贮藏温度对鲜枸杞类胡萝卜素和氨基酸的影响及调控机制

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摘要 本研究探讨了4和-4°C贮藏对鲜枸杞类胡萝卜素水平、类胡萝卜素合成、存储、降解相关基因以及氨基酸代谢相关基因表达的调控作用。结果表明, -4°C贮藏的枸杞果实中 β -胡萝卜素、玉米黄素和玉米黄素双棕榈酸酯含量显著高于4°C, 贮藏末期玉米黄素双棕榈酸酯含量比4°C贮藏高42.14%。枸杞果实低温贮藏过程中类胡萝卜素合成、存储和降解相关基因普遍上调表达, 特别是类胡萝卜素裂解酶基因*LbCCD4*在贮藏过程中表达量升高超过70倍。4°C贮藏枸杞果实类胡萝卜素合成基因表达显著高于-4°C, 但是存储蛋白基因*LbHSP21*、*LbOR2*表达极显著低于-4°C, 降解酶基因*LbNCED6*、*LbCCD1*、*LbCCD4*则极显著高于-4°C, 可能是导致其类胡萝卜素含量低于-4°C的主要原因。另一方面, 4°C贮藏上调脯氨酸合成相关的*LbOAT*表达、维持脯氨酸降解相关的*LbProDH*在较低水平, 从而有利于脯氨酸的积累。上述结果说明, -4°C贮藏更有利于枸杞类胡萝卜素的积累、保持较好的营养品质, 并延缓果实衰老劣变。

关键词 贮藏温度, 玉米黄素双棕榈酸酯, 类胡萝卜素, 氨基酸, 代谢, 调控

枸杞(*Lycium barbarum* L.)是茄科枸杞属多年生落叶灌木。枸杞果实富含多糖、类胡萝卜素、类黄酮、维生素、氨基酸等营养物质和生物活性物质, 具有清肝、明目、补气, 提高免疫力, 预防心血管疾病、癌症等作用, 因此被认为是一种“超级食品”^[1]。

枸杞果实的色泽主要由枸杞果实中类胡萝卜素的种类和含量决定。枸杞果实中主要的类胡萝卜素成分为玉米黄素双棕榈酸酯、玉米黄素和 β -胡萝卜素。其中, 玉米黄素双棕榈酸酯(zeaxanthin dipalmitate)是枸杞中最具特征性的类胡萝卜素, 其含量占枸杞类胡萝卜

素总量的80%以上, 因此又被称为“枸杞红素”^[2]。玉米黄素双棕榈酸酯因其抗氧化和抗视网膜病变、黄斑变性、非酒精性脂肪肝、急性肝损伤、肝癌等疾病的作用, 近年来备受关注^[3,4]。目前植物中类胡萝卜素的生物合成途径已经比较清晰, 玉米黄素双棕榈酸酯的合成在类胡萝卜素下游 β -胡萝卜素分支途径中, β -胡萝卜素和玉米黄素是玉米黄素双棕榈酸酯合成的上游物质。因此, 该下游分支合成途径可能是枸杞果实类胡萝卜素代谢的主要途径; 并且, 枸杞果实类胡萝卜素水平受合成、存储和降解酶基因的共同调控^[5]。

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氨基酸是枸杞果实的重要营养成分和药效成分,其种类和含量是决定枸杞品质的关键因素^[6,7]。枸杞中的游离氨基酸可以直接被人体吸收利用,也可以合成蛋白质,参与维持机体新陈代谢、生长、繁殖以及免疫反应^[8]。果蔬等农产品中的氨基酸,如缬氨酸、亮氨酸、异亮氨酸、蛋氨酸、苯丙氨酸和丙氨酸的合成或分解代谢形成的嗅感物质和味感物质,使果蔬呈现特有的香味/甜味。其他氨基酸作为这些产生气味的氨基酸的前体物质发挥作用,例如天冬氨酸作为前体物质参与多种氨基酸的合成,包括赖氨酸、蛋氨酸、苏氨酸等^[9-11]。

