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High-Resolution Mass Spectrometry-Based Untargeted Metabolite Profiling of Raisins

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ABSTRACT: Raisins (Vitis vinifera L.) are widely consumed nutrient-rich dried fruits and important sources of health-promoting phytochemicals. However, comprehensive information on metabolite alterations associated with varietal differences and biotic stress, particularly mealybug infestation, remains limited. Therefore, this study aimed to characterize and compare the metabolomic profiles of healthy and naturally mealybug-infested raisins from two commercially important grape cultivars, 'Thompson Seedless' and 'Manik Chaman', and to identify metabolites associated with cultivar characteristics and stress responses. We extracted metabolites using acidified methanol and analyzed them by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (HPLC–QTOF–MS) in positive ionization mode. Untargeted metabolomics analysis putatively identified 23 metabolites across diverse chemical classes, including phenolic acids, flavonols, amino acid derivatives, fatty acids, hydroxycinnamic acid glucosides, flavanones, volatile compounds, and lysine derivatives. Distinct cultivar- and infestation-dependent metabolic variations were observed. Healthy raisins exhibited relatively higher levels of flavonols, hydroxycinnamic acid derivatives, and aroma-associated volatile compounds, whereas infested raisins showed accumulation of stress-responsive metabolites, including L-pipecolic acid, proline, hydroxy fatty acids, and branched-chain amino acid derivatives. 'Thompson Seedless' demonstrated greater metabolic plasticity, characterized by enhanced accumulation of defense-associated metabolites, whereas 'Manik Chaman' exhibited pronounced depletion of phenolic and flavonoid compounds following mealybug infestation. Overall, the findings demonstrate the utility of HRMS-based untargeted metabolomics for comprehensive characterization of raisin metabolites and identification of potential biomarkers associated with cultivar discrimination and biotic stress responses. These metabolic signatures may improve raisin quality assessment, stress-management strategies, and grape breeding programs. To the best of our knowledge, this is the first study to investigate metabolomics profiling in naturally mealybug-infested 'Manik Chaman' raisins, using an untargeted HPLC-QTOF-MS approach.

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1. INTRODUCTION

Raisins, the dried fruits of *Vitis vinifera* L., are widely consumed because of their nutritional value, pleasant flavour, and health-promoting properties (Di Lorenzo et al., 2016; Olmo-Cunillera et al., 2020). They are rich sources of natural sugars, dietary fibre, essential minerals such as potassium, magnesium and boron, together with vitamins and phytochemicals, making them valuable functional foods (Olmo-Cunillera et al., 2020). Raisins also possess abundant phenolic compounds with considerable antioxidant activity, which further enhance their nutritional quality (Breksa et al., 2010; Di Lorenzo et al., 2016). Regular consumption of raisins has been associated with multiple health benefits, including improved cardiovascular health and other physiological benefits attributed to their polyphenol content (Williamson & Carughi, 2010). Furthermore, population-based dietary studies have shown that raisin consumption is associated with improved nutrient intake, better diet quality, reduced risk factors for metabolic syndrome, improved satiety, enhanced fat metabolism and better glycaemic control (Fulgoni et al., 2017).

The chemical and nutritional quality of raisins is largely determined by their metabolite composition. Raisins contain diverse primary metabolites, including amino acids, organic acids and fatty acids, together with a wide range of secondary metabolites that contribute to their nutritional and functional properties (Parker et al., 2007). Phenolic compounds constitute one of the major classes of bioactive metabolites in raisins and grapes and include phenolic acids, flavan-3-ols, flavonols, anthocyanins, tannins and stilbenes (Savalekar et al., 2019). The antioxidant capacity of raisins has been closely associated with their phenolic composition, highlighting the importance of these metabolites in determining fruit quality and biological activity (Parker et al., 2007). In addition to antioxidant, antimicrobial, and anti-inflammatory effects, these compounds influence flavor, color, stability, and nutritional value. Phenolic compounds are prone to oxidation, causing pigment instability and quality loss, especially in white raisins (Kelebek et al., 2013). Metabolite composition in raisins is strongly influenced by grape cultivar, drying process, geographical origin, storage conditions and environmental stresses (Nale, 2024; Olivati et al., 2019). Cultivar-dependent variation in phenolic composition has been well documented in grapes, with 'Thompson Seedless' exhibiting differences in phenolic accumulation under varying cultivation conditions (Satisha et al., 2008). Drying treatments further modify the phenolic composition of raisins, resulting in significant changes in metabolite profiles (Olivati et al., 2019; Somkuwar R, 2023). Studies on raisin cultivars have also demonstrated considerable variation in phytochemical composition and antioxidant properties among different grape genotypes (Mnari et al., 2016). Phenolic metabolites commonly reported in grapes and raisins include caftaric acid, coumaric acid, rutin, catechins, quercetin derivatives and procyanidins, which contribute significantly to fruit quality and biological activity (Escobar-Avello et al., 2019). Metabolomics is a systems-level approach for analyzing low-molecular-weight metabolites in biological samples. In food science, it helps study nutritional profiles, verify authenticity, determine origins, monitor quality, assess processing effects, and examine biochemical responses to stresses. Analyzing multiple metabolites simultaneously provides deeper insights into food quality, nutrition, and biological impacts. Untargeted metabolomics is especially valuable for its comprehensive, unbiased profiling without prior analyte selection (Chatterjee, 2024). Unlike targeted methods that focus on specific compounds, untargeted approaches enable detection of known, unknown, low-concentration, and potentially novel metabolites. This is helpful for complex food matrices like raisins, where many metabolites influence cultivar identity, nutritional value, processing traits, and stress responses.

High-resolution mass spectrometry (HRMS), especially quadrupole time-of-flight (Q TOF-MS), is popular for untargeted metabolomics due to its high sensitivity, precise mass, excellent resolution, broad coverage, and retrospective data analysis, aiding metabolite annotation and structure determination (López-Gutiérrez et al., 2016; Narduzzi et. al., 2015). Quadrupole time-of-flight mass spectrometry (Q-TOF-MS) combines quadrupole-based ion selection with time-of-flight detection, enabling rapid analysis, high mass accuracy and reliable metabolite identification over a broad mass range (López-Gutiérrez et al., 2016). Compared with conventional analytical techniques, high-resolution mass spectrometry (HRMS) provides superior sensitivity for detecting trace-level and previously uncharacterized metabolites, thereby expanding the coverage of metabolome analysis (Escobar-Avello et al., 2021). HRMS-based untargeted metabolomics has become an important tool for food authentication and adulteration detection (Selamat et al., 2021). In addition, it has been widely applied for nutritional assessment, quality control and comprehensive profiling of bioactive compounds in food matrices (Salo et al., 2021). In grapes and related products, it differentiates cultivars, monitors biochemical changes during processing, and assesses metabolic variations caused by environmental factors or stress. Although raisins are valued nutritionally and economically, their chemical diversity is not well characterized by metabolomics. Traditional targeted techniques like HPLC and LC–

