, Sigma-Aldrich) was used as solvent for each extract. The NMR data were processed using TopSpin 3.2 software.

All samples were run at 300 K, using the zg pulse sequence; the relaxation delay was 1.0 s, with 80 number scans and data collected into 64 k data points. Each free induction decay (FID) was zero-filled to 128 k data points. After the acquisition, the spectra were analyzed by MestreNova 10 software. CD_3OD (δ_H 3.31; δ_C 49.0 ppm) was selected as deuterated solvent. Standard pulse sequences were employed for the respective classes of spectra. All NMR data reported in the text were derived from 1D ^{1}H NMR and ^{13}C NMR spectra and from 2D experiments including COZY, ROESY, HMBC, and HSQC. NMR-based quantitative

analyses were conducted on a Varian Unity Inova 700 MHz spectrometer equipped with a 13C Enhanced HCN Cold Probe and by using a Shigemi 5 mm NMR tubes. ^{1}H NMR spectra were run in CD_3OD (δ_H 3.31; δ_C 49.0 ppm) as a deuterated solvent by using a standard Varian pulse sequence and adjusting the d1 (relaxation delay) value at 7.0 s to allow a complete relaxation of pyridine to equilibrium (5 μL of pyridine were added to the sample as an internal standard). By means of the inversion-recovery T1 relaxation experiment, for pyridine a T1 value of 0.70 s was determined. Quantitation of oleanolic acid was conducted according to the analytical procedure detailed in Forino et al. (2020).

2.5. LC-ESI linear trap quadrupole (LTQ) orbitrap MS/MS

The grape hydroethanolic extracts were analyzed using liquid chromatography coupled to electrospray ionization and multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQ Orbitrap/MS/MS). A quaternary Accela 600 pump (ThermoScientific, San Jose, CA) and an Accela autosampler coupled to a LTQ Orbitrap XL (linear io-trap-orbitrap high resolution mass spectrometer) (ThermoScientific, San Jose, CA), operating in both positive and negative electrospray ionization mode were used. Data were collected and analyzed using the Xcalibur software (version 2.1, Dreieich, Germany) provided by the manufacturer (Masullo et al., 2019).

LC-MS analyses were carried out on a Luna MS C18 5 μm (150 mm × 2.00 mm) column (Phenomenex) (Aschaffenburg, Germany) using a flow rate of 0.2 mL/min. A binary solvent system was used: eluent A = water with 0.1% formic acid (99.9:0.1, v/v), eluent B = acetonitrile with 0.1% formic acid (99.9:0.1, v/v). The autosampler was set to inject 8 μL of each extract (1.0 mg/mL). In the negative ion mode, the following experimental conditions for the ESI source were adopted: sheath gas at 15 (arbitrary units), auxiliary gas at 5 (arbitrary units), source voltage at 3.5 kV, capillary temperature at 280 °C, capillary voltage at –48 V and tube lens at –176.47 V.

LC-MS analyses were carried out on a Luna MS C18 5 μm (1.5 mm × 2.00 mm) column (Phenomenex) (Aschaffenburg, Germany) using a flow rate of 0.2 mL/min. A binary solvent system was used: eluent A = water with 0.1% formic acid (99.9:0.1, v/v), eluent B = acetonitrile with 0.1% formic acid (99.9:0.1, v/v).

The HPLC gradient started at 5% B and remained at 5% B for 5 min; after 5 min% B reached 50% and remained at 50% for 5 min; after 5 min it was at 55%, holding this percentage for 5 min; in 15 min% B arrived at 100% and remained at this percentage for 5 min before returning to the starting percentage.

In the positive ion mode, the following experimental conditions for the ESI source were adopted: sheath gas at 30 (arbitrary units), auxiliary gas at 5 (arbitrary units), source voltage at 3.0 kV, capillary temperature at 280 °C, capillary voltage at 49 V and tube lens at 120 V.

The HPLC gradient started at 5% B and remained at this percentage for 5 min.; after 25 min,% B reached 100% and remained so for 5 min, before returning to the starting percentage.

The mass range was from 120 to 1600 m/z with a resolution of 30,000. For the data-dependent scan, the first and the second most intense ions from the HRMS scan event were selected, in order to offer their tandem mass (MS/MS) product ions with a normalization collision energy of 30%, a minimum signal threshold at 250, and an isolation width at 2.0; multiple-stage tandem mass (MS^n with n = 3, 4…) experiments on selected product ions were carried out by the same collision energy. The same Xcalibur software as that already reported above was used for instrument control, data acquisition, and data analysis (Cerulli et al., 2020).

2.6. Quantitative analysis by LC-ESI/QTrap/MS

Quantitative analysis was performed on liquid chromatography coupled to tandem mass spectrometry with ESI source and hybrid triple quadrupole-linear ion trap mass analyzer (LC-ESI/QTrap/MS) (AB Sciex, Milan, Italy) system working in Multiple Reaction Monitoring (MRM) mode (Cerulli et al., 2021). HPLC separation was conducted by Kinetex Omega 2.6 μm RP C18 column (100 mm × 2.1 mm i.d) at a flow rate of 0.3 μL/min.