枸杞在干制过程中往往伴随着营养成分,如类胡萝卜素、氨基酸等的流失^[12]。相比干枸杞,不经任何处理的新鲜枸杞能够更好地保留枸杞中的营养成分。近年来,随着消费者对食物的营养、健康以及自然属性的更高追求,鲜食枸杞越来越受欢迎^[13]。但是枸杞鲜果由于其结构的特殊性(皮薄质嫩、中空、含水量高),采后极易发生糖化和腐烂,在室温下贮藏1~2 d即失去商品价值^[14]。枸杞鲜果的贮藏保鲜不仅决定鲜食枸杞产品质量,同时对降低制干过程中油果(油籽)率,提高干制枸杞产品质量具有重要意义。低温贮藏可以降低果蔬呼吸速率、降低物质消耗、抑制微生物繁殖、延缓腐烂,是农产品保鲜最有效的途径^[15,16]。

本研究在前期工作^[17]基础上,以“宁杞9号”鲜枸杞为实验材料,研究不同贮藏温度(4和-4℃)对鲜枸杞β-胡萝卜素、玉米黄素、玉米黄素双棕榈酸酯水平,类胡萝卜素合成、存储、降解相关基因以及氨基酸代谢相关基因表达的影响,以期探讨贮藏温度对鲜枸杞类胡萝卜素和游离氨基酸的调控作用,明确其营养品质衰变规律,为鲜枸杞的贮藏保鲜提供理论依据。

1 材料和方法

1.1 植物材料

实验材料为宁夏特色大果型枸杞品种“宁杞9号”,商业采收成熟度(80%~90%成熟),7月中旬采自中国科学院西北生物农业中心枸杞种植园(银川市平吉堡)。果实采收后,在13.5 h内(平均温度26.5℃)空运到广州的实验室。挑选大小一致、色泽均一、无机械损伤、无肉眼可见病斑的枸杞果实,用自来水漂洗,晾干,装入塑料饭盒,每盒150 g,共24盒,分别贮藏于4和-4℃冷库,每个温度放置12盒。在0、7、14、21 d每个温度随机选取3盒,

观察拍照,样品冻存于-80℃用于后续实验。每个实验设置3个生物学重复,结果取3次实验数据的平均值。

1.2 外观品质分级

枸杞果实按外观品质由好到差分为5级:0级,无明显皱缩、糖化、霉变斑点;1级,可见皱缩、糖化,无明显霉变斑点,或霉变斑点直径 $d \leq 1$ mm;2级,霉点 $1 < d \leq 2$ mm,轻度皱缩、糖化;3级,霉点 $2 < d \leq 4$ mm,中度皱缩、糖化;4级,霉点 $d > 4$ mm,严重皱缩、糖化。其中,2~4级果被认为无商品价值(图S1)。

1.3 玉米黄素、β-类胡萝卜素和玉米黄素双棕榈酸酯含量测定

参照李新^[17]的研究方法,采用非皂化的方法提取枸杞果实类胡萝卜素。将干燥的类胡萝卜素提取物加入流动相至10 mL,用0.45 μm注射器过滤。用高效液相色谱(HPLC)分析类胡萝卜素、玉米黄素和玉米黄素双棕榈酸酯含量。检测器为二极管阵列检测器(PDA),样品经C18反相柱(25 cm × 4.6 mm,粒径5 μm)纯化。流动相由甲醇:乙腈:二氯甲烷:正己烷=15:40:20:20(体积比)组成,柱温30℃,流速为1 mL/min,检测波长为450 nm。采用玉米黄素和β-胡萝卜素标准品绘制标准曲线,计算枸杞果实中玉米黄素和β-类胡萝卜素含量。

玉米黄素双棕榈酸酯的定量分析参照Karioti等人^[3]的方法,用HPLC-ESI-MS法。以β-胡萝卜素作为外标物制作标准曲线,结果用分子量校正系数(玉米黄素双棕榈酸酯的分子量1045.8与β-胡萝卜素的分子量536.8的比值)进行校正。

1.4 RNA提取和基因表达分析

使用总RNA提取试剂盒(Magen, 中国)从不同温度、不同贮藏时间的枸杞果实中提取总RNA。提取的RNA用无RNase的DNase I(TaKaRa, 日本)在37℃处理30 min,以去除基因组DNA。采用NanoDrop检测RNA质量,采用RNA凝胶电泳检测RNA纯度。第一链cDNA使用PrimeScript第一链cDNA合成试剂盒(TaKaRa, 日本)合成。

本研究所用基因序列主要参考刘永亮^[18]的研究。利用Primer 5.0软件,根据目标基因全长序列设计荧光定量引物(表S1)。采用枸杞Actin作为内参基因。采用Tecan Spark™ Multi-function Microwell Plate Detector (Dobio, 上海)测定合成的cDNA含量。以cDNA为模板,