MS/MS have identified key phenolic compounds such as quercetin, kaempferol, catechin, rutin, gallic acid, and resveratrol (Karadeniz et al., 2000; Mnari et al., 2016). However, these approaches might miss minor or unknown metabolites that can affect raisin quality, nutrition, and stress responses. Limited knowledge exists of metabolomic changes induced by biotic stressors such as mealybug infestations and sooty mould growth. Mealybugs harm grapevines by feeding on sap, reducing yield and quality, and promoting sooty mould through honeydew. Heavy infestations cause leaf loss, shriveled berries, lower photosynthesis, vine decline, and economic loss, especially in India. Biotic stress from mealybugs impacts phenylpropanoid metabolism, triggers stress-related metabolites, and alters primary and secondary processes, affecting berry development, nutrition, and post-harvest quality (Krstić et al., 2023). Consequently, detailed metabolomic studies are essential for understanding these biochemical changes and identifying putative metabolic biomarkers associated with varietal traits and stress responses.

This study performed untargeted HRMS metabolite profiling on healthy and infested 'Thompson Seedless' and 'Manik Chaman' raisins to identify metabolites and potential biomarkers. Limited data exist on HRMS-based metabolomics of naturally mealybug-infested raisins. The study explores metabolite diversity and infestation -related changes in these key grape cultivars.

2. MATERIALS AND METHODS

2.1 Study Material

All chemicals and solvents used in the study were of analytical grade. LC–MS–grade water, acetonitrile and methanol (99.8%, HPLC grade) were procured from Fisher Chemicals™ (NJ, USA) and NICE Chemicals™ Pvt. Ltd. (Kochi, India), respectively. Formic acid (90%, p.a.) was obtained from J.T. Baker™ (NJ, USA).

2.2 Sample Collection, Raisin Preparation, and Metabolite Extraction

Grape berries of *Vitis vinifera* cultivars 'Thompson Seedless' (TS) and its mutant 'Manik Chaman' (MC) were collected during February 2025 from commercial vineyards located in Sangli district, Maharashtra, India (17.03°N, 74.6°E; 560 m above sea level) (Figure 1a, b). Both healthy and naturally infested grape berries were harvested at full maturity with total soluble solids (TSS) exceeding 22°Brix. Infestation was confirmed based on the presence of mealybugs, honeydew secretion, and characteristic black sooty mould growth on berry surfaces. Naturally infested berries exhibiting visible symptoms of mealybug infestation and associated black sooty mold deposition were collected directly from vineyard sites. Healthy berries without visible signs of infestation, collected from the same geographic region, served as controls. No artificial inoculation procedures were performed. Infestation status was determined based on field-level symptom assessment, including visible mealybug infestation and sooty mold formation on berry surfaces. Raisin preparation from healthy and naturally infested grape berries was performed according to the standardized protocol developed by the ICAR-National Research Centre for Grapes, Pune (Sharma et al., 2018). The resulting raisin samples were stored at −20°C until metabolomics analysis.

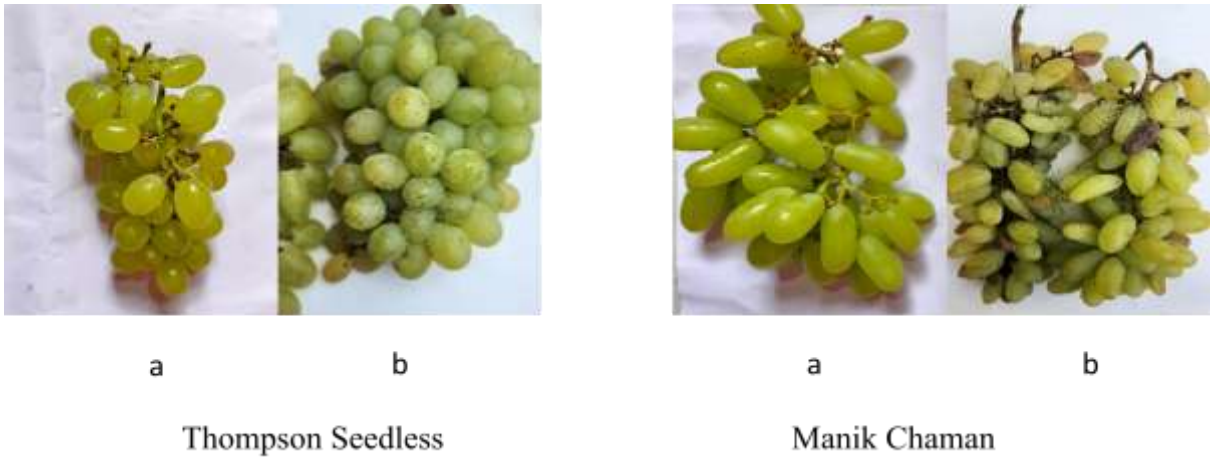

Figure 1. Healthy (a) and naturally mealybug-infested (b) grape samples collected from the field

Metabolite extraction was conducted following the procedure described by (Koley et al., 2020). Briefly, 5 ± 1 g of homogenized raisin sample was extracted with 20 mL of 80% methanol acidified with 1% formic acid, vortexed for 2 min, and centrifuged at 10,000 rpm for 10 min at 10°C. The supernatant was diluted with water and filtered through a 0.2 µm PTFE membrane filter. The clarified extract was transferred into LC vials and analyzed directly by HPLC–HRMS/QTOF. Three independent biological replicates were included for each group: TS-H, TS-I, MC-H, and MC-I. Each biological replicate was analyzed in triplicate (technical replicates) under identical HPLC–HRMS conditions to ensure analytical reproducibility and data reliability.

