

不同海拔对刺葡萄果实风味物质的影响

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摘要:【目的】以湖南省中方县的‘紫秋’和‘涩葡萄’2个刺葡萄品种(品系)为试材,研究其位于3个不同海拔(240、620、700 m)葡萄园的果实风味物质含量的变化,为湖南省优质酒用刺葡萄果实生产及葡萄酒酿造提供理论依据。【方法】采用高效液相色谱-电喷雾-质谱(HPLC-ESI-MS)法对2个刺葡萄品种(品系)成熟期果皮花色苷及非花色苷酚类物质组分及含量进行测定分析,采用顶空固相微萃取气质联用(HS-SPME-GC-MS)法测定成熟期果皮香气物质含量。【结果】(1)‘紫秋’葡萄在海拔240 m,其果实还原糖、花色苷单体含量、非花色苷单体酚类物质种类及含量和果实香气物质含量在3个园中最高。(2)‘涩葡萄’在海拔700 m,其果皮花色苷单体、非花色苷酚类物质单体含量和果实香气物质种类和含量均显著高于其他2个海拔的葡萄。【结论】海拔240 m的‘紫秋’葡萄及海拔700 m的‘涩葡萄’风味物质含量最佳,更有利于葡萄酒酿造。

关键词: 刺葡萄;海拔;花色苷;香气;风味物质

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Effects of altitude on berry flavor compounds in spine grapes

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Abstract: 【Objective】Spine grape (*Vitis davidii* Foex) is a wild variety cultivated in southern China. It has strong tolerances to high-temperatures and drought and a strong disease-resistance. In recent years, because of its good adaptability in southern China, it has been widely cultivated in Hunan province and Jiangxi province, and not only brings high economic benefits to local growers but also offers high-quality grape berries for fresh consumption and for fermentation in Hunan province. Flavor compounds are important quality indexes for evaluating wine, and wine flavors are mainly derived from grape berries. The flavor of grape berry consists of sweetness, sourness and aroma. Soil, altitude, slope and cultivation management are important factors affecting the types and contents of metabolites in grape berry such as carbohydrates, phenols, amino acids and volatile substances. Altitude is one of the important factors affecting metabolism of the winemaking grape berry. In this study, we evaluated the flavor compounds of ‘Ziqiu’ and ‘Seputao’ berries growing in vineyards at 3 different altitudes (240 m, 620 m, 700 m) in Zhongfang county, Hunan province. 【Methods】Samples each with 20 clusters were randomly collected from the vineyards when grape reached commercial maturity. Samples were stored at -40 °C for laboratory analysis. Reducing sugars in the berries were determined using direct titration method with Folin’s solution; total acids of berries determined with alkali titration method. Extraction and analyses of individual anthocyanin were conducted on frozen grape berries with pedicel removed. Skin was taken from the frozen berries and grinded into powder. Grape powder (0.5 g) and 5 mL methanol solution containing 2% formic acid were added into a test tube, sonicated for 10 min (30 °C, 40 Hz), shaken in an orbit-

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al shaker at 25 °C for 30 min at a rate of 150 r · min⁻¹, and centrifuged at 8 000 r · min⁻¹ for 10 min. The supernatant was collected, and the above extraction operation was repeated 4 times. The supernatants were pooled in a 50 mL centrifuge tube and evaporated in a rotary evaporator at 30 °C until dry. The residues were re-dissolved in 10 mL of the solution of the mobile phase for high performance liquid chromatography (HPLC). All the steps were operated in the dark. Detection of anthocyanins was carried out using an Agilent 1100 series HPLC-MSD trap VL instrument, equipped with a diode array detector and a reverse phase column (Zorbax Eclipse SB C-18 250 mm×4.6 mm, 5 μm). The mobile phase A was $V_{\text{water}} : V_{\text{formic acid}} : V_{\text{acetonitrile}} = 92 : 2 : 6$; the mobile phase B was $V_{\text{water}} : V_{\text{formic acid}} : V_{\text{acetonitrile}} = 44 : 2 : 54$. Elution program was as follows: 0-18 min, 10%-25% B; 18-20 min, 25% B; 20-30 min, 25%-40% B; 30-35 min, 40%-70% B; 35-40 min, 70%-100% B. The flow rate was 1.0 mL · min⁻¹; the column temperature was 50 °C; the detection wavelength 525 nm; the wavelength range was 200-900 nm and injection volume was 30 μL. MS conditions were as follows: Electrospray ionization (ESI) interface, positive ion model, 35 psi nebulizer pressure, 10 mL · min⁻¹ dry gas flow rate, 325 °C dry gas temperature, and scans between m/z 100 and 1 000. Anthocyanins were quantified at 525 nm using malvidin-3-*O*-glucoside as standard and calibration curves obtained within a concentration range between 0.5 and 500 mg · L⁻¹, with a linear correlation coefficient greater than 0.999. For extraction and analyses of individual non-anthocyanin phenols, 2 g grape powder was macerated with 5 mL distilled water and 45 mL ethyl acetate in a conical flask, and shaken for 30 min in darkness. The supernatant was transferred in a 250 mL round bottom flask and the residue was extracted four times. The pooled supernatant was evaporated to dryness in a rotary evaporator at 33 °C, and then re-dissolved in methanol (2 mL). The non-anthocyanin phenols were measured by Agilent 1200 series HPLC-ESI-MS, equipped with a G1322A degasser, a G1312B bin pump, a G1367C HiP-ALS autosampler, a G1316B TCC, a G1314C VWD and a reversed phase column (ZORBAX SB-C18, 3 mm × 50 mm, 5 μm). The mobile phases were (A) aqueous 1% acetic acid and (B) acetonitrile containing 1% acetic acid. The wavelength was 280 nm and the injection volume was 2 μL. The gradient was 0-15 min, 10%-26% B; 30-50 min, 40%-65% B; 50-60 min, 65%-95% B; 60-63 min, 95%-10% B; 63-66 min, 10% B at a flow rate of 1.0 mL/min. The aroma components were detected by GC-MS. **【Results】**Nine anthocyanins were detected in ‘Seputao’ and ‘Ziqiu’ berry skins. The total amount of anthocyanins, non-acylated anthocyanins, caffeoyl anthocyanins and coumarin acylated in ‘Ziqiu’ grape decreased with increase of altitude (240 m > 700 m > 620 m). Non-acylated anthocyanins were the dominate anthocyanins in ‘Seputao’ and highest in samples from the altitude of 240 meters (66.93%) followed by those from 700 m (55.88%) and lowest in those from 620 m (50.22%). Eighteen non-anthocyanin phenols were detected in ‘Seputao’ and ‘Ziqiu’ berry skins from different altitudes, including hydroxybenzoates, flavanols, hydroxycinnamic acids and flavonols. Myricetin was not detected in the skin of ‘Ziqiu’ grape, while rhamnosyl-3-*O*-galactoside and clovene-galactoside were not detected in the skin of ‘Seputao’. The total non-anthocyanin phenols content in ‘Ziqiu’ grape at different altitudes were 240 m > 620 m > 700 m and 700 m > 240 m > 620 m in ‘Seputao’. 19, 21 and 21 volatile aroma compounds were detected in ‘Ziqiu’ grape at the altitude of 240 m, 620 m and 700 m, respectively, and the total amount of aroma was 3 016.30 μg · L⁻¹ at 240 m, 2 705.20 μg · L⁻¹ at 620 m and 1 681.35 μg · L⁻¹ at 700 m. But volatile components of aroma of ‘Seputao’ were 3 148.65 at 700 m, 2 967.19 μg · L⁻¹ at 240 m and 1 908.53 μg · L⁻¹ at 620 m. Overall, at the height of 700 meters above sea level, the contents of anthocyanins, non-anthocyanin phenolic compounds, and aroma compounds in ‘Seputao’ were significantly higher than those at the other two altitudes. At the altitude of 240 meters, the contents of reducing sugars, anthocyanins, non-anthocyanin phenolic compounds