A binary solvent system was used (eluent A: water with 0.1% formic acid (99.9:0.1, v/v), eluent B: acetonitrile with 0.1% formic acid (99.9:0.1, v/v)). The HPLC gradient started at 5% B and remained at 5% B for 0.48 s; after 1.06 min,% B was at 50%, remained at this% for 1.07 min, changed from 50% B to 55% B in 1.07 min, remained at 55% B for 1.06; reached in 3.30 min 100% B and remained at this percentage for 1.07 min before returning to the starting percentage. The instrument operated in negative ion mode for internal standard (chlorogenic acid, 1 µg/mL for each solution), and external standards; specific values of declustering potential, focusing potential, entrance potential, collision energy, and collision cell exit potential were used (SI.1). Extracts were diluted by using methanol, and 8 μL (1.0 mg/mL) were injected in triplicate; solutions of different external standards with the following concentrations: 0.001, 0.01, 1.0, 2.5, 5.0, 7.0, 10.0, 15.0, and 20.0 µg/mL, were used. Linear regression analysis was performed using the Analyst 1.6.2 Software provided by the manufacturer (AB Sciex, Milan, Italy).

Linearity was evaluated by correlation values of calibration curves. The limit of quantification (LOQ; equivalent to sensitivity) was evaluated by injecting a series of increasingly diluted standard solutions until the signal-to-noise ratio was reduced to 10. The limit of detection (LOD) was estimated by injecting a series of increasingly diluted standard solutions until the signal-to-noise ratio was reduced to 3 (SI.1) (Cerulli et al., 2018).

2.7. Grapes amino acids analysis

Standard solutions of each amino acid were prepared in HCl 0.1 N. HPLC-based analyses of amino acids were conducted on an HPLC Agilent 1260 Infinity II LC apparatus (Santa Clara, USA) equipped with a binary pump with an integrated two-channel degasser unit and a diode array detector-DAD (G7114A). Data collection and analyses were performed through the software OpenLAB CDS ChemStation Edition (Agilent Technologies, Santa Clara, USA).

Amino acids were extracted from 25-g berries and derivatized with DEEMM according to the procedure described by Gomez-Alonso et al. (2007) and Ortega-Heras et al. (2014). HPLC-based identification of amino acids was carried out by comparison with amino acid standards. For HPLC analyses, an Agilent Infinity Lab Poroshell 120 EC–C18 (3.0 × 150 mm - 2.7 μm) was used and eluted with the following gradient. The gradient started at 5% B and remained at 5% B for 20.0 min; from 20.0 to 30.0 min, B became 10%; from 30.0 to 33.0 min, eluent B reached 17%; from 33.0 to 65.0 min%B became 40 and changed to 72% from 65.0 to 73.0 min. From 73.0 min to 78.0 min%B became 82 and finally it reached 100% value at min 82.0 to remain at this% for 5.0 min, before returning to the starting percentage.

Eluent A: 25 mM acetate buffer, pH 5.8 with 0.02% sodium azide; Eluent B: acetonitrile:methanol 8:2 (v/v). Flow rate: 0.5 mL/min. For detection, λ=280 nm was used.

Amino acids were identified and quantified on the basis of the retention times and UV spectral properties of the derivatives of the respective standards (Gómez-Alonso et al., 2007).

2.8. Statistical data analysis

Concentrations of identified metabolites were subjected to ANOVA test using the Turkey method for the significant differences procedure (p < 0.05) and to Principal Component Analysis (PCA). Statistical analysis was performed using XLSTAT 2017 statistical software (Addinsoft, Paris, France). All data are means of six values (3 experimental replicates x 2 analytical replicates).

3. Results and discussion

3.1. NMR-based analysis of grapes skins

Skins and seeds from each grape variety were extracted with a hydroethanolic mixture. All of the extracts were subjected to NMR analysis. As expected, the ^{1}H NMR spectra of the seed extracts highlighted the occurrence of resonances typical of flavan-3-ols, mainly (+)-catechin and (–)-epicatechin; the significant drift of the spectra baselines was indicative of a high concentration of polymers, likely condensed tannins. These extracts were no further investigated by NMR and subjected to LC-MS/MS analysis as detailed below.

