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# 枇杷果实品质形成过程中差异表达蛋白的变化

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**摘 要:【目的】**研究枇杷果实成熟过程中不同发育阶段(青果期、转色期和成熟期)的生理代谢变化及果实品质形成机制。**【方法】**以‘解放钟’枇杷品种为试验材料,采用蛋白质双向凝胶电泳及MALDI-TOF/TOF-MS技术分离并鉴定蛋白质,采用RT-qPCR技术分析基因表达水平变化,同时应用生理生化技术分析生理代谢变化。**【结果】**成功鉴定出55个差异表达蛋白,这些蛋白主要参与糖代谢(16%)、三羧酸循环与能量代谢(9%)、抗坏血酸-谷胱甘肽循环(14%)、类胡萝卜素合成及乙烯合成(11%)、蛋白质合成(13%)、细胞结构(8%)、防御与胁迫(9%)以及其它调控(13%)等生物学过程。随着果实成熟,参与糖代谢、三羧酸循环与能量代谢以及类胡萝卜素合成及乙烯合成过程的相关蛋白均上调表达;RT-qPCR的结果与蛋白质丰度变化趋势相一致;同时,糖类物质、类胡萝卜素以及维生素C等的含量不断积累,有机酸含量不断减少。**【结论】**在蛋白质丰度、生理生化水平以及mRNA水平上的结果共同表明糖代谢、三羧酸循环及能量代谢、类胡萝卜素合成以及乙烯合成等代谢途径在枇杷果实品质形成过程中起着重要作用。这些结果为枇杷果实品质改良提供重要的理论依据,并为今后枇杷新品种的培育奠定分子生物学基础。

**关键词:**枇杷;发育时期;蛋白质组;品质形成

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## Study on differentially expressed proteins during the fruit quality formation in loquat (*Eriobotrya japonica*)

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**Abstract:**【Objective】Loquat [*Eriobotrya japonica* (Thunb.) Lindl.] is a species of *Eriobotrya* plants belonging to Maloideae in family Rosaceae, which is an important fruit in Asia that ripens in late spring and early summer. Its fruit contains rich nutrients such as carotenoids, vitamins, minerals and amygdalin in flesh. It also has some health-beneficial effects, for example, expelling phlegm, arresting coughing, moistening lung, promoting appetite and helping digestion. Different regulation metabolisms in 3 different development periods (green fruit period, color changing period and yellow ripening period) were revealed during the fruit quality formation in loquat.【Methods】‘Jiefangzhong’ loquat trees were planted in the National Germplasm Resources Nursery for Loquat in Fruit Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou, China. The fruits were harvested during 3 different fruit development periods from 7-year-old loquat trees. Total 27 fruits at each period were randomly divided into three groups, with each group having 9 fruits. The fruits were peeled and the pulps were immediately frozen in liquid nitrogen, and stored at −80 ℃ for further analysis. The proteins of loquat pulp were ex-

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tracted using the Tris-buffered phenol method. The total proteins were separated by using two-dimensional gel electrophoresis (2-DE) with 24 cm, pH 4-7 linear immobilized pH gradient (IPG) strip in the first dimension, and after isoeletric focusing (IEF), with 12% polyacrylamide gels in the second dimension (namely SDS-PAGE). Differentially expressed protein spots with more than 2-fold differences in abundance at the three fruit development periods were found from the 2-DE gels by using the ImageMaster software. These differentially expressed proteins were identified using the matrix-assisted laser desorption/ionization tandem time-of-flight mass spectroscopy (MALDI-TOF/TOF-MS) and protein database search. The nutrient contents, including the total soluble sugar, reducing sugar, sucrose, carotenoid and ascorbic acid (AsA), were measured by plant biochemistry methods at three fruit development periods. Meanwhile, the total RNA was extracted from the fruit pulp using the CTAB-LiCl method. The relative mRNA expression was analyzed using the RT-qPCR method in a CFX Connect™ (Bio-Rad) platform. The relative mRNA expression level of each gene was quantified using the $2^{-\Delta\Delta Ct}$ method. 【Results】To identify the proteins involved in the fruit development periods, 2-DE analysis was performed in 3 different development periods, in which more than 1 200 protein spots were detected for each period by using Image Master 2D Platinum 5.0, respectively. Statistical analysis ($p < 0.05$) revealed a total of 56 protein spots with at least 2-fold differences in abundance as differentially expressed proteins in the green fruit period, color changing period, and yellow ripening period. Among them, 36 proteins were found to be up-regulated, 12 were down-regulated and the rest were up-regulated or down-regulated at the color changing period and yellow ripening period. These differentially expressed protein spots were excised from the gels, digested, and then analyzed by matching MALDI-TOF–TOF/MS data to determine the biochemical characteristics of proteins. Fifty-five proteins were successfully identified, which were divided into 9 classes: sugar metabolism (16%), tricarboxylic acid (TCA) cycle and energy metabolism (9%), AsA-GSH cycle (14%), carotenoid biosynthesis and ethylene formation (11%), amino acid and protein synthesis (13%), cell structure (8%), defense stress (9%), other regulatory proteins (13%) and so on, according to their physiological function. The proteins from sugar metabolism, TCA cycle energy metabolism and carotenoid biosynthesis and ethylene formation were up-regulated, while the ones in the AsA-GSH cycle pathway were down-regulated. Visual and biochemical analysis in the fruit development of loquat revealed that the contents of total soluble sugar, reducing sugar, sucrose, carotenoid, and AsA increased with the fruit development, while the organic acid content decreased. To ascertain whether the protein expression levels were correlated with their mRNA contents, several genes encoding proteins related to carotenoids synthesis and ethylene formation metabolism (namely phytoene synthase (*PSY*), chromoplaste beta lycopene cyclase (*CYCB*), lycopene beta cyclase (*LCYb*), lycopene epsilon cyclase (*LCYe*), beta carotene ring hydroxylase (*BCH*), zeaxanthin cyclase (*ZEP*), 1-aminocyclopropane-1-carboxylate oxidase (*ACO*)) were examined through quantitative RT-PCR analyses. The result of RT-qPCR was similar to protein data. Compared with green fruit period, the relative mRNA expression levels of *PSY*, *BCH*, *ZEP*, *LCYb*, *CYCB*, and *ACO* were remarkably up-regulated in both color changing period and yellow ripening period. 【Conclusion】The proteomic analysis of the loquat fruits at different development periods was performed in the present study. Fifty-five differential expressed protein spots were identified by MALDI-TOF/TOF-MS. The consistent results in the physiological analysis and relative mRNA expression level with the protein data reinforced each other to demonstrate an important scientific basis during the quality formation of loquat fruits. The quality formation of loquat fruits was closely related to these pathways including sugar metabolism, TCA cycle energy metabolism, carotenoid biosynthesis and ethylene formation.