and aroma compounds in 'Ziqiu' were the highest. 【Conclusion】'Ziqiu' grape at the altitude of 240 meters and 'Seputao' at the altitude of 700 meters have the highest flavor content, which is beneficial to wine making.

Key words: *Vitis davidii* Foex; Altitude; Anthocyanin; Aroma; Flavor compounds

刺葡萄(*Vitis davidii* Foex)是葡萄科葡萄属真葡萄亚属的一个种,在湖南、江西等南方地区野生较多,树势强,新梢、叶柄和叶脉上均生小皮刺^[1],有一定的抗病虫能力,对黑痘病、白腐病、炭疽病和葡萄灰霉病等具有很强的抗性^[2]。果实在8月下旬成熟,挂果留树期长,成熟后可挂果留树至10月下旬,且果实含糖量较高,在野生葡萄中果粒最大。近年来,湖南省中方县利用其独特的地理特征,从刺葡萄中选择优良株系大量种植,有“中国南方最大葡萄沟”之称,给当地农民带来较高的经济效益,也为湖南省优质酒用刺葡萄果实生产及葡萄酒酿造提供无限可能。

果实的风味物质由呈香和呈味物质组成,包括糖、酸、酚及芳香性物质等,风味物质是衡量葡萄酒的一个重要质量指标,而葡萄酒风味物质主要来源于葡萄果实。目前,已有大量研究证实诸多影响因素可对葡萄的风味物质产生影响^[3-7]。风土(Terroir)如土壤、地貌、海拔、坡度、坡向和栽培管理等是影响葡萄浆果代谢物种类和含量的重要原因^[8-9],代谢物如糖类、酚类、氨基酸和挥发性物质是葡萄适应环境的产物,这些代谢物参与多条代谢通路的调节和控制,是重要的营养物质^[10-11]。海拔是影响酿酒葡萄果实和代谢组的重要限制因子之一^[12]。有研究发现葡萄园温度差异与欧亚种酿酒葡萄品种‘赤霞珠’和‘西拉’等葡萄果实糖、酸和酚类物质含量有相关性^[13-14],紫外辐射强度影响‘黑比诺’‘赤霞珠’等葡萄果实酚类物质和香气物质的积累^[15-16],而光辐射显著影响葡萄果实糖、酸、色素的积累^[17-18]。Alessandrini等^[19]研究发现,意大利DOCG地区的‘歌蕾拉’葡萄在200~380 m海拔之间香气差异显著,高海拔葡萄特征香气含量显著高于低海拔葡萄特征香气。Mateus等^[20]针对圣路易斯杜罗葡萄园的弗兰克‘多瑞加’品种(250~300 m)和国产‘多瑞加’(300~350 m)2个红色葡萄品种研究发现,海拔对不同聚合度的原花青素影响不同,而低海拔的葡萄更有利于酿造葡萄酒。不同海拔的葡萄园,光照强度、温度、成熟期气候等的差异,导致不同海拔园区的葡萄果实品质和葡萄酒质量存

在差异。

目前,国内外关于海拔对葡萄果实品质影响的研究主要集中在鲜食葡萄^[21-22]、酿酒葡萄^[23-24]方面,而对既可鲜食又可酿酒的刺葡萄风味物质影响的研究报道较为鲜见。笔者通过研究不同海拔对刺葡萄品种(品系)果实风味物质的影响,为酒用刺葡萄生产基地建设提供依据。

1 材料和方法

1.1 试验地概况

试验于2015年在湖南省怀化市中方县桐木镇(N 27° 19' 3.18", E 109° 52' 5.08")和花桥镇(N 27° 42' 6.67", E 110° 08' 54.63")的刺葡萄园进行。怀化地区位于湖南省西部雪峰山脉和武陵山脉之间,境内气候温和,雨量充沛,无霜期长。年平均气温16.4~17.0 °C,绝对最高气温41.9 °C,绝对最低气温-13 °C,10 °C以上有效积温5 000~5 300 °C;年无霜期287 d;年降水量1 160~1 600 mm,年日照时数1 300~1 500 h。

1.2 试验材料

供试品种(品系)为刺葡萄(*V. davidii* Foex)中的‘紫秋’和‘涩葡萄’。‘紫秋’是2005年通过湖南省农作物品种审定委员会认定并登记的刺葡萄新品种^[1],‘涩葡萄’是怀化地区广泛种植的刺葡萄品系。均为2008年定植,棚架,土肥水及病虫害管理同常规。

