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采后NO处理提高冷藏期间板栗果实抗病相关酶活性

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摘 要:【目的】探究采后NO处理对冷藏期间板栗果实抗病相关酶活性变化的影响。**【方法】**以‘金优2号’板栗果实为材料,采用外源NO供体硝普钠(SNP)溶液浸泡处理果实,观察处理对冷藏条件下板栗果实腐烂率的影响,分析测定SNP处理后果实抗病相关酶、果胶酶活性及丙二醛含量等贮藏期间生理指标的变化。**【结果】**与对照相比,SNP处理能有效地降低冷藏期间板栗果实腐烂率,贮藏180 d时,SNP处理果实腐烂率为18.89%,显著低于对照,为对照的43.60%;生理指标测定结果表明,SNP处理不同程度地提高了板栗果实贮藏期间超氧化物歧化酶、过氧化氢酶、过氧化物酶、几丁质酶和β-1,3-葡聚糖酶等抗病相关酶活性,降低了贮藏期果胶酶和多酚氧化酶活性,减少了果实丙二醛的积累。**【结论】**NO采后处理可提高板栗果实冷藏期间抗病相关酶活性,抑制果实PG酶和PPO酶活性,减少果实MDA的积累,进而降低板栗果实的腐烂率。

关键词:板栗;一氧化氮;采后;抗病相关酶

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Effect of postharvest nitric oxide treatment on the activities of disease-resistant related enzymes in chestnuts during cold-storage

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Abstract:【Objective】Chestnut (*Castanea mollissima* BL.) is well known for its nutritional value and pleasant flavor. Chestnut fruits usually ripe in late summer when temperatures are still higher, so that the processes of water loss, microbial attack and decay are accelerated after harvest. As a result, the fresh nut has a short shelf life, losing marketability within a few days at ambient temperatures. Cold storage could delay ripening and improve the resistance of nuts to pathogens effectively, but the nut qualities decline obviously after storage. Nitro oxide (NO) is a signaling molecule in plant and animal tissues, and has an important function of regulating postharvest physiology of fruits. It has been reported that NO treatment could reduce decay and extend storage life of various fruits. According to the current results, sodium nitroprusside (SNP, NO donor) treatment could reduce the decay of chestnut. The study aimed to investigate the effects of postharvest NO treatment on the activities of disease-resistant related enzymes of chestnut during low temperature storage.【Methods】‘Jinyou 2’ nuts at mature stage were used as the material. The fruits were harvested from an orchard in Luotian county of Hubei province and immediately transferred to laboratory on the same day. Nuts with uniform size, free of visible injuries and defects, were selected and treated with 0.4 mmol·L^{-1} SNP solution for 5 minutes, while water treatment was used as the control. After air-dried and pre-cooling, all the nuts were packed and stored at cold temperatures (0-3 ℃) with 80%-85% relative humidity for 180 days. The rot rate of nuts was investigated every 20 d, and meanwhile pulp samples were stored in a ultra-low temperature freez-