^{1}H NMR spectra of the skin extracts, recorded on a 600 MHz spectrometer, contained resonances attributable to carbohydrates (mainly glucose and fructose), to organic acids (malic and tartaric acid) and amino acids. Additionally, singlets typical of triterpenoid methyl groups were detected in the spectrum region ranging from 0.7 to 1.5 ppm. In order to identify the occurring triterpenoid(s), the skin hydroethanolic extracts from each variety were partitioned first against dichloromethane and then against ethyl acetate. The dichloromethane extracts from Fenile, Pepella, and Ripoli turned out to contain a triterpenoid with a high degree of purity (>90%). Particularly, the Pepella extract (SI.2) was selected for a comprehensive 1D and 2D NMR-based structural investigation (^{13}C NMR, COZY, TOCSY, ROESY, HSQC and HMBC) that was conducted on a 700 MHz spectrometer equipped with a 13C Enhanced HCN Cold Probe. The same spectrometer was used for quantitative purposes as detailed in Material and methods. By comparison with data available in literature (Forino et al., 2019), the isolated triterpenoid was identified as oleanolic acid. The NMR-driven structural hypothesis was corroborated by the detection of an intense High Resolution ESIMS $[M+H]^{+}$ ion peak centered at m/z 457.3667 corresponding to $C_{30}H_{49}O_{3}$. NMR-based quantitation ascertained that the content of oleanolic acid in the hydroethanolic extracts from Pepella, Fenile, and Ripoli was 104.7 μg/mL, 93.4 μg/mL, and 75.8 μg/mL, respectively. In the Ginestra hydroethanolic extract oleanolic acid was detected but it was not doable a reliable quantitation by NMR. The occurrence of oleanolic acid in the grape extracts is not surprising as it is a main constituent of the skin protective waxy bloom (Szakiel et al., 2012). Beyond its physiological role, oleanolic acid is of great interest on account of its numerous beneficial effects to human health, including antitumor, anti-inflammatory, antibiotic and antiviral properties (Lin et al., 2016). Lately, together with glycyrrhetinic acid and bile acids, oleanolic acid was shown to inhibit interaction of the Spike's protein receptor binding domain with the carboxypeptidase domain of the Angiotensin Converting Enzyme (ACE2), thus suggesting potential novel approaches to treat COVID-19 (Carino et al., 2020). On account of the above interesting bioactivities, it will be worth investigating how to recover oleanolic acid - preferentially through a green chemistry approach in view of circular economy - from grape skins, since it is expected to constitute an important part of grape pomaces as vinification of white grapes does not employ skins.

3.2. LC-MS/MS-based analysis of grapes skins and seeds

Samples of skins and seeds from Fenile, Ginestra, Pepella and Ripoli grapes were separately extracted with a hydroethanolic mixtures as described in Materials and methods and submitted to qualitative and quantitative analysis by LC-ESI/LTQOrbitrap/MS/MS and LC-ESI/QTrap/MS, respectively. Such analyses led us to define the samples phenolic profiles from a qualitative and quantitative standpoint. Tables 1 and 2 report all the phenolics identified in the hydroethanolic extracts of both skins and seeds. The compounds displayed in table 1 have been grouped according to their chemical structures into flavonols, flavanols, stilbenes and phenolic acids.

The flavonol content was qualitatively the same in all of the varieties, but significant differences emerged in terms of the relative abundance of

Table 1
Quantitative results of identified compounds are expresses as mg / g of extract from Fenile (F), Ginestra (G), Pepella (P) and Ripoli (R) grape skins. Different letters in the same row indicates that the values are significantly different.

Flavonols								
	quercetin-3-(6″-rhamnosyl)hexoside	kaempferol 3-(6″-rhamnosyl)hexoside	quercetin-3-hexoside	kaempferol-3-hexoside	quercetin-3-hexuronide	kaempferol-3-hexuronide		
F	0.1910 ± 0.0165b	0.1504 ± 0.0141a	1.2381 ± 0.0764c	1.02642 ± 0.0045c	0.6753 ± 0.0622a	0.0366 ± 0.0098d		
G	0.1700 ± 0.0028bc	0.1033 ± 0.0059c	2.5391 ± 0.0416b	1.4212 ± 0.0228b	0.6806 ± 0.1007a	0.0750 ± 0.0148c		
P	0.1615 ± 0.0015c	0.0982 ± 0.0001c	2.2187 ± 0.1396b	2.0655 ± 0.0573a	0.4460 ± 0.0527b	0.2425 ± 0.0044a		
R	0.3123 ± 0.0101a	0.1234 ± 0.0006b	5.1000± 0.0600a	2.0919 ± 0.1293a	0.3807 ± 0.0482b	0.1708 ± 0.0017b		
Hydroxycinnamic acids, hydroxybenzoic acids and stilbenes								
	coumaric acid	ferulic acid-O-hexoside	caffeic acid	caffeic acid-O-hexoside	resveratrol	piceid	protocatechuic acid	dihydroxybenzoic acid-O-hexoside
F	0.0085 ± 0.0001c	0.0085 ± 0.0001c	0.0669 ± 0.0008a	0.0839 ± 0.0013a	0.007 ± 0.0001a	0.3299 ± 0.1986a	0.0026 ± 0.0001c	0.0085 ± 0.0001c
G	0.0100 ± 0.0001a	0.0100 ± 0.0001a	0.0640 ± 0.0003c	0.0486 ± 0.0016b	0.007 ± 0.0001b	0.2461 ± 0.0005a	0.0036 ± 0.0001a	0.0099 ± 0.0001a
P	0.0089 ± 0.0001b	0.0089 ± 0.0001b	0.0653 ± 0.0002b	0.0177 ± 0.0005d	n.d.	0.3386 ± 0.0089a	0.0032 ± 0.0001b	0.0089 ± 0.0001b
R	0.0078 ± 0.0001d	0.0078 ± 0.0001d	0.0668 ± 0.0002a	0.0224 ± 0.0005c	n.d.	0.2675 ± 0.0036a	0.0031 ± 0.0001b	0.0078 ± 0.0001d
Flavanols								
	(-)-epicatechin	(+)-catechin	procyanidin B type*					
F	0.1931± 0.0008d	0.4021 ± 0.0026a	1.3983 ± 0.0006c					
G	0.2095 ± 0.0005c	0.3542 ± 0.0002c	1.4867 ± 0.0328c					
P	0.2580 ± 0.0010a	0.3919 ± 0.0058b	2.9516 ± 0.0063a					
R	0.2281 ± 0.0004b	0.2280 ± 0.0004d	2.0962 ± 0.1665b					

* Procyanidin trimers, tetramers and pentamers were also detected but below the quantitation limit.