采用TaKaRa公司的TB Green[®] Premix Ex Taq (Tli RNaseH Plus)试剂盒(TaKaRa, 日本)进行qRT-PCR扩增。所用仪器为ABI7500荧光定量PCR仪, 采用 $2^{-\Delta\Delta C_t}$ 法分析基因的相对表达水平。本研究中所有qRT-PCR步骤均符合Minimum Information for Publication of Quantitative Real-Time PCR Experiments(MIQE)规范。

1.5 数据分析

使用Excel 2010进行初步数据统计, 使用SPSS Statistics 13软件进行差异显著性分析, 利用Sigmaplot 12.5软件进行绘图。

2 实验结果

2.1 玉米黄素、 β -类胡萝卜素和玉米黄素双棕榈酸酯含量

枸杞果实从变色期开始逐渐积累玉米黄素直至果实成熟。在4和-4℃贮藏过程中玉米黄素含量总体呈上升趋势(图1(a))。在第7天, 4℃贮藏的枸杞玉米黄素含量高于-4℃贮藏, 贮藏14 d后-4℃贮藏的枸杞果实玉米黄素含量始终高于4℃的果实。贮藏末期, -4℃贮藏的枸杞果实玉米黄素从 $4.9 \pm 0.2 \mu\text{g/g}$ 上升到 $10.4 \pm 0.1 \mu\text{g/g}$, 而4℃贮藏仅为 $8.0 \pm 0.2 \mu\text{g/g}$, 二者之间差异显著($P < 0.05$)。-4和4℃贮藏21 d枸杞玉米黄素含量分别增加了112.2%和63.3%, 说明-4℃贮藏显著增加枸杞果实玉米黄素的积累。

枸杞采后4℃贮藏初期 β -胡萝卜素含量稍有增加, 在第7天达到峰值, 此后持续下降(图1(b))。但-4℃贮藏的枸杞果实贮藏14 d时 β -胡萝卜素仍保持较高的水平(比4℃贮藏高16.67%, 差异显著), 说明-4℃贮藏可

以显著延缓枸杞果实 β -胡萝卜素的降解。

新鲜采摘的枸杞果实玉米黄素双棕榈酸酯含量为 $340.6 \pm 33.7 \mu\text{g/g}$, 约占总类胡萝卜素含量的85.75%^[17]。在贮藏过程中玉米黄素双棕榈酸酯的含量呈先降后升的趋势。4和-4℃贮藏7 d玉米黄素双棕榈酸酯含量均降到最低水平, 分别比贮藏前降低36.3%和48.6%, 差异显著(图1(c))。此后, -4℃贮藏枸杞玉米黄素双棕榈酸酯含量迅速上升, 贮藏末期, -4℃贮藏的枸杞果实玉米黄素双棕榈酸酯含量高达 $593.5 \pm 52.2 \mu\text{g/g}$, 比4℃贮藏的枸杞高42.14%, 差异极显著($P < 0.01$), 表明-4℃贮藏显著增加枸杞果实玉米黄素双棕榈酸酯的积累。

2.2 类胡萝卜素合成途径关键酶基因表达

类胡萝卜素的合成受多种关键酶基因的共同催化, 其中1-脱氧木酮糖-5-磷酸合成酶(DXS)是甲羟戊酸MEP途径中第一个关键酶^[19]。如图2所示, *LbDXS1*和*LbDXS2*在贮藏前期表达均显著上调。在整个贮藏过程中4℃贮藏的枸杞果实的*LbDXS1*表达量始终高于-4℃。在4℃贮藏条件下其表达量在14 d达到峰值(16.6 ± 2.5), 随之迅速下降; 而在-4℃贮藏条件下, *LbDXS2*表达量在7 d达到峰值, 比4℃贮藏提前了7 d。在贮藏第14天时, *LbDXS2*基因的表达量在4和-4℃下相比贮藏初期分别提高了17和5倍, 二者存在极显著差异($P < 0.01$)。

八氢番茄红素合成酶(PSY)、八氢番茄红素脱氢酶(PDS)、 ζ -胡萝卜素异构酶(ZISO)、 ζ -胡萝卜素脱氢酶(ZDS)和类胡萝卜素异构酶(CRTISO)是类胡萝卜素合成途径上游关键合成酶^[20]。从图2中可以看出, 这些基因在贮藏过程中均呈现先升高后降低的趋势; 并且, 除*LbZDS*以外, 4℃贮藏条件下基因的表达量均显著高于-4℃。此外, *LbCRTISO*基因上调表达的增幅最为突