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er after liquid nitrogen freezing. The physiological indexes were measured during storage. The indexes included malondialdehyde (MDA) content and the activities of peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), polygalacturonase (PG), chitinase (CHT) and β-1,3-glucanase (GLU). Microsoft Excel 2007 software was used to calculate the mean and standard deviation. The SAS 8.0 software was used to analyse the significance of difference.【Results】The decay rate of nuts increased significantly during the long period storage. Compared with the control, SNP treatment effectively inhibited the rot of nuts. After 180 days of storage, the rot rate of SNP treatment was 18.89%, which was just 43.6% of the control. During the whole storage period, the activities of POD and CAT increased slightly at the beginning and then decreased, but SOD activity kept increasing until the 100th day and then decreased. Compared with the control, SNP treatment kept a higher POD activity remarkably during the storage, and the POD activity was 12.32% higher than the control at 120th day. SNP treatment also kept a higher SOD activity significantly, which was 9.17% higher than the control at 160th day. The CAT activity of SNP treatment was 40.19% higher than the control after 180 days of storage. The PPO activities kept increasing until the 120th day and then decreased, SNP treatment inhibited the PPO activity, and the activity was lower than the control significantly from day 60 to 120. The PPO activity of SNP treatment was 75.43% of the control at day 60, and 73.33% at day 120. The PG activity kept increasing during the storage, SNP treatment kept a lower PG activity significantly, which was 74.71% of the control at day 60, and 84.54% at day 140. The CHT activities increased to the maximum at 80th day, and then decreased. SNP treatment enhanced the CHT activity obviously, the maximum value was 16.10% higher than the control at 80th day, and the activity was 46.85% higher than the control at 120th day. The GLU activity was also enhanced by SNP treatment, especially from day 60 to 120. The GLU activity of SNP treatment was 27.14% higher than the control at day 60, and 16.34% at day 180. The MDA content in nuts increased during the storage, and SNP treatment decreased the MDA content significantly. After 180 days of storage, the MDA content with the SNP treatment was 84.26% of the control.【Conclusion】Postharvest NO treatment might enhance the activities of disease-resistant related enzymes in chestnut. Under low temperature conditions, postharvest SNP treatment increased the activities of SOD, CAT, POD, CHT and GLU, reduced MDA content and the activities of PG and PPO, and then effectively inhibited the rot of nuts.

Key words: Chestnut; Nitric oxide; Postharvest; Disease-resistant related enzymes

板栗(*Castanea mollissima* BL.)是我国特有的栗属(*Castanea*)植物,在大部分省份均有产业化栽培,果实风味突出,保健功能显著,经济价值高。但板栗果实成熟期较为集中,采后贮藏保鲜难度大,贮运过程中容易腐烂,增加了鲜果的销售压力,限制了鲜果供应期和板栗加工制品的生产,制约了板栗生产效益的提高。低温是影响板栗果实品质的主要因素,冷藏能有效延长板栗果实的贮藏寿命[1],是目前板栗生产中广泛应用的贮藏方式,但随着冷藏时间的延长,果实品质下降明显且贮后果实货架期短。采后处理是提高果实贮藏保鲜性能的重要环节,开展相关研究对提升板栗果实贮藏品质、促进板栗生产的健康发展具有重要的作用。

一氧化氮(nitric oxide,NO)是一种广泛存在于动植物各组织中的具有生物活性的气体分子,自1980年被证明是血管内皮释放的可扩散物质后,就成为研究热点。直到科学家1988年发现NO是一种植物中的信号分子,NO的功能的地位才重新被人们所认知[2]。研究表明,NO对动植物体内的免疫反应、生理功能、信号转导、细胞凋亡、防御反应及酶调节等过程都起着重要作用[3-4]。Leshem等[5]1996年首次报道了经济作物的内源NO的产生以及作用,提出了以NO作为果实成熟过程中的调控因子,进一步研究发现,NO可以影响果实中乙烯的生物合成,从而抑制果实的成熟和衰老[6]。随着研究的深入,NO对果实的保鲜研究日益受到关注,被应用于多

种果实的采后处理，大量研究证明，用适当浓度的NO处理，可调节果实采后多个生理代谢途径，从而有效延长果实的贮藏寿命[7-8]。笔者实验室前期工作也发现，NO处理能明显地抑制贮藏期板栗果实的腐烂，推断NO可能对板栗果实采后生理代谢有一定的调节作用，而目前有关NO应用于板栗果实采后处理的研究较少，尚不清楚NO对板栗果实采后生理代谢的影响。笔者以‘金优2号’（*Castanea mollissima* ‘Jinyou 2’）板栗果实为试材，以硝普钠（sodium nitroprusside，SNP）为外源NO供体用于板栗果实采后处理，初步探讨采后NO处理对板栗果实冷藏期间抗病相关酶等生理指标的影响，为NO处理在板栗果实贮藏保鲜中的应用和相关基础研究提供理论依据。