Table 2
Flavanols identified in seeds extracts from Fenile, Ginestra, Pepella and Ripoli. Concentrations are expressed as mg/gr of extract. Different letters in the same row indicates that the values are significantly different. All of the identified polymers belong to the B-type class.

Seeds flavanols	FENILE				GINESTRA				PEPELLA				RIPOLI			
Compounds																
Catechin	33.57	±	0.10	c	27.60	±	0.03	d	43.00	±	0.08	b	74.30	±	0.07	a
Epicatechin	30.27	±	0.03	c	17.85	±	0.07	d	40.10	±	0.06	b	97.90	±	0.04	a
(Epi)catechin monogallate	2.48	±	0.08	c	0.52	±	0.06	d	9.10	±	0.10	a	8.36	±	0.08	b
(Epi)catechin monoglycoside	2.43	±	0.03	c	3.25	±	0.01	b	0.63	±	0.03	d	4.43	±	0.01	a
Procyanidin dimers	33.49	±	0.07	c	23.52	±	0.07	d	40.78	±	0.11	b	73.16	±	0.10	a
(Epi)catechin diglycoside	1.40	±	0.11	b	2.89	±	0.04	a	0.52	±	0.03	c	3.09	±	0.06	a
Procyanidin dimers monogallate	4.34	±	0.06	c	0.89	±	0.10	d	11.37	±	0.04	a	9.35	±	0.04	b
(Epi)catechin dimers monoglycoside	0.64	±	0.06	a	0.59	±	0.06	a	0.01	±	0.00	c	0.31	±	0.03	b
Procyanidin trimers	22.82	±	0.06	b	15.27	±	0.01	c	8.04	±	0.04	d	47.66	±	0.08	a
Procyanidin dimers digallate	0.47	±	0.06	c	1.13	±	0.03	b	0.35	±	0.04	d	0.40	±	0.04	a
(Epi)catechin dimers diglycoside	0.72	±	0.07	c	1.48	±	0.10	b	0.10	±	0.08	d	1.94	±	0.03	a
Procyanidin trimers monogallate	2.84	±	0.06	c	0.76	±	0.07	d	10.48	±	0.04	a	8.71	±	0.07	b
Procyanidin tetramers	10.22	±	0.03	b	8.93	±	0.04	c	1.59	±	0.06	d	22.48	±	0.11	a
Procyanidin trimers digallate	0.40	±	0.04	b	0.92	±	0.03	a	0.28	±	0.04	b	0.34	±	0.01	b
Procyanidin tetramers monogallate	2.08	±	0.07	c	0.56	±	0.06	d	8.91	±	0.03	a	7.93	±	0.03	b
Procyanidin pentamers	4.82	±	0.06	c	6.72	±	0.01	a	0.59	±	0.04	d	6.40	±	0.01	b
Procyanidin tetramers digallate	0.36	±	0.06	a	0.46	±	0.07	a	0.24	±	0.03	a	0.32	±	0.07	a
Procyanidin pentamers monogallate	2.04	±	0.04	c	0.51	±	0.06	d	2.45	±	0.06	b	4.66	±	0.01	a
Procyanidin hexamers	2.97	±	0.01	c	4.64	±	0.03	a	0.42	±	0.01	d	3.65	±	0.03	b
Procyanidin pentamers digallate	0.24	±	0.06	ab	0.35	±	0.04	a	0.15	±	0.04	b	0.28	±	0.03	ab
Procyanidin hexamers monogallate	1.96	±	0.04	a	0.43	±	0.04	d	0.86	±	0.10	c	1.54	±	0.04	b
Procyanidin heptamers	2.66	±	0.06	a	1.90	±	0.07	b	0.29	±	0.04	c	2.84	±	0.03	a
Procyanidin hexamers digallate	0.21	±	0.01	a	0.29	±	0.10	a	0.10	±	0.07	a	0.14	±	0.06	a
Procyanidin heptamers monogallate	1.88	±	0.01	a	0.29	±	0.06	c	0.25	±	0.04	c	1.06	±	0.04	b
Procyanidin octamers	2.53	±	0.04	a	0.93	±	0.04	c	0.16	±	0.03	d	1.25	±	0.04	b
Procyanidin heptamers digallate	0.15	±	0.06	a	0.22	±	0.07	a	0.06	±	0.06	a	0.13	±	0.10	a
Procyanidin octamers monogallate	1.56	±	0.04	a	0.18	±	0.06	c	0.03	±	0.03	c	0.87	±	0.01	b
Procyanidin nonamers	0.98	±	0.04	a	0.20	±	0.07	b	0.05	±	0.03	b	1.14	±	0.01	a
Procyanidin octamers digallate	0.13	±	0.07	a	0.15	±	0.01	a	0.03	±	0.01	a	0.10	±	0.07	a
Procyanidin nonamers monogallate	1.22	±	0.04	a	0.08	±	0.03	c	0.01	±	0.01	c	0.35	±	0.03	b
Procyanidin nonamers digallate	0.11	±	0.01	a	0.14	±	0.04	a	0.03	±	0.03	a	0.10	±	0.06	a
Procyanidin decamers digallate	0.10	±	0.04	a	0.08	±	0.03	a	0.01	±	0.00	a	0.02	±	0.01	a
Total (mg/g)	*172.10*	±	1.53	b	*123.73*	±	1.47	c	*181.00*	±	0.94	b	*385.22*	±	1.36	a
% Galloylation	*13.1*	±	1.31	a	*6.4*	±	0.75	b	*24.7*	±	1.48	c	*11.6*	±	0.93	a

