

石榴褐变、保鲜及生物活性物质研究进展

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摘要: 石榴风味独特,鲜嫩多汁,能够延缓衰老、预防疾病,深受大众喜爱。果实褐变是制约石榴贮藏期限和运输距离的突出问题,克服褐变、延长保鲜期是科研工作者努力的方向。石榴的皮、叶、汁、籽等富含生物活性物质,对肿瘤、糖尿病、心脑血管等疾病疗效显著,其含量测定、成分分析、提取工艺和抗氧化活性分析近年来得到深入研究和进展。笔者总结了国内外近5年来石榴褐变、保鲜及生物活性物质方面的研究和取得的成就,并对石榴今后果品保鲜、品质调控、良种选育和产业发展方向提出了建议。

关键词: 石榴;褐变;保鲜;生物活性物质

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Recent advances in the pomegranate fruit browning, storage and bioactive substances of the pomegranate

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Abstract: Pomegranate is loved by the public because of its fresh and juicy unique taste and health care function of delaying senility and preventing disease. Fruit browning is a severe problem that restricts the storage time and transportation distance of pomegranate, so overcoming browning and prolonging shelf life are the research directions of scientific researchers. Pomegranate is rich in bioactive substances such as skin, leaves, juice and seeds, which has excellent curative effect on tumor, diabetes, cardio cerebral vascular disease and so on. The analysis about content determination, component analysis, extraction process and antioxidant activity of bioactive substances have been further studied and developed in recent years. This article summarizes the research and achievements of browning, preservation and bioactive substances of pomegranate in recent five years, besides, it puts forward some suggestions for future preservation, quality control, breeding and industrial development of pomegranate.

Key words: Pomegranate; Browning; Storage; Bioactive substances

石榴(*Punica granatum* L.),为石榴科(Punicaceae)石榴属(*Punica* L.)落叶果树。石榴起源于中亚地区,在我国栽培历史悠久,汉代^[1]由使者传入我国,至唐代进入种植的全盛时期,清时期栽培生产技术趋于成熟,如今石榴栽培区域纵贯南北,温带和亚热带均有种植。石榴^[2]以其鲜嫩多汁的独特口感,喜庆祥和的深远寓意,花红似火的繁荣景象,获得国内外广大消费者的青睐。石榴的用途越来越广泛,石榴

皮、石榴汁、石榴籽中含有丰富的保健^[3]和药用^[4]成分,能有效对抗人体衰老,进行免疫调节,对肿瘤、糖尿病、心脑血管等疾病^[5]具有极好的疗效,在保健和医疗行业发挥着不可替代的重要作用。

石榴果实采后贮藏期间,果皮部分极易发生褐变,有时为部分,有时为整果,极大削弱了石榴的外观品质和商业价值,严重制约着石榴产业的发展。开展果实褐变原因和机制研究对推动整个石榴产业

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发展具有重要的意义。前人研究^[6]表明,植物果实的褐变是一种氧化反应,有酶促反应和非酶促反应两种类型。传统的减缓石榴褐变、保持果实新鲜的方法有低温、气调、化学药剂处理等,但易造成果实品质下降、贮藏期间易发生冷害、存在农药残留等问题。保鲜剂保鲜法,因不受地域、设备限制,操作简单高效,对人类和环境无害、环保^[7]、健康等优点受到越来越多的重视。保鲜剂的种类和配方因果品种类和保存目的不同而异。

石榴富含多种生物活性物质,包括鞣质类、酚酸类、单宁类、花色苷类、黄酮类等,使果实呈现绚丽多彩的颜色,并具有抗炎、抑菌、抗衰老、治疗多种慢性病等重要作用。石榴活性物质的提取、含量测定、成分确定和抗氧化活性分析,是国内外学者普遍研究的热点。石榴活性物质成分、含量及抗氧化活性,在不同品种、不同发育期^[8]、不同部位^[9]有不同表现和特点。