图1 (网络版彩色)不同贮藏温度鲜枸杞玉米黄素、 β -类胡萝卜素和玉米黄素双棕榈酸酯的含量。(a) 玉米黄素含量;(b) β 类胡萝卜素含量;(c) 玉米黄素双棕榈酸酯含量。“*”、“**”分别表示同一贮藏时间不同贮藏温度之间差异显著($P < 0.05$)或极显著($P < 0.01$), 下同

Figure 1 (Color online) Contents of zeaxanthin, β -carotenoid and zeaxanthin dipalmitate, in fresh Goji berry during storage at different temperatures. (a) Content of zeaxanthin; (b) contents of β -carotenoid; (c) contents of zeaxanthin dipalmitate. “*” and “**” indicated that there was significant difference ($P < 0.05$) or extremely significance ($P < 0.01$) between different storage temperatures at the same storage time, the same below

图2 (网络版彩色)不同贮藏温度鲜枸杞类胡萝卜素合成途径关键酶基因的表达。DXS1, 1-脱氧木酮糖-5-磷酸合成酶1基因; DXS2, 1-脱氧木酮糖-5-磷酸合成酶2基因; PSY1, 八氢番茄红素合成酶1基因; PSY2, 八氢番茄红素合成酶2基因; PDS, 八氢番茄红素脱氢酶基因; ZDS, ζ -胡萝卜素脱氢酶基因; CRTISO, 类胡萝卜素异构酶基因; LCY-E, 番茄红素 ϵ -环化酶基因; LCY-B, 番茄红素 β -环化酶基因; CYP97-A29, 细胞色素P450 α 环羟化酶基因; CYP97-C11, 细胞色素P450 ϵ 环羟化酶基因; CRTR-B1, β -胡萝卜素羟化酶基因1基因; CRTR-B2, β -胡萝卜素羟化酶基因2基因; ZEP, 玉米黄素环氧化酶基因; VDE, 紫黄质脱环氧化酶基因

Figure 2 (Color online) Gene expression of key enzymes in carotenoid biosynthesis pathway in fresh Goji berry during storage at different temperatures. DXS1, 1-deoxy-D-xylulose 5-phosphate-synthase 1 gene; DXS2, 1-deoxy-D-xylulose 5-phosphate-synthase 2 gene; PSY1, phytoene synthase 1 gene; PSY2, phytoene synthase 2 gene; PDS, phytoene desaturase gene; ZDS, ζ -carotene desaturase gene; CRTISO, carotene isomerase gene; LCY-E, lycopene ϵ -cyclase gene; LCY-B, lycopene β -cyclase gene; CYP97-A29, cytochrome P450 α cyclohydroxylase gene; CYP97-C11, cytochrome P450 ϵ cyclohydroxylase gene; CRTR-B1, beta-carotene hydroxylase 1 gene; CRTR-B2, beta-carotene hydroxylase 2 gene; ZEP, zeaxanthin epoxidase gene; VDE, violaxanthin de-epoxidase gene

出, 在4和-4°C贮藏第7天时表达量分别比贮藏初期上调28.0和23.9倍, 说明枸杞果实在收获后仍进行着活跃的类胡萝卜素的合成和积累, 并且4°C贮藏条件更利于类胡萝卜素合成基因的表达。

类胡萝卜素合成途径下游 β -胡萝卜素分支途径的合成酶包括: 番茄红素 β -环化酶(LCY-B)、有色体特异的番茄红素 β -环化酶(CYC-B)、 β -胡萝卜素羟化酶(CRTR-B)、玉米黄素环氧化酶(ZEP)和紫黄质脱环氧化酶

(VDE)^[20]。由图2可以看出, 4°C贮藏条件下LbLCY-E、LbLCY-B、LbCYCB、LbCRTR-B1、LbCRTR-B2表达量均高于-4°C。其中LbLCY-B、LbCYCB作为分支上游的两个关键酶在贮藏第7天时其基因表达量均显著上调($P<0.01$), 与贮藏初期 β -胡萝卜素和玉米黄素含量升高相一致(图1)。

玉米黄素是 β -胡萝卜素分支合成途径的中间物质。一方面主要酯化合成玉米黄素双棕榈酸酯, 另一方面

又可在玉米黄素环氧化酶(ZEP)的作用下氧化为视紫黄质, 玉米黄素的积累受多个酶调控. 如图2, *LbZEP*和*LbVDE*在贮藏14~21 d显著上调表达, 并且4°C条件下表达量高于-4°C. 这与枸杞果实玉米黄素含量的变化趋势相同.