**Key words:** Loquat; Development period; Proteomics; Quality formation

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果实的成熟过程是包括涩酸味的消失、甜味的增加、香味的产生以及色泽的变化等一系列复杂生理生化反应[1]。有研究发现，在苹果、桃子、杨梅、荔枝、甜瓜、柑橘、草莓等果实中甜味的增加与蔗糖等糖类代谢关系密切[2-8]。在果实细胞中，有机酸主要在线粒体中通过三羧酸循环（TCA）形成，但线粒体中只含有极少的有机酸，大部分有机酸储存在液泡中。不同果实中有机酸的形成受到不同代谢酶的调控，如在苹果酸型的杏果实中，苹果酸质量分数与苹果酸脱氢酶活性呈正相关[9]，而在柠檬酸型的脐橙柑橘果实中，柠檬酸积累与磷酸烯醇式丙酮酸羧化酶和柠檬酸合成酶活性呈显著正相关[10]。一般情况下，在果实接近成熟时类胡萝卜素等果实色素开始累积，使果实色泽变得鲜艳。有研究发现类胡萝卜素含量与糖含量的变化呈现出一定的相关性，在番茄果实中，番茄红素和β-胡萝卜素与果糖及葡萄糖的积累表现出显著的正相关性，而与蔗糖的积累变化呈负相关性[11]。因此，果实品质的形成与糖类、有机酸以及类胡萝卜素等果实色素代谢密切相关，这些代谢途径的调控对果实品质的形成至关重要。

枇杷[*Eriobotrya japonica* (Thunb.) Lindl.]属于非呼吸跃变型果实。根据果实成熟过程中果皮颜色的变化，枇杷果实成熟过程可分为青果期、转色期和成熟期三个发育阶段。目前，国内外对枇杷的研究主要集中在果实成熟过程、采后以及逆境胁迫下的生理生化和差异蛋白特性[12-13]；类胡萝卜素生物合成基因的表达分析以及积累分子机制研究[14-15]；在高温强光胁迫下枇杷果皮的差异表达蛋白分析[16-18]。然而，对果实成熟过程中不同发育阶段的枇杷果实品质形成机制的研究较少，尤其是对枇杷果肉中的蛋白丰度变化的研究还未见报道。笔者以著名的枇杷品种——‘解放钟’枇杷（*Eriobotrya japonica* Lindl. cv. Jiefangzhong）为试验材料，应用蛋白质双向凝胶电泳（2-DE）及质谱分析技术研究枇杷果实品质形成过程差异蛋白的变化，从蛋白质水平上揭示枇杷果实品质形成过程的分子生理机制。

## 1　材料和方法

### 1.1　试验材料

供试材料采自福建省农业科学院果树研究所国家果树种质福州龙眼枇杷圃中青果期、转色期和成熟期3个不同发育阶段的‘解放钟’枇杷果实（图1）。每个时期均选择大小色泽一致，且无病虫害的健康果实进行试验，随机选取27个果实削除果皮去核后取果肉（3次生物学重复，每组重复9个果实），液氮速冻，于−80 ℃冰箱储存备用。

**图1　三个不同发育阶段的枇杷果实外观图（A）和横向剖面图（B）**

**Fig. 1 The appearance (A) and transverse cross section (B) of loquat fruit in three different developmental period**

### 1.2　枇杷果肉蛋白质的提取与2-DE

枇杷果肉蛋白质的提取使用Tris-饱和酚抽提方法[19]。蛋白干粉溶解于蛋白裂解液[7 mol·L$^{-1}$尿素、2 mol·L$^{-1}$硫脲、40 mmol·L$^{-1}$二硫苏糖醇、40 g·L$^{-1}$ CHAPS、0.5%（V/V）IPG buffer pH 4~7]，应用Bradford[20]的方法测定蛋白质含量。将含有1.3 mg蛋白质的溶液上样到IPG胶条（24 cm，pH 4~7）；室温下水化处理12 h后，第一向等电聚焦（IEF）在Amersham Ettan IPGphor Ⅲ系统（GE Healthcare Amersham Biosciences）上运行，其运行参数设置为：30 V（12 h）、200 V（1 h）、500 V（1 h）、1 000 V（1 h）、1 000~8 000 VGradient（30 min）、8 000 V（6 h）、1 000 V（2 h），运行温度20 ℃，最大电流50 μA·Strip$^{-1}$；IEF完成后，参考You等[21]的方法对IPG胶条平衡2次，每次平衡时间15 min。经平衡后的IPG胶条转至12% (*m*/*V*) SDS－PAGE凝胶上，在EttanTM DALT Ⅱ垂直板电泳系统（GE Healthcare Amersham Biosciences）进行第二向电泳，先在15 mA·Strip$^{-1}$下电泳30 min后增大电流至30 mA·Strip$^{-1}$，待溴酚

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蓝迁移至胶底0.5 cm处结束电泳。电泳结束后，应用考马斯亮蓝R-250对SDS-PAGE凝胶进行染色。染色结束后应用脱色液[含有40% (V/V)甲醇和10% (V/V)乙酸]对凝胶进行脱色处理，直至背景清晰可见。然后，应用EPSON PERFECTION 2480 PHOTO仪器扫描脱色后的凝胶，同时应用Image-Master 2D platinum 5.0软件分析凝胶图像。

### 1.3 蛋白质谱鉴定

差异蛋白点（蛋白丰度差异2倍及以上，而且$p < 0.05$）从凝胶上切割下来，参考You等[21]的方法，应用12.5 mg·L$^{-1}$ trypsin在37 ℃下孵育12 h进行酶解。酶解后的蛋白质在API 5800串联飞行时间质谱仪（MALDI-TOF/TOF）上进行质谱分离，然后应用GPS Explore（ABI）分析软件和MASCOT（Matrix-Science，London，UK）搜索软件进行蛋白点的鉴定。当蛋白的Protein score大于77时，就说明这个蛋白被成功鉴定。

### 1.4 枇杷果实品质形成过程中生理生化指标的测定

枇杷果肉中可溶性糖含量的测定采用蒽酮-硫酸比色法[22]。还原糖含量的测定应用3,5-二硝基水杨酸比色法[22]。蔗糖含量测定参照李利军等[23]的紫外分光光法。有机酸含量参照陈建勋等[24]的滴定法测定。总类胡萝卜素含量测定参考高慧颖等[25]的方法，分别在663 nm、646 nm和470 nm处测定吸光值计算总类胡萝卜素含量。维生素C（抗坏血酸）含量的测定采用2,6-二氯酚靛酚滴定法[22]。

### 1.5 枇杷果实品质形成过程中相关基因的转录水平分析

应用CTAB-LiCl沉淀法提取枇杷果实总RNA[26]。按照PrimeScriptTM RT Reagent Kit产品使用说明书（TaKaRa，东京，日本）进行cDNA合成。参照枇杷品质形成过程中相关基因的荧光定量PCR（RT-qPCR）分析根据试剂盒TaKaRa SYBR Premix Ex TaqTM（Tli RNaseH Plus）的使用说明书，在Bio-Rad CFX96 Real-Time PCR仪进行操作，以β-actin为内参基因。荧光定量分析所需的引物根据NCBI中枇杷属的mRNA序列设计（表1）。