2.3 HPLC–HRMS/QTOF Analysis

HPLC-HRMS analysis was performed using an Agilent 6540B QTOF Mass Spectrometer coupled with an Agilent 1260 Infinity II HPLC System. Data acquisition was carried out using Agilent MassHunter software. Chromatographic separation was achieved on an Agilent Eclipse XDB-C18 Column using water and acetonitrile containing 0.1% formic acid as mobile phases. Gradient elution was programmed as 0–2 min, 95% A and 5% B; 2-25 min a linear gradient from 95% A to 5% A; 25–28 min, 5% A and 95% B; and 28.1–30 min, 95% A and 5% B for column re-equilibration with a flow rate of 0.3 mL min^{-1}. The injection volume was 20 µL, and the column temperature was maintained at 40°C. Blank injections were included between samples to minimize carryover, and all samples were analyzed in triplicate. The QTOF mass spectrometer was operated under the following conditions: sheath gas (N_2), 11 L min^{-1} at 350°C; drying gas (N_2), 8 L min^{-1} at 300°C; nebulizer pressure, 35 psig; nozzle voltage, 1000 V; and capillary voltage, 3500 V. Full-scan mass spectra were acquired in positive ion mode over an m/z range of 100–1700. Mass calibration was performed using a standard calibration mixture. Raw data processing and compound annotation were conducted using Agilent MassHunter™ Qualitative Analysis software (version B.09.00, B9044.0). Putative metabolite annotation was performed using accurate mass determination, isotopic distribution patterns, and database matching of HRMS data. Metabolite assignments were classified as MSI Level 2 (putatively annotated compounds) according to Metabolomics Standards Initiative guidelines, since confirmation by authentic standards, retention time matching, and MS/MS spectral library verification was not conducted. Identification confidence was expressed as a weighted overall identification score calculated using isotopic pattern parameters (W mass = 100, W abundance = 60, W spacing = 50)(Soltana et al., 2018). Acceptance thresholds included mass accuracy tolerances of 2.0 mDa + 5.6 ppm for expected masses, 7.5% isotope abundance variation, and 0.0025 m/z + 7.0 ppm isotope spacing tolerance (Biswal et al., 2022; Plumb et al., 2010).

2.4 Statistical Analysis and Data Processing

Peak areas obtained from triplicate technical injections were averaged for each biological replicate prior to statistical analysis. The averaged dataset was subsequently subjected to multivariate and univariate analyses using MetaboAnalyst 6.0. The mean values of the biological replicates (n = 3 per group) were used for downstream statistical analyses, including principal component analysis (PCA), heat map analysis, and one-way analysis of variance (ANOVA). Pooled quality control (QC) samples were not included in the analytical workflow. Nevertheless, analytical reproducibility was evaluated through triplicate technical injections of each biological replicate under identical HPLC-HRMS conditions. Peak areas from technical replicates showed consistent responses with low analytical variation, indicating acceptable instrument stability during data acquisition. In addition, all samples were processed using the same extraction procedure, chromatographic conditions, mass spectrometric settings, and data-processing workflow to minimize analytical bias. Although the inclusion of pooled QC samples is recommended for large-scale untargeted metabolomics studies, the relatively small sample set and controlled analytical conditions employed in the present study provided sufficient reproducibility for comparative metabolomics profiling. Future studies will incorporate pooled QC samples and comprehensive system suitability assessments to further strengthen analytical quality assurance. Metabolomics data were log-transformed to improve normality and reduce heteroscedasticity, followed by Pareto scaling to minimize the influence of highly abundant metabolites while preserving biological variation. Significant differences among groups were determined by one-way ANOVA, with FDR-adjusted p values ($q < 0.05$) considered statistically significant. PCA was performed on log-transformed and Pareto-scaled data without class assignment. Heat map analysis was performed using Euclidean distance as the similarity measure and Ward's linkage (ward.D) method as the clustering algorithm. A feature matrix (samples × metabolites) was generated and exported in .csv format for downstream analyses. Putative metabolite annotation was supported using the METLIN Metabolomics Database, and relative metabolite abundance (%) was calculated for comparison among groups.

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$$\text{Relative Abundance} = \frac{\text{Abundance of Compound}}{\text{Total Abundance of All Compounds}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Putative Identification and Semi-Quantitative Analysis of Metabolites in Healthy and Mealybug-Infested Raisins

The metabolic variations detected in raisins may be attributed to natural mealybug infestation and the associated development of sooty mold. These biotic stresses may have triggered significant alterations in primary and secondary metabolism, indicative of plant defense responses and compromised fruit physiology. High-resolution mass spectrometry (HRMS) profiling of raisins obtained from healthy and naturally infested berries of TS and MC cultivars resulted in the putative annotation of multiple metabolite classes, including polyphenols, amino acid derivatives, fatty acids, and volatile compounds, with distinct abundance patterns observed between healthy and infested samples (Tables 1 and 2). Overall, twenty-three metabolites were putatively identified across both cultivars.

Table 1. Putatively annotated metabolites in healthy and mealybug-infested raisins of 'Thompson Seedless' cultivar using HRMS-based untargeted metabolomics

Compound name Name	Formula	Adduct	Expected mass (m/z)	Observed mass (m/z)	RT (min)	Mass error (ppm)	'Thompson Seedless' healthy		'Thompson Seedless' infested	
							Abundance mean ± S.D.	Relative abundance (%)	Abundance mean ±S.D.	Relative abundance (%)
Hydroxybenzoic acid derivative										
4-hydroxybenzoic acid	$C_7H_6O_3$	$(M+NH_4)^+[-H_2O]$	137.04827	138.05553	2.522	0.56	86278.38± 7246.28	10	114145.59 ±5973.217	8.82
Amino acid derivative										
Arginine derivative	$C_{12}H_{24}N_4O_7$	$(M+H)^+$	336.16449	337.17179	2.652	0.01	17758.34± 549.31	2.05	14949.71± 438.423	1.15
Isoleucine derivative	$C_{12}H_{23}NO_7$	$(M+H)^+$	293.14809	293.14809	2.541	-0.64	10567.50± 493.132	11.60	562974.75 ±1076.224	43.54
Phenylalanine derivative	$C_{15}H_{21}NO_7$	$(M+H)^+$	327.13264	328.13996	4.704	-0.83	128430.57 ±6736.6351	14.89	174475.04 ±6654.359631	13.49

Tyrosine derivative	$C_{15}H_{21}NO_8$	$(M+H)^+$	343.12714	344.13448	2.892	-0.7	91026.41±621.563	10.55	19240.82±757.689	1.48
Proteinogenic amino acid										
L-proline	$C_5H_9NO_2$	$(M+H)^+$	115.0637	116.07097	2.585	-0.45	191197.45±5203.756	22.17	240055.81±4019.634	18.56
Volatile compound										
2-methylbutanal	$C_5H_{10}O$	$(M+NH_4)^+$	86.0734	104.1072	2.581	-0.23	31597.70±1775.076	3.66	45074.85±3155.239	3.48
Ethyl decanoate	$C_{12}H_{24}O_2$	$(M+NH_4)^+$	200.17811	218.21193	13.97	-0.48	11997.19±457.193	1.39	10124.9133±225.620	0.78
Methyl cinnamate	$C_{10}H_{10}O_2$	$(M+H)^+$	162.06851	163.07581	26.305	0.52	86255.52±4197.451	10	67072.9667±11031.985	5.18
Flavonol										
Quercetin	$C_{15}H_{10}O_7$	$(M+H)^+$	302.0428	303.0501	13.105	-0.16	9338.12±812.534	1.08	12299.83±298.577	0.95
Hydroxycinnamic acid glucoside										
Trans-cinnamoyl β-d-glucoside	$C_{15}H_{16}O_6$	$(M+NH_4)^+$	292.09526	310.12913	4.675	-0.57	108220.66±7702.851	12.55	0.00±0	0.00
Hydroxy fatty acids										
9,10-dihydroxy stearic acid	$C_{18}H_{36}O_4$	$(M+NH_4)^+$	316.26044	334.29437	19.855	0.92	0.00±0	0.00	6581.07±127.72	0.50
Lysine derivative										
L-pipecolic acid	$C_6H_{11}NO_2$	$(M+H)^+$	129.079	130.087	2.604	-0.39	0.00±0	0.00	25871.9133±4019.634	2.00