the identified compounds. In more details, Ripoli turned out to be by far the richest in quercetin derivatives (Table 1). This variety possessed a kaempferol derivatives content half that of quercetins, comparable with that occurring in Pepella and slightly more abundant than those in either Fenile and Ginestra. Additionally, Pepella possessed almost the same amounts of quercetins and kaempferols; whilst, Ginestra and Fenile featured a quercetins abundance twice that of kaempferols (Table 1). Interestingly, myricetins were not detected in any varieties. The above results are of great interest, because flavonols, secondary metabolites playing a key role as UV-protectors in grape skins, have been often regarded as authenticity and varietal differentiation tools (Hermosín-Gutiérrez et al., 2011). Hence, the significant differences detected in terms of relative abundance of flavonols among the analyzed samples are indeed instrumental for the characterization of the grapes under investigation in the present work.

Flavanols constitute a class of flavonoids occurring in grapes as monomers, dimers and even as compounds with higher degree of polymerization, generally referred to as procyanidins. As monomers, (+)-catechin and (–)-epicatechin are the most common molecules detected in grapes. Apart from Ripoli, in all of the skin hydroethanolic extracts of the analyzed grape varieties, the (+)-catechin content was higher than (–)-epicatechin's, even if with some significant differences (Table 1). Indeed, in Fenile and Ginestra skin extracts, (+)-catechin was by far more abundant than (–)-epicatechin, while (+)-catechin concentration was only slightly higher than (–)-epicatechin in Pepella. In Ripoli the amounts of the two flavan-3-ols monomers were comparable. Additionally, procyanidins belonging to the B-type, as inferred on the basis of their m/z values, turned out to be occurring in Pepella and Ripoli in higher concentrations than in Fenile and Ginestra. Trimers, tetramers and pentamers were also detected in the skins of all of the grape varieties, but they were occurring in concentrations below the quantification limit.

Higher procyanidins amounts than either catechin or epicatechin have previously been found in Moscadel, Gewurztraminer, Saubignon and Chardonnay by Montealegre et al. (2006) suggesting that this is a recurring pattern for flavanols in white grape skins. From an enological perspective, an excessive presence of flavanols and procyanidins can be detrimental since they are responsible for chemical oxidation of wine (Waterhouse & Laurie, 2006) as well as for their bitterness and astringency (Brossaud et al., 2001). For this reason, it is advisable that during the winemaking of Pepella and Ripoli, and to a lesser extent of Fenile and Ginestra, avoiding or limiting as much as possible the extraction of these compounds from skins.

The seeds extracts mainly contained flavanols that were identified up to decamers (Table 2; SI.4). It is to be underlined that monocharged ion peaks were detected up to pentamers, while polymers with higher molecular masses were only detected as doubly charged ion peaks. Also, monomers including (+)-catechin and (–)-epicatechin, and procyanidin dimers were quantified by employing pure standards; all of the other flavanol polymers were quantified by assuming for them the same molar response as procyanidin dimer standard. Also, when different extracted ion peaks (XICs) were detected for structural isomers of the same flavan-3-ol derivative (SI.4), quantification was conducted by taking into account the sum of the areas of each XIC. Ripoli seeds turned out to be the richest in terms of flavanols with a total content twice that of the three other varieties. In particular, they showed significant high contents of (+)-catechin, (–)-epicatechin, and procyanidin dimers. Conversely, the galloylation percentage of flavanols in Pepella (24.7%) was twice that of either Fenile or Ripoli; while significantly lower was the galloylation percentage in Ginestra (6.4%).

Consistently with previous reports (Gu et al., 2003), A-type procyanidins were not detected in any grape variety analyzed apart from the dimer at *m/z* 575. In SI.4, identification of A-type procyanidin based on LC-MS/MS analysis and its quantification in the four analyzed grape varieties are reported.

Consistently with our results, greater amounts of flavanols and flavanol polymers in seeds with respect to skins were detected in numerous international white varieties (Montealegre et al., 2006). It is also well known that the higher the amount of flavanol polymers and galloylation percentage of flavanols, the higher the astringency of oligomeric structures (Brossaud et al., 2001). Thus, although these compounds are not easily extracted from seeds (Torchio et al., 2014), it is clear that a quick separation of musts from skins and seeds of Pepella and Ripoli grape varieties is necessary to avoid the appearance of deleterious sensory defaults in wines.

