葡萄与葡萄酒文摘
（2011）

主编 王华

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葡萄酒抗氧化功能与结合态香气研究

STUDY ON WINE'S ANTIOXIDANT CAPACITY AND BOUND VOLATILES

2011

目前葡萄酒研究领域的两大热点为葡萄酒的抗氧化功能与葡萄酒中结合态香气化合物，二者是葡萄酒消费者与爱好者最为关心的问题，本论文分别对葡萄酒抑制由自由基引发的胆固醇氧化的抑制能力与葡萄酒中结合态香气化合物以及影响其固相萃取回收率的因子进行了研究。

适量饮用葡萄酒对心脏病、动脉粥样硬化、血小板凝聚等心血管疾病以及中风、某些癌症都具有一定的预防与抑制的作用，主要是因为葡萄酒中所含具有抗氧化、清除自由基功能的各类多酚类物质；同时也有研究证明，胆固醇与胆固醇的氧化产物在人体中含量与心血管疾病紧密相关，故抑制由自由基引发的胆固醇氧化对降低上述疾病的发病率以及死亡率都具有重要意义。本研究采用了与健康人体中胆固醇含量基本一致的体外由自由基引发的胆固醇氧化模型调查了圆叶(Vitis Muscadinia rotundifolia）红葡萄酒，欧亚种(Vitis Vihifera)红，白葡萄酒抑制胆固醇氧化的能力。将供试样品葡萄酒与胆固醇悬浮液按照不同的体积比例混合，在该反应体系中加入 2,2'-偶氮(2-甲基丙基脒)二盐酸盐（AAPH）作为自由基引发剂，加速胆固醇氧化，保持 37℃ 的反应温度（与健康人体温度一致），利用搅拌子使其反应体系维持在均匀混合的状态，反应 0 h、24 h、48 h、72 h 后，利用高效液相色谱 HPLC 测试胆固醇氧化的主要终端产物 7-酮基胆固醇（7-ketocholesterol）的含量。对欧亚种红葡萄酒与白葡萄酒进行研究，结果表明欧亚种梅尔诺（Merlot）与赤霞珠（Cabernet Sauvignon）红葡萄酒二者拥有类似的抑制由自由基引发的胆固醇氧化的能力；欧亚种白诗南（Chenin Blanc）与缩味浓（Sauvignon Blanc）白葡萄酒二者也具有类似的抑制由自由基引发的胆固醇氧化的能力，但是欧亚种红葡萄酒抑制胆固醇氧化能力远远大于欧亚种白葡萄酒，红葡萄酒抑制胆固醇氧化的能力是白葡萄酒抑制胆固醇氧化能力的 50 倍；同时发现梅尔诺与赤霞珠红葡萄酒中儿茶素含量分别为 140.6mg/L, 132.5mg/L; 白藜芦醇的含量分别为 0.7 mg/L, 1.0mg/L; 而欧亚种白诗南与缩味浓白葡萄酒中儿茶素含量分别为 10.6mg/L, 11.0mg/L; 白藜芦醇的含量均为 0.3mg/L。同时本部分研究还证明了儿茶素抑制胆固醇的氧化的能力强于白藜芦醇。对圆叶红葡萄酒与欧亚种红葡萄酒进行研究，结果表明圆叶（Muscadine）与欧亚种梅尔诺红葡萄酒对由自由基引发的胆固醇氧化都具有很强的抑制作用，其中梅尔诺红葡萄酒抑制由自由基引发的胆固醇氧化的能力强于圆叶红葡萄酒。二者的主要抗氧化物质为儿茶素与花青素，在本部分实验中，样品酒中没有检测到白藜芦醇，圆叶与梅尔诺红葡萄酒花青素含量分别为 150.1mg/L 和 150.3mg/L；儿茶素含量分别为 15.6mg/L 与 140.6mg/L。相比较花青素而言，儿茶素与白藜芦醇在人体中消化后不易发生降解，同时更容易进入到人体血液系统中，而葡萄酒中白藜芦醇的含量又很低，由此可以推断出，在红葡萄酒抑制由自由基引发的胆固醇氧化儿茶素比白藜芦醇过程中发挥了更大的作用。依照实验测定结果，普通的佐餐红葡萄酒中儿茶素含量为 150 mg/L 左右，那么健康人每天饮用 60-70mL 红葡萄酒就能有效抑制人体中低密度脂蛋白胆固醇的氧化。