1　材料和方法

1.1　材料及处理

试验材料为本单位从湖北省罗田县板栗主产区实生优株中选育出的大果形品种‘金优2号’板栗，果实采自罗田县平湖乡黄家湾村金玉家庭农场板栗品种示范园内，高接换种后14 a（年）生板栗树，种植密度4 m×4 m。在前期试验中，研究了不同浓度SNP处理对板栗果实贮藏保鲜效果的影响，确定0.4 mmol·L^{-1}处理保鲜效果最佳，并应用于本试验。

试验用板栗果实于完熟时采收，当天运抵实验室，挑选无机械伤、无病虫害、中等大小的果实用于低温贮藏试验，贮藏温度为0~3℃、环境相对湿度80%~85%。试验设置2组处理，分别为0.4 mmol·L^{-1} SNP溶液和清水（CK），浸泡时间5 min。处理后的果实自然晾干，打孔保鲜袋包装，入库贮藏。每组处理果实10 kg，设置3个生物学重复，冷藏前取样1次，之后每20 d取样1次，每个重复取样的果实数量为30个。统计腐烂率后，将栗仁经液氮速冻后保存于−80 ℃冰箱，用于果实生理指标测定。

1.2　指标测定

1.2.1　*腐烂率的测定*　十字法切开果实，栗仁出现明显病斑的果实为腐烂果，腐烂率为腐烂果个数占总果数的百分率。

1.2.2　*过氧化物酶（peroxidase，POD）、超氧化物歧化酶（superoxide dismutase，SOD）和过氧化氢酶（catalase，CAT）活性的测定*　取样品1 g，加入50 mmol·L^{-1} pH 7.0的磷酸缓冲液（内含1% PVP），冰浴下研磨成匀浆，分四次洗入10 mL离心管中，在4 ℃下10 000 r·min^{-1}离心30 min，取上清液作为粗酶提取液用于测定酶活性。POD活性采用愈创木酚法测定，SOD活性采用氮蓝四唑（NBT）法[9]测定。CAT活性采用过氧化氢法[10]测定。

1.2.3　*丙二醛（malondialdehyde，MDA）含量的测定*　样品处理同1.2.2，MDA含量采用硫代巴比妥酸（TBA）法[11]测定。

1.2.4　*多酚氧化酶（polyphenol oxidase，PPO）活性的测定*　称取2 g果实样品，加入0.2 mol·L^{-1}磷酸缓冲液（pH 6.5），研磨后转入10 mL离心管中，5 000×*g*下4 ℃离心10 min，取上清液用于PPO酶活性测定。酶活性测定反应体系和方法采用邻苯二酚法[12]。

1.2.5　*果胶酶（polygalacturonase，PG）活性的测定*　称取10.0 g果实样品，加入预冷的95%乙醇，在冰浴条件下研磨匀浆后，4 ℃低温放置10 min，于4 ℃、9 000 r·min^{-1}离心15 min。弃去上清液，在沉淀中加入预冷的80%乙醇，低温放置10 min，离心，弃去上清液。在沉淀中加入预冷的提取缓冲液，于4 ℃放置20 min，同条件下离心，上清液即为酶提取液。酶活性测定反应体系和方法采用比色法[13]。

1.2.6　*几丁质酶（chitinase，CHT）活性的测定*　取液氮研磨样品0.5 g，加入预冷的柠檬酸-磷酸氢二钠缓冲液（pH 6.7），涡旋后于4 ℃冷冻离心机12 000 *g*离心20 min，取上清液测定酶活性。酶活性测定反应体系和具体方法参照刘普[14]的方法。

1.2.7　*β-1，3-葡聚糖酶（β-1,3-glucanase，GLU）活性的测定*　取液氮研磨样品0.5 g，加入4 ℃预冷的柠檬酸-磷酸氢二钠缓冲液（pH 4.8），充分涡旋振荡后于4 ℃冷冻离心机12 000 *g*离心20 min，取上清液测定酶活性。酶活性测定反应体系和具体方法参照刘普[14]的方法。