上述结果表明, 参与类胡萝卜素合成的基因在贮藏过程中总体上呈现上调表达的趋势, 与玉米黄素及玉米黄素双棕榈酸酯含量的总体变化趋势一致, 但与不同贮藏温度下各种类胡萝卜素组分、含量的变化趋势不符说明类胡萝卜素积累不仅取决于合成相关酶基因的表达, 还受到类胡萝卜素存储和降解相关酶基因的共同调控.

2.3 类胡萝卜素存储相关酶基因表达

类胡萝卜素的积累不仅与合成速率有关, 还与体内质体结构和存储相关蛋白基因的表达水平有关. 鲜枸杞贮藏期间有色体特异的类胡萝卜素结合蛋白(CHRC)、热激蛋白(HSP)和橙色类胡萝卜素蛋白OR1、OR2基因表达总体呈上调趋势, 并且, -4°C贮藏条件下枸杞果实类胡萝卜素存储相关基因的表达量显著高于4°C(图3). 特别是在贮藏期第14~21天上调最为

显著($P<0.01$). 这一变化趋势与枸杞果实总类胡萝卜素含量在贮藏末期显著上升^[17]以及-4°C贮藏条件下类胡萝卜素含量高于4°C(图1)的结果相一致.

2.4 类胡萝卜素降解相关酶基因表达

类胡萝卜素可被类胡萝卜素裂解双氧化酶(carotenoid cleavage dioxygenase, CCD)催化降解为脱辅基类胡萝卜素(apocarotenoid). 虽然目前CCD酶降解类胡萝卜素的具体机制尚不清楚, 但是现有证据表明, CCD家族基因对类胡萝卜素积累可能具有重要调控作用^[21]. 枸杞低温贮藏过程中*LbNCED1*、*LbNCED6*、*LbCCD1*和*LbCCD4*均显著上调表达; 4°C贮藏7 d, *LbCCD1*、*LbCCD4*和*LbNCED6*表达量达到高峰, 尤其是*LbCCD4*表达量比贮藏前提高70多倍, -4°C贮藏也提高了近30倍(图4).

2.5 氨基酸代谢相关基因表达

氨基酸是枸杞果实的主要营养成分, 同时也是重要的功效成分, 其种类和含量决定果实营养品质和药用价值. 我们前期在枸杞中共检测到23种游离氨基酸, 总氨基酸含量约为 9.607 ± 0.5 mg/g, 必需氨基酸的含量

图3 (网络版彩色)不同贮藏温度鲜枸杞类胡萝卜素存储基因的表达. *CHRC*, 有色体特异的类胡萝卜素结合蛋白基因; *HSP21*, 热激蛋白21基因; *OR1*, 橙色类胡萝卜素蛋白1基因; *OR2*, 橙色类胡萝卜素蛋白2基因

Figure 3 (Color online) Gene expression of carotenoid storage protein in fresh Goji berry during storage at different temperatures. *CHRC*, chromoplast-specific carotenoid-associated protein gene; *HSP21*, heat shock protein 21 gene; *OR1*, orange carotenoid protein 1 gene; *OR2*, orange carotenoid protein 2 gene

图4 (网络版彩色)不同贮藏温度鲜枸杞类胡萝卜素降解酶基因的表达。LbNCED1, 环氧类胡萝卜素双氧化酶1基因; LbNCED6, 环氧类胡萝卜素双氧化酶6基因; LbCCD1, 类胡萝卜素裂解双氧化酶1基因; LbCCD4, 类胡萝卜素裂解双氧化酶4基因

Figure 4 (Color online) Gene expression of carotenoid degrading enzyme in fresh Goji berry during storage at different temperatures. LbNCED1, 9-cis-epoxycarotenoid dioxygenase 1 gene; LbNCED6, 9-cis-epoxycarotenoid dioxygenase 6 gene; LbCCD1, carotenoid cleavage dioxygenase 1 gene; LbCCD4, carotenoid cleavage dioxygenase 4 gene