Table 2. Putatively annotated metabolites in healthy and mealybug-infested raisins of 'Manik Chaman' cultivar using HRMS-based untargeted metabolomics

Compound name	Formula	Adduct	Expected mass (m/z)	Observed mass (m/z)	RT (min)	Mass error (ppm)	'Manik Chaman' healthy		'Manik Chaman' infested	
							Abundance Mean ± S.D	Relative abundance (%)	Abundance Mean ± S.D	Relative abundance (%)
Hydroxybenzoic acid derivatives										
4-hydroxybenzoic acid	$C_7 H_6 O_3$	$(M+NH_4)^+[-H_2O]$	137.0482	138.05553	2.522	0.60	86494.24±7241.454	7.26	89134.63±3014.008	9.80
Amino acid derivative										
Arginine derivative	$C_{12} H_{24} N_4 O_7$	$(M+H)^+$	336.16445	337.17175	2.634	0.04	8792.40±250.938	0.73	10257.42±669.287	1.12
Isoleucine derivative	$C_{12} H_{23} N O_7$	$(M+H)^+$	293.14866	294.15599	3.076	-1.21	526305.02±613.952	44.19	422089.47±1694.696	46.42
Phenylalanine derivative	$C_{15} H_{21} N O_7$	$(M+H)^+$	327.13264	328.13996	4.704	-0.83	147787.97±10206.864	12.41	118500.75±3139.479	13.03
Tyrosine derivative	$C_{15} H_{21} N O_8$	$(M+H)^+$	343.12722	344.13448	2.864	-0.5	29787.51±2206.482	2.50	18426.47±322.623	2.02
Flavonol										
Quercetin	$C_{15} H_{10} O_7$	$(M+H)^+$	302.04318	303.0505	13.126	-0.52	19711.45±1499.812	1.65	7679.66±186.952	0.84
Quercetin 3-o-glucoside	$C_{21} H_{21} O_{12}$	M^+	465.10348	465.10303	13.117	-0.18	49038.95±734.034	4.11	0.00±0	0.00
Kaempferol	$C_{15} H_{10} O_6$	$(M+H)^+$	286.04815	287.05543	13.789	-0.42	5714.65±514.318	0.47	0.00±0	0.00
Hydroxycinnami										

c acid glucoside										
Trans-cinnamoyl β-d-glucoside	$C_{15}H_{16}O_6$	$(M+NH4)^+$	292.095	310.12913	4.675	-0.57	14914.67±2088.053	1.25	0.00±0	0.00
P-coumaroyl-d-glucose	$C_{15}H_{16}O_7$	$(M+NH4)^+$	308.08951	326.12335	2.856	0.1	8519.93±1002.212	0.71	7458.60±327.776	0.82
Proteinogenic amino acid										
L-proline	$C_5H_9NO_2$	$(M+H)^+$	115.06378	116.07097	2.585	-0.45	237965.85±13863.510	19.98	217189.44±4375.650	23.88
Saturated fatty acid										
Palmitic acid	$C_{16}H_{33}NO$	$(M+H)^+$	255.25619	256.26347	19.601	0.02	4667.03±700.054	0.39	0.00±0	0.00
Unsaturated fatty acids										
Linoleic acid	$C_{18}H_{32}O_2$	$(M+H)^+$	280.2398	281.24708	27.375	0.43	3773.82±490.596	0.31	0.00±0	0.00
Dihydrocaffeic acid derivative										
Dihydrocaffeic acid 3-o-glucuronide	$C_{15}H_{18}O_{10}$	$(M+Na)^+$	358.09044	381.08002	2.558	-0.44	26580.39±3721.254	2.23	0.00±0	0.00
Hydroxycinnamic acid										
Cis-p-coumaric acid	$C_9H_8O_3$	$(M+H)^+$	164.04793	165.0552	2.882	-0.88	3644.85±400.933	0.30	0.00±0	0.00
Phenolic acid derivative										
Quercetin 3-o-glucuronide	$C_{21}H_{18}O_{13}$	$(M+H)^+$	478.07434	479.08147	13.4	0.4	5243.10±734.0340	0.44	0.00±0	0.00

Lysine derivative										
L-pipecolic acid	$C_6 H_{11} N O_2$	$(M+H)^+$	129.079	130.08652	2.625	-0.28	0.00±0	0.00	14685.41±1321.686	1.61
Flavanones										
Naringenin 5-o-glucuronide	$C_{21} H_{20} O_{11}$	$(M+H)^+$	448.10022	449.10756	13.789	0.34	7721.05 ±1256.914	0.64	0.00±0	0.00
Benzoate ester										
Ethyl 2-aminobenzoate	$C_9H_{11}NO_2$	$(M+H)^+[-H_2O]$	183.0902	166.0869	4.521	-0.04	4078.25 ±486.8800	0.34	3830.94 ±47.234	0.42