**表1 RT-qPCR所用引物**

**Table 1 The primers used for RT-qPCR**

| 基因<br>Gene | 引物<br>Primer | 引物序列5’—3’<br>Sequence 5’—3’ | 编号<br>Gene No. |
|---|---|---|---|
| 八氢番茄红素合成酶<br>Phytoene synthase (*PSY*) | *PSY-S*<br>*PSY-A* | ACAGATGAGCTAGTGGATGG<br>CATTCCTTCTACCATGTCT | JN004210 |
| 有色体特异β番茄红素环化酶<br>Chromoplaste beta lycopene cyclase (*CYCB*) | *CYCB-S*<br>*CYCB-A* | AACCATGGATATCAGATTGCTC<br>GAATTACTAGTGCGCAAATAAGG | JN004215 |
| 番茄红素β环化酶<br>Lycopene beta cyclase (*LCYb*) | *LCYb-S*<br>*LCYb-A* | CAAACGGTGTTAAATTTCACCA<br>CCACTTGGTAACCTGGATTGTA | JN004214 |
| 番茄红素ε环化酶<br>Lycopene epsilon cyclase (*LCYe*) | *LCYe-S*<br>*LCYe-A* | ACTAGATTGTTCTTTGAGGA<br>CAACCGGAATCCAAGACCA | JN004216 |
| 胡萝卜素β环羟化酶<br>Beta carotene ring hydroxylase (*BCH*) | *BCH-S*<br>*BCH-A* | GAGAAGGTCCGTTCGAGCT<br>AGGCCATCATGGACAAACAT | JN004217 |
| 玉米黄质环化酶<br>Zeaxanthin cyclase (*ZEP*) | *ZEP-S*<br>*ZEP-A* | TACACTGGTATCGCAGATTT<br>TGAAACGCATACCACTGCAT | JN004219 |
| 1-氨基环丙烷-1-羧酸氧化酶<br>1- aminocyclopropane -1- carboxylate oxidase (*ACO*) | *ACO-S*<br>*ACO-A* | GGTGATTGCCCAAACAGATG<br>ACTGGACCCAAACCAAAACTG | GQ377219 |
| 内参基因<br>Reference gene | *β-actin-S*<br>*β−actin−A* | ATCCTTCGTCTGGACCTTGC<br>TTCCCGTTCAGCAGTAGTGG | JN004223 |

## 2 结果与分析

### 2.1 枇杷果实品质形成过程中差异蛋白变化

2.1.1 *蛋白表达图谱分析* 为确保试验的准确性，对果实成熟过程中3个不同发育阶段（即青果期、转色期和成熟期）的枇杷果肉蛋白质的2-DE分离都进行3次。将所得到的蛋白分离凝胶图谱应用Image-Master软件进行分析，结果显示每个样品的图谱均检测到1 200个以上的蛋白点，检测出56个蛋白丰度变化在两倍以上的差异蛋白点（图2）。

2.1.2 *差异蛋白质的质谱鉴定结果及功能分析* 与青果期相比，在转色期和成熟期的枇杷果肉有36个蛋白的丰度均呈上调趋势，12个蛋白丰度下调，还有8个蛋白的丰度呈现复杂变化（表2和图3）。其

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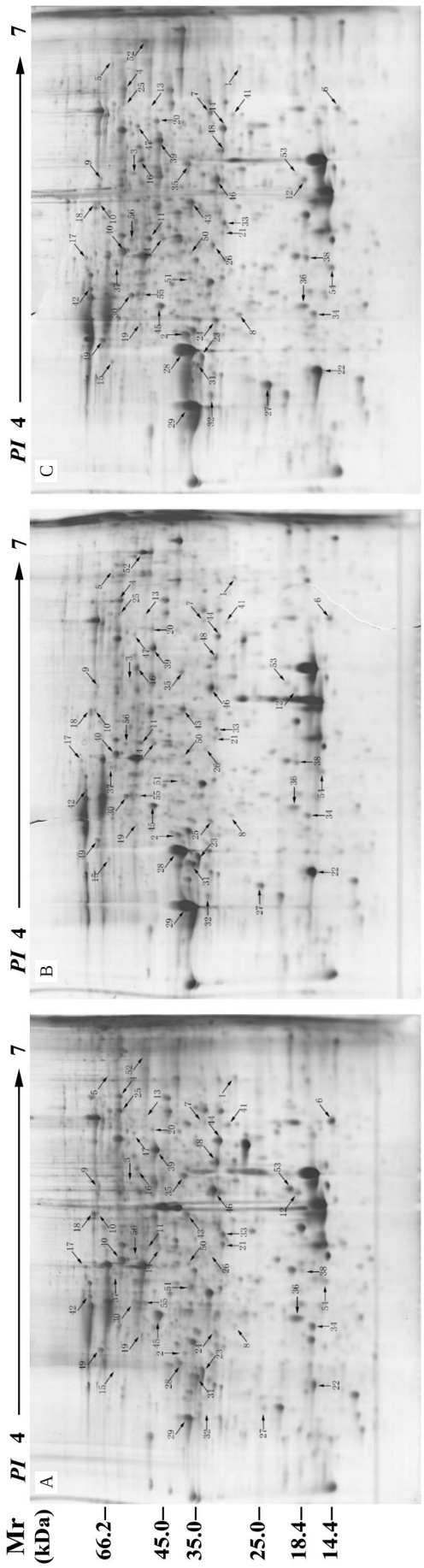


A. 青果期；B. 转色期；C. 成熟期
A. Green fruit period; B. Color changing period; C. Yellow ripening period

**图 2　枇杷果实品质形成过程中果肉蛋白质双向电泳**

**Fig. 2　Two-dimensional electrophoresis gel images of pulp proteins during the fruit quality formation of loquat**

中，按照蛋白得分大于77且$p < 0.05$的标准，有55个蛋白经MALDI-TOF/TOF-MS及数据库搜索后被成功鉴定，鉴定成功率达到98.1%。

对成功鉴定的55个差异表达蛋白进行功能注释，这些差异蛋白可分为9大类（表2），分别为糖类代谢（9个）、三羧酸循环与能量代谢（5个）、抗坏血酸-谷胱甘肽循环（AsA-GSH，8个）、类胡萝卜素合成及乙烯合成（6个）、蛋白质合成（7个）、细胞结构（4个）、防御与胁迫（5个）以及其他调控过程（7个）。在糖代谢途径中，参与淀粉降解和蔗糖形成有关酶的蛋白丰度上调，参与三羧酸循环的有关酶的蛋白丰度也上调，涉及类胡萝卜素形成的蛋白以及乙烯合成及防御胁迫抗病性的蛋白丰度都是上调的；而参与AsA-GSH循环相关蛋白的丰度下调，参与蛋白质合成的相关蛋白丰度变化较为复杂。