1.3 试验方法

1.3.1 不同海拔选取 在240、620、700 m 3个不同海拔,分别选择‘紫秋’和‘涩葡萄’2个品种进行标记(依据现有的葡萄园来设计试验品种及海拔高度)(表1),在果实达到最佳成熟期后,分别随机采集30个果穗,进行果实相关指标的分析。

1.3.2 果实风味物质的测定 (1)果实还原糖、可滴定酸的测定。果实还原糖含量采用斐林试剂热滴定法测定^[25],可滴定酸含量采用酸碱滴定法测定^[26]。

(2)果皮单体花色苷组分的测定。采用HPLC-

表 1 不同海拔试验设计

Table 1 Experimental design of different altitudes

品种(品系) Variety(Strain)	葡萄园海拔和地形 Vineyard altitude and terrain	重复次数 Repeat times
紫秋 Ziqiu	240 m 谷地 240 m valley	3
紫秋 Ziqiu	610~622 m 西北方向坡地 610~622 m northwest slope	3
紫秋 Ziqiu	695~703 m 东南方向坡地 695~703 m southeast slope	3
涩葡萄 Seputao	240 m 谷地 240 m valley	3
涩葡萄 Seputao	610~622 m 西北方向坡地 610~622 m northwest slope	3
涩葡萄 Seputao	695~703 m 东南方向坡地 695~703 m southeast slope	3

ESI-MS 分析,参照 He 等^[27]的方法进行。葡萄干粉制备:随机选取 200 粒葡萄,冷冻状态下迅速将果皮与果肉分离,葡萄果皮液氮冷冻打粉,置于冷冻干燥机 24 h,冻干后备用。提取液制备:葡萄干粉称样 0.5 g,放于离心管中,加入 10 mL 甲酸甲醇(甲酸含量 2%)溶液,超声提取 10 min(水温 30 °C、功率 40 Hz),25 °C 下摇床(150 r·min⁻¹)避光提取 30 min,然后以 8 000 r·min⁻¹ 转速低温离心 10 min,收集上清液于 50 mL 离心管,重复以上提取操作 3 次,于旋转蒸发仪蒸干(30 °C),再用流动相(A 相为水:甲酸:乙腈=92:2:6, B 相为水:甲酸:乙腈=44:2:54)按 A:B=9:1 的比例定容至 10 mL, -80 °C 下保存待测。采用 Agilent1100 系列配有二极管阵列检测器(DAD)的 LC/MSD Trap-VL 液相色谱离子阱质谱联用仪进行花色苷的检测。MSD 包括电喷雾离子源和离子阱质谱检测器,所有部件均由安捷伦 v.5.2 化学工作站控制。色谱条件如下:色谱柱采用 Zorbax Eclipse SB C-18(250×4.6 mm, 5 μm)柱,流动相 A: V_水:V_{甲酸}:V_{乙腈}=92:2:6;流动相 B: V_水:V_{甲酸}:V_{乙腈}=44:2:54。洗脱程序:0~18 min, 10%~25%B; 18~20 min, 25%B; 20~30 min, 25%~40%B; 30~35 min, 40%~70%B; 35~40 min, 70%~100%B; 流速 1.0 mL·min⁻¹, 柱温 50 °C, 检测波长 525 nm, 波长扫描范围 200~900 nm, 进样量 30 μL。质谱采用电喷雾离子源(ESI), 正离子模式, 离子扫描范围 100~1 500 m/z, 雾化器压力 35 psi (241.32 kPa), 干燥气流速 10 L·min⁻¹, 干燥气温度 350 °C。

(3) 非花色苷酚类物质组分的测定。采用 HPLC-ESI-MS 分析,参照 He 等^[27]的方法进行。提取

液制备:称取葡萄干粉 2 g,加入 5 mL 蒸馏水和 45 mL 乙酸乙酯于锥形瓶中,25 °C 下摇床(150 r·min⁻¹)避光提取 30 min,转移上清液于 250 mL 圆底烧瓶,4 次重复,离心蒸干(温度 33 °C),用色谱甲醇定容至 2 mL, -80 °C 下保存待测。采用 Agilent1200 系列 LC-UV-MS 仪分析标准样品和葡萄皮提取物中的非花色苷单体酚类物质(单体酚)。色谱条件:色谱柱, Zorbax SB C-18, 50 m×3 mm, 5 μm 柱;柱温:25 °C;进样量:10 μL,检测波长:280 nm。流动相 A:1.0% 的醋酸水溶液;流动相 B:1.0%醋酸甲醇溶液1流速:1.0 mL·min⁻¹;梯度洗脱:0~15 min, 10%~26% B; 30~50 min, 40%~65%B; 50~60 min, 65%~95%B; 60~63 min, 95%~10%B; 63~66 min, 10%B。MSD 参数:离子源为 ESI, 负离子模式;雾化器压力:30 psi (206.85 kPa);干燥气流速为 10 mL·min⁻¹;干燥气温度:325 °C;离子扫描范围为 100~1 500 m·z⁻¹;CID 的 MS/MS 诱导碰撞电压:1.0 V。

(4) 葡萄果实挥发性香气物质成分的测定。葡萄果实挥发性香气物质的提取由自动进样器完成,提取参照吴玉文等^[28]的方法进行。随机选取 100 g 左右葡萄,液氮速冻,迅速去除果梗,在液氮保护下破碎并去除种子,果实剩余部分加入 0.5 g D-葡萄糖内酯和 2 g 聚乙烯吡咯烷酮(PVPP),用破碎机打成粉末。葡萄粉末混匀,于 4 °C 静置浸提至少 4 h,在 4 °C、8 000 r·min⁻¹ 转速下离心 10 min,取上清葡萄汁。此过程中分别记录葡萄果实粒数、鲜质量、种子质量、葡萄粉质量和葡萄汁质量。试验葡萄汁保存于 -40 °C 冰箱,以备香气物质的提取与检测。提取方法:准确称取 1.00 g NaCl 和 5 mL 充分解冻的葡萄汁于 15 mL 的样品瓶中,加入磁力搅拌子(1 cm),加入 10 μL 内标物质 4-甲基-2-戊醇(4M2P, 1.008 3 g·L⁻¹),迅速用带有聚四氟乙烯的隔热盖子拧紧。准备上机检测。将样品瓶放在磁力加热搅拌台上,在 40 °C 条件下平衡振动 30 min,然后将已经活化好的萃取头(50/30 μm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA, USA)插入样品的顶空部分,在 40 °C 的条件下萃取 30 min,然后去除萃取头插入 GC 进样口,在进样口解析 8 min,然后按照中国农业大学葡萄与葡萄酒研究中心建立的葡萄果实香气物质检测方法进行检测。