Finally, in skins some compounds belonging to the hydroxycinnamic acid class were also identified and quantified (Table 1). The highest amount of caffeic acid-*O*-hexoside was found in Fenile and Ginestra. These compounds are critical for the management of the first phases of winemaking because, already during crushing and pressing of white grapes, cell rupture brings polyphenol oxidases (PPOs), stored within chloroplasts, and hydroxycinnamic acids, stored within vacuoles, together in the presence of oxygen (Toivonen & Brummell, 2008). In must conditions PPOs quickly oxidize phenolic acids into highly reactive quinones, responsible for browning reactions. In addition, these quinones are prone to nucleophilic attacks by sulfur dioxide and sulfur containing aromatic compounds, resulting in musts and future wines less rich in varietal aroma and less protected by sulfur dioxide (Nikolantonaki & Waterhouse, 2012). Data obtained in this study suggest that, especially for Fenile and Ginestra grapes, during the first phases of winemaking, it is important to limit the negative activity of PPOs by a proper management of temperature and sulfur dioxide. From a human health-related point of view, these compounds are of some interest on account of their antioxidant and anti-inflammatory properties (Alam et al., 2016). Also, hydroxycinnamic acids are key intermediates in the biosynthetic pathways leading to other phenolic compounds including stilbenes. In regards to this latter class of compounds, *trans*-resveratrol was identified and quantified only in Fenile and Ginestra, while *trans*-piceid, the major glucoside derivative of resveratrol in grapes, was detected in all of the analyzed varieties in slightly higher concentrations in Fenile and Pepella. *Trans*-piceid is a critical compound to protect grapevines from fungal infection (Chong et al., 2009). The piceid *cis* stereoisomer was not detected in any skin extract. Conversely, resveratrol dimer, trimer and tetramer were detected in Fenile, but below the quantification limit.

3.3. HPLC-DAD analyses of amino acids

The amino acid composition of grapes in combination with the polyphenol profile is often used for differentiating varieties. It has been indeed demonstrated that each grape variety shows a more or less specific amino acid profile, even though variations can naturally occur depending on geographical factors, soil characteristics as well as on climate fluctuations. Defining the amino acid content of a given grape variety is strategic also from an enological perspective in that amino acids are taken by yeasts in orders that are strictly linked to the specific aminoacidic profile of the environment the microorganisms thrive in. This can have significant impacts on the ratio of some secondary metabolites, including those determining the aroma profile of the finished wines such as higher alcohols, aldehydes, ketones and esters. Fenile turned out to be the variety with the highest total content of amino acids expressed as mg/g of berries (fresh weight) (Table 3). Tyrosine, proline and arginine were, in the order, the most abundant amino acids in the analyzed sample. Tyrosine is a good source of tyrosol, a powerful bioactive compound (Karković Marković et al., 2019) which is produced by yeasts during alcoholic fermentation through the Ehrlich pathway. Arginine and proline are among the most abundant amino acids in grapes (Stines et al., 2000). Proline protects plants against water stress caused by drought and salinity, arginine instead is the main storage component of nitrogen in vineyards, and it has long been considered an indicator of vine nitrogen status. Therefore, the proline/arginine ratio is referred to as a good indicator of the nitrogen nutritional status of the musts from different grape varieties. Concerning yeasts fermentation, proline cannot be assimilated by yeast whereas arginine is one of the most important amino acid for yeasts in the must because it enhances the synthesis of aromatic acetate and ethyl esters during the stationary phase of fermentation (Gutierrez et al., 2015).

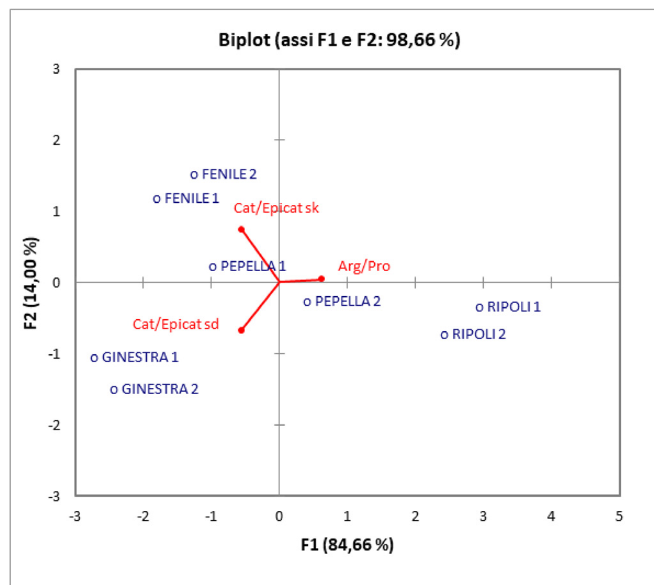

Fig. 1. PCA classification of grapes by varietal origin based on the ratios between amino acids and flavanols. (Arg/Pro= arginine/proline; CATsd/EPICATsd= catechin from seeds/epicatechin from seeds; CATsk/EPICATsk= catechin from skins/epicatechin from skins.