约为 0.58 ± 0.03 mg/g^[17]。在4°C贮藏过程中, 总游离氨基酸含量和必需氨基酸含量较为恒定。-4°C贮藏7 d, 总氨基酸含量和必需氨基酸含量均显著增加, 说明-4°C贮藏条件下有利于枸杞果实游离氨基酸的积累。

植物脯氨酸主要通过谷氨酸和鸟氨酸这两条途径合成, 其中 Δ 1-二氢吡咯-5-羧酸合成酶(P5CS)和鸟氨酸转氨酶(OAT)分别是这两条途径中的关键限速酶。脯氨酸脱氢酶(ProDH)催化脯氨酸的分解代谢, 在脯氨酸降解中起重要作用^[22]。由图5可知, LbP5CS在两个贮藏

温度下的表达趋势不同, 在4°C贮藏条件下先升高后降低, 而在-4°C贮藏条件下随着时间的延长持续增加。4°C贮藏条件下LbOAT表达量始终显著高于-4°C, 但是-4°C条件下LbProDH的表达量则极显著高于4°C ($P < 0.01$)。结合枸杞贮藏期间氨基酸组分和含量变化的结果, 说明4°C低温贮藏上调LbOAT表达、维持LbProDH在较低水平, 因而有利于脯氨酸的积累。

天冬氨酸激酶(AK)是赖氨酸、苏氨酸等合成途径中的关键酶, 受到代谢产物赖氨酸与苏氨酸的协同抑

图5 (网络版彩色)不同贮藏温度鲜枸杞脯氨酸代谢酶基因的表达。P5CS, Δ 1-二氢吡咯-5-羧酸合成酶基因; OAT, 鸟氨酸转氨酶基因; ProDH, 脯氨酸脱氢酶基因

Figure 5 (Color online) Gene expression of proline metabolism enzyme in fresh Goji berry during storage at different temperatures. P5CS, Δ 1-dihydropyrrrole-5-carboxylic acid synthetase gene; OAT, ornithine aminotransferase gene; ProDH, proline dehydrogenase gene

制。天冬氨酸激酶(AK)催化天冬氨酸磷酸化产生天冬氨酰磷酸,后者在天冬氨酸半醛脱氢酶(ASADH)催化下转变成天冬氨酸半醛;天冬氨酸半醛分别在高丝氨酸激酶(HK)和其他合成酶的催化进一步合成苏氨酸、赖氨酸等^[23]。由图6可知, *LbAK*在整个贮藏过程中表达持续增加。 *LbASADH*和*LbHK*表达先升后降, -4℃贮藏的枸杞果实7 d达到峰值,而4℃贮藏的枸杞果实第14 d达到峰值(2.94±0.37)。与苏氨酸和赖氨酸的含量增加相一致。

苯丙氨酸解氨酶(PAL)和肉桂酸-4-羟基化酶(C4H)是苯丙氨酸代谢途径中的限速酶^[24]。在4℃贮藏条件下*LbPAL*表达水平持续下降,在-4℃条件下其表达跌宕起伏。 *LbC4H1*表达在整个贮藏过程中呈上升趋势,并且4和-4℃之间无显著性差异(图7)。

3 讨论

枸杞鲜果采后贮藏期短是限制产业发展的瓶颈问

题。低温是延缓生鲜产品衰老劣变、延长其贮藏寿命最安全有效的技术手段。类胡萝卜素种类和含量是决定枸杞果实色泽、品质的关键因素。宁夏枸杞中主要的类胡萝卜素成分为玉米黄素双棕榈酸酯、玉米黄素和β-胡萝卜素^[25,26]。本研究表明,枸杞果实中富含玉米黄素双棕榈酸酯(含量超过85%), -4℃贮藏显著增加枸杞果实玉米黄素双棕榈酸酯的积累;作为玉米黄素双棕榈酸酯合成的前体物质,玉米黄素和β-胡萝卜素在-4℃度贮藏条件下积累相对较多或者降解相对延缓,有利于玉米黄素双棕榈酸酯的合成和积累(图1)。但是4℃贮藏条件却更有利于多数类胡萝卜素合成关键酶基因的表达(图2),说明类胡萝卜素在植物体内的积累是一个动态变化的过程,受合成、降解以及存储等多方面因素共同调控(图8)。在-4℃贮藏条件下,枸杞果实类胡萝卜素存储关键基因*LbCHRC*、*LbOR2*、*LbHSP21*的表达量显著高于4℃(图3)。此外,以*LbCCD4*为代表的类胡萝卜素降解酶在枸杞果实类胡萝卜素合成与积累调控中扮演着