### 2.2　枇杷果实品质形成过程中的生理生化水平变化

糖类参与植物体内重要的能量代谢和细胞内结构物质的形成，如图4-A~C所示，在枇杷果实成熟过程中，可溶性总糖、还原糖和蔗糖含量都显著上升，而且在转色期到成熟期过程中，各糖分含量上升的幅度最大。有机酸是果实风味形成的重要因素之一。在枇杷果实品质形成过程中，有机酸含量呈下降趋势，到了成熟期有机酸含量下降的幅度最大（图4-D）。随着枇杷果实的成熟，类胡萝卜素和维生素C的含量均呈显著上升趋势（图4-E、F）。

### 2.3　枇杷果实品质形成过程中相关基因的mRNA水平变化

在枇杷果实品质形成过程中，类胡萝卜素的含量是显著上升的，因此选取类胡萝卜素代谢通路中的6个关键基因进行mRNA水平分析，结果如图5所示。与青果期的果实相比，在转色期和成熟期的果实中，除了*LCYe*基因的表达水平下调外，其他6个参与类胡萝卜素代谢的基因即*PSY*、*ZEP*、*LCYb*、*CYCB*、*BCH*以及*ACO*的表达水平均上调；与转色期的果实相比，成熟期果实中的*PSY*、*CYCB*、*BCH*以及*LCYe*的基因的表达上调，其他基因即*ZEP*、*LCYb*以及*ACO*的表达量均下调。

## 3　讨　论

本研究发现枇杷果实成熟过程中，参与糖代谢、三羧酸循环与能量代谢、类胡萝卜素合成以及乙烯

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表 2 枇杷果实品质形成过程中果肉差异蛋白的质谱鉴定结果

**Table 2 Identification of differentially expressed pulp proteins during the fruit quality formation of loquat**

| 蛋白点 Spot No. | 蛋白名称 Protein name | gi 号 gi Number | 物种 Species | 覆盖率 Coverage of matched peptides/% | 得分 Mascot score | 理论蛋白点相对分子质量和等电点 Theor. Mr (kDa)/pI | 实际蛋白点相对分子质量和等电点 Exp. Mr (kDa)/pI |
|---|---|---|---|---|---|---|---|
| 糖类代谢 Carbohydrate metabolism | | | | | | | |
| 4 | UDP 葡萄糖焦磷酸化酶 UDP-glucose pyrophosphorylase | 3107931 | 沙梨 *Pyrus pyrifolia* | 39 | 751 | 51.81/5.99 | 62.81/6.59 |
| 10 | 磷酸甘油酸变位酶 Phosphoglycerate mutase | 474170 | 蓖麻 *Ricinus communis* | 15 | 376 | 60.78/5.52 | 79.81/5.82 |
| 25 | 核酮糖-1,5-二磷酸羧化酶 Ribulose-1,5-biphosphate-carboxylase | 18477574 | 肖鸾尾 *Moraea herrei* | 23 | 742 | 47.22/6.78 | 62.80/6.50 |
| 30 | α-淀粉酶 Alpha-amylase | 229610885 | 大麦 *Hordeum vulgare* subsp. *vulgare* | 14 | 86 | 47.30/5.80 | 59.24/6.05 |
| 33 | 磷酸丙糖异构酶 Triose phosphate isomerase | 546735 | 长须银柴胡 *Stellaria longipes* | 8 | 171 | 27.48/5.54 | 33.08/5.70 |
| 44 | 磷酸丙糖异构酶 Triose phosphate isomerase | 211906460 | 陆地棉 *Gossypium hirsutum* | 17 | 152 | 27.19/6.00 | 33.39/6.34 |
| 45 | 醛/酮还原酶家族蛋白 Aldo/keto-reductase family protein | 355501454 | 蒺藜苜蓿 *Medicago truncatula* | 14 | 270 | 26.31/5.35 | 50.29/5.19 |
| 46 | 假定：醛酮还原酶 PREDICTED: probable aldo-keto reductase 1-like | 470132346 | 草莓 *Fragaria vesca* subsp. *vesca* | 41 | 435 | 38.23/5.53 | 35.00/6.02 |
| 50 | 磷酸甘露糖变位酶 Phosphomannomutase | 464895779 | 碧桃 *Prunus persica* | 23 | 358 | 28.12/6.33 | 41.12/5.53 |
| TCA 循环及能量代谢 Krebs cycle and energy metabolism | | | | | | | |
| 3 | 酮戊二酸脱氢酶复合体--二氢硫辛酰转琥珀酰酶组分 Dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex | 226532024 | 玉米 *Zea mays* | 9 | 179 | 48.75/8.95 | 59.24/6.05 |
| 5 | 二氢硫辛酰胺脱氢酶 Dihydrolipoamide dehydrogenase | 984678 | 豌豆 *Pisum sativum* | 34 | 116 | 53.34/6.67 | 68.72/6.78 |
| 9 | 琥珀酸脱氢酶 Succinate dehydrogenase | 326467055 | 荔枝 *Litchi chinensis* | 26 | 365 | 69.00/6.65 | 75.93/6.02 |
| 36 | ATP 合酶 D 链，线粒体 ATP synthase D chain, mitochondrial | 508726652 | 可可 *Theobroma cacao* | 35 | 139 | 19.65/5.21 | 19.95/5.18 |
| 40 | ATP 合酶β链 ATP synthase beta chain | 226493589 | 玉米 *Zea mays* | 46 | 1210 | 58.94/5.90 | 63.17/5.55 |
| 抗坏血酸-谷胱甘肽循环 AsA-GHS cycle | | | | | | | |
| 1 | Mn-超氧化物歧化酶 Manganese-superoxide dismutase, partial | 378724804 | 慈竹 *Neosinocalamus affinis* | 7 | 81 | 18.51/ 5.80 | 29.78/6.74 |
| 6 | 铜/锌超氧化物歧化酶 Copper/zinc-superoxide dismutase | 378724812 | 加拿大杨 *Populus* × *canadensis* | 25 | 144 | 14.41/5.42 | 15.35/6.48 |
| 18 | 多酚氧化酶 Polyphenol oxidase | 71738563 | 柰李 *Prunus salicina* var. *cordata* | 9 | 244 | 67.29/6.72 | 83.89/5.82 |
| 21 | 胞质抗坏血酸盐过氧化物酶 Cytosolic ascorbate peroxidase | 161778778 | 葡萄 *Vitis vinifera* | 21 | 191 | 27.98/5.43 | 33.39/5.67 |
| 34 | 硫氧还蛋白 Thioredoxin | 146454812 | 海桑 *Sonneratia apetala* | 56 | 115 | 66.44/5.69 | 18.90/5.12 |
| 41 | 谷胱甘肽 S-转移酶 Glutathione S-transferase GST | 358248536 | 大豆 *Glycine max* | 20 | 110 | 24.81/5.73 | 31.22/6.44 |
| 48 | 抗坏血酸过氧化物酶 Ascorbate peroxidase | 288188862 | 苹果 *Malus pumila* | 40 | 523 | 16.40/4.66 | 33.84/6.18 |