葡萄酒中香气主要是源自于酿酒葡萄（Vitis. vinifera）果实，在葡萄酒的酿造与陈酿过程中结合态香气

前体物质对香气的释放，其主要是糖结合的半萜类化合物。在葡萄酒的生产过程中，葡萄果实中的一部分结合态香气化合物在其葡萄果实自身的酶，以及酵母释放的酶类物质的作用下，会被水解一部分，构成最初的葡萄酒香气，例如里拉醇；同时，另一类结合态香气化合物在酸作用下水解，发生化合物结构重排，释放出来游离态，阈值很低的葡萄酒香气主要贡献物，例如大马酮、TDN。由于葡萄酒中的结合态香气化合物始终影响到葡萄酒协调及优雅的香气的形成，故准确定量定性葡萄酒中结合态香气化合物至关重要。本研究采用 Lichrolut EN 商业包装固相萃取柱，利用固相萃取（SPE）提取方法提取葡萄酒中结合态香气化合物，然后在 100℃温度下酸水解 1h 提取到的结合态葡萄酒香气化合物，再利用 GC-MS 和标准样品鉴定葡萄酒中的结合态香气化合物。在本部分研究中，雷司令（Riesling）白葡萄酒与赤霞珠红葡萄酒中 37 与 36 种结合态香气化合物被鉴定，按照葡萄酒中香气化合物的不同生物来源前体物质，将其分为脂肪类香气化合物、异戊二烯类香气化合物、萜类香气化合物以及莽草酸类香气化合物。同时对可能影响葡萄酒结合态香气化合物固相萃取回收率的因子进行了研究，采用统计分析软件 MODDE 6.0，利用响应面与全因子实验设计方法对实验进行了设计。研究结果表明葡萄酒参数乙醇含量、葡萄糖浓度、pH 值以及单宁含量等对不同类型的结合态香气化合物固相萃取回收率影响不一样。总体上来讲，在雷司令白葡萄酒中，当乙醇浓度为 3.75 mg /ml 到 123.75 mg /ml 时，乙醇是影响其固相萃取结合态香气化合物回收率最主要的因素，第二是葡萄糖含量，第三是葡萄糖含量的平方；当乙醇含量降为 3.75 mg /ml 到 63.75 mg /ml 时，乙醇仍然是最重要的影响葡萄酒中固相萃取结合态香气化合物回收率最主要的因素，第二是 pH 值与乙醇含量的交互作用，第三是 pH 值。在赤霞珠红葡萄酒中，当乙醇浓度为 3.75 mg /ml 到 123.75 mg /ml 时，乙醇是影响其固相萃取结合态香气化合物回收率最主要的因素，第二是乙醇与单宁的交互作用，第三是 pH 值与单宁的交互作用；当乙醇含量降为 3.75 mg /ml 到 63.75 mg /ml 时，乙醇仍然是最重要的影响葡萄酒中固相萃取结合态香气化合物回收率最主要的因素，第二是乙醇的平方的作用，第三是 pH 值。当供试红白葡萄样酒中的乙醇浓度降为 23.75 mg/ ml 后，其葡萄酒中结合态香气化合物固相萃取的回收率不受上述因子的影响；通过本研究，弄清楚了影响葡萄酒结合态香气固相萃取（SPE）回收率的因子；同时也建立了一种简单、准确、快速定性定量检测葡萄酒中结合态香气化合物的方法。

关键词   HPLC   GC-MS

Abstract

At present, wine antioxidant function and bound aroma attracted many studies. In this research, we focused on the capability of red and white wine to prevent cholesterol oxidation induced by free radical, the bound aroma of wine and the wine parameters which affect the wine bound volatiles SPE extraction recovery.

Moderate consumption of wine can reduce the incident of Cardiovascular diseases, platelet aggregation, stroke and some cancers, cause wine contain resveratrol and different kinds of phenols, and their antioxidant and capability. So it is very necessary to prevent cholesterol oxidation inducing by free radicals, which will decrease the incidence of above disease and mortality. In this study, capabilities of Muscadine (MCD), Merlot (MLT), Cabernet Sauvignon red wine, Chenin Blanc and Sauvignon white wine in inhibiting cholesterol oxidation were evaluated using cholesterol emulsion (CE) system, a free radical generator, 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), was used to accelerate cholesterol oxidation at 37℃, the production of 7-ketocholesterol was detected after 0h, 24h, 48h and 72 h oxidation. In the research about white wine and red wine, the results show that Merlot and Cabernet Sauvignon red wines have the similar capability in reducing cholesterol oxidation reducing by free radical; and the Chenin Blanc and Sauvignon Blanc white wines have the similar capability of anti-cholesterol-oxidation, too; however, the red wines' activity are almost equal to 50 times