笔者总结了国内外学者近5年来石榴褐变、保鲜及生物活性物质方面的研究和取得的成就,并对石榴今后果品保鲜、品质调控、良种选育和产业发展方向提出建议。

1 石榴果实褐变影响因子与机制

石榴果实褐变为非呼吸跃变型,随着贮藏时间^[10]的延长,果皮迅速衰老、变软并发生褐变,外观品质下降,营养成分流失,严重制约石榴产业的发展。果皮褐变的本质^[11]是果皮内的酚类成分发生氧化:由氨基酸和糖类引起的美拉德等反应,为非酶促反应;由多酚氧化酶参与调节的褐变,为酶促反应。两种反应共同存在、同时影响果皮的褐变进程。

1.1 果实褐变影响因子

外部环境因素方面,果实采收方法、采收后水分含量(果皮水分和环境水分)、贮藏温度和时间、外压与创伤、保鲜剂的种类与用量等,都影响果皮褐变速率;内部生理方面,果皮褐变与酶的种类与活性^[12-13]、多酚的种类(鞣质类、酚酸类、花色素类、黄酮类)^[14]与含量^[15-16]以及膜结构^[17]等密切相关。

1.2 果实褐变机制

齐笑笑等^[6]发现‘白花玉石籽’石榴,采后果皮水分含量与褐变程度呈负相关;氨基酸态氮和还原糖通过非酶促反应途径影响褐变,在采后初期呈下降趋势,5-HMF作为反应产物,整个贮藏期间含量一

直增加;多酚氧化酶(PPO)活性采后呈逐渐下降趋势,与其参与酶促反应促进褐变有关,过氧化物酶(POD)和苯丙氨酸解氨酶(PAL)^[8]也参与酶促褐变;总酚含量,贮藏初期含量下降,后期急剧上升,是因为初期酶促反应消耗酚类的同时、破坏了果皮中氧化反应和还原反应平衡,致使酚类氧化产物发生累积;果皮褐变造成膜结构的破坏,底物与酶区域化^[9]遭到破坏,加剧了酶促反应,膜质过氧化产物丙二醛(MDA)随褐变加剧含量大幅增加;DPPH(1,1-二苯基-2-三硝基苯肼)自由基清除水平较低,表明果皮的抗氧化体系已被损坏。

1.3 果实褐变特点

果实褐变度因发育时期和品种而异。不同石榴品种^[8]之间,果皮褐变与相关生理生化指标之间的相关性表现各异,‘临选2号’、‘胭脂红’和‘泰山金红’比较抗褐变。在果实发育过程中,成熟期褐变度低于成熟前期;参与褐变反应的总酚含量、相关酶(POD、PPO和PAL)活性、DPPH自由基清除率随果实发育逐渐降低,均表明适当推迟采收时期,能有效减缓褐变;此外,‘泰山三白甜’的褐变度^[9]在各时间段均高于‘泰山红’。

2 石榴保鲜贮藏

2.1 果实保鲜常用技术及存在问题

果实保鲜常用技术包括:(1)低温技术:低温冷藏^[18-19]、气调贮藏(CA)^[20]等;(2)气调技术:臭氧保鲜技术、充氮保鲜技术、自发气调包装技术(MAP)^[21]等;(3)物理技术:辐照保鲜^[22]、超声波保鲜等;(4)化学药剂技术:杀菌剂、植物生长调节剂等;(5)涂膜保鲜:乙烯膜保鲜、乙酰水杨酸膜保鲜、壳聚糖膜保鲜、羧甲基纤维素钠(CMC)、茉莉酸甲酯、草酸、水杨酸等;(6)生物保鲜:微生物菌株、抗菌素类^[23]等;(7)复合保鲜:多种方法组合使用。

传统石榴保鲜方法存在的问题有:果实品质下降、风味寡淡、营养成分损失;贮藏期间易发生病害^[24]和冷害^[25]、籽粒变褐;农药残留或药剂毒性,药剂作用不均匀;气调成本高、操作复杂等。

2.2 涂膜保鲜剂的应用

2.2.1 涂膜保鲜剂原理和要求 气调法^[21]保存果实,成本较高,操作复杂。果实涂保鲜剂,是石榴延长贮藏期、保持品质的更好方法,其机制为在大分子液体涂在果实表面干燥后形成均匀半透膜,可以调

节膜内外的气体交换,使膜内成为低氧微环境,抑制脂膜过氧化、乙烯合成、酶促褐变、呼吸作用等,同时防止水分和物质损耗,减少病原菌侵染,保持果实品质良好。涂膜剂本身需无异味且安全、绿色、环保、无毒,与果皮接触后的产物也要对人体无害。

