柠檬果实不同发育时期柠檬苦素及主要类黄酮分析

朱春华[1,2]，周先艳[1]，李进学[1]，杜玉霞[1]，董美超[1]，沈正松[1]，高俊燕[1]，潘思轶[2]，岳建强[1]
（1.云南省农业科学院热带亚热带经济作物研究所，云南保山 678600）
（2.华中农业大学食品科学技术学院，湖北武汉 430000）

摘要：瑞丽柠檬是国家地理标志产品，柠檬果实富含维生素、矿物质和类黄酮等物质，是一种营养和药用价值较高的水果。本文采用 HPLC 分析了云南湿热气候条件下栽培的云柠 1 号柠檬果实三个不同发育期具有降血脂、降血压和抗癌防癌功效的柠檬苦素和 9 种类黄酮物质，研究表明，柠檬苦素在果实发育过程中含量逐渐下降，但至花后 210 d 时，果实中柠檬苦素含量仍高出人体苦味阈值 6.3 倍；类黄酮中地奥司明含量最高，占类黄酮总含量的 83.26%~84.61%，其次是以橙皮苷和新橙皮苷为主的黄烷酮类，占类黄酮总含量的 14.18%~15.45%，含量最低的为多甲氧基黄酮，含量仅占类黄酮总含量的 0.008%~0.89%，未检测出橘黄酮。推测云南湿热环境下有利于地奥司明成分的累积，类黄酮的累积转化机理有待进一步研究。

关键词：柠檬；品质；类黄酮；功效；代谢
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Determination of Limonin and Flavonoids in the Lemon Fruit at Different Development Stages

ZHU Chun-hua[1,2], ZHOU Xian-yan[1], LI Jin-xue[1], DU Yu-xia[1], DONG Mei-chao[1], SHEN Zheng-song[1], GAO Jun-yan[1], PAN Si-yi[2], YUE Jian-qiang[1]
(1.Institute of Tropical and Subtropical Cash Crops, YAAS, Baoshan 678600, China)(2.College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430000, China)

Abstract: Ruili lemon is China geographical indication product with high nutritive and medicinal value, which is rich in vitamins, minerals, flavonoids and other nutritional substances. High-performance liquid chromatography was applied to determine Limonin and 9 flavonoids in lemon fruit widely cultivated in hot and humid climates of Yunnan Province. Limonin and Flavonoids exhibited a wide range of biological effects, including hypolipidemic effect, lowering blood pressure and anti-cancer. The results showed that there were significant differences in limonin and flavonoids at different Development stages.The content of limonin decreased gradually during the development stoags, but the content of limonin in the fruit was still higher than the threshold of human bitterness by 6.3 times in 210 days after the flowers. Diosmin in flavonoids was the highest among all maturity stages, which accounted for 83.26% ~ 84.61% of total flavonoids, followed by hesperidin and neo-hesperidin as the main flavanone accounting for 14.18%~15.45% of the total flavonoids. PMFs were detected at lowest levels in lemon fruit, which was only 0.008% ~ 0.89% of total flavonoids, and no tangeretin were detected. It is deduced that Yunnan hot and humid environment is conducive to the accumulation of diosmin components, but the accumulation and transformation mechanism of flavonoids needs further study.

Key words: lemons; quality; flavonoids; function; metabolism

柠檬是芸香科柑橘属的常绿果树，在世界柑橘产业中，柠檬产量次于橙和宽皮橘，位居第三位。柠檬在大航海时代就用于治疗坏血症，不仅能够补充人体必需的维生素 C，而且具有美容养颜、清除自由基、降尿酸、降血脂、降血压和防癌抗癌等功效[1~5]。柠檬果实中具有特殊功效的成分主要有圣草枸橼苷、柚皮苷、多甲氧基黄酮、D-柠檬烯、橙皮苷、地奥司明、香豆素及单萜物质橙皮油素类、柠檬醛和柠檬苦素类等物质[6]。

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作者简介：朱春华（1985-），女，副研究员，在读博士，主要从事柑橘功效及产品加工研究
通讯作者：岳建强（1967-），男，研究员，硕士，主要从事柑橘育种与栽培研究