图6 (网络版彩色)不同贮藏温度鲜枸杞天冬氨酸代谢酶基因的表达。AK, 天冬氨酸激酶基因; ASADH, 天冬氨酸半醛脱氢酶基因; HK, 高丝氨酸激酶基因

Figure 6 (Color online) Gene expression of aspartic acid metabolism enzyme in fresh Goji berry during storage at different temperatures. AK, aspartate kinase gene; ASADH, aspartate semialdehyde dehydrogenase gene; HK, homoserine kinase gene

图7 (网络版彩色)不同贮藏温度鲜枸杞苯丙氨酸代谢酶基因的表达。PAL, 苯丙氨酸解氨酶基因; C4H1, 肉桂酸-4-羟基化酶基因

Figure 7 (Color online) Gene expression of phenylalanine metabolism enzyme in fresh Goji berry during storage at different temperatures. PAL, phenylalanine ammonia lyase gene; C4H1, cinnamic acid-4-hydroxylase 1 gene

图 8 (网络版彩色)枸杞类胡萝卜素合成、降解通路图. 热图表示枸杞类胡萝卜素合成、降解通路相关基因在采后贮藏0、7、14、21 d时表达水平的变化, 取 $\log_2$ (基因相对表达量)绘制热图; GA-3-P, 3-磷酸甘油醛; pyruvate, 丙酮酸; GGPP, 牻牛儿苗基焦磷酸; phytoene, 八氢番茄红素; ζ -carotene, ζ -胡萝卜素; lycopene, 番茄红素; δ -carotene, δ -胡萝卜素; α -carotene, α -胡萝卜素; lutein, 芦丁; γ -carotene, γ -胡萝卜素; β -carotene, β -胡萝卜素; β -cryptoxanthin, β -隐黄质; zeaxanthin, 玉米黄素; zeaxanthin dipalmitate, 玉米黄素双棕榈酸酯; violaxanthin, 紫黄质; neoxanthin, 新黄素; abscisic acid, 脱落酸; apocarotenoid, 脱辅基类胡萝卜素

Figure 8 (Color online) Schematic of carotenoid biosynthesis and degradation pathways in Goji berry. The heat map shows expression levels of genes participate in carotenoid biosynthesis and degradation pathways of Goji berry stored for 0, 7, 14 and 21 d, respectively. $\log_2$ (relative gene expression) was used to draw the graph; GA-3-P, glyceraldehyde-3-phosphate; GGPP, geranylgeranyl diphosphate

极其重要的角色, 黑果枸杞中*LbCCD4*异常高表达被认为是其无法积累类胡萝卜素、最终呈现黑色的主要因素^[18]. 4°C贮藏条件下类胡萝卜素降解途径的关键基因*LbNCED6*、*LbCCD1*、*LbCCD4*表达量显著高于-4°C, 特别是*LbCCD4*在4和-4°C贮藏7 d表达量分别比新鲜采收时提高70和30倍(图4). 上述结果说明, 虽然4°C贮藏的枸杞果实类胡萝卜素合成基因表达量较高, 有利于类胡萝卜素的合成, 但类胡萝卜素存储基因的下调表达和降解基因的超高表达显著影响其积累. 同时, -4°C

条件枸杞类胡萝卜素的存储相关基因表达增加, 使得枸杞类胡萝卜素含量的增加有稳定的保障(图3).

天冬酰胺、丙氨酸和脯氨酸是枸杞果实中含量最高的3种氨基酸. 低温贮藏使脯氨酸合成相关的*LbP5CS*和*LbOAT*基因表达水平持续上调, 但维持脯氨酸降解相关的*LbProDH*在较低水平, 从而有利于脯氨酸的积累(图5); 4°C贮藏条件下*LbOAT*表达量始终显著高于-4°C, 但是*LbProDH*的表达量则极显著低于-4°C ($P<0.01$), 从而导致脯氨酸大量积累^[15]. 同时, 天冬氨

酸激酶基因*LbAK*的持续高表达则有利于天冬酰胺的合成和积累(图6)。在-4℃条件下,*LbC4H1*随着贮藏时间的延长逐渐升高(图7),与果实中苯丙氨酸含量的变化趋势一致。