of white wines' activity. The level of catechin in Merlot and Cabernet red wines are 140.6mg/L and 132.5mg/L, respectively; the resveratrol are 0.7 mg/L, 1.0mg/L, respectively; at the same time, the level of catechin in Chenin Blanc and Sauvignon Blanc are 10.6mg/L, 11.0mg/L, respectively; and the resveratrol level of the two white sample wines are 0.3 mg/L. also, the results demonstrated that the antioxidant capability of catechin are much stronger than the resveratrol's capability. In the research about Muscadine and Merlot red wine, the results demonstrated that the both of sample red wines can strongly prevent cholesterol oxidation inducing by free radicals, meanwhile, the activity anti-cholesterol-oxidation of Merlot red wines is stronger than Muscadine red wines'; The mainly antioxidant compounds are catechin and anthocyanins, meanwhile, resveratrol was not determined, the content of anthocycanin in Muscadine and Merlot red wine are 150.1mg/L and 150.3 mg/L, respectively; and the content of catechin are 15.6 mg/L and 140.6mg/L, respectively. Compared with anthocyanins, catechin and resveratrol are more readily absorbed in the bloodstream without degradation after ingestion, it can be known that catechin rather than resveratrol may significantly contribute to the antioxidant capability of red wine in retarding cholesterol oxidation. Based on the results of that study, catechin level of normal table red wine is about 150 mg/L, consuming 60-70mL of the red wine would be helpful in inhibiting the oxidation of LDL cholesterol of a healthy person for 24 hours.

The aromas of a finished wine are absent in many wine-grape cultivars (*Vitis. L. vinifera*), but are instead generated during fermentation and storage from non-volatile precursors such as the glycoconjugates. Glycoconjugates (glycosides) posses an aglycone covalently bound to one or four sugar moieties via an ether linkage. During wine production, glycosides may be hydrolyzed enzymatically by yeasts and exogenous glycosidases or non-enzymatically by acid-catalyzed hydrolysis. Following hydrolysis, the liberated aglycone can have immediate odor impact (e.g. linalool) or else may rearrange under acid conditions to form odor-active compounds (e.g. β-damascenone, 1,1,6-trimethyldihydronapHthalene). Because the bound volatiles always effect on the wine's desirable aroma, it is very important to determine the bound volatiles. In this research, the bound volatiles of wine was extracted by   solid-phase extraction (SPE), with LiChrolut EN polymeric packing resin, then those bound compound were hydrolyzed in water bath at 100℃. In Riesling white wine and Cabernet Sauvignon red wine, there are 37 and 36 different bound volatiles were identified. Those aroma compounds are grouped according to the biological origin of the precursors which presumably generated the volatiles, using this criterion, four large groups can be differentiated: lipid derivatives, norisoprenoid, terpenes and shikimic acid derivates. In generally, the different wine parameters: ethanol level, glucose, pH and tannin have the different response to those aroma compounds of SPE extraction recovery. In white wine 'Riesling', when then ethanol level is between 3.75 mg/ml and 123.75mg/ml, ethanol is the most important parameter which affects SPE of bound volatiles recovery , the following is glucose and Glucose×Glucose; when the ethanol level decreased, between 3.75mg/ml and 63.75mg/ml, the ethanol is still the most important factor, the following is pH×Eth and pH; In Cabernet Sauvignon red wine, when the ethanol level is between 3.75mg/ml and 123.75mg/ml, ethanol also is the most important parameter which affects SPE of bound volatiles recovery, the following is ethanol × tannin and pH ×tannin; when the ethanol decreased, between 3.75mg/ml and 63.75mg/ml, the ethanol is still the most important parameters, the following is Eth×Eth and pH. When the ethanol level is under 23.75mg/ml, there are no bound volatiles compounds will be affected by wine parameters. According to our study, we figured out the wine parameters affect the bound aroma's SPE recovery; Meanwhile, a quickly, precise method was developed to determine those wine bound volatiles.

Key words　Wine　Cholesterol oxidation　Bound Volatiles　HPLC　GC-MS

前体物和诱导子对悬浮培养葡萄细胞多酚
生物合成影响的研究

EFFECTS OF PRECURSOR AND ELICITORS ON THE PHENOLIC
COMPOUNDS BIOSYNTHESIS IN SUSPENSION CULTURES OF GRAPE
(*VITIS ROMANETII* ROMAN)

2011

利用植物细胞作为生物反应器生产高附加值次生代谢产物的技术，近些年来取得很大进展，该技术不但可以保护濒危物种，解决人类医药和保健问题，而且不占用耕地，不受地理环境和气候条件等的影响。本研究首先调整培养基与植物生长调节物质浓度配比，诱导葡萄茎和叶片外植体产生愈伤组织，通过筛选出高酚含量愈伤组织和优化细胞悬浮培养条件建立稳定的悬浮细胞系。以此悬浮细胞系为模式体系，添加前体物或诱导子，以 *Actin* 为内参基因，用实时定量 PCR(RTq-PCR)方法检测苯丙氨酸解氨酶(PAL)、查尔酮合成酶(CHS)和二苯乙烯合成酶(STS)基因相对表达量变化；测定细胞生长量，分析 PAL 活性变化，定量分析总酚、原花色素和白藜芦醇的含量和产量，研究前体物和诱导子对悬浮培养葡萄细胞中多酚生物合成的影响，为葡萄次生代谢物实现大规模工业化生产提供理论依据。获得主要研究结果如下：