柠檬苦素类化合物是引起芸香科果实“后苦”的主要原因[7,8]，存在于果实的不同部位[9]，具有抗肿瘤[10]及白血病细胞[11]等功效。橙皮苷、地奥司明可抑制肿瘤细胞增殖，引起细胞凋亡[12,13]、具有降血压、降血脂等功效[14~16]、国外对柠檬、莱檬果实中黄酮物质含量及功效研究有较多报道[2,13,17,18]，黄酮含量与品种[19,20]、果实不同部位[21]、成熟度、生长环境等因素有关[22~24]。于玉涵分析了四川安岳柠檬果实中 13 种类黄酮含量[25]，Wanpeng Xi 报道了重庆 5 个柠檬品种不同部位的主要酚类物质及其抗氧化能力[26]，王红静建立了尿酸模型，验证了云南柠檬具有降尿酸的功效[27]，云南德宏和四川安岳是国内柠檬主产区，瑞丽柠檬是国家地理标志产品，柠檬鲜果较国内市场提前 1~2 月上市，作者曾分析了德宏地区柠檬不同发育时期基本营养品质的变化[28~30]，但对云南柠檬果实中柠檬苦素、圣草枸橼苷、橙皮苷和地奥司明等具有特殊功效的功能成分未见报道。本文分析瑞丽三个不同生长发育时期的柠檬果实中柠檬苦素及主要类黄酮物质含量，可为柠檬深加工和健康饮食指导提供理论依据。

1 材料、方法

1.1 试验品种、气候条件

本文选择云南省德宏州瑞丽市勐秀乡勐典栽培的尤力克柠檬为研究对象，砧木为枳壳砧。种植地海拔 950 mm，平均气温 18.3~20 ℃，最高气温 38.8 ℃，最低气温-2.1 ℃，1 月份平均气温 10.9 ℃~12.5 ℃，年均日照 2218~2453 h，年均降雨量 1400~1800 mm，年积温 6400~7300 ℃。样品分析在中国农业科学院柑桔研究所农业部柑桔及苗木质量监督检验测试中心完成。

1.2 试验方法

1.2.1 标准品溶液的制备

准确称取圣草枸橼苷、芦丁、柚皮苷、橙皮苷、新橙皮苷、地奥司明、甜橙黄酮、川皮苷、橘黄酮和柠檬苦素各 5.00 mg，分别用色谱纯甲醇溶解并定容至 10.00 mL 容量瓶中，配成 500 mg/L 标准品的母液备用。采用逐级稀释法用色谱纯甲醇溶液将标准品溶液配制成一系列质量浓度的混合标准品溶液。

1.2.2 样品的制备

柠檬汁样品：新鲜柠檬洗净，采用手动压榨法制取新鲜柠檬汁，用双层纱布过滤后备用。准确吸取制好果汁样品 2.00 mL 置于 50 mL 离心管中，加入 10.00 mL 甲醇振荡 1 min，以 10000 r/min 离心 10 min，分离上清液，残渣以 10 mL 提取剂重复提取一次，合并上清液定容至 25 mL，过 0.22 μm 微孔滤膜后待测。

1.2.3 色谱条件

色谱柱：ACQUITY UPLC BEH C1 8 分析柱(2.1 mm×100 mm，1.7 μm)；流动相：甲醇和 0.2%乙酸溶液，采用梯度洗脱（见表 1）；柱温：35 ℃；流速：0.3 mL/min；定量波长为 283 nm 和 330 nm，波长扫描范围 200~400 nm，进样量为 3.0 μL。以保留时间结合光谱扫描图定性，采用外标法定量。

表 1 流动相及流速

Table 1 Mobile phase and flow rate

时间/min	流速/(mL/min)	0.2%乙酸水/%	甲醇/%	曲线
	0.30	90	10	-
5.00	0.30	70	10	6
10.00	0.30	20	90	6
15.00	0.30	20	90	6
16.00	0.30	90	10	1