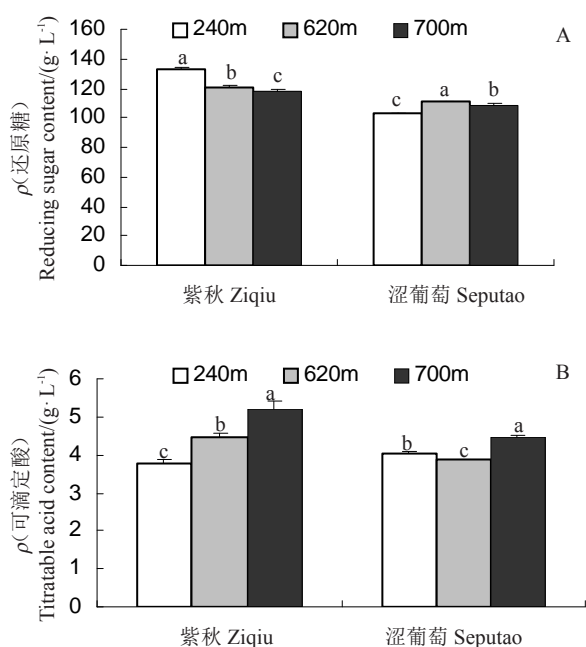
1.3.3 数据统计与分析 试验数据采用 DPS 软件进行处理,进行 Duncan's 测验,用 Excel 软件制作图

表。

2 结果与分析

2.1 海拔对‘紫秋’和‘涩葡萄’果实糖酸含量的影响

由图1可以看出,‘紫秋’和‘涩葡萄’果实含糖量在3个不同海拔下均存在显著性差异。在240 m谷地‘紫秋’葡萄含糖量最高,比其他2个坡地葡萄园中的果实含糖量分别高出10.3%(620 m)、12.8%(700 m)。生长在620 m坡地的‘涩葡萄’含糖量最高(111.02 g·L⁻¹),700 m坡地的‘涩葡萄’次之,240 m谷地的‘涩葡萄’含糖量最低,且种植在2个坡地葡萄园的‘涩葡萄’果实含糖量之间的差异小于它们分别与谷地葡萄园中‘涩葡萄’果实含糖量的差异。图1显示,生长在不同海拔的‘紫秋’和‘涩葡萄’果实可滴定酸含量之间均存在显著的差异。与上述‘紫秋’葡萄果实含糖量相反,240 m谷地的‘紫秋’葡萄果实可滴定酸含量最低,比其他2个坡地葡萄园中的果实分别低了15.1%(620 m)、27.5%(700 m)。3个不同海拔‘涩葡萄’果实可滴定酸含量最低的为620 m的坡地,最高的为700 m的坡地。



不同小写字母表示处理间差异显著($p < 0.05$)。下同。

Different small letters indicate significant difference among treatments at $p < 0.05$. The same below.

图1 不同海拔的葡萄果实还原糖(A)、可滴定酸(B)含量

Fig. 1 Reducing sugar(A), titratable acid(B) content in grapes at different altitudes

2.2 海拔对‘紫秋’和‘涩葡萄’果皮酚类物质的影响

2.2.1 海拔对‘紫秋’和‘涩葡萄’果皮单体花色苷的影响 由表2可知,‘紫秋’和‘涩葡萄’果皮中均检测出9种花色苷,包括3种单糖花色苷和6种双糖花色苷;且这2种葡萄中检出的花色苷均包括4种非酰化花色苷,1种咖啡酰化花色苷和4种香豆酰化花色苷。‘紫秋’和‘涩葡萄’双糖花色苷含量均显著高于单糖花色苷,且双糖花色苷达到了花色苷总量的94.5%以上。

‘紫秋’葡萄果皮花色苷总量、非酰化花色苷、咖啡酰化花色苷和香豆酰化花色苷总量均为240 m谷地>700 m坡地>620 m坡地。240 m海拔葡萄园中的‘紫秋’葡萄果皮中非酰化花色苷占花色苷总量的比例最高为54.99%,其次为620 m(50.41%);而生长在700 m坡地的‘紫秋’葡萄非酰化花色苷占花色苷总量的比例(46.82%)低于酰化花色苷。‘紫秋’葡萄果皮花色苷组分中,含量最多的是二甲花翠素-3,5-二葡萄糖苷,其次是二甲花翠素-3,5-香豆酰化二葡萄糖苷,这2种双糖花色苷的总量占总花色苷总量的比例高达92.6%;其中240 m海拔高度的二甲花翠素-3,5-二葡萄糖苷和二甲花翠素-3,5-香豆酰化二葡萄糖苷含量均最高。

‘涩葡萄’果皮花色苷总量和咖啡酰化花色苷总量均为700 m>620 m>240 m,非酰化花色苷总量为240 m>700 m>620 m,香豆酰化花色苷总量为620 m>700 m>240 m。3个海拔葡萄园内的‘涩葡萄’均为非酰化花色苷占花色苷总量比例最高,其中240 m谷地(66.93%)所占比例最高,700 m坡地(55.88%)次之,620 m坡地(50.22%)最低。由表2可知,不同海拔的‘涩葡萄’果实花色苷总量之间存在显著性差异,但不同海拔的‘紫秋’葡萄果实花色苷总量差异比‘涩葡萄’的差异更大。

2.2.2 海拔对‘紫秋’和‘涩葡萄’果皮非花色苷酚类物质含量的影响 不同海拔的紫外辐射强度差异对‘紫秋’和‘涩葡萄’非花色苷酚类物质含量有一定影响。表3显示,不同海拔的‘紫秋’和‘涩葡萄’共检测出18种非花色苷酚类物质,包括羟基苯甲酸类、黄烷醇类、羟基肉桂酸类和黄酮醇类,未检测到芪类物质;其中‘紫秋’葡萄果皮中未检测到杨梅酮、‘涩葡萄’果皮中未检测到鼠李糖素-3-O-半乳糖苷和丁香亭-半乳糖苷。不同海拔的非花色苷酚类物质总