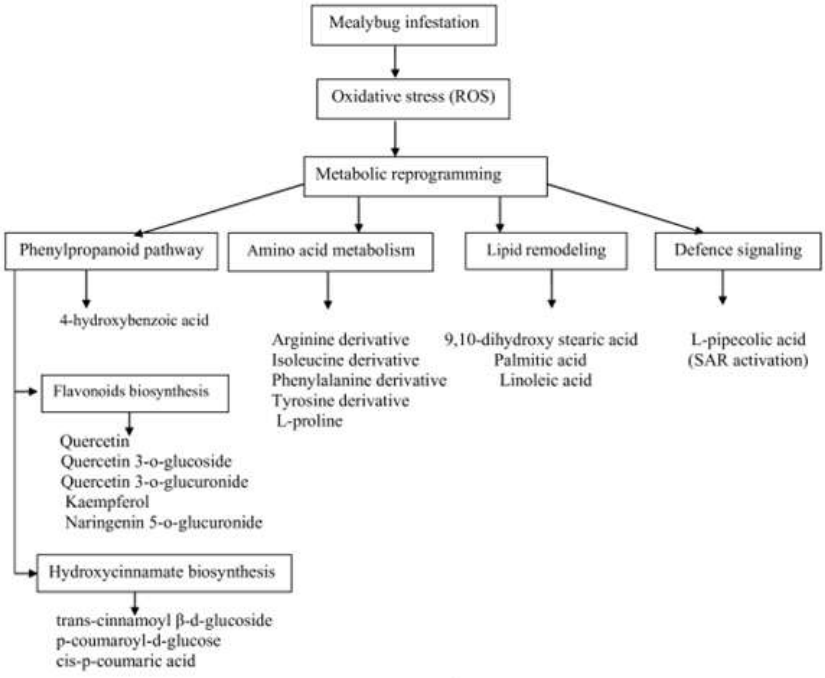
Semi-quantitative untargeted metabolomic analysis of TS and MC raisins revealed pronounced varietal and infestation - induced differences in metabolite profiles. As shown in Tables 1 and 2, several polyphenolic compounds, amino acid derivatives, fatty acids, and flavonols showed differential abundance between healthy and infested samples, indicating distinct biochemical adaptations and stress-responsive metabolic regulation in the two cultivars. The semi-quantitative differences reported in the present study were obtained using an untargeted HRMS metabolomics workflow, consistent with established approaches for semi-quantitative non-targeted metabolite profiling (Malm et al., 2021).

Untargeted HRMS based metabolomic profiling revealed significant cultivar-specific metabolic responses to mealybug infestation in TS and MC raisins. Hydroxybenzoic acid derivatives are important phenolic antioxidants that contribute to fruit quality and antioxidant capacity in grapes and raisins (Kelebek et al., 2013; Mnari et al., 2016). The contrasting changes observed in the present study therefore suggest cultivar-specific regulation of phenolic metabolism in response to mealybug infestation (Escobar-Avello et al., 2019). Hydroxybenzoic acid derivatives play a crucial role in antioxidant activity and fruit quality. The relative abundance of 4-hydroxybenzoic acid decreased in TS raisins from 10.00 to 8.82%, whereas it increased in MC raisins from 7.26 to 9.80%, indicating differential regulation of phenolic metabolism under biotic stress. Amino acid–derived metabolites exhibited significant changes linked to infestation. An isoleucine derivative increased substantially in TS raisins (11.60 to 43.54%), while consistently high levels were maintained in MC raisins. Similarly, arginine derivatives decreased in TS but increased in MC, while tyrosine derivatives showed a pronounced decline in TS with only slight fluctuations in MC. These results indicate a significant increase in an isoleucine derivative in TS raisins together with the contrasting behaviour of arginine and tyrosine derivatives between TS and MC cultivars indicates activation of amino acid metabolism during infestation. L-proline, a well-known osmoprotectant involved in osmotic adjustment and reactive oxygen species scavenging, exhibited cultivar-dependent responses. Its relative abundance decreased in TS raisins following infestation (22.17 to 18.56%) but increased in MC raisins (19.98 to 23.88%). Generally, amino acids function as important metabolic constituents in grape tissues, contributing to stress adaptation and overall fruit biochemical composition (Mnari et al., 2016). Volatile compounds associated with aroma and flavour quality, including 2-methylbutanal, ethyl decanoate and methyl cinnamate, declined following infestation in TS raisins. These volatile metabolites are important contributors to the sensory quality of raisins and grapes (Kelebek et al., 2013; Styger et al., 2011). Their reduction following infestation suggests disruption of volatile biosynthetic pathways, indicating that mealybug-induced stress may alter metabolic processes associated with aroma production.

Flavonol profiling further demonstrated distinct cultivar-specific responses. Healthy MC raisins contained a richer flavonoid composition, including quercetin 3-O-glucoside and kaempferol, whereas these compounds were no longer detected

following infestation. The occurrence of these flavonoids is consistent with previous LC-MS-based characterization of grape phenolic metabolites (Escobar-Avello et al., 2019). In contrast, TS raisins exhibited only moderate reductions in quercetin abundance. This observation suggests that mealybug infestation may cause a greater disruption of flavonoid biosynthesis in MC than in TS raisins. Genotype-dependent variation in flavonoid composition has earlier been reported in *Vitis vinifera*, supporting the cultivar-specific differences observed in the present study (Pérez-Navarro et al., 2021). Hydroxycinnamic acid metabolism was also affected by infestation. The absence of trans-cinnamoyl β-D-glucoside after infestation in both cultivars, together with the persistence of p-coumaroyl-D-glucose in MC raisins, indicates selective regulation of hydroxycinnamate metabolism during stress. Hydroxycinnamic acid derivatives are important intermediates in phenylpropanoid metabolism and contribute to defence-related cell wall strengthening and antioxidant activity, suggesting differential regulation of these pathways between cultivars (Teixeira et al., 2013).

Furthermore, accumulation of 9,10-dihydroxystearic acid in TS raisins indicates activation of hydroxy fatty acid metabolism and membrane remodelling processes, whereas the disappearance of palmitic acid and linoleic acid from infested MC raisins reflects disruption of lipid homeostasis. Differential regulation of membrane lipid metabolism is a characteristic metabolic response of plants subjected to biotic stress, supporting the contrasting lipid profiles observed between the two cultivars (Beckles & Roessner, 2011). In the present study, several metabolites showed pronounced cultivar specificity. Healthy MC raisins contained dihydrocaffeic acid 3-O-glucuronide, quercetin 3-O-glucuronide, cis-p-coumaric acid, and naringenin 5-O-glucuronide, all of which were not detected after infestation under applied analytical conditions. The selective loss of these conjugated phenolic metabolites indicates considerable disruption of phenolic conjugation and flavonoid metabolism in the MC cultivar (Aydoğan, 2020 ; Darwish et al., 2021). A notable defense response was the accumulation of L-pipecolic acid, a lysine-derived metabolite associated with systemic acquired resistance. This metabolite was not detected in healthy raisins but was found exclusively in infested TS (2.00%) and MC (1.61%) samples. L-pipecolic acid is recognized as a signalling molecule involved in systemic acquired resistance and plant immune responses. Its accumulation following infestation suggests activation of stress signalling pathways associated with defence against insect attack (Hartmann & Zeier, 2019; Jogaiah et al., 2010). A schematic overview of the proposed metabolic reprogramming induced by mealybug infestation in raisins is presented in Figure 2 a,b.