1.3 数据分析

数据分析采用 SPASS 20.0，图形制作采用 origin 8.0 完成。

2 结果与分析

2.1 柠檬主要黄酮物质色谱图及含量

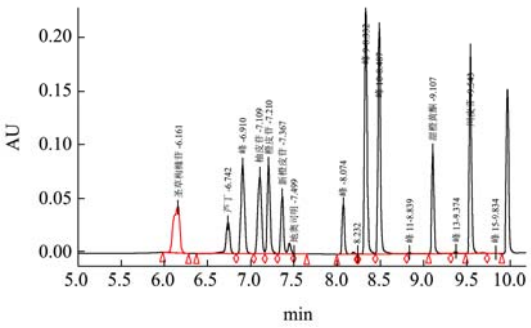

图 1 类黄酮标准色谱图

Fig.1 HPLC chromatogram of 9 flavonoids standards

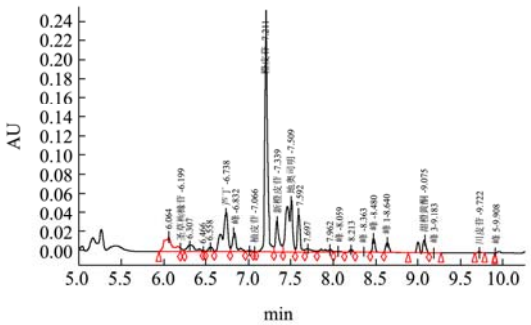

图 2 花后 60 d 柠檬果实类黄酮色谱图

Fig.2 HPLC chromatogram of flavonoids in lemon fruit in 60 days

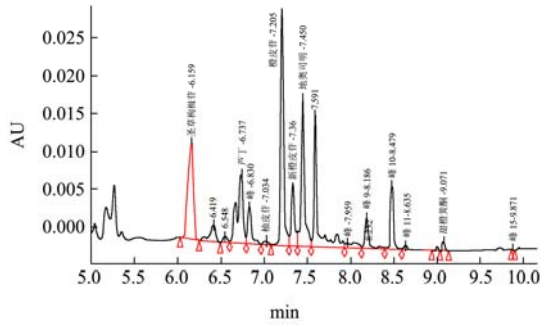

图 3 花后 150 d 柠檬果实类黄酮色谱图

Fig.3 HPLC chromatograms of flavonoids in 150 days

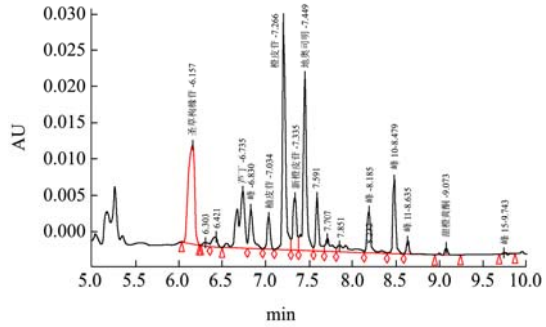

图 4 花后 210 d 柠檬果实类黄酮色谱图

Fig.4 HPLC chromatogram of flavonoids in lemon fruit in 210 days

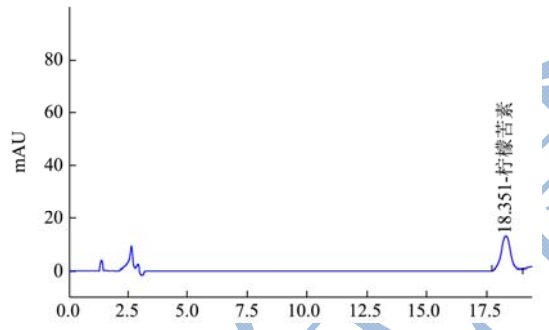

图 5 柠檬苦素标准色谱图

Fig.5 HPLC chromatogram of limonin standard

柠檬果实中类黄酮混合标准品的 HPLC 色谱图见图 1。混合标准品中各类黄酮成分目标峰形尖锐，对称性好。

柠檬果实中主要类黄酮成分含量结果见表 2，由表中可见，不同发育时期柠檬果实中类黄酮种类和含量差异显著。随着柠檬果实的成熟，类黄酮含量总体呈下降趋势，花后 60 d 总含量为 83.15 mg/g，花后 210 d 下降至 20.94 mg/g。柠檬果实中地奥司明含量最高，含量占总类黄酮含量的 83.26%~84.61%、其次为黄烷酮类，占 14.18%~15.45%，含量最低的为多甲氧基黄酮，含量仅为 0.01%~0.89%。4 种黄烷酮在不同发育时期均达到极显著水平（p<0.01），橙皮苷、新橙皮苷随着果实的成熟，含量呈下降趋势，从花后 60 d 至花后 210 d，分别下降了 85.74%和 76.11%；圣草枸橼苷、柚皮苷则与之相反，随着成熟度的增加，其含量增多，圣草枸橼苷从 0.02 mg/g 增加至 0.98 mg/g；柚皮苷在花后 60 d 未检出，花后 210 d 含量为 0.01 mg/g。

在柑橘中，柚皮素及产物在 7-O-葡萄糖苷转移酶的作用下先形成 7-O-葡萄糖苷类，经 1,2-鼠李糖基转移酶催化形成带苦味的柚皮苷、新橙皮苷、新圣草次苷和枳属苷，然后在 1,6-鼠李糖基转移酶的作用下分别形成无苦味的柚皮芸香苷、橙皮苷、圣草次苷和香风草苷[31,32]。本实验中，新橙皮苷在花后 150 d、210 d 分别下降了 75.6%、76.14%，圣草枸橼苷在花后 150 d、210 d 分别增加了 61.1%、64.2%，推测可能是新橙皮苷转化为圣草枸橼苷。