2.2.2 保鲜剂的使用与效果分析 张润光等^[7]研究出一种复合保鲜剂,能有效对抗石榴贮藏期腐烂软化、果皮和籽粒褐变、易生病害等情况,其中含有壳寡糖、柠檬酸、那他霉素等成分,先将石榴在低温下预冷 3 d,再浸入保鲜剂中 10~20 s,塑料袋独立包装,与对照相比,果实色泽、硬度、口感、气味等均表现良好,差异显著。杨雪梅等^[26]采前对‘泰山红’石榴分别用壳聚糖^[27]和石榴皮提取复合液进行涂膜,与对照相比,120 d 后石榴外观品质良好,腐烂率低,色彩鲜亮;涂膜使类黄酮和总酚等活性物质含量保持在较高水平,多酚氧化酶活性峰值出现时间推迟并增高;在贮藏前期,可降低石榴籽粒可滴定酸含量、提高可溶性固形物含量;两种涂膜保鲜效果均良好,复合膜效果更佳。冯立娟等^[28]研究发现,2%柠檬酸处理石榴果皮,能够缓解果实褐变,抑制褐变相关酶活性,使石榴活性物质的抗氧化活性维持在较高水平。杨宗渠等^[29]先用 1000 倍液的杀菌剂浸果 60 s,再用 1%的壳聚糖进行涂膜,聚乙烯袋单果独立包装,贮藏温度设为 6 ℃,120 d 后发现 96.54%的石榴外观品质和籽粒品质均表现良好,褐变指数在 0.15 以下,质量损失率约为 2%。

3 生物活性物质提取工艺与检测方法

石榴活性成分,种类繁多,包括鞣质类、酚酸类、单宁类、花色苷类、黄酮类等,它们使植物呈现多彩的颜色,并具有极强的抗氧化^[30]、抗炎和抑菌^[31]能力,对延缓衰老、预防和治疗慢性病(肿瘤、糖尿病、心血管病等)起到重要作用^[32]。石榴叶片、果皮、石榴汁、石榴籽等多个部位均富含活性物质。

3.1 生物活性物质提取工艺的改进

活性物质的提取方法^[33]有微波辅助提取、生物酶解提取、超临界流体萃取和超高压辅助提取等方法。王占一等^[34]在石榴籽原花青素的提取过程中,首次采用了超声波辅助溶剂法,该法环境友好、操作简单、快速高效,有效避免了提取过程中活性成分因氧化而丧失功效。闫林林等^[35]首次将亚临界水提取法应用于石榴叶总酚的提取过程,提取含量明显增

加,提取时间也大大缩短。邓娜等^[36]通过响应面试验^[37]优化并筛选出高效的石榴皮多酚提取条件:以 40%乙醇为提取介质,60 ℃、250 W 超声波浸提 39.3 min,提取多酚量为 $(1.13\pm 0.26)\text{mg}\cdot\text{g}^{-1}$;为获得经济实用、高效清洁的提取方法。黄菊等^[38]以响应面法结合单因素试验,采用微波辅助提取法,得到石榴皮黄酮类的最佳工艺:乙醇 60%体积比,提取 2.7 min,提取率 $(4.424\pm 0.168)\%$,与模型预测 4.523%相吻合。

3.2 生物活性物质检测方法

生物活性物质的检测分析方法^[39]包含光谱和色谱两种途径。王常喜等^[40]建立的超高效液相色谱-串联四级杆质谱(UPLC-QQQMS)内标法,可以同时检测新鲜石榴、中药石榴和全石榴的皮和籽中诃子裂酸、没食子酸和五没食子酰葡萄糖等 7 种成分,并针对含量差异进行统计分析,为食品质量控制监测方法提供了理论依据。冯立娟等^[41]将 HPLC 法进行优化,以快速准确地测定叶片中鞣花酸、莽草酸和没食子酸等的含量;刘振平等^[42]采用 RP-HPLC 法建立了适合石榴汁中安石榴苷和没食子酸等 4 种酸的精密检测方法。王琼等^[43]和王晓琴等^[44]应用近红外光谱(NIR)检测技术,筛选出最优数学模型,实现了多酚类物质检测的高效、环保和无损,对石榴科研和生产具有重要意义。