综上所述,枸杞在采后贮藏过程中类胡萝卜素和氨基酸的组分和含量均发生动态变化,这些变化不仅受到内部的基因表达调控还受到外界温度的影响。4℃贮藏条件下枸杞果实类胡萝卜素裂解酶基因*LbCCD4*的超高表达和存储蛋白基因的低水平表达,可能是其

类胡萝卜素含量低于-4℃贮藏的主要原因。

结合贮藏过程中枸杞类胡萝卜素含量的变化认为,4℃贮藏7 d时,枸杞积累较多的玉米黄素、β-胡萝卜素和玉米黄素双棕榈酸酯,此时食用可能对人体更有益。-4℃低温贮藏则有利于延缓脯氨酸积累,并且-4℃贮藏14~21 d积累较多的玉米黄素、β-胡萝卜素和玉米黄素双棕榈酸酯。生产上可以根据设备和物流运输条件,采用4℃贮藏结合气调和包装等手段进一步改善鲜枸杞的外观品质和口感,从而满足鲜枸杞物流保鲜的技术需求。

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补充材料

图S1 不同贮藏温度鲜枸杞外观品质

表S1 基因qRT-PCR引物

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Summary for “贮藏温度对鲜枸杞类胡萝卜素和氨基酸的影响及调控机制”

Effects of storage temperature on carotenoids and amino acids in fresh Goji berry and the related regulation mechanism

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Fresh Goji berry has become more and more popular in recent years with consumers' increasing demand for nutritional, healthy, and natural food. However, freshly harvested Goji berry is highly perishable, deteriorating and quickly losing its commercial value in 1–2 d at room temperature due to its unique structural and biological characteristics (tender, hollow with thin pericarp and high water content). In order to optimize the storage conditions for fresh Goji berry and better retain its nutritional components, Goji berry was stored at 4 and –4°C for up to 21 d. The changes in fruit appearance, the evolution of the contents of zeaxanthin, β -carotene, and zeaxanthin dipalmitate, and the expressions of genes related to the synthesis, storage, and degradation of carotenoids and those related to the metabolism of amino acids were investigated in the present study. The results showed that the contents of zeaxanthin, β -carotene, and zeaxanthin dipalmitate were relatively higher in Goji berry stored at 4°C for 7 d or at –4°C for 21 d. As the most characteristic carotenoid in Goji berry, zeaxanthin dipalmitate was 42.14% higher at –4°C than at 4°C at the end of storage. The carotenoid synthesis genes were expressed at higher levels, while the carotenoid storage protein genes, *LbHSP21* and *LbOR2*, were expressed at significantly lower levels at 4°C. At the same time, the carotenoid cleavage enzyme genes, *LbNCED6*, *LbCCD1*, and *LbCCD4*, were expressed at significantly higher levels in Goji berry stored at 4°C. In particular, the expression level of *LbCCD4* on day 7 was 70 and 30 times higher than that on day 0 at 4 and –4°C, respectively, which may explain the lower carotenoids contents at 4°C by the end of storage.

The expressions of proline synthesis genes, *LbP5CS* and *LbOAT*, increased, while that of proline degradation gene *LbPRODH* declined during storage. The expression level of *LbOAT* was significantly higher, while that of *LbProDH* was significantly lower at 4 than at –4°C ($P < 0.01$), which might facilitate the accumulation of proline in Goji berry stored at 4°C. At the same time, the continuously higher expression of aspartate kinase gene *LbAK* was conducive to the synthesis and accumulation of asparagine. The expression of *LbC4H1* increased gradually during storage, which was consistent with the changing trend of phenylalanine content.

In conclusion, the contents of carotenoids and amino acids in Goji berry changed dynamically during postharvest storage, which were not only regulated by the internal gene expression but also affected by the external environment such as temperature. The results of the present study showed that Goji berry stored at –4°C had higher contents of carotenoids, a lower content of proline, and therefore, a better nutritional quality with postponed senescence and deterioration. The significantly higher expression of *LbCCD4* and lower expressions of carotenoid storage protein genes may be the main reason for the lower carotenoids contents at 4°C compared with at –4°C. The presented study preliminarily elucidated the possible molecular mechanism of why –4°C storage is more conducive to the accumulation of carotenoids and the delay of senescence and deterioration in Goji berry. This study provides new insights for the preservation and circulation of fresh Goji berry in the supply chain.

storage temperature, zeaxanthin dipalmitate, carotenoid, amino acid, metabolism, regulation

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