(a)

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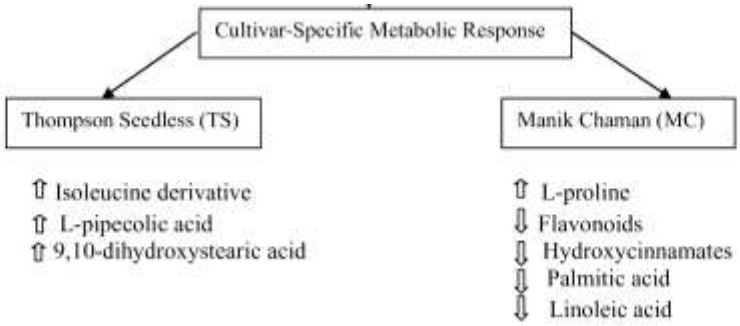

(b)

Figure 2. Proposed Schematic representation of metabolite remodeling associated with mealybug infestation in raisins. Upward solid arrow (⇧) shows increased abundance and downward solid arrow (⇩) shows decreased abundance after infestation.

Overall, TS raisins appeared to exhibit greater metabolic flexibility, as evidenced by increased levels of stress-responsive metabolites such as L-pipecolic acid, isoleucine derivatives, and hydroxy fatty acids. In contrast, MC raisins showed substantial depletion of flavonoids, fatty acids, and hydroxycinnamic acid derivatives following infestation. These results highlight several metabolites like quercetin derivatives, proline, L-pipecolic acid, and 9,10-dihydroxystearic acid as potential biomarkers for cultivar differentiation and responses to biotic stress, as demonstrated by untargeted metabolomics studies in grapes (Darwish et al., 2021). Collectively, these findings demonstrate the utility of HRMS-based metabolomics for elucidating biochemical responses to mealybug infestation and identifying potential metabolite markers associated with raisin quality and stress adaptation. The application of semi-quantitative untargeted HRMS workflows and HRMS-based metabolite characterization provides a robust platform for biomarker discovery in complex plant matrices (Aydoğan, 2020; Koulis et al., 2021; Malm et al., 2021).

3.2 Statistical Evaluation of Metabolite Variation

The metabolite data were further subjected to multivariate Statistical approaches including PCA and heat map, using MetaboAnalyst 6.0 online software (https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml). One-way ANOVA followed by FDR correction indicated significant differences in the relative abundances of the putatively annotated metabolites among the experimental groups ($q < 0.05$). Notably, 9,10-dihydroxystearic acid showed the strongest significance among the detected metabolites, with quercetin-3-O-glucoside and ethyl decanoate were also prominent. In addition, several phenolic compounds, volatile metabolites, and defense-related metabolites like L-pipecolic acid and amino acid derivatives displayed significant differences across treatments which were subsequently validated by FDR correction. Overall, these metabolites may serve as potential biomarkers for differentiating cultivars and understanding infestation-induced metabolic changes. Ultimately, this emphasizes their potential as biomarkers for raisin quality and biotic stress.

3.2.1 PCA Biplot

Principal component analysis (PCA) was conducted to visualize metabolic differences between healthy and mealybug-infested raisins from 'Thompson Seedless' (TS) and 'Manik Chaman' (MC) cultivars. The PCA biplot showed that the first two components accounted for 87.7% of the total variation, with PC1 and PC2 explaining 62.4% and 25.3%, respectively (Figure 3). The high cumulative variance explained by PC1 and PC2, together with the clear separation of the experimental groups in the score plot, indicating substantial variation in the metabolite profiles associated with cultivar and infestation status. The correlation between L-pipecolic acid and infested 'Thompson Seedless' raisins is especially noteworthy. As L-pipecolic acid plays a key role as a signalling molecule in systemic acquired resistance (SAR) and plant immune responses, its accumulation in infested raisins indicates that plants are activating defense mechanisms in response to mealybug infestation. These findings are consistent with previous reports demonstrating increased pipecolic acid accumulation during pathogen and insect attack (Hartmann et al., 2017). Overall, the PCA results show that mealybug infestation was associated with significant metabolic changes in raisins, marked by increased levels of defense-related metabolites and shifts in flavor- and antioxidant-related

compounds. These results align with earlier metabolomics research, which indicates that biotic stress notably alters primary and secondary metabolism in grape tissues and can impact fruit quality and nutritional value (Darwish et al., 2021).

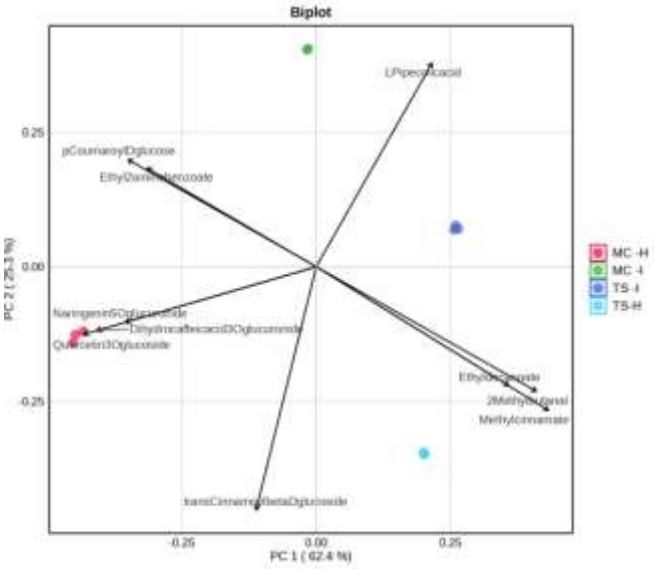

Figure 3. Principal component analysis (PCA) biplot

3.2.2 Heat Map

Heat map analysis based on Euclidean distance and Ward's linkage revealed distinct clustering patterns among raisin samples according to cultivar and mealybug infestation status (Figure 4). The results showed clear grouping of TS and MC cultivars, indicating cultivar -dependent differences in their metabolite profiles associated with mealybug infestation. Moreover, healthy and mealybug-infested samples exhibited distinct clustering within each cultivar, suggesting that the mealybug infestation was associated with substantial alterations in the metabolite profile of raisins. Overall, the present clustering pattern highlights the utility of metabolomics-based profiling and hierarchical clustering for discriminating cultivars and identifying potential stress-responsive metabolic biomarkers, in agreement with previous transcriptomics and metabolomics studies in grapevine (Savoi et al., 2016).