1.筛选出中国野生葡萄高酚含量愈伤组织并建立稳定的悬浮细胞系

以中国野生的毛葡萄(*V. quinquangularis* Rehd)、秋葡萄(*V. romanetii* Roman)、刺葡萄(*V. davidii* Roman)和酿酒葡萄品种赤霞珠(*V. vinifera* L. cv. Cabernet Sauvignon)半木质化茎在附加 30 g/L 蔗糖、6 g/L 琼脂、2.0 mg/L 6-BA 和 0.05 mg/L NAA，pH=6.0 的 B5 培养基上诱导产生愈伤组织生长量最大，诱导率最高；赤霞珠产生愈伤组织较其他种容易；在同样消毒与培养条件下，半木质化茎诱导产生愈伤组织的能力较叶片强。对秋葡萄愈伤组织生长影响大小的因素依次是：培养基＞植物生长调节物质浓度＞植物生长调节物质组合＞培养基体积，最佳组合为每瓶 35 mL B5 培养基加入 0.2 mg/L KT＋0.1 mg/L NAA。

以高酚含量秋葡萄愈伤组织培养稳定的悬浮细胞系；愈伤组织的继代次数、生长天数和初始接种量是悬浮培养成功的关键；继代培养时新旧培养基体积比控制在 4:1～8:1 之间，可以培养出稳定的悬浮细胞系。

2.前体物对 3、4 和 5 d 龄悬浮细胞生长有促进作用，诱导子对悬浮细胞生长有抑制作用

悬浮细胞生长迟滞期随着蔗糖浓度增加而延长；40 g/L 的蔗糖培养细胞的生长量最大。L-苯丙氨酸抑制 1、2 d 龄细胞生长，促进 3、4 和 5 d 龄细胞生长；4 种诱导子对葡萄细胞生长均有抑制作用，且处理细胞龄越小，抑制作用越大。前体物和诱导子多因素协同作用时，各因素对悬浮培养细胞生长影响大小依次是：蔗糖浓度＞L-苯丙氨酸＞MJ＞真菌诱导子。其中最佳组合是 40 g·L^{-1} 蔗糖、0.2 mmol/L L-苯丙氨酸、10 μmol/L MJ 和 1.5 mL 真菌诱导子。

3.前体物和诱导子显著提高 *PAL*、*CHS* 和 *STS* 基因的表达量和 PAL 的活性

加入 L-苯丙氨酸后，前期 *PAL*、*CHS* 和 *STS* 基因的表达量迅速增加，后期三个基因的表达量均下降。诱导子对 *PAL*、*CHS* 和 *STS* 三个基因表达具有较大的刺激作用，MJ、UV-B 和 UV-C 在处理后，细胞生长初期对三个基因的表达影响较大，真菌诱导子则在后期影响较大。

L-苯丙氨酸促使细胞 PAL 活性在 12 h 时达到最大，而后下降。4 种诱导子处理细胞的 PAL 活性呈双 S 形变化：第一个峰值出现在 12 h 时，第二个高峰则在 3 d 后的不同时间出现。前体物和诱导子联合处理后，PAL 活性在 12 h 内迅速上升，然后持续下降；12 h 时 L-苯丙氨酸对 PAL 活性影响最大，到第 3 d 时 MJ 对 PAL 活性影响大于 L-苯丙氨酸。

4.前体物和诱导子显著提高细胞总酚、原花色素和白藜芦醇的含量及产量

总酚、原花色素和白藜芦醇含量随着蔗糖浓度的增加而升高。40 g/L 蔗糖培养细胞的总酚、原花色素和白藜芦醇产量最高，其总酚产量和其他处理之间的差异显著($p < 0.05$)；原花色素和白藜芦醇产量与其他处理之间的差异极显著($p < 0.01$)。30 g/L 蔗糖添加水解酪蛋白(Casein hydrolysate, CH)的细胞生长量、总酚、原花色素和白藜芦醇的含量和最大产量都比无 CH 的高，但生长趋势相同，最大产量出现的时间不变。

L-苯丙氨酸处理 3、4 和 5 d 龄细胞总酚、原花色素和白藜芦醇的产量增加，但 1、2 d 龄细胞的产量都下降。4 种诱导子对细胞的总酚、原花色素和白藜芦醇含量有促进作用，不同处理的最高产量之间差异达到极显著水平($p < 0.01$)，MJ 处理的白藜芦醇产量在 4 种诱导子处理中最低，真菌诱导子处理的产量最高。