表2 不同海拔的刺葡萄果皮花色苷组分和含量

Table 2 Composition and content of anthocyanin in spine grape skins at different altitudes

(mg·kg⁻¹)

花色苷 Anthocyanins	保留 时间 Retention time/min	分子离子/ 碎片离子 [M ⁺](Frag MS ² m/z)	紫秋 Ziqiu			涩葡萄 Seputao		
			240 m	620 m	700 m	240 m	620 m	700 m
花翠素-3,5-二葡萄糖苷 Dephinidin-3,5-O-diglucoside	3.8	627(303)	110.50± 0.12 a	99.35± 0.20 b	38.72± 3.56 c	203.28± 10.06 c	256.77± 9.64 b	365.52± 3.84 a
甲基花翠素-3,5-二葡萄糖苷 Petunidin-3,5-O-diglucoside	4.7	641(317)	2.59± 1.47 c	12.69± 0.81 b	20.19± 5.20 a	34.57± 2.06 a	16.16± 0.16 b	18.69± 0.07 b
二甲花翠素-3,5-二葡萄糖苷 Malvidin-3,5-O-diglucoside	5.6	655(331)	12 122.59± 89.25 a	6175.99± 218.92 c	7 666.56± 120.84 b	13 117.33± 117.48 a	9 934.59± 74.09 b	12 135.61± 34.79 a
二甲花翠素-3-葡萄糖苷 Malvidin-3-O-glucoside	10.8	493(331)	330.52± 6.52 a	121.67± 0.11 c	269.40± 0.56 b	213.72± 1.08 c	243.03± 0.09 b	327.36± 0.70 a
小计 Subtotal			12 566.20	6 409.69	7 994.87	13 568.89	10 450.55	12 847.17
比例 Proportion			54.99	50.41	46.82	66.93	50.22	55.88
花翠素-3-(6-咖啡酰化)-葡萄糖苷 Dephinidin-3-O-(6-caffeoyl)-glucoside	12.3	627(303)	429.28± 4.23 a	228.53± 1.49 c	306.26± 0.39 b	207.29± 4.43 b	382.36± 1.87 a	395.01± 0.99 a
小计 Subtotal			429.28	228.53	306.26	207.29	382.36	395.01
比例 Proportion			1.88	1.80	1.79	1.02	1.84	1.72
花翠素-3-(6-香豆酰化)-葡萄糖苷 Dephinidin-3-O-(6-coumaryl)-glucoside	15.1	611(303)	109.93± 0.62 b	143.13± 0.41 a	43.5± 0.67 c	79.00± 0.75 c	390.68± 7.55 b	464.63± 9.73 a
甲基花翠素-3,5-(6-香豆酰化)-二葡萄糖苷 Petunidin-3,5-O-(6-coumaryl)-diglucoside	18.8	787(317)	294.38± 2.69 a	229.44± 3.11 b	180.42± 1.95 c	162.43± 8.09 c	439.23± 9.01 b	466.50± 3.93 a
花翠素-3,5-(6-香豆酰化)-二葡萄糖苷 Dephinidin-3,5-O-(6-coumaryl)-diglucoside	20.1	773(303)	82.58± 0.85 c	103.81± 0.22 b	135.40± 0.38 a	5.98±0.06 b	86.47± 0.57 a	90.26± 0.98 a
二甲花翠素-3,5-(6-香豆酰化)-二葡萄糖苷 Malvidin-3,5-O-(6-coumaryl)-diglucoside	22.1	801(331)	9 371.12± 328.41 a	5 599.87± 372.71 c	8 415.60± 125.80 b	6 249.28± 238.68 b	9 060.49± 96.42 a	8 726.18± 77.99 a
小计 Subtotal			9 858.01	6 076.25	8 774.96	6 496.69	9 976.87	9 747.59
比例 Proportion			43.14	47.79	51.39	32.05	47.94	42.40
总量 Total			22 853.49± 202.54 a	12 714.46± 147.83 c	17 076.08± 109.58 b	20 272.87± 60.01 b	20 809.77± 44.61 b	22 989.77± 30.44 a

注:不同小写字母表示相同品种(品系)各海拔之间数据差异显著($p < 0.05$)。下同。Note: Different small letters mean significant difference ($p < 0.05$) among altitudes in the same varieties (strains). The same below.

量差异显著,‘紫秋’葡萄果皮非花色苷酚类物质总量为240 m>620 m>700 m,‘涩葡萄’为700 m>240 m>620m。

羟基苯甲酸类:不同海拔的‘紫秋’和‘涩葡萄’果皮中均检测到2种羟基苯甲酸,‘紫秋’葡萄果皮中羟基苯甲酸含量均为700 m>620 m>240 m,‘涩葡萄’为240 m>620 m>700 m。2个品种(品系)在不同海拔的羟基苯甲酸含量占非花色苷酚类物质总量的比例均较低,不到0.3%,不同处理间差异不大。

黄烷醇类:儿茶素是不同海拔的‘涩葡萄’果皮中均检测到的唯一一种黄烷醇,且黄烷醇含量占非花色苷酚类物质总量的比例均较低。不同海拔的‘紫秋’葡萄中,700 m‘紫秋’葡萄果皮未检测到黄烷醇,240 m检测到痕量,620 m检测到黄烷醇,但占非花色苷酚类物质总量比例低,只有0.01%。

羟基肉桂酸类:不同海拔‘涩葡萄’果皮中检测

到2中羟基肉桂酸,且与上述羟基苯甲酸一样,‘涩葡萄’果皮羟基肉桂酸占非花色苷酚类物质总量的比例为240 m>620 m>700 m;240 m和700 m两个海拔的‘紫秋’葡萄均有部分羟基肉桂酸为痕量。

黄酮醇类:不同海拔‘紫秋’葡萄果皮中检测到13种黄酮醇,‘涩葡萄’果皮中检测到12种黄酮醇,且2个品系的葡萄果皮中黄酮醇占非花色苷酚类物质总量的比例高,在99.6%以上。不同海拔黄酮醇含量差异显著,‘紫秋’葡萄黄酮醇含量为240 m>620 m>700 m,‘涩葡萄’为700 m>240 m>620 m。槲皮素-3-O-鼠李糖苷在黄酮醇中含量最高,占黄酮醇总量的62.5%以上。