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表2（续） **Table 2（continued）**

| 蛋白点 Spot No. | 蛋白名称 Protein name | gi号 gi Number | 物种 Species | 覆盖率 Coverage of matched peptides/% | 得分 Mascot score | 理论蛋白点相对分子质量和等电点 Theor. Mr (kDa)/pI | 实际蛋白点相对分子质量和等电点 Exp. Mr (kDa)/pI |
|---|---|---|---|---|---|---|---|
| 55 | 脱氢抗坏血酸还原酶 Dehydroascorbate reductase | 110083901 | 苹果 *Malus × domestica* | 46 | 357 | 23.50/6.18 | 55.87/5.28 |
| 类胡萝卜素合成及乙烯合成 Carotenoids synthetic and ethylene formation | | | | | | | |
| 13 | 隐花色素2 Cryptochrome 2 | 261876463 | 大豆 *Glycine max* | 21 | 87 | 68.07/6.15 | 53.94/6.32 |
| 14 | 1- 氨基环丙烷-1-羧酸氧化酶 1-aminocyclopropane-1-carboxylate oxidase | 283101128 | 枇杷 *Eriobotrya japonica* | 36 | 390 | 36.34/5.35 | 52.39/5.45 |
| 16 | S-腺苷甲硫氨酸合成酶 3s-adenosylmethionine synthetase 3 | 222852116 | 大叶杨 *Populus trichocarpa* | 29 | 474 | 43.16/5.50 | 56.86/6.13 |
| 47 | 5′-甲硫基核苷激酶 5-methylthioribose kinase | 452090896 | 碧桃 *Prunus persica* | 14 | 298 | 47.33/5.75 | 58.21/6.36 |
| 52 | 乙醇脱氢酶 Alcohol dehydrogenase | 342851389 | 西洋梨 *Pyrus communis* | 35 | 1020 | 41.40/6.51 | 55.87/6.89 |
| 56 | 异戊烯焦磷酸异构酶 Isopentenyl pyrophosphate isomerase | 330376138 | 紫苜蓿 *Medicago sativa* | 22 | 178 | 33.07/6.22 | 59.93/5.59 |
| 蛋白质合成 Protein synthesis | | | | | | | |
| 2 | 同型半胱氨酸S-甲基转移酶 Homocysteine S-methyltransferase | 222863714 | 大叶杨 *Populus trichocarpa* | 16 | 78 | 36.71/5.54 | 42.74/5.00 |
| 12 | 真核翻译起始因子5-A4 Eukaryotic translation initiation factor 5A-4 | 42565381 | 风信子 *Hyacinthus orientalis* | 20 | 168 | 17.19/5.51 | 19.95/6.01 |
| 17 | 假定氨肽酶 Cputative aminopeptidase C | 51091128 | 水稻 *Oryza sativa Japonica Group* | 12 | 260 | 74.80/5.02 | 91.54/5.45 |
| 37 | 丙氨酸氨基转移酶alanine Aminotransferase | 37953301 | 辣椒 *Capsicum annuum* | 4 | 124 | 52.76/5.29 | 65.43/5.43 |
| 38 | 真核翻译起始因子5A1 Eukaryotic translation initiation factor 5A1 | 333108555 | 毛白杨 *Populus tomentosa* | 20 | 253 | 17.42/5.60 | 19.73/5.51 |
| 39 | 胞质谷氨酰胺合成酶 Cytosolic glutamine synthetase | 113171384 | 香瓜 *Cucumis melo* | 17 | 336 | 39.17/5.88 | 51.18/6.25 |
| 49 | 蛋白质二硫键异构酶 Protein disulphide isomerase PDI | 1134968 | 蓖麻 *Ricinus communis* | 2 | 128 | 55.26/4.95 | 74.43/4.95 |
| 细胞结构 Cellular structure | | | | | | | |
| 11 | 肉桂醇脱氢酶Cinnamyl alcohol dehydrogenase | 474300 | 土当归 *Aralia cordata* | 20 | 138 | 39.12/5.44 | 53.32/5.60 |
| 20 | α-1,4-葡聚糖合成酶1 Alpha-1,4-glucan- protein synthase 1 | 474042704 | 图小麦 *Triticum urartu* | 23 | 323 | 63.21/5.55 | 52.09/6.39 |
| 26 | 咖啡酰辅基-*O*-甲基转移酶 Caffeoyl-CoA-*O*-methyltransferase | 429142523 | 枇杷 *Eriobotrya japonica* | 48 | 833 | 27.79/5.43 | 35.68/5.58 |
| 51 | 微管蛋白 3 beta tubulin 3 | 146760211 | 大麦 *Hordeum vulgare* subsp. *vulgare* | 23 | 226 | 50.21/4.70 | 41.12/5.35 |
| 防御与胁迫 Defense and stress | | | | | | | |
| 22 | 病原相关蛋白4 Pathogenesis-related protein 4 | 384236234 | 苹果 *Malus × domestica* | 22 | 145 | 15.85/5.12 | 18.49/4.75 |
| 28 | β-1,3-葡聚糖酶 Beta-1,3-glucanase | 399137110 | 沙梨 *Pyrus pyrifolia* | 13 | 306 | 37.49/5.07 | 42.74/4.89 |
| 32 | 内源几丁质酶 Class III endo-chitinase | 33347391 | 沙梨 *Pyrus pyrifolia* | 12 | 203 | 17.66/4.12 | 35.91/4.60 |
| 53 | 温度诱导脂质运载蛋白 Temperature-induced lipocalin | 77744861 | 大豆 *Glycine max* | 21 | 126 | 21.22/7.79 | 21.05/6.05 |