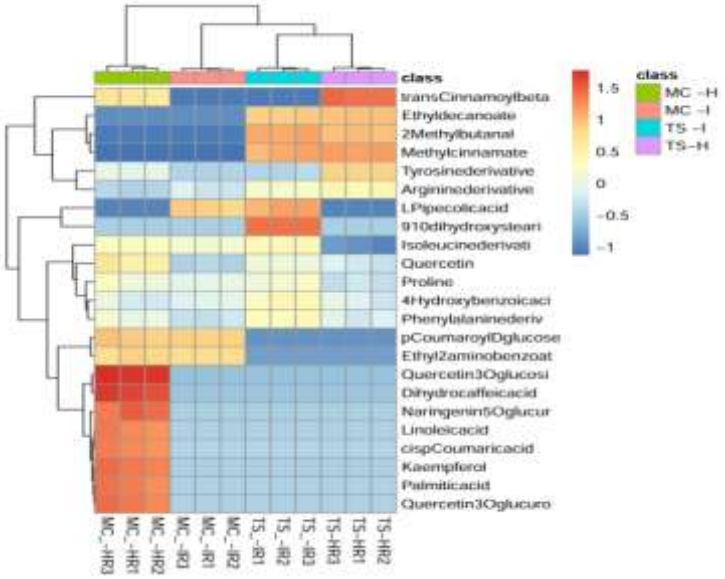

Figure 4. Heat Map

4. STUDY LIMITATIONS

The metabolites reported in this study were putatively annotated based on accurate mass, isotopic distribution, and database matching. Structural confirmation using authentic standards and MS/MS spectral matching was not performed. Metabolite identities should therefore be interpreted as putative annotations consistent with MSI Level 2 confidence. Future studies incorporating targeted validation and larger biological populations are warranted. Another limitation of the present study is the absence of pooled quality control (QC) samples, which are commonly used in untargeted metabolomics to monitor instrument drift and analytical variability. Therefore, the reported metabolite differences should be interpreted within the context of the controlled experimental design and technical replicate reproducibility. Future investigations incorporating pooled QC samples and larger biological populations will provide additional validation of the observed metabolic trends.

5. CONCLUSION

This study established a comprehensive HRMS-based metabolite profile of healthy and naturally mealybug-infested raisins of the 'Thompson Seedless' and 'Manik Chaman' cultivars. A total of 23 metabolites, including phenolic acids, flavonols, amino acid derivatives, fatty acids, volatile compounds, and hydroxycinnamic acid glucosides, were putatively identified. Multivariate analyses clearly distinguished the metabolic profiles of healthy versus infested raisins and differentiated between 'Thompson Seedless' and 'Manik Chaman' cultivars. Mealybug infestation was associated with increased relative abundances of defense-related metabolites such as L-pipecolic acid, proline, and amino acid derivatives, while several flavonoids, phenolic compounds, and aroma-related metabolites decreased. 'Thompson Seedless' showed greater metabolic adaptability by inducing stress-responsive metabolites, whereas 'Manik Chaman' experienced significant depletion of flavonoids under infestation. ANOVA highlighted several metabolites such as 9,10-dihydroxystearic acid, quercetin-3-O-glucoside, and ethyl decanoate as potential biomarkers for varietal differences and biotic stress responses. To the best of our knowledge, this is the first semi-quantitative untargeted metabolomics study of naturally mealybug-infested 'Manik Chaman' raisins using HPLC-QTOF-MS. Overall, these results offer valuable insights into the metabolic changes associated with mealybug infestation and their possible influence on raisin composition and quality. Additionally, HRMS-based metabolomics proved to be a valuable analytical tool for raisin quality assessment and potential biomarker discovery. These findings may also support stress tolerance and grape breeding programmes.

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AUTHOR CONTRIBUTIONS

Komal Ramchandra Pawar: Conceptualization, methodology, investigation, sample collection, experimental work, Data curation, Data analysis, manuscript writing.

Bharat Shinde: Supervision, scientific guidance, critical review of the manuscript.

Archana Dhole: Data interpretation, critical review of the manuscript.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Biswal, R.P., Dandamudi, R.B., Patnana, D.P., Pandey, M., Vutukuri, V.N.R.K., 2022. Metabolic fingerprinting of *Ganoderma spp.* using UHPLC-ESI-QTOF-MS and its chemometric analysis. Phytochemistry 199, 113169. https://doi.org/10.1016/j.phytochem.2022.113169

[2] Breksa, A.P., Takeoka, G.R., Hidalgo, M.B., Vilches, A., Vasse, J., Ramming, D.W., 2010. Antioxidant activity and phenolic content of 16 raisin grape (*Vitis vinifera* L.) cultivars and selections. Food Chemistry 121, 740–745. https://doi.org/10.1016/j.foodchem.2010.01.029

[3] Di Lorenzo, C., Frigerio, G., Colombo, F., de Sousa, L.P., Altindişli, A., Dell'Agli, M., Restani, P., 2016. Phenolic profile and antioxidant activity of different raisin (*Vitis vinifera* L.) samples. BIO Web of Conferences 7, 04006. https://doi.org/10.1051/bioconf/20160704006

[4] Escobar-Avello, D., Lozano-Castellón, J., Mardones, C., Pérez, A.J., Saéz, V., Riquelme, S., Von Baer, D., Vallverdú-Queralt, A., 2019. Phenolic profile of grape canes: Novel compounds identified by LC-ESI-LTQ-orbitrap-MS. Molecules 24, 3763. https://doi.org/10.3390/molecules24203763

[5] Escobar-Avello, D., Mardones, C., Saéz, V., Riquelme, S., von Baer, D., Lamuela-Raventós, R. M., & Vallverdú-Queralt, A., 2021. Pilot-plant scale extraction of phenolic compounds from grape canes: Comprehensive characterization by LC-ESI-LTQ-Orbitrap-MS. Food Research International 143, 110265. https://doi.org/10.1016/j.foodres.2021.110265

[6] Fulgoni, V.L., Painter, J., Carughi, A., 2017. Association of raisin consumption with nutrient intake, diet quality, and health risk factors in US adults: National health and nutrition examination survey 2001–2012. Food and Nutrition Research 61, 1378567. https://doi.org/10.1080/16546628.2017.1378567

[7] Karadeniz, F., Durst, R.W., Wrolstad, R.E., 2000. Polyphenolic composition of raisins. Journal of Agricultural and Food Chemistry 48, 5343–5350. https://doi.org/10.1021/jf0009753

[8] Kelebek, H., Jourdes, M., Selli, S., Teissedre, P.L., 2013. Comparative evaluation of the phenolic content and antioxidant capacity of sun-dried raisins. Journal of the Science of Food and Agriculture 93, 2963–2972. https://doi.org/10.1002/jsfa.6125

[9] Koley, T.K., Khan, Z., Oulkar, D., Singh, B.K., Maurya, A., Singh, B., Banerjee, K., 2020. High resolution LC-MS characterization of phenolic compounds and the evaluation of antioxidant properties of a tropical purple radish genotype. Arabian Journal of Chemistry 13, 1355–1366. https://doi.org/10.1016/j.arabjc.2017.11.007