In Ginestra, Pepella and Ripoli glutamic acid was the main amino acid followed by tyrosine that in Ripoli was almost as abundant as glutamic acid (SI.3). In regards to glutamic acid quantitation, it is worth to underline that the HPLC peak of such amino acid appeared broad and dissymmetric. Nonetheless, also the peak relative to a pure standard of glutamic acid injected under the same chromatographic conditions resembled that attributed to glutamic acid in the analyzed grapes extracts in terms of shape and broadness (SI.3).

Fairbairn et al. (2017) showed that glutamate, one of preferred nitrogen source for *Saccharomyces cerevisiae*, resulted in the shortest lag phase, a rapid exponential growth rate, large biomass formation and short fermentation duration. Glutamine, arginine, alanine, serine, aspartate, phenylalanine, and valine also generally supported similar patterns but with less emphasis.

3.4. PCA analysis

To explore the distribution pattern of the samples and to the aim of differentiating the grape samples by variety, principal component analysis (PCA) was applied to the obtained data. The best separation along first and second axis was achieved when the ratios between the most abundant amino acids (Arg/Pro) and catechin and epicatechin (Cat/Epicatsd from seeds and Cat/Epicatsk from skins) were analyzed. The Arg/Pro-ratio has been already used to classify grape varieties according to their ability to accumulate these amino acids (Garde-Cerdán et al., 2009; Stines et al., 2000). This index is a useful indication of the likely nutritional value of the must to yeasts. Concerning

Table 3
Amino acids detected in Fenile, Ginestra, Pepella and Ripoli whole berries. Concentrations are expressed as mg/gr of berries (FW). Different letters in the same row indicates that the values are significantly different.

Amino acid	FENILE			GINESTRA			PEPELLA			RIPOLI		
	R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*		R_t (min)	*(mg/g)*	
Aspartic acid	21.68	0.005 ± 0.00	a	22.73	0.002 ± 0.00	c	22.63	0.001 ± 0.00	d	23.25	0.004 ± 0.00	b
Glutamic acid	25.86	0.049 ± 0.00	d	27.44	0.158 ± 0.00	c	27.62	0.259 ± 0.00	a	27.43	0.158 ± 0.00	b
Serine	30.44	0.006 ± 0.00	b	31.11	0.002 ± 0.00	c	31.01	0.001 ± 0.00	d	31.17	0.010 ± 0.00	a
Asparagine	31.38	0.011 ± 0.00	b	31.95	0.012 ± 0.00	a	31.87	0.006 ± 0.00	c	32.05	0.003 ± 0.00	d
HO-proline	33.73	0.025 ± 0.00	a	34.12	0.005 ± 0.00	d	34.05	0.006 ± 0.00	c	33.26	0.012 ± 0.00	b
Glutamine	34.36	0.008 ± 0.00	a	34.69	0.002 ± 0.00	b	34.62	0.002 ± 0.00	c	nd	0.000 ± 0.00	d
Histidine	34.95	0.006 ± 0.00	b	35.40	0.003 ± 0.00	c	35.32	0.002 ± 0.00	d	34.32	0.006 ± 0.00	a
Glycine	35.35	0.003 ± 0.00	b	35.70	0.005 ± 0.00	a	35.60	0.002 ± 0.00	d	35.04	0.002 ± 0.00	c
Threonine	36.23	0.019 ± 0.00	a	36.40	0.004 ± 0.00	c	36.36	0.004 ± 0.00	d	35.93	0.009 ± 0.00	b
β-alanine	36.59	0.021 ± 0.00	a	36.82	0.003 ± 0.00	b	36.77	0.002 ± 0.00	c	36.68	0.002 ± 0.00	d
Arginine	37.15	0.185 ± 0.00	a	37.37	0.009 ± 0.00	d	37.33	0.030 ± 0.00	c	37.13	0.110 ± 0.00	b
α-alanine	38.54	0.034 ± 0.00	a	38.79	0.013 ± 0.00	c	38.74	0.008 ± 0.00	d	38.47	0.027 ± 0.00	b
GABA	39.09	0.048 ± 0.00	a	39.35	0.021 ± 0.00	d	39.30	0.024 ± 0.00	c	39.01	0.044 ± 0.00	b
Proline	39.71	0.211 ± 0.00	a	40.04	0.087 ± 0.00	b	40.05	0.023 ± 0.00	d	39.73	0.024 ± 0.00	c
Tyrosine	42.12	0.213 ± 0.00	a	42.53	0.102 ± 0.00	c	42.44	0.097 ± 0.00	d	42.01	0.136 ± 0.00	b
Valine	46.44	0.011 ± 0.00	a	46.64	0.007 ± 0.00	b	46.55	0.006 ± 0.00	d	46.08	0.006 ± 0.00	c
Methionine	48.46	0.015 ± 0.00	a	49.21	0.003 ± 0.00	c	48.61	0.002 ± 0.00	d	48.29	0.008 ± 0.00	b
Cysteine	50.41; 56.80	0.012 ± 0.00	d	50.78; 56.88	0.015 ± 0.00	a	50.66; 56.84	0.015 ± 0.00	b	50.58; 56.85	0.014 ± 0.00	c
Isoleucine	51.83	0.014 ± 0.00	a	52.02	0.005 ± 0.00	b	51.94	0.004 ± 0.00	d	51.80	0.004 ± 0.00	c
Tryptophan	52.34	0.004 ± 0.00	b	52.49	0.001 ± 0.00	d	52.33	0.002 ± 0.00	c	52.51	0.007 ± 0.00	a
Leucine	52.90	0.014 ± 0.00	a	53.16	0.010 ± 0.00	b	53.08	0.008 ± 0.00	c	52.99	0.008 ± 0.00	d
Phenylalanine	53.54	0.007 ± 0.00	a	53.98	0.004 ± 0.00	c	53.87	0.004 ± 0.00	b	53.85	0.002 ± 0.00	d
Ornithine	58.62	0.010 ± 0.00	d	58.80	0.038 ± 0.00	b	58.68	0.043 ± 0.00	a	58.91	0.023 ± 0.00	c
Lysine	59.68	0.004 ± 0.00	a	59.73	0.003 ± 0.00	b	59.70	0.003 ± 0.00	c	59.72	0.003 ± 0.00	bc
Tyramine	69.65	0.001 ± 0.00	d	70.04	0.004 ± 0.00	b	70.06	0.003 ± 0.00	c	70.08	0.020 ± 0.00	a