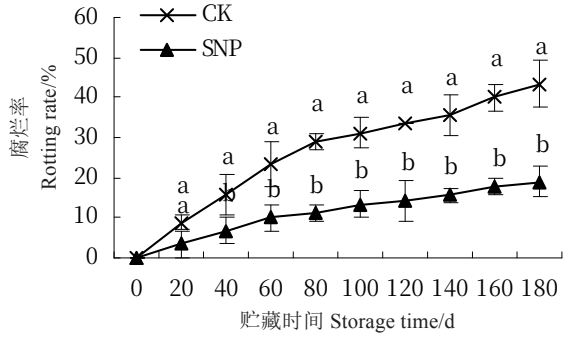
1.3　数据分析

试验数据用Microsoft Excel软件进行初步处理，利用数据统计软件SAS 8.0进行差异显著性分析。

2　结果与分析

2.1　SNP处理对板栗果实采后腐烂率的影响

贮藏期间，板栗果实腐烂率逐渐升高（图1）。对照果实腐烂率上升速率较快，贮藏180 d时果实腐烂率为43.33%。SNP处理明显抑制了果实的腐烂，



不同小写字母表示同一时间不同处理在α= 0.05 水平差异显著。下同。

Different small letters indicate significant difference at α = 0.05 between different treatments at the same time. The same below.

图 1 SNP 处理对板栗果实冷藏期腐烂率的影响

Fig. 1 Effect of SNP treatment on rotting rate of chestnut fruit during cold storage

呈较为平缓的上升趋势，贮藏期间果实腐烂率显著低于对照，贮藏 180 d时，果实腐烂率为18.89%，为对照的43.6%。

2.2 SNP处理对板栗果实贮藏期POD、SOD、CAT酶活性的影响

板栗果实3种酶活性在贮藏期间的变化趋势见图2~图4。对照板栗果实POD酶活性在贮藏初期略有上升，随后逐渐下降；SOD酶活性贮藏后先缓慢上升，至100 d之后呈下降趋势；CAT酶活性在贮藏期间先上升再下降。

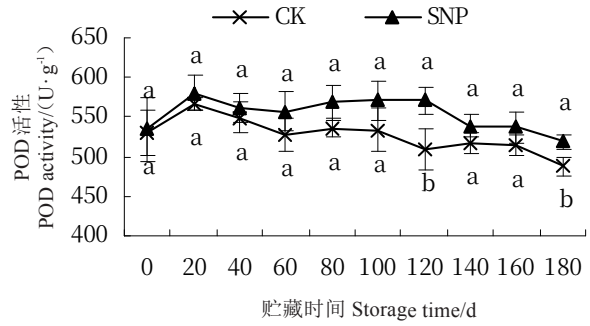


图 2 SNP 处理对冷藏板栗果实 POD 活性的影响

Fig. 2 Effect of SNP treatment on POD activity in chestnut fruit during cold storage

与对照相比，SNP处理对3种酶活性表现出不同程度的增强效果。SNP处理延缓了果实POD酶活性的下降趋势，贮藏期间保持了较高的POD酶活性，贮藏120 d和180 d时的酶活性显著高于对照，贮藏120 d时SNP处理果实POD酶活性比对照高12.32%。SNP处理果实SOD酶活性在贮藏100 d后

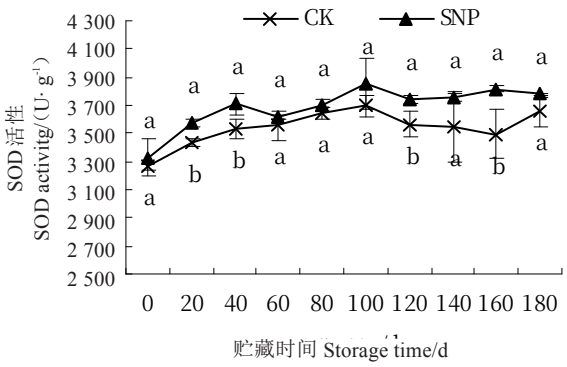


图 3 SNP 处理对冷藏板栗果实 SOD 活性的影响

Fig. 3 Effect of SNP treatment on SOD activity in chestnut fruit during cold storage