2.3 海拔对‘紫秋’和‘涩葡萄’果实挥发性物质的影响

表4显示,通过对不同海拔刺葡萄果实挥发性香气GC/MS分析可以看出,3个海拔的‘紫秋’葡萄

表 3 不同海拔的刺葡萄果皮非花色苷酚类物质种类与含量

Table 3 Non-anthocyanins phenolic compositions and contents in spine grape skins at different altitudes (mg·kg⁻¹)

非花色苷酚类物质 Non-anthocyanin phenolic compounds	保留时间 Retention time/min	分子离子 /碎片离子 [M-H](Frag. MS ² m/z)	紫秋 Ziqiu			涩葡萄 Seputao		
			240 m	620 m	700 m	240 m	620 m	700 m
羟基苯甲酸类 Hydroxybenzoic acids								
原儿茶酸 Protocatechuic acid	2.0	153(109)	0.17±0.01 b	0.22±0.00 a	0.20±0.01 a	0.28±0.05 a	0.16±0.01 b	0.14±0.02 b
香草酸 Vanillic acid	4.4	167(152)	1.79±0.11 b	1.90±0.10 b	2.12±0.03 a	3.48±0.32 a	2.28±0.51 b	1.79±0.30 c
小计 Subtotal			1.96±0.00 b	2.12±0.01 ab	2.32±0.01 a	3.76±0.04 a	2.44±0.58 b	1.93±0.22 c
比例 Proportion/%			0.14	0.17	0.21	0.26	0.18	0.12
黄烷醇类 Flavan-3-ols								
儿茶素 Catechin	3.2	289(123)	tr	0.17±0.01 a	--	0.13±0.01 c	0.20±0.01 b	0.43±0.02 a
小计 Subtotal			tr	0.17±0.01 a	0.00	0.13±0.02 c	0.20±0.01 b	0.43±0.10 a
比例 Proportion/%			tr	0.01	0.00	0.01	0.01	0.03
羟基肉桂酸类 Hydroxybenzoic acids								
阿魏酸 Ferulic acid	9.2	193(134)	tr	0.31±0.01 a	0.17±0.01b	0.46±0.02 a	0.04±0.00 b	0.05±0.00 b
3-羟基肉桂酸 3-Hydroxycinnamic acid	9.7	163(119)	tr	0.15±0.01 a	tr	0.60±0.03 a	0.61±0.03 a	0.61±0.02 a
小计 Subtotal			0.00	0.46±0.04 a	0.17±0.02 b	1.06±0.12 a	0.65±0.23 b	0.66±0.19 b
比例 Proportion/%			tr	0.04	0.02	0.07	0.05	0.04
黄酮醇类 Flavonols								
杨梅酮-半乳糖苷 Myricetin-galactoside	8.6	479(316)	tr	tr	tr	tr	0.05±0.01 b	0.46±0.01 a
杨梅酮-葡萄糖苷 Myricetin-glucoside	9.5	479(316)	tr	tr	tr	tr	1.62±0.04 b	18.20±1.87 a
槲皮素-3-O-半乳糖苷 Quercetin-3-O-galactoside	10.6	463(300)	53.28±5.06 a	38.31±2.77 b	24.65±3.92 c	18.78±1.53 b	18.68±1.48 b	29.65±3.51 a
槲皮素-3-O-葡萄糖醛酸酐 Quercetin-3-O-glucuronide	11.8	477(301)	110.61±5.73 a	57.14±4.02 b	54.19±5.11 b	61.60±1.00 b	57.95±2.36 b	81.01±0.89 a
槲皮素-葡萄糖苷 Quercetin-glucoside	12.0	463(300)	51.60±1.83 a	43.18±4.33 b	21.84±3.90 c	79.53±3.33 b	76.83±3.61 b	119.67± 5.80 a
二氢山奈酚 Dihydrokaempferol	12.2	287(259)	0.21±0.01 a	0.18±0.01 a	0.09±0.01 b	0.39±0.10 a	0.37±0.12 a	0.12±0.04 b
山奈酚-3-O-半乳糖苷 Kaempferol-3-O-galactoside	12.9	447(255)	9.72±0.09 a	8.53±0.06 b	1.67±0.02 c	3.95±1.21 b	3.39±1.17 b	7.55±0.26 a
山奈酚-3-O-葡萄糖苷 Kaempferol-3-O-glucoside	14.2	447(255)	118.49±5.80 a	103.71±2.34 b	90.00±2.89 c	117.37±0.10 b	119.95±0.21 b	141.70± 3.74 a
杨梅酮 Myricetin	14.3	317(151)	--	--	--	0.53±0.02 b	0.51±0.01 b	0.97±0.02 a
槲皮素-3-O-鼠李糖苷 Quercetin-3-O-rhamnoside	14.5	447(300)	918.62± 50.84 a	796.85± 32.93 b	699.57± 78.55 c	899.22± 20.80 b	929.59± 17.39 b	1089.88± 29.05 a
鼠李糖素-3-O-半乳糖苷 Isorhamnetin-3-O-galactoside	14.9	477(314)	2.07±0.07 b	2.94±0.13 a	1.31±0.11 c	--	--	--
丁香亭-半乳糖苷 Syringetin-galactoside	15.7	507(344)	3.75±0.20 b	3.60±0.18 b	5.08±0.03 a	--	--	--
丁香亭-3-O-葡萄糖苷 Syringetin-3-O-glucoside	16.0	507(344)	145.90± 2.84 b	139.22± 3.34 b	197.23± 6.73 a	210.77± 7.99 a	112.20± 3.74 b	112.18± 4.08 b
小计 Subtotal			1 424.06± 19.94 a	1 209.82± 21.40 b	1 107.62± 43.02 c	1 433.91± 0.38 b	1 342.08± 30.04 c	1 627.58± 67.41 a
比例 Proportion/%			99.86	99.77	99.78	99.66	99.76	99.81
种类 Kind			18	18	17	17	17	17
总量 Total			1 426.02± 58.72 a	1 212.57± 12.88 b	1 110.11± 31.35 c	1 438.87± 1.88 b	1 345.37± 66.82 c	1 630.60± 39.01 a

注: tr 表示痕量, -- 表示未检测到。下同。

Note: "tr" stands for trace quantity, "--" stands for not detected. The same below.