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表2（续） Table 2（continued）

| 蛋白点 Spot No. | 蛋白名称 Protein name | gi号 gi Number | 物种 Species | 覆盖率 Coverage of matched peptides/% | 得分 Mascot score | 理论蛋白点相对分子质量和等电点 Theor. Mr (kDa)/pI | 实际蛋白点相对分子质量和等电点 Exp. Mr (kDa)/pI |
|---|---|---|---|---|---|---|---|
| 54 | 过敏原 Mal dallergen Mal d 1.06C | 333471423 | 苹果 *Malus × domestica* | 39 | 466 | 17.53/5.74 | 16.29/5.43 |
| 其他调控蛋白 Other regulation proteins | | | | | | | |
| 15 | 泛素激活酶 Ubiquitin-like modifier-activating enzyme | 355491858 | 蒺藜苜蓿 *Medicago truncatula* | 10 | 239 | 46.76/4.62 | 67.03/4.82 |
| 19 | 假定:DNA损伤诱导蛋白 PREDICTED: DNA damage-inducible protein 1-like | 356527193 | 大豆 *Glycine max* | 11 | 291 | 44.53/5.01 | 56.53/5.06 |
| 23 | 14-3-3 蛋白家族 14-3-3 family protein | 55375985 | 苹果 *Malus × domestica* | 55 | 553 | 29.68/4.75 | 38.06/4.86 |
| 24 | 生长素诱导蛋白 Auxin-induced protein | 475526794 | 山羊草 *Aegilops tauschii* | 15 | 84 | 38.72/6.15 | 35.00/5.09 |
| 29 | 14-3-3 蛋白家族 14-3-3 family protein | 3153902 | 番茄 *Solanum lycopersicum* | 23 | 124 | 28.93/4.65 | 41.38/4.54 |
| 31 | 14-3-3 蛋白家族 14-3-3 family protein | 55375985 | 苹果 *Malus × domestica* | 38 | 240 | 29.68/4.75 | 39.05/4.78 |
| 42 | 高分子量热休克蛋白 High molecular weight heat shock protein | 6969976 | 苹果 *Malus × domestica* | 23 | 787 | 71.17/5.17 | 86.00/5.29 |
| 未分类蛋白 Unclassified proteins | | | | | | | |
| 7 | Hypothetical protein PRUPE_ppa010816mg | 462402991 | 碧桃 *Prunus persica* | 14 | 151 | 27.33/5.85 | 36.61/6.45 |
| 8 | 铁结合蛋白 Iron-binding protein | 89276797 | 沙梨 *Pyrus pyrifolia* | 25 | 263 | 29.20/5.32 | 29.58/5.13 |
| 35 | Hypothetical protein PRUPE_ppa009165mg | 462405128 | 沙梨 *Pyrus pyrifolia* | 21 | 193 | 32.92/5.07 | 41.92/6.12 |
| 43 | Hypothetical protein PRUPE_ppa009165mg | 462405128 | 碧桃 *Prunus persica* | 16 | 190 | 32.92/5.07 | 41.38/5.85 |

合成等生物学过程的蛋白丰度均发生显著变化，因此这些代谢过程共同参与果实品质形成的调节作用。

### 3.1 糖代谢在枇杷果实品质形成中的作用

糖代谢与果实品质形成关系密切。α-淀粉酶具有糖苷水解酶和糖基转移酶的催化活性，在淀粉水解中扮演着重要的角色[27]。研究发现，在苹果发育早期，α-淀粉酶的活性很低，随着苹果果实的成熟，α-淀粉酶活性持续升高，在果实成熟后期，其活性达最大[28]。在枇杷果实成熟过程中，α-淀粉酶（spot 30）的含量不断增加，有利于淀粉的水解，可溶性糖的合成。

UDP-葡萄糖焦磷酸化酶是糖代谢中的一种关键酶，催化葡萄糖-1-磷酸和三磷酸脲苷UTP形成UDP-葡萄糖（UDPG）和焦磷酸的可逆反应[29]。UDP-葡萄糖焦磷酸化酶是一种位于细胞质的可溶性蛋白，负责UDPG的合成，可以参与蔗糖的形成和降解[30]。随着枇杷果实的成熟，细胞质中的磷酸丙糖异构酶（spot 33）丰度是先下调后上调，而UDP-葡萄糖焦磷酸化酶（spot 4）丰度在成熟期最大。从枇杷果实的转色期至成熟期，磷酸丙糖异构酶丰度的上调说明磷酸二羟丙酮和磷酸甘油醛之间的转化活跃，大量形成蔗糖合成代谢的前体物质磷酸丙糖，而此时UDP-葡萄糖焦磷酸化酶的丰度达到最大，说明该酶向着UDPG的合成方向进行，有利于代谢向蔗糖合成的方向进行，促进了蔗糖的合成。同时，在成熟期，蔗糖含量最大，与蛋白质丰度变化趋势相一致，故枇杷果实变甜，风味形成。

### 3.2 三羧酸循环及能量代谢在枇杷果实品质形成中的作用

三羧酸循环是植物有氧呼吸以及能量供应的重要生物学过程。随着枇杷果实的成熟，参与三羧酸循环的二氢硫辛酰转琥珀酰酶（spot 3）、二氢硫辛酸

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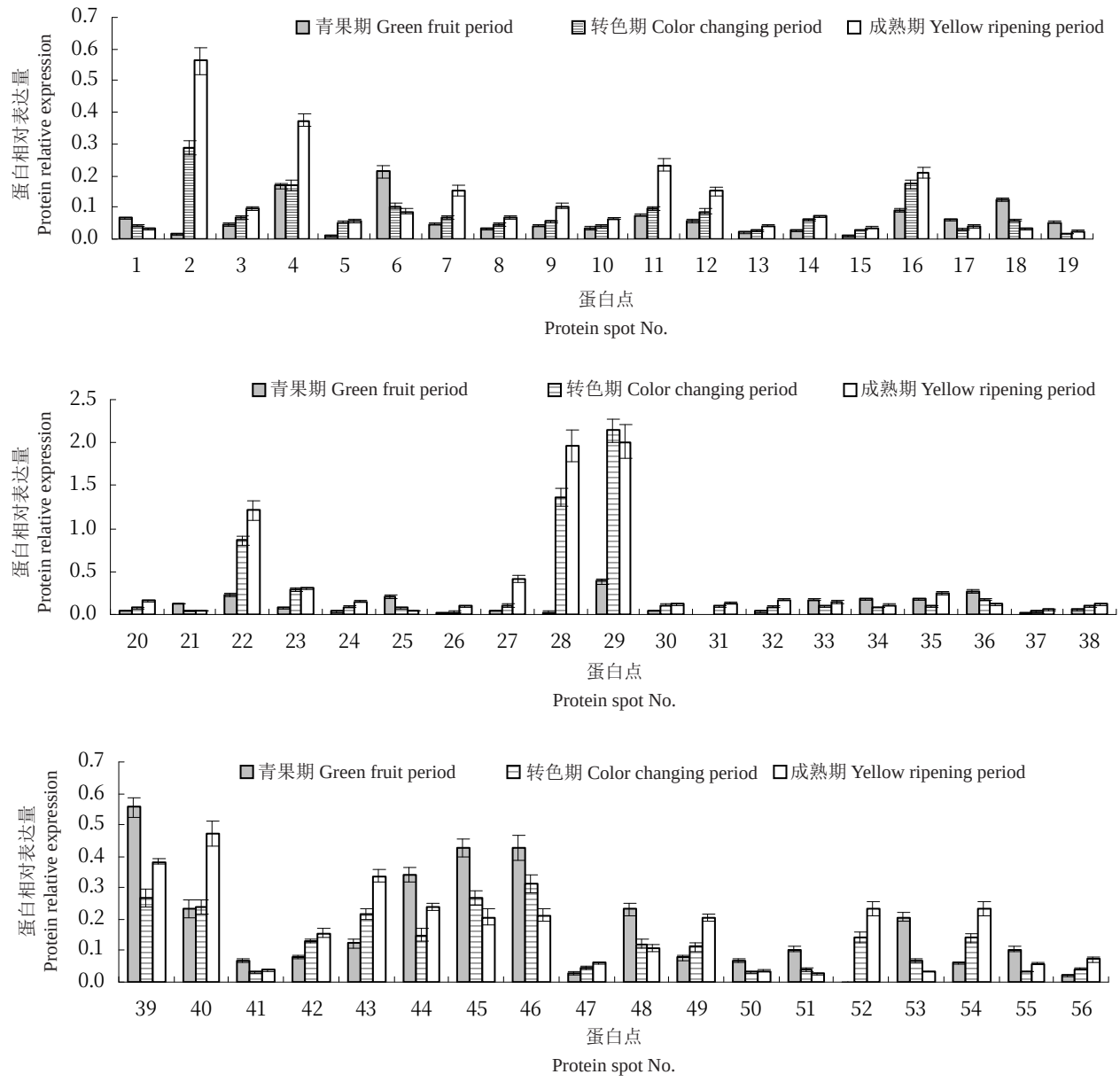