前体物和诱导子协同处理时，30 g/L 蔗糖、1.2 mmol/L L-苯丙氨酸、50 μmol/L MJ 和 1.5 mL 真菌诱导子处理细胞的总酚和白藜芦醇含量最高，与其他处理之间的差异极显著($p < 0.01$)。50 g/L 蔗糖、1.2 mmol/L L-苯丙氨酸、30 μmol/L MJ 和 0.5 mL 真菌诱导子处理的原花色素含量最高，与其他处理之间的差异极显著($p < 0.01$)。蔗糖、MJ 和真菌诱导子分别对总酚、原花色素和白藜芦醇的产量影响较大。结果是 40 g/L 蔗糖、0.2 mmol/L L-苯丙氨酸、30 μmol/L MJ 和 1.5 mL 真菌诱导子组合的总酚和原花色素产量最高；40 g/L 蔗糖、0.7 mmol/L L-苯丙氨酸、30 μmol/L MJ 和 1.5 mL 真菌诱导子组合白藜芦醇总产量最高。

本文的创新之处在于，以中国秋葡萄悬浮培养细胞为基础，通过添加前体物或诱导子处理，得到细胞 *PAL*、*CHS* 和 *STS* 三个基因的表达变化和 PAL 活性变化；该法显著提高葡萄内天然功能性成分原花色素和白藜芦醇含量。此项研究在未来天然食品添加剂工业化生产中具有较大的应用前景。

关键词

Abstract

Great advances have been achieved in producing desirable plant secondary metabolites by the technology of plant cell bioreactors in recent years. It both protects endangered species and solves human healthcare and welfare issues, while neither impropriates cultivated land nor depends on geographical location, climatic conditions, seasonal variations and growth conditions. In this study, callus were induced from the stems and leaves of grape through adjusting the medium and the hormone concentrations, and a stable cell suspension culture system was developed by selecting callus with high phenolic content, optimizing the conditions of cell suspension culture. Precursor and elicitors were added into the model system that established cell suspension culture system. The relative expression changes of phenylalanine ammonia lyase (PAL), chalcone synthase (CHS) and stilbene synthase (STS) genes in cells were monitored by a real-time quantitative PCR. The PAL activity, cell biomass and

the contents of total phenols, proanthocyanidins and resveratrol were also investigated during cell suspension culture. The study aims at examining the effects of precursors and elicitors on phenolic compounds biosynthesis in cell suspension cultures of grape (*Vitis romanetii* Roman), so as to provide theoretical foundation for the industrialized mass production of grape second metabolites in future. The main results are as follows:

1. A stable cell suspension culture system was developed by selecting callus with high phenolic content and optimizing the conditions of cell suspension culture

Callus were induced using semi-lignification stems as explants of four grape varieties (Cabernet Sauvignon, hairy grape, romanet grape and brier grape), and the best culture medium was B5 with sucrose 30 g/L, agar 6 g/L, 6-BA 2.0 mg/L and NAA 0.05 mg/L, with the highest frequency above 85%. The induced callus of Cabernet Sauvignon was easily than other varieties and the better was with semi-ligification stems as explants than leaves. Factors influencing the growth of romanet grape callus were: medium > hormone concentrations > hormone combination > medium volume, and the optimum combination medium for its growth was 35 ml B5 medium per bottle with 0.2 mg/L KT and 0.1 mg/L NAA.

The Romanet Grapes Callus was chosen as the stable cell suspension culture system after comprehensively comparing the contents of total phenolics, proanthocyanidins and resveratrol in the four varieties. The key to succeeding suspension culture were callus passages, cell age and original inoculation sizes. The establishment of primary suspension cultural cell line of grape, required that the transferring–culturing callus should subculture at least 5 times, cell age 11 to 15-day and original inoculation amount 150 g/L and the volume ratio of the new and the old medium for subculture arranged from 4:1 to 8:1.

2. The precursors promoted the growth of the suspension cultural cells that growth 3, 4, 5 days, while the elicitors inhibited the growth of suspension cultural cells

The growth lag phases of suspension culture cells extended with the increasing concentrations of sucrose. The largest cell biomass was achieved when grown in B5 medium with 40 g/L sucrose. The cell growth was inhibited by L-phenylalanine when the cell at 1, 2-day-cell age, but promoted when the cell at 3-, 4- and 5-day-cell age. The grape cell growth was inhibited by four elicitors, and the greater inhibition existed in the cell with smaller age. Different factors affecting the cell biomass of grape suspension culture were: sucrose> L-phenylalanine> MJ> fungal elicitor, when synergistic effects of precursors and elicitors were considered. The best combination of precursors and elicitors was: 40 g/L sucrose, 0.2 mmol/L L-phenylalanine, 10 μmol/L MJ and 1.5 ml fungal elicitor.

3. The precursors and elicitors significantly promoted the PAL activity and the gene expression of *PAL*, *CHS* and *STS*

The addition of L-phenylalanine enhanced the expression of *PAL*, *CHS* and *STS* genes in the 12 hours, then *PAL* and *STS* began to decrease, while the expression of *CHS* gene increased continuously until reached the peak at the 7th day. Three genes' expression was decreased with the cell entering a decline phase after 7 days. The elicitors showed marked effects on the expression of the three genes: MJ, UV-B and UV-C had significant influence in the early stage, but the fungal elicitor was markedly in the late stage.