表 4 不同海拔的刺葡萄果实香气组成与含量

Table 4 Composition and contents of aroma compounds in spine grapes at different altitudes

 $(\mu\text{g} \cdot \text{L}^{-1})$

化合物 Compounds	保留时间 Retention time/min	紫秋 Ziqiu			涩葡萄 Seputao		
		240 m	620 m	700 m	240 m	620 m	700 m
高级醇 High Alcohols							
3-甲基-2-丁烯-1-醇 3-methyl-2-buten-1-ol	19.9	1.9±0.01 c	3.51±0.10 b	11.80±1.06 a	--	--	6.84±0.02 a
1-己醇 1-hexanol	21.2	39.34±2.31 b	44.37±0.65 a	42.51±0.39 a	34.42±1.08 b	17.69±3.49 c	38.60±0.24 a
(E)-3-己烯-1-醇 (E)-3-hexen-1-ol	21.7	--	4.82±0.02 a	4.93±0.10 a	4.85±0.21 a	--	--
(Z)-3-己烯-1-醇 (Z)-3-hexen-1-ol	22.6	55.19±5.43 a	47.98±0.92 b	42.00±2.19 c	45.28±0.68 c	48.30±0.11 b	68.92±2.40 a
壬醇 Nononal	23.2	--	--	--	0.38±0.01 b	0.08±0.00 c	0.57±0.01 a
(E)-2-己烯-1-醇 (E)-2-hexen-1-ol	23.5	166.70±6.67 a	87.97±10.03 c	138.16±7.09 b	128.22±0.59 b	74.28±3.52 c	166.30±9.87 a
(Z)-2-己烯-1-醇 (Z)-2-hexen-1-ol	23.6	28.27±4.56 a	9.87±0.07 b	11.02±0.56 b	20.11±0.03 b	11.12±0.11 c	30.12±1.47 a
1-辛烯-3-醇 1-octen-3-ol	25.4	3.36±0.02 b	3.93±0.02 a	3.78±0.01 a	3.67±0.00 b	3.67±0.01 b	4.10±0.09 a
1-庚醇 1-heptanol	25.6	2.21±0.01 c	3.02±0.05 a	2.55±0.03 b	3.35±0.14 a	2.99±0.08 b	3.57±0.12 a
2-乙基-1-己醇 2-ethyl-1-hexanol	27.1	1.77±0.15 c	2.32±0.32 b	4.31±1.23 a	2.85±0.14 a	1.80±0.06 c	2.49±0.03 b
(S)3-乙基-4-甲基戊醇 (S)3-ethyl-4-methylpentanol	27.9	3.50±0.21 c	18.34±2.33 b	23.62±1.14 a	7.02±1.68 c	15.22±4.52 b	24.66±0.95 a
1-辛醇 1-octanol	29.8	2.35±0.22 b	3.08±0.08 a	2.11±0.02 b	2.83±0.16 c	4.04±0.99 b	6.15±1.12 a
1-壬醇 1-nonanol	33.8	0.43±0.06 b	0.80±0.01 a	0.36±0.04 b	1.02±0.02 c	1.80±0.01 b	3.23±0.02 a
种类 Kinds		11	12	12	12	11	12
小计 Subtotal		305.10±2.07 a	230.00±7.85 c	287.15±12.44 b	254.00±10.31 b	180.97±1.09 c	355.54±6.37 a
比例 Proportion/%		10.12	8.50	17.08	8.55	9.48	11.29
脂肪酸 Fatty Acids							
己酸 Hexanoic acid	41.0	59.22±0.08 b	55.56±1.56 b	67.95±3.77 a	60.80±5.45 a	40.68±0.93 b	40.68±1.85 b
种类 Kinds		1	1	1	1	1	1
小计 Subtotal		59.22±1.33 b	55.56±2.09 b	67.95±0.03 a	60.80±2.36 a	40.68±0.48 b	40.68±3.99 b
比例 Proportion/%		1.96	2.05	4.04	2.05	2.13	1.29
醛、酮类 Aldehydes and ketones							
2,6-二甲基-4-庚酮 2,6-dimethyl-4-heptanone	14.2	202.42±5.82 a	198.48±3.09 a	175.08±7.71 b	199.08±1.89 b	187.49±2.03 c	207.82±3.44 a
2-己烯醛 2-hexenal	16.0	2 338.92± 34.72 a	2 076.73± 78.35 b	923.08± 120.03 c	2 321.11± 109.37 a	1 256.74± 163.03 c	2 262.11± 99.78 b
2,4-己二烯醛,(E,E) 2,4-hexadienal,(E,E)	23.8	40.67±5.98 a	35.60±6.77 b	22.68±8.87 c	40.47±3.71 a	28.62±7.20 b	39.48±4.95 a
(E,E)-2,4-庚二烯醛 (E,E)-2,4-heptadienal	25.8	7.48±0.78 b	7.98±0.80 a	8.05±0.34 a	7.82±0.02 a	7.72±0.03 b	7.99±0.01 a
种类 Kinds		4	4	4	4	4	4
小计 Subtotal		2 589.50± 79.09 a	2 318.80± 103.84 b	1 128.90± 210.37 c	2 568.48± 153.08 a	1 480.56± 50.72 b	2 517.39± 178.48 a
比例 Proportion/%		85.85	85.72	67.14	86.57	77.58	79.95
挥发性苯类衍生物 Volatile benzene-derivatives							
甲苯 Toluene	9.7	31.73±4.36 b	35.55±5.67 a	21.82±3.95 c	50.61±2.73 a	27.46±6.08 b	30.38±7.79 b
苯乙烯 Styrene	17.7	4.95±0.84 c	33.22±4.69 a	6.33±0.98 b	10.02±2.05 a	5.41±1.47 b	5.58±1.63 b
苯,1-甲基-2-(1-甲基乙基) benzene, 1-methyl-2-(1-methylethyl)	17.8	--	5.73±0.31 a	--	--	--	--
苯甲醛 benzaldehyde	28.9	25.80±2.22 a	26.34±1.98 a	26.50±2.63 a	23.28±3.81 b	18.15±4.01 c	27.38±1.05 a
苯甲醇 benzyl alcohol	42.3	--	--	142.70±32.09 a	--	155.30±21.89 b	171.70±37.98 a
种类 Kinds		3	4	4	3	4	4
小计 Subtotal		62.48±6.37 c	100.84±10.60 b	197.35±41.79 a	83.91±12.43 c	206.31±20.11 b	235.04±10.19 a
比例 Proportion/%		2.07	3.73	11.74	2.83	10.81	7.46
种类 Kind		19	21	21	20	20	21
总量 Total		3 016.30± 122.45 a	2 705.20± 209.39 b	1 681.35± 45.95 c	2 967.19± 55.01 b	1 908.53± 241.36 c	3 148.65± 72.96 a