**图 3　枇杷果实品质形成过程中 56 个差异蛋白相对表达量变化**

**Fig. 3 Relative expression quantities of 56 differentially expressed proteins during the fruit quality formation of loquat**

脱氢酶（spot 5）和琥珀酸脱氢酶（spot 9）3个差异表达蛋白丰度均呈现上调趋势。前两个酶是三羧酸循环限速酶酮戊二酸脱氢酶复合体的重要组成部分[31]。有研究发现，在番茄等果实成熟过程中，酮戊二酸脱氢酶异构体大量存在，而且抑制酮戊二酸脱氢酶复合体会使呼吸作用显著下降[32-33]。在枇杷果实品质形成过程中，酮戊二酸脱氢酶复合体中二氢硫辛酰转琥珀酰酶和二氢硫辛酸脱氢酶的蛋白丰度的上调，说明有氧呼吸增强，可使枇杷果实细胞内各种有机酸被氧化分解，致使果肉中有机酸含量下降，糖酸比升高，促进了果实的成熟，有利于枇杷果实风味形成。同时维生素C含量的增加，使枇杷果实的营养价值提高。

### 3.3　类胡萝卜素代谢在枇杷果实品质形成中的作用

类胡萝卜素对于提升果实的外观品质和营养质量方面都发挥着重要作用。在枇杷果实成熟过程中，参与类胡萝卜素代谢的3,3-二甲基丙烯基焦磷酸异构酶（IPI，spot 56）和隐花色素（spot 13）的丰度均上调。IPI位于类胡萝卜素代谢的上游，促使二甲基烯丙基焦磷酸和异戊烯焦磷酸的直接相互转化。研究发现玉米白色体中，在光刺激下IPI的活性提

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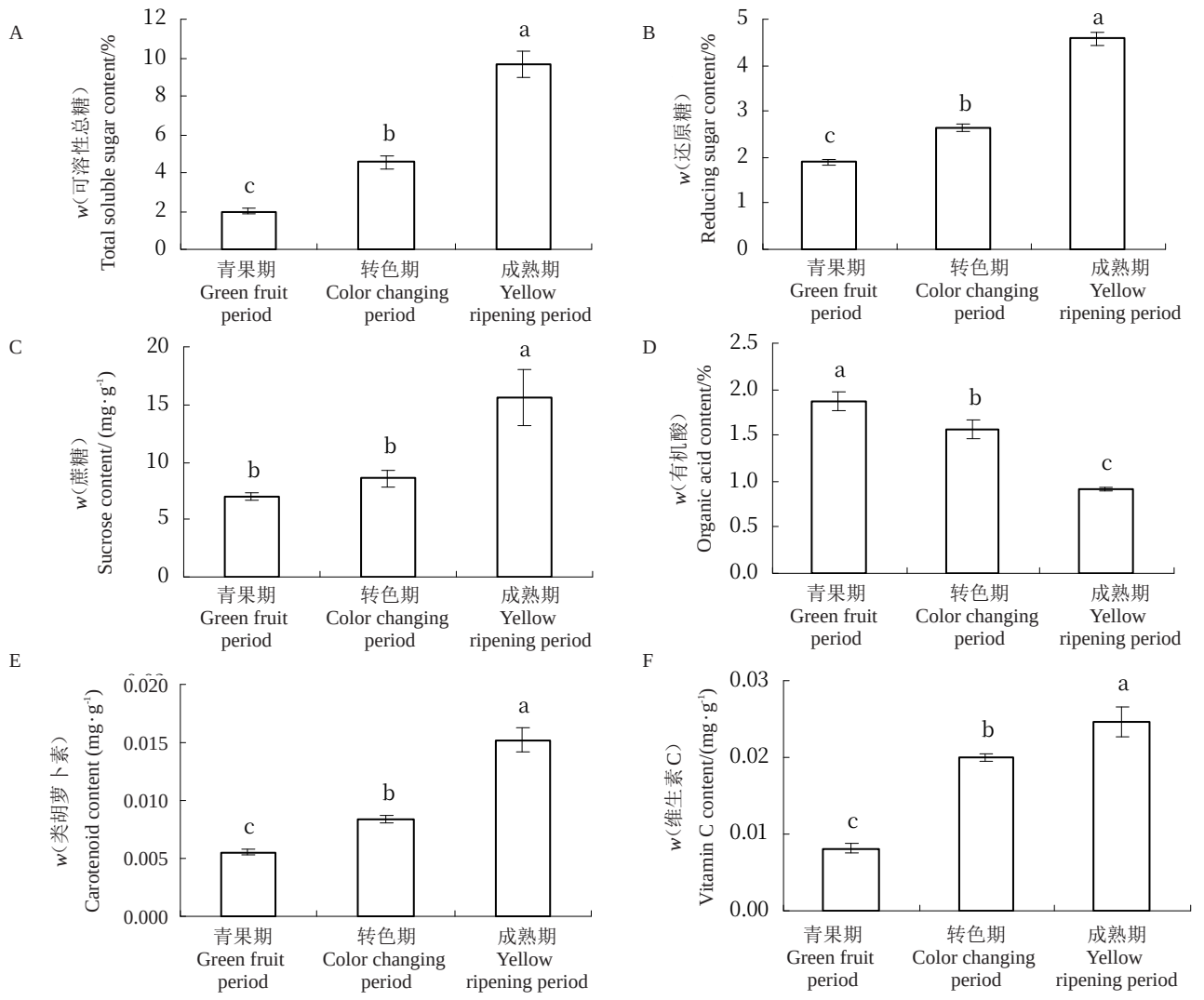