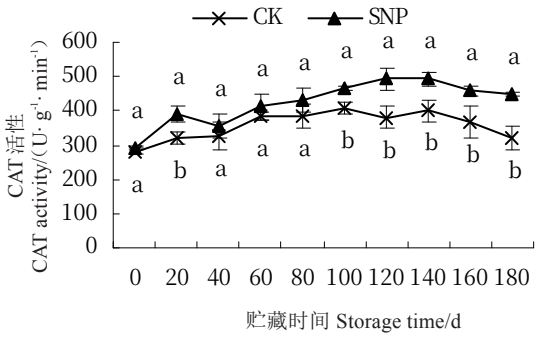


图 4 SNP 处理对冷藏板栗果实 CAT 活性的影响

Fig. 4 Effect of SNP treatment on CAT activity in chestnut fruit during cold storage

仍保持缓慢上升趋势，SOD酶活性显著高于对照，贮藏160 d时SOD酶活性比对照高9.17%（图3）。SNP处理还提高了整个贮藏期间果实的CAT活性，表现出差异显著，贮藏180 d时，CAT酶活性比对照高40.19%（图4）。

2.3 SNP对板栗果实贮藏期PPO活性的影响

对照板栗果实PPO酶活性在贮藏期间先上升，至120 d时达最大值，之后开始下降，SNP处理抑制了果实PPO酶活性的上升，保持了较低的PPO酶活性，在果实贮藏60~120 d期间显著低于对照，贮藏60 d和120 d时的PPO酶活性分别为对照的75.43%和73.33%（图5）。

2.4 SNP对板栗果实贮藏期PG活性的影响

随着板栗贮藏时间的延长，果实PG酶活性逐渐上升（图6）。与对照相比，SNP处理明显地抑制了果实PG酶活性的上升，在果实贮藏0~140 d时，PG酶活性上升较为缓慢，PG酶活性显著低于对照，贮藏60 d和140 d时酶活性分别为对照的74.71%和84.54%。

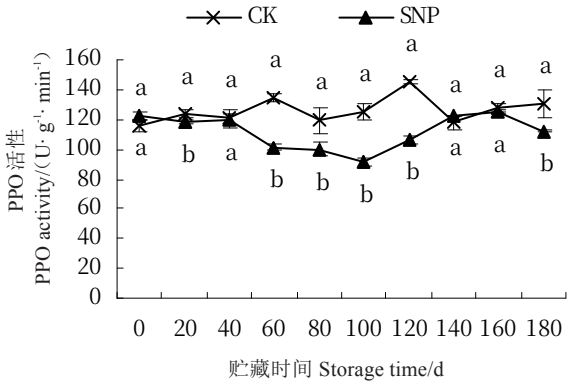


图5　SNP处理对冷藏板栗果实PPO酶活性的影响

Fig. 5　Effect of SNP treatment on PPO activity in chestnut fruit during cold storage

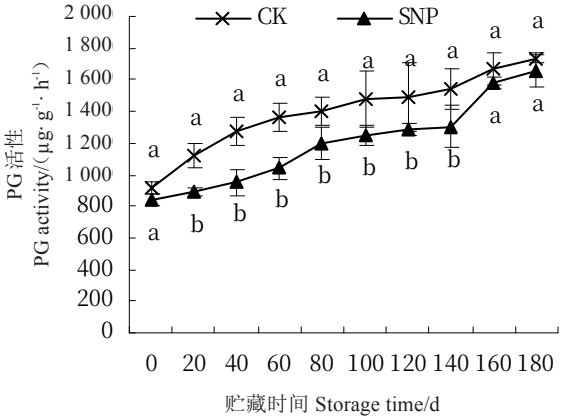


图6　SNP处理对冷藏板栗果实PG酶活性的影响

Fig. 6　Effect of SNP treatment on PG activity in chestnut fruit during cold storage

2.5　SNP对板栗果实贮藏期CHT酶和GLU活性的