The PAL activity reached the peak at 12th hour after added L-phenylalanine, and then decreased. The PAL activity with the treatment time showed a double sigmoid curve after induced with the four elicitors: the first peak was at the 12th hour and the second peak occurred at different times after 3 days. When the synergistic effects between precursors and elicitors were studied, the PAL activity increased rapidly within 12 hours and then decreased, which was influenced most at 12 hour by L-phenylalanine. The effect of MJ on PAL activity was more

than L-phenylalanine's at the 3rd day.

4. The precursor and elicitors significantly increased the production of total polyphenol, proanthocyanidins and resveratrol in the suspension cultural cells

The contents of total polyphenol, proanthocyanidins and resveratrol in the cells increased with the increase of sucrose concentration. Total polyphenol, proanthocyanidins and resveratrol all had highest yields when sucrose content in medium at 40 g·l^{-1}, and the total polyphenol yield reached significant differences between treatments ($p < 0.05$). The production of proanthocyanidins and resveratrol had extremely significant differences between treatments ($p < 0.01$). The cell biomass and the contents of total polyphenol, proanthocyanidins and resveratrol were higher than those without CH (Casein hydrolysate), but the growth trends seemed similar and the time with the maximum yield appeared unchanged, when sucrose concentration was 30 g/L.

The production of total polyphenol, proanthocyanidins and resveratrol was increased when adding L-phenylalanine at 3-, 4- and 5-day-cell age, but the production was decreased at 1- and 2-day-cell age. Four elicitors had greatly positive influence on the contents of total polyphenol, proanthocyanidins and resveratrol, and the differences between the maximum yields of different treatments seemed significant ($p < 0.01$). MJ treatment induced resveratrol production was the lowest, while the fungal elicitor treatment had the highest yield among the four treatments.

Synergistic effect between the precursors and elicitors, the highest content of total polyphenol and resveratrol was achieved with 30 g/L sucrose, 1.2 mmol/L L-phenylalanine, 50 μmol/L MJ and 1.5 mL fungal elicitor, and the total polyphenol content was 3.5 times of the control with significant differences between treatments ($p < 0.01$); the highest content of proanthocyanidin was obtained with 50 g/L sucrose, 1.2 mmol/L L-phenylalanine, 30 μmol/L MJ and 0.5 ml fungal elicitor, and significant differences were also found between treatments ($p < 0.01$). Meanwhile, the factors affecting the content of total polyphenol were: sucrose> fungal elicitor> MJ > L-phenylalanine, ones affecting proanthocyanidin production were: MJ >sucrose> L-phenylalanine > fungal elicitor, and those affecting resveratrol production were: fungal elicitor> sucrose> MJ > L-phenylalanine. As a result, the conditions with the highest production of total polyphenol and proanthocyanidin were: 40 g/L sucrose, 0.2 mmol/L L-phenylalanine, 30 μmol/L MJ and 1.5 ml fungal elicitor. The conditions with the highest production of resveratrol were: 40 g/L sucrose, 0.7 mmol/L L-phenylalanine, 30 μmol/L MJ and 1.5 ml fungal elicitor.

The innovation of this paper is that obtained expression changes of *PAL*, *CHS* and *STS* genes and PAL activity, based on the suspension cultural cells by adding the precursors and elicitors; and significantly improved the proanthocyanidins and resveratrol production in the suspension cultural cells, Thus possessing broad application foreground in the natural food additives.

Key words Suspension culture Grape cell Precursor Elicitor Total polyphenol Proanthocyanidin Resveratrol

中国四个地区酿酒葡萄果实及其葡萄酒质量特征的研究

STUDY ON CHARACTERISTIC QUALITY OF GRAPE BERRIES AND WINES FROM FOUR WINE-GROWING REGIONS IN CHINA

2011

本论文以酿酒葡萄品种赤霞珠、梅鹿辄、蛇龙珠、品丽珠和霞多丽为试材,采用 HPLC 和 SPME-GC/MS 等方法对我国 4 个地区(宁夏玉泉营、山西乡宁、河北沙城和昌黎地区)、乡宁不同地形条件及年份间葡萄与葡萄酒的花色苷物质、非花色苷酚类物质、香气成分及抗氧化能力进行系统、深入的研究,阐明了 4 个地区葡萄与葡萄酒的品质特征。主要获得以下结论:

(1)4 个地区赤霞珠和梅鹿辄果皮及相应葡萄酒中花色苷的组成和含量有一定差异,分别检测到 22 和 23 种花色苷物质,相应葡萄酒中分别检测到 37 和 36 种花色苷物质,其中二甲花翠素葡萄糖苷含量最高。玉泉营地区赤霞珠和梅鹿辄果皮花色苷含量最高,沙城和昌黎地区含量较低;此外,玉泉营地区赤霞珠和梅鹿辄葡萄酒中 5 种基本花色苷及二甲花翠素葡萄糖苷含量均占花色苷总量的比例最大,其次是乡宁地区。葡萄酒中花色苷组成不同的物质含量均低于 23.9 mg ME/L,产地因素对赤霞珠葡萄酒花色苷的影响程度大于梅鹿辄葡萄酒。乡宁地区 3 种地形条件下的赤霞珠和品丽珠果皮花色苷组成基本相同,赤霞珠果皮花色苷总量随葡萄园海拔的上升而增加,但地形间的差异程度较地区间相对较小;坡地葡萄酒的花色苷总量高于谷地和平地。年份对乡宁地区赤霞珠和蛇龙珠果皮花色苷组分的影响较小,果皮花色苷含量受年份影响的程度低于产地和地形,葡萄酒陈酿后花色苷含量下降幅度因品种而异。

(2)在 4 个地区的赤霞珠和梅鹿辄果皮中均检测到 29 种非花色苷酚类物质,相应葡萄酒中分别检测到 25 和 27 种非花色苷酚类物质,地区间葡萄酒非花色苷酚类物质在组成和含量上的差异较其果皮明显。黄酮醇在葡萄果皮的 5 类非花色苷酚类物质中含量最高,所占比例也最大,其次是黄烷-3-醇。对葡萄酒中儿茶素和表儿茶素含量而言,玉泉营地区赤霞珠是乡宁地区的 10 倍,其他 2 个地区彼此相差不大;沙城地区梅鹿辄是乡宁地区的 2 倍,地区间的差异程度较赤霞珠低。乡宁地区 3 种地形条件下的赤霞珠和品丽珠果皮及相应葡萄酒的非花色苷酚类物质组成基本相同,表明地形对非花色苷酚类物质组成的影响程度要小于地区因素。坡地赤霞珠和品丽珠果皮黄烷-3-醇、黄酮醇和羟基苯甲酸含量均高于谷地和平地;儿茶素和表儿茶素是葡萄酒中含量最高的非花色苷酚类物质,其含量表现为坡地低于谷地和平地。年份对非花色苷酚类物质组成的影响较小,但对其含量有不同程度的影响。2009 年的陈酒与 2010 年的新酒相比,其黄酮醇含量彼此间互有高低,而黄烷-3-醇含量有所下降。

(3)在 4 个地区的赤霞珠和梅鹿辄果实中分别检测到 36 和 45 种香气成分,其葡萄酒中均检测出 52 种香气物质,其中酯类和醇类是主要成分,含量较高的香气物质是异戊醇、异丁醇、苯乙醇、丙醇、乙酸

乙酯和辛酸乙酯等。通过香气活性值的分析，沙城地区赤霞珠和乡宁地区梅鹿辄葡萄酒的主要香气特征物质是己酸乙酯，其他 3 个地区的葡萄酒主要的香气特征物质是辛酸乙酯；另外，己酸乙酯是谷地、高海拔坡地赤霞珠和 3 种地形条件的品丽珠葡萄酒主要的香气特征物质，低海拔坡地赤霞珠葡萄酒的特征香气物质是辛酸乙酯。2009 年赤霞珠、蛇龙珠和霞多丽果实中醇类占果实香气总量的比例最大，醇类和酯类是其葡萄酒的主要香气成分。

（4）玉泉营地区赤霞珠和梅鹿辄葡萄酒的酚类物质含量最高、抗氧化能力最强，其次是沙城地区，乡宁和昌黎地区较低。除花色苷外，谷地赤霞珠和平地品丽珠果实及相应葡萄酒的酚类物质含量较高、抗氧化能力较强，这种关系在其葡萄酒中更为明显；随葡萄园海拔的上升酚类物质含量有下降的趋势。2 个年份的研究表明谷地赤霞珠果实和相应葡萄酒的酚类物质含量最高，抗氧化能力也最强；霞多丽含量最低，抗氧化能力也最弱；坡地赤霞珠和蛇龙珠互有高低。

（5）酚类物质含量在葡萄果实和相应葡萄酒中呈对应关系；葡萄果实和相应葡萄酒中酚类物质的含量与其抗氧化能力存在正相关；除超氧阴离子清除法（SRSA）外，其它 2 种方法（DPPH 和 CUPRAC 法）存在较好的协同性。

关键词

Abstract

In this dissertation, Cabernet Sauvignon, Merlot, Cabernet Gernischet, Cabernet Franc and Chardonnay were selected to study the effect of regions, terrains, vintages to the anthocyanins, non-anthocyanin phenolic substances, aroma composition and antioxidant activities of the grapes and wines by HPLC and SPME-GC/MS. Elucidating the quality characteristic of grape berries and wines from regions of Yuquanying, Xiangning, Changli and Shacheng. The main conclusions as followed:

(1) The total 22 and 23 kinds of anthocyanins were detected from grape skin of Cabernet Sauvignon and Merlot respectively in four regions, from wines of Cabernet Sauvignon and Merlot, 37 and 36 kinds of anthocyanins were detected respectively in these regions, the malvidin-3-*O*-glucoside among anthocyanins detected had the highest amount. To Cabernet Sauvignon and Merlot wines, the highest contents of anthocyanins was found in Yuquanying region of Ningxia, their content in Shacheng and Changli regions of Hebei was lower; the ratioes of five essential anthocyanins/total anthocyanins and the malvidin-3-*O*-glucoside/ total anthocyanins in Cabernet Sauvignon and Merlot wines from Yuquanying region were higher, secondly was Xiangning region. Their contents with different composition in wines were lower than 23.9 mg ME/L, moreover, regional factor had less effect on the composition of anthocyanins of Cabernet Sauvignon wines than that of Merlot wines. The composition of anthocyanins in Cabernet Sauvignon and Cabernet Franc wines from three different terrain conditions was the same, the higher altitude vineyard had, the more content of anthocyanins Cabernet Sauvignon skin contained, the degree of discrepancy of anthocyanins which existed in different terrain conditions was less than that of different regions; the hillside vineyard had more contents of anthocyanins in wines than the flat and valley lands. There was little difference between two years grape skins about anthocyanins composition, and total contents of anthocyanins in skin was closely between two years, and with aging period of increasing, the composition of anthocyanins had no change, but total contents of anthocyanins was decreased obviously.

(2) The total 29 kinds of non-anthocyanins phenolic substances are detected both in Cabernet Sauvignon and Merlot grape skins coming from four regions, the corresponding wines contained 25 and 27 kinds of

non-anthocyanins phenolic substances detected, respectively, the discrepancy of the content and composition of non-anthocyanins phenolic substances in 4 regional wines was less than that of their grape skins. The contents of flavonols among five kinds of essential non-anthocyanins phenolic substances were the highest in grape skins, secondly was the flavan-3-ols content. The content of catechin and epicatechin in Cabernet Sauvignon wine from Yuquanying region was almost 10 times than that of Xiangning region, their content in other 2 regional wines was not significant difference; their content in Merlot wine from Shacheng region was 2 times than that of Xiangning wine. Those grape skins and wines contained the same compositions of non-anthocyanidin phenolic substances from 3 different terrain conditions. It appeared that the contents of flavan-3-ols, flavonols and hydroxybenzoic acid in grape skins of slope are higher than those of flat and valley lands. The contents of catechin and epicatechin among non-anthocyanins phenolic substances were the highest in different wines, and their contents in slope land were lower than those of valley and flat lands. Years that had less influence on the compositions of non-anthocyanins phenolic substances, but influenced those contents in different degree. Compared to the 2010 wines, the contents of flavan-3-ol in the 2009 wines was lower, but the contents of flavonol was irregular each other.

(3) It had been detected 36 and 45 aroma compounds in the two kinds of grape berries which made from Cabernet Sauvignon and Merlot respectively, their corresponding wines contained 52 aroma compounds. Among these compounds, the most high content were isoamyl alcohol, isobutyl alcohol, phenethyl alcohol, isobutyl alcohol, ethyl acetate, ethyl caprylate, etc. Based on the OAVs values of aroma compounds，ethyl hexanoate was able to exert a strong influence on wine aroma: it was responsible for a major part of the aroma characteristics substance of Yuquanying Cabernet Sauvignon wine and Xiangning Merlot wine, ethyl octanoate was the aroma characteristics substance of other regional wines. Compared to the other two terrain conditions. The alcohol had the greatest proportion in total content of aroma in Cabernet Sauvignon, Cabernet Gernischet and Chardonnay berries in 2009, of which the esters and alcohols were the main components of the wines' aroma.

(4) The contents of phenolic substances in Cabernet sauvignon and Merlot wines from Yu quanying region were the highest, secondly was Changli region; three antioxidant methods all indicated that the Cabernet sauvignon and Merlot wines from Yuquanying region had the highest antioxidant activity, followed by those of Shacheng of Hebei Province, while those of Xiangning and Changli regions had lower antioxidant activities. According to these results, except for the contents of anthocyanins, both the antioxidant activity and the contents of phenolic substances of grape berries and wines from sloping lands were lower than those of grape berries and wines from valley and flat lands. Besides, the contents of phenolic substances drop with the increase of cultural altitude. The phenolic contents of Cabernet Sauvignon berries from valley land and corresponding wine were the highest, followed by Cabernet Gernischet and Cabernet Sauvignon berries and wine from sloping land, Chardonnay is the lowest.

(5) The contents of phenolic substances between grape berries and wines exhibited corresponding relationship; the results showed that there was a positive correlation between phenolic content and antioxidant activity of grape berries and wines; except for SRSA method, a relatively tight coupling of the DPPH and CUPRAC assays.

Key words   Wine   Loess Plateau region   Phenolic compound   Antioxidant activity   Aroma