**图 4 枇杷果实品质形成过程中生理生化结果**

**Fig. 4 Physiological and biochemical traits of loquat pulp during the fruit quality formation**

高，促进八氢番茄红素的合成，有利于类胡萝卜素的形成[34]。隐花色素的过表达可通过调节番茄红素环化酶（*LCYe*）基因从而增加果实中番茄红素的积累量[35]。在转录水平上，本研究发现除*LCYe*基因外，参与类胡萝卜素代谢的相关基因*PSY*、*LCYb*、*CYCB*、*BCH*和*ZEP*的表达水平均在转色期上调，而到了成熟期，*PSY*、*CYCB*和*BCH*的基因表达水平继续上调（图5）。在柑橘和脐橙中，β-胡萝卜素、β-隐黄质以及玉米黄质含量的累积与*PSY*、*LCYb*、*CYCB*和*ZEP*基因的上调表达和*LCYe*基因的下调表达关系密切[36]，本文的发现与此结论相一致。随着枇杷果实的成熟，类胡萝卜素总含量逐步上升（图4），其趋势与IPI和隐花色素的蛋白丰度变化及*CYCB*和*BCH*基因的转录水平表达趋势基本一致。这些结果表明IPI和隐花色素的含量增加可促进果实中番茄红素的合成，*LCYb*、*CYCB*、*BCH*和*ZEP*基因表达水平的提高可促进番茄红素向胡萝卜素、隐黄质、玉米黄质等色素转化，直接调控枇杷中总类胡萝卜素的合成。

### 3.4 乙烯合成代谢在枇杷果实品质形成中的作用

在植物果实的成熟过程中，乙烯发挥着重要的作用，它可促进果实香味的产生和果皮的软化。甲硫氨酸是乙烯生物合成所需要的最初底物，而在S-腺苷甲硫氨酸合成酶、5'-甲硫基腺苷激酶和甲基转移酶等的作用下完成一次甲硫氨酸循环代谢。同时，甲硫氨酸循环产生的乙烯合成前体物质S-腺苷甲硫氨酸（SAM）在1-氨基环丙烷-1-羧酸合成酶和1-氨基环丙烷-1-羧酸氧化酶（ACO）作用下生成乙烯。在枇杷果实成熟过程中，参与乙烯合成代谢的S-腺苷甲硫氨酸合成酶（spot 16）、5'-甲硫基核苷激

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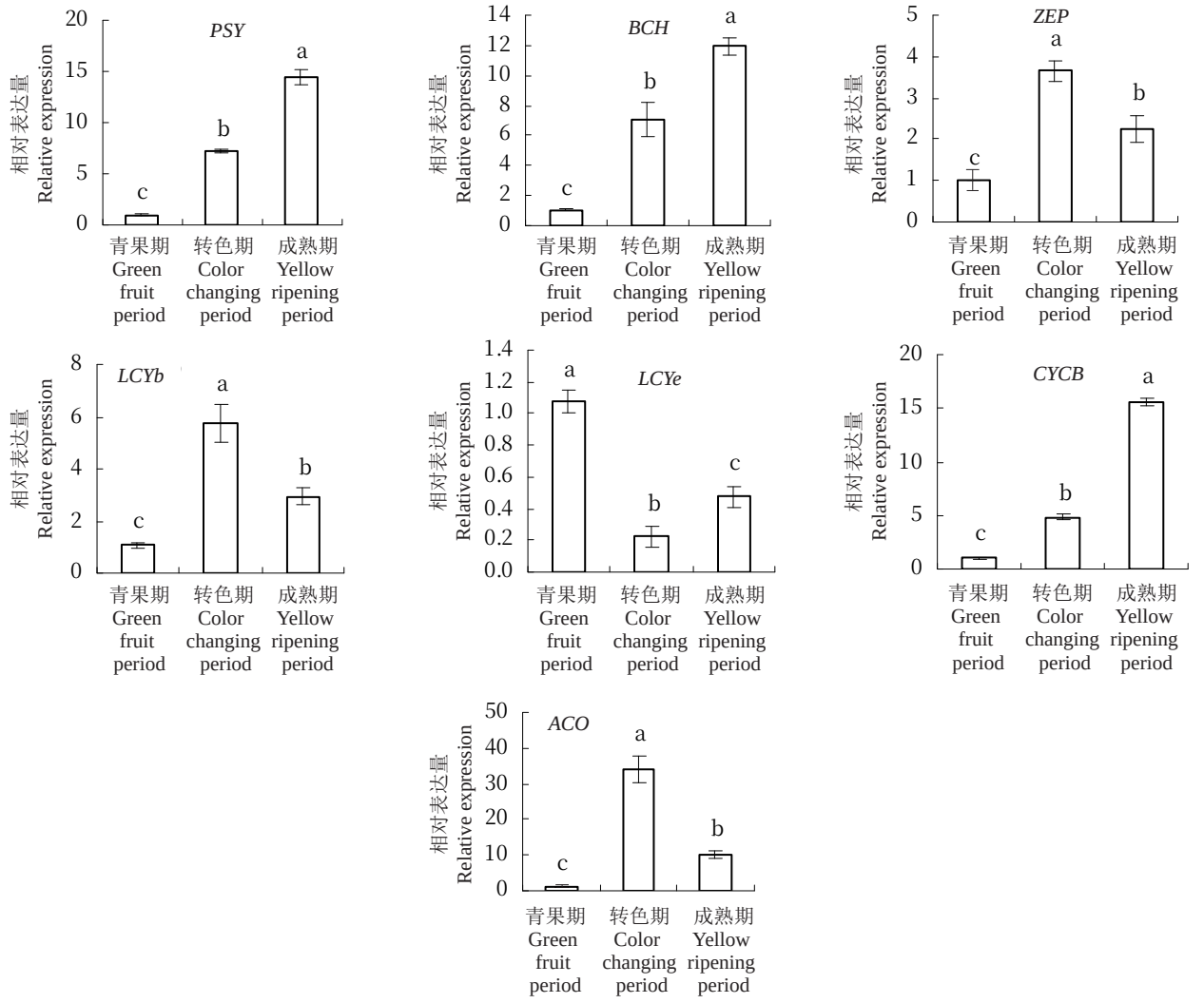


**图 5　枇杷果实品质形成过程中相关基因的表达水平**

**Fig. 5 Relative expression levels of genes related to loquat fruit quality formation**

酶（spot 47）和1-氨基环丙烷-1-羧酸氧化酶（spot 14）的蛋白丰度均上调。表明甲硫氨酸循环代谢十分活跃，有利于SAM的生成，促使乙烯的生物合成。在乙烯合成的后期，枇杷果实中催化乙烯合成的最后一步反应所需的关键酶ACO的蛋白丰度随着果实的成熟不断上调，在转色期和成熟期的*ACO*表达水平均显著高于青果期，这与梨果实成熟过程的变化结果一致[37]。这些结果表明随着枇杷果实的成熟，与乙烯合成代谢相关酶的丰度增加有利于乙烯的合成，促进了枇杷果实品质的形成。

综上所述，通过对青果期、转色期和成熟期的枇杷果肉的总蛋白质含量变化分析，我们发现枇杷果实成熟与糖代谢、三羧酸循环与能量代谢、类胡萝卜素合成及乙烯合成过程相关蛋白（酶）的上调表达密切相关，并调控着果实品质的形成。

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