果实中分别检测出 19(240 m)、21(620 m)、21(700 m)种挥发性香气组分,香气物质总量为 240 m ($3\ 016.30\ \mu\text{g}\cdot\text{L}^{-1}$)>620 m($2\ 705.20\ \mu\text{g}\cdot\text{L}^{-1}$)>700 m ($1\ 681.35\ \mu\text{g}\cdot\text{L}^{-1}$);‘涩葡萄’果实中分别检测出 20(240 m)、20(620 m)、21(700 m)种挥发性香气组分,香气物质总量为 700 m($3\ 148.65\ \mu\text{g}\cdot\text{L}^{-1}$)>240 m ($2\ 967.19\ \mu\text{g}\cdot\text{L}^{-1}$)>620 m($1\ 908.53\ \mu\text{g}\cdot\text{L}^{-1}$)。不同海拔的挥发性香气物质总量有显著差异,2个品种(品系)的葡萄果实香气物质总量最高均为种植在 700 m 坡地葡萄园中葡萄。

2个品种(品系)的葡萄果实挥发性香气物质中醛、酮类物质含量和占香气物质总量的比例比较高,占香气物质总量的67%以上。‘紫秋’葡萄果实中醛、酮类物质含量为 240 m($2\ 589.50\ \mu\text{g}\cdot\text{L}^{-1}$)>620 m ($2\ 318.80\ \mu\text{g}\cdot\text{L}^{-1}$)>700 m($1\ 128.90\ \mu\text{g}\cdot\text{L}^{-1}$),‘涩葡萄’果实中醛、酮类物质含量为 240 m($2\ 568.48\ \mu\text{g}\cdot\text{L}^{-1}$)>700 m($2\ 517.39\ \mu\text{g}\cdot\text{L}^{-1}$)>620 m($1\ 480.56\ \mu\text{g}\cdot\text{L}^{-1}$)。醛、酮类物质中 2,6-二甲基-4-庚酮、2-己烯醛等含量较高。

挥发性苯类衍生物是葡萄酒中重要的香气物质,不同海拔的‘涩葡萄’果实中均未检测到苯-1-甲基-2-(1-甲基乙基),而检测出的苯甲醇在挥发性苯类衍生物中含量最高,但 240 m‘涩葡萄’和 240 m、620 m‘紫秋’葡萄中均未检测到这种物质。高级醇在香气物质中种类最多,不同海拔的‘紫秋’刺葡萄均未检测到壬醇。而(*E*)-2-己烯-1-醇、(*Z*)-3-己烯-1-醇、1-己醇和(*S*)-3-乙基-4-甲基戊醇在高级醇中所占比例较高。

3 讨论

随着葡萄园海拔的变化,光照和温度对园内植物生长的间接影响也发生改变,一般情况下,海拔越高,紫外线强度越高,增强的紫外线可影响蛋白质、核酸及细胞的形态、结构及功能^[29]。同时,紫外线强度越高,葡萄园内的植株产生越多的能够吸收紫外线的次生代谢物质,主要包括黄酮类化合物(花色素、黄烷-3-醇、黄酮醇类等)、类胡萝卜素和氨基酸,其含量的增加使酿酒葡萄及葡萄酒的酚类物质构成增加,也使葡萄酒香气更加丰富,对葡萄酒质量的提高起到了一定的作用^[30]。

本研究发现‘涩葡萄’果实风味物质随着海拔梯度的升高呈现整体上升趋势,且果皮花色苷单体、非

花色苷酚类物质单体含量和果实香气物质种类和含量均显著高于其他两个海拔,这与毛如志等^[31]的研究结果一致,其对高海拔和低海拔产区的欧亚种葡萄‘美乐’果实品质指标测定发现,高海拔产区的‘美乐’果实累积的氨基酸、有机酸、酚类物质和糖类物质显著高于低海拔产区;通过对香格里拉河谷区 4 个海拔(1 117、1 860、2 102、2 797 m)‘玫瑰蜜’葡萄进行果实指标的测定,发现位于中间 2 个海拔(1 860、2 102 m)的葡萄园生产的‘玫瑰蜜’葡萄果实品质更高^[31],认为高海拔更有利于葡萄果实品质的提高,类似的报道很多^[21,33]。而本研究中‘紫秋’葡萄在低海拔葡萄园里果实风味物质表现较好,这可能是由于‘紫秋’葡萄品种特性原因,即该品种植株生长势强、适应性强、耐旱、耐粗放管理,适合在南方高温、多湿气候条件下生长^[1]。

怀化地区地处丘陵地带,由于地形地势的不同,不同海拔园区的微气候有很大的差异。本研究中,位于 620 m 和 700 m 高度的葡萄园地处山坡,2 个园区的海拔最大差异仅有 93 m,但可能是两个葡萄园坡向不同,增大了光照和温度的差异,使试验结果中这两个海拔的果实风味物质存在显著差异。

4 结论

综合各风味物质种类及含量,‘紫秋’葡萄在海拔 240 m 时,其果实酿酒品质指标明显优于其他海拔,更有利于葡萄酒酿造;而‘涩葡萄’在海拔 700 m 时,其果实酿酒品质指标明显优于其他海拔,更有利于葡萄酒酿造。

致谢:本研究中果实酚类和香气物质组成的测定在农业部葡萄加工重点实验室完成。

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