flavonoid compounds, several studies showed that, also when subjected to the same extrinsic environmental factors, the flavanol pattern resulted mainly driven by genetic divergences among cultivars (Wei et al., 2017). Therefore Cat/Epicat ratio was useful to differentiate international and national grape varieties (Goldberg et al., 1998; Muccillo et al., 2014).

Fig. 1 shows the projection of variables, obtained for the three grape varieties, in the plane composed by principal axes F1 and F2, containing 98.66% of the total variance. The first axis, representing 84.66% of the total variance, was mainly associated with the Arg/Pro-ratio. Ripoli differed from Fenile and Ginestra by the Arg/Pro-and Cat/Epicatsd ratios. The second component explained only 14.0% of the total variance and is mainly associated with Cat/Epicatsd and Cat/Epicatsk ratios. Fenile and Ginestra slightly ddiverged by these latter ratios. On the basis of PC, it was not possible to clearly differentiate Pepella grapes.

4. Conclusions

Four ancient and autochthonous white grape varieties (Fenile, Ginestra, Pepella and Ripoli) from the Amalfi coast were thoroughly analyzed by means of an experimental approach based on three different yet complementary techniques: NMR, LC-MS and DAD HPLC. For the first time, we provided insightful details on the metabolic compositions of the above ancient grape varieties. More specifically, by LC-MS the phenolic profile of either seeds and skins from each variety was defined from both a qualitative and quantitative standpoint, along with the whole berry amino acidic content that was analyzed by DAD HPLC. Also, NMR led us to conduct an untargeted investigation that brought to light the occurrence in skins of oleanolic acid, an interesting bioactive constituent of wax berries that would have been overlooked otherwise. In conclusion, our study provided a useful characterization of the grape varieties analyzed, in that especially the flavonol and amino acidic contents are usually regarded as tools of variety differentiation. Also, data on phenolics and amino acids presented in this paper are crucial to winemakers to develop specific viticultural and enological practices, in order to address the climate change-related challenges. In fact, the metabolic profiles and in particular that of the so-called specialized metabolites may hold the secret of the high survival rates of the grapevines in the harsh Amalfi Coast pedoclimatic environment. Hence, winemakers might trace back the possible viticultural variables determining the accumulation of specific phytochemicals in grapes, with the purpose of understanding how to modulate their transfer into wines by appropriate vinification procedures. This is ultimately expected to ameliorate the quality of the wines characterized by a high territorial identity, thus increasing the reputation of the wineries along with that of the whole territory.

D'Amato Maria: Experimental work: extraction, sample preparation; NMR studies; Antonietta Cerulli: Mass Spectrometry Analysis; Francesco Errichiello: Sampling and enological studies; Angelita Gambuti: Data curation, statistical analyses; Luigi Moio: Reviewing and Editing; Martino Forino: Data analyses, Supervision and Writing- Original draft preparation. Sonia Piacente: Supervision and reviewing.

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

No data was used for the research described in the article.

Acknowledgments

This piece of work was financed by FEARS – PSR Campania 14–20. Misura 19 sviluppo locale di tipo partecipativo – LEADER – sottomisura 19.2 – tipologia di intervento 19.2.1 – strategie di sviluppo locale – GAL Terra Protetta S.c.a.r.l. - Misura 16.1 – Tipologia di Intervento 16.1.1 Az.2 "Sostegno per la costituzione e il funzionamento dei Gruppi Operativi del PEI in materia di produttività e sostenibilità dell'agricoltura", VI.B.RI.S. (Vini bianchi a ridotto contenuto di solfiti, longevi e di elevata qualità sensoriale) (CUP E51B20001120009), Capofila Cantine Marisa Cuomo Gran Furor Divina Costiera SRL.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100201.

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