[10] Krstić, Đ.D., Ristivojević, P.M., Gašić, U.M., Lazović, M., Fotirić Akšić, M.M., Milivojević, J., Morlock, G.E., Milojković-Opsenica, D.M., Trifković, J., 2023. Authenticity assessment of cultivated berries via phenolic profiles of seeds. Food Chemistry 402, 134184. https://doi.org/10.1016/j.foodchem.2022.134184

[11] López-Gutiérrez, N., Romero-González, R., Martínez Vidal, J.L., Frenich, A.G., 2016. Determination of polyphenols in grape-based nutraceutical products using high resolution mass spectrometry. Lwt-Food Science and Technology 71, 249–259. https://doi.org/10.1016/j.lwt.2016.03.037

[12] Lucci, P., Saurina, J., Núñez, O., 2017. Trends in LC-MS and LC-HRMS analysis and characterization of polyphenols in food. TrAC - Trends in Analytical Chemistry 88, 1–24. https://doi.org/10.1016/j.trac.2016.12.006

[13] Malm, L., Palm, E., Souihi, A., Plassmann, M., Liigand, J., Kruve, A., 2021. Guide to semi-quantitative non-targeted screening using LC/ESI/HRMS. Molecules 26, 3524. https://doi.org/10.3390/molecules26123524

[14] Mnari, A.B., Harzallah, A., Amri, Z., Dhaou Aguir, S., Hammami, M., 2016. Phytochemical Content, Antioxidant Properties, and Phenolic Profile of Tunisian Raisin Varieties (*Vitis Vinifera* L.). In International Journal of Food Properties 19, 578-590. https://doi.org/10.1080/10942912.2015.1038720

[15] Nale, R., Somkuwar, R.G., Singh, A. Sharma, A.K., 2024. Grapes raisin: its nutritional profile and health benefits- A review. Plant archives 24, 1837-1842. https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.263

[16] Narduzzi, L., Stanstrup, J. and Mattivi, F., 2015. Comparing wild American grapes with Vitis vinifera: a metabolomics study of grape composition. Journal of Agricultural and Food Chemistry 63, 6823-6834. https://doi.org/10.1021/acs.jafc.5b01999

[17] Olivati, C., de Oliveira Nishiyama, Y.P., de Souza, R.T., Janzantti, N.S., Mauro, M.A., Gomes, E., Hermosín-Gutiérrez, I., da Silva, R., Lago-Vanzela, E.S., 2019. Effect of the pre-treatment and the drying process on the phenolic composition of raisins produced with a seedless Brazilian grape cultivar. Food Research International 116, 190–199. https://doi.org/10.1016/j.foodres.2018.08.012

[18] Olmo-Cunillera, A., Escobar-Avello, D., Pérez, A.J., Marhuenda-Muñoz, M., Lamuela-Raventós, R.M., Vallverdú-Queralt, A., 2020. Is eating raisins healthy? Nutrients 12, 54. https://doi.org/10.3390/nu12010054

[19] Parker, T.L., Wang, X.H., Pazmiño, J., Engeseth, N.J., 2007. Antioxidant capacity and phenolic content of grapes, sun-dried raisins, and golden raisins and their effect on ex vivo serum antioxidant capacity. Journal of Agricultural and Food Chemistry 55, 8472–8477. https://doi.org/10.1021/jf071468p

[20] Plumb, R.S., Mather, J., Little, D., Rainville, P.D., Twohig, M., Harland, G., Kenny, D.J., Nicholson, J.K., Wilson, I.D., Kass, I.J., 2010. A novel LC-MS approach for the detection of metabolites in DMPK studies. Bioanalysis 2, 1767–1778. https://doi.org/10.4155/bio.10.115

[21] Salo, H.M., Nguyen, N., Alakärppä, E., Klavins, L., Hykkerud, A.L., Karppinen, K., Jaakola, L., Klavins, M., Häggman, H., 2021. Authentication of berries and berry-based food products. Comprehensive Reviews in Food Science and Food Safety 20, 5197–5225. https://doi.org/10.1111/1541-4337.12811

[22] Satisha, J., Doshi, P., Gundappa Adsule, P., 2008. Influence of Rootstocks on Changing the Pattern of Phenolic Compounds in Thompson Seedless Grapes and Its Relationship to the Incidence of Powdery Mildew. Turkish Journal of Agriculture and Forestry 32, 1-9. https://journals.tubitak.gov.tr/agriculture/vol32/iss1/1

[23] Savalekar, K., Ahammed Shabeer, T.P., Khan, Z., Oulkar, D., Jain, P., Patil, C., Banerjee, K., 2019. Targeted phenolic profiling of Sauvignon blanc and Shiraz grapes grown in two regions of India by liquid chromatography-tandem mass spectrometry. Journal of Food Science and Technology 56, 3300–3312. https://doi.org/10.1007/s13197-019-03802-w

[24] Selamat, J., Rozani, N.A.A., Murugesu, S., 2021. Application of the metabolomics approach in food authentication. In Molecules 26, 7565. https://doi.org/10.3390/molecules26247565

[25] Sharma, A.K., Somkuwar, R.G., Upadhyay, A.K., Sawant, S.D., Naik, S., 2018. Production of quality and safe dried grapes. Extension folder No.9. ICAR-National Research Centre for Grapes, Pune, p.12.

[26] Soltana, H., De Rosso, M., Lazreg, H., Vedova, A.D., Hammami, M., Flamini, R., 2018. LC-QTOF characterization of non-anthocyanic flavonoids in four tunisian fig varieties. Journal of Mass Spectrometry 53, 817–823. https://doi.org/10.1002/jms.4209

[27] Somkuwar, R.G., Ghule, V.S., Sharma, A.K. Naik, S., 2023. Evaluation of grape varieties for raisin purposes under tropical conditions of India. Grape Insight, 75-80. https://doi.org/10.59904/gi.v1.i2.2023.14

[28] Chatterjee, S., Shaikh, N., Chatterjee, N.S., Kassouf, A., Dhole, A. Banerjee, K., 2024. Unraveling varietal differences and nutraceutical potentials of Manjari Medika grape hybrid and its parents: An untargeted metabolomics study. Food and Humanity 2, 100285. https://doi.org/10.1016/j.foohum.2024.100285

[29] Teixeira, A., Eiras-Dias, J., Castellarin, S.D., Gerós, H., 2013. Berry phenolics of grapevine under challenging environments. In International Journal of Molecular Sciences 14, 18711–18739. https://doi.org/10.3390/ijms140918711

[30] Williamson, G., Carughi, A., 2010. Polyphenol content and health benefits of raisins. Nutrition Research 30, 511–519. https://doi.org/10.1016/j.nutres.2010.07.005