4 生物活性物质定性定量及抗氧化能力分析

4.1 活性物质定性定量分析

冯立娟等^[45]以全国 4 个石榴主栽区的 8 个品种为试材,发现酚类物质含量从高到低依次为果皮>果汁>种子;果皮酚类主要成分为安石榴苷,也是石榴果实中酚类主要来源,占 48.94%;果汁酚类主要成分为花青苷;鞣花酸、总黄酮、安石榴苷、花青苷和总酚间互为正相关。韩玲玲等^[46]发现石榴汁、石榴籽、果皮中多酚含量品种间差异显著,各品种果皮中多酚含量均最高,主要成分为儿茶素、表儿茶素和对羟基苯甲酸。刘安成等^[47]在石榴果实中发现了 10 种花青苷,其中芍药花素 3-己糖苷和飞燕草素 3-戊糖苷为首次检出;花青苷主要存在于石榴果皮和果肉中。冯立娟等^[41]首次以石榴叶片为试材,研究安石榴苷及鞣花酸、莽草酸和没食子酸等 5 种合成相关物质在营养生长期内的含量变化,发现差异性和相关性均显著;没食子酸的合成与 3-脱氢莽草酸、莽

草酸密切相关;五没食子酰葡萄糖为没食子酸代谢产物,推测安石榴苷的合成与其有关;安石榴苷降解后生成鞣花酸。以上研究为调控石榴果实品质、提示石榴生长发育库源关系、良种选育等,奠定了理论基础。

4.2 活性物质抗氧化能力分析

石榴的药用^[48]和保健^[49]功能,体现了其活性物质具有极强的抗氧化能力。石榴各部位的抗氧化活性差异显著,邓娜等^[36]通过比较石榴不同部位羟自由基、DPPH 自由基和总还原能力,发现石榴皮的抗氧化能力最强,石榴籽次之,果肉最弱;抗氧化活性在人工模拟胃液中升高,在模拟肠液中会降低,说明活性物质在不同pH值环境和蛋白酶作用下,结构和功能会发生不同的变化:苷类水解为苷元时抗氧化力提高,酚类水解为没食子酸等小分子时抗氧化力提高减弱。冯立娟等^[45]发现我国不同产区不同品种的石榴中鞣花酸、总黄酮、安石榴苷、花青苷含量与DPPH 自由基清除率均呈显著正相关;果皮的抗氧化能力最强;‘千籽红’‘喀什甜’和‘红玛瑙’是全国石榴品种中保健功能最突出的品种。徐云凤等^[50]发现石榴皮单宁由鞣花酸、没食子酸和安石榴苷等组成,安石榴苷为主要成分,也是发挥抑菌作用的主要物质。黄菊等^[38]对石榴皮中提取的黄酮类物质进行自由基清除力试验,发现半抑制浓度值 $IC_{50} = 0.187 \text{ g} \cdot \text{L}^{-1}$,表明其抗氧化能力是非常强的。

5 展 望

石榴果实褐变表现与机制因种类、品种及发育期不同而呈多样性,根据每种石榴本身的褐变特点,建立对应的保鲜贮藏方法,是一项具有长期性、艰巨性和必要性的工作。分子生物学的蓬勃发展,将带动学者们在分子水平上揭示石榴果皮褐变机制,通过基因改良调控石榴褐变相关基因,将是今后石榴褐变的研究重点。石榴保鲜贮藏过程中,容易出现外观品质良好但内部籽粒变褐和风味品质下降的问题,目前尚无这方面的系统研究,应是以后着重研究的方向。石榴低温保鲜容易发生冷害,且受设备限制,深入研究简便高效、成本经济的常温保鲜技术,对石榴的贮存、运输和销售具有重要的意义。石榴保鲜剂应减少或杜绝有害化学物质的使用,研制环保、绿色、健康和生态的保鲜剂,是食品和化工行业的重要工作。加强生物活性物质合成与代谢方面的

研究,结合基因调控技术,是果实品质调控与良种选育方面的工作重点。继续改进石榴活性物质的提取工艺,获得回收率高、纯度高的活性物质,开发新的生物活性物质,对充分发挥石榴在医药、保健领域的抗肿瘤、抗炎和抑菌等作用,具有极其重要的意义。

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