芦丁在不同果实发育时期均达到了极显著水平（p<0.01），含量随着果实的成熟逐渐降低，花后 60 d 含量为 1.31 mg/g，花后 210 d 下降至 0.25 mg/g，降幅为 81.02%。地奥司明是柠檬果实中含量最高的黄酮类物质，花后 60 d 含量为 69.69 mg/g，占总类黄酮含量的 83.81%，花后 150 d 降至 14.91 mg/g，但仍是同一时期含量最高的物质，发育至花后 210 d，含量为 17.72 mg/g，占总类黄酮含量的 84.61%。柠檬果实中未检测出橘黄酮，甜橙黄酮和川皮苷均呈现先升后降的趋势，花后 150 d 均达到最高值，二者均为 0.01 mg/g。甜橙黄酮含量在不同时期达到了极显著水平（p<0.01），花后 60 d 未检出，花后 150 d 增至 0.01 mg/g，花后 210 d 降低至 0.001 mg/g，川皮苷未达到显著水平。

本文中除了圣草枸橼苷、柚皮苷在果实发育过程中含量呈上升趋势，柠檬苦素、地奥司明和橙皮苷等物质伴随果实的发育和成熟其含量总体呈现下降的趋势，这与 Kim 等人研究结果相似[33,34]，J Chen 等也表明 4 种甜橙中尽管黄烷酮在果实的绿熟期和转色期其积累量有少许波动，但黄烷酮糖苷类在不同种质果实的不同组织中都呈现总体下降的趋势[12]，说明黄酮类在柑橘果实中的代谢存在明显的规律性变化，但柠檬果实中黄酮物质具体代谢途径有待进一步研究。

J Chen 表明，橘、甜橙和柠檬中无苦味的芸香糖苷类尤其是橙皮苷异常丰富[35]，Delourdesmatabilbao 指出芦丁是柑橘中含量最为丰富的黄酮醇糖苷，且以柠檬中的含量居高[36]，Escriche 等也指出黄酮醇则主要存在于柠檬中[37]，于玉涵表明四川尤力克柠檬果实中含量最高的类黄酮物质为橙皮苷和圣草枸橼苷[16]，Wanpeng Xi 也报道了四川柠檬果实中橙皮苷是主要的黄酮类物质[17]，Vincenzo Sicari 指出意大利卡拉布里亚区柠檬果实中新橙皮苷含量最高、柚皮苷次之[16]。本文检测结果表明，在整个柠檬果实发育过程中，地奥司明含量最高，其次为橙皮苷和新橙皮苷为主的

黄烷酮类，含量最低的为多甲氧基黄酮，但未检出橘黄酮，芦丁为代表的黄酮醇类物质含量在柠檬果实中并不高，该结果与现有文献的差异可能由于地理气候、土壤条件有关，推测云南德宏气候条件下更有利于地奥司明成分积累，具体原因有待进一步研究。

表 2 柠檬果实中主要功能成分含量

Table 2 The content of main functional components in lemon fruit

序号	分类	检测指标/(mg/g)	花后 60 d	花后 150 d	花后 210 d
1	黄烷酮类	圣草枸橼苷	0.02±0.01^{aA}	0.93±0.01 ^{bB}	0.98±0.01^{cC}
2		柚皮苷	0.00±0.00^{aA}	0.01±0.00^{bB}	0.01±0.00^{bB}
3		橙皮苷	9.58±0.01^{aA}	1.20±0.00^{bB}	1.37±0.02^{cC}
4		新橙皮苷	2.55±0.01^{aA}	0.62±0.01^{bB}	0.61±0.00^{cC}
5	黄酮醇类	芦丁	1.31±0.01 ^{aA}	0.22±0.01^{bB}	0.25±0.01 ^{cC}
6	黄酮类：糖苷或配糖体	地奥司明	69.69±0.02^{aA}	14.91±0.00^{bB}	17.72±0.00^{cC}
7	多甲氧基黄酮	甜橙黄酮	0.00±0.00^{aA}	0.01±0.00^{bB}	0.01±0.00^{cC}
8		川皮苷	0.01±0.00^{aA}	0.01±0.00^{aA}	0.01±0.00^{aA}
9		橘黄酮	-	-	-
		合计	83.15	17.91	20.94

2.2 柠檬苦素色谱图及含量分析

三个不同时期的柠檬果实中均检测到柠檬苦素，含量随着果实的成熟而逐渐降低，如图 5 所示。柠檬苦素在柠檬果实中含量见图 9，柠檬苦素在果实发育过程中呈下降趋势，含量差异达到显著水平（$p<0.05$），花后 60 d 含量为 0.11 mg/g，花后 150 d 下降至 0.05 mg/g，花后 210 d 含量最低，仅为 0.04 mg/g。

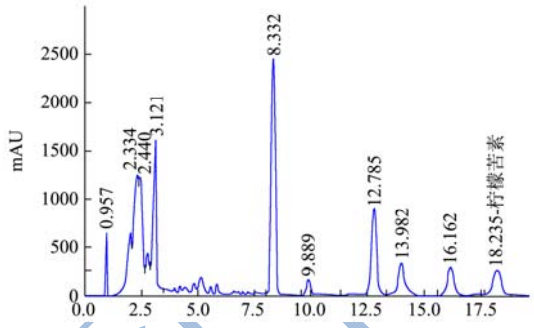

图 6 花后 60d 柠檬果实柠檬苦素色谱图

Fig.6 HPLC chromatogram of limonin in lemon fruit in 60 days

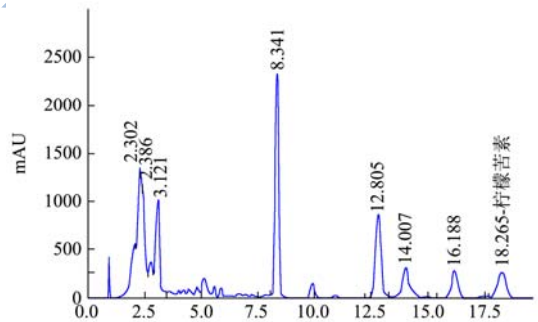

图 7 花后 150d 柠檬果实柠檬苦素色谱图

Fig.7 HPLC chromatogram of limonin in lemon fruit in 150 days

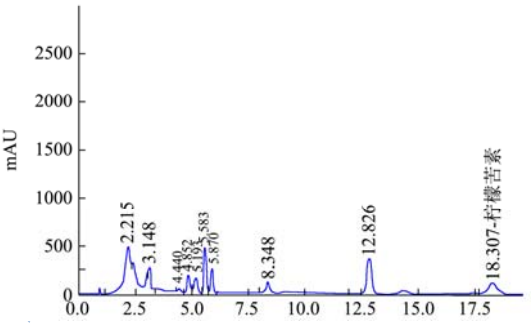

图 8 花后 210d 柠檬果实柠檬苦素色谱图

Fig.8 HPLC chromatogram of flavonoids in lemon fruit in 210 days

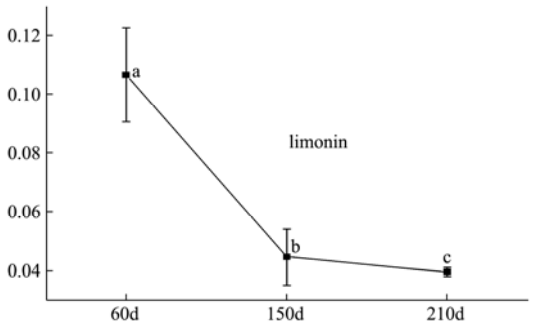

图 9 不同时期柠檬苦素含量变化图

Fig.9 Limonin content in lemon fruit at different mature stages

根据人口感柠檬苦素阈值在 6 mg/L 以下，花后 210 d 时仍有较明显的苦味。今后应完善和深入其在代谢及调控方面的研究，为高效提升柠檬果实特殊功效成分调控提供便捷之径，并为果实品质的提升提供理论依据。

3 结论

本文研究表明，在柠檬果实发育过程中，类黄酮

含量总体呈下降趋势，花后 60 d 总含量为 83.15 mg/g，花后 210 d 下降至 20.94 mg/g。柠檬果实中含有大量类黄酮，尤其是地奥司明和橙皮苷，地奥司明含量占总类黄酮含量的 83.26%~84.61%，其次为黄烷酮类（橙皮苷和新橙皮苷为主），占 14.18%~15.45%，含量最低的为多甲氧基黄酮，含量仅为 0.01%~0.89%，未检测出橘黄酮。除了川皮苷，其余物质均达到极显著水平（p<0.01）。花后 210 d 时，柠檬苦素含量仍为 0.04 mg/g，有较明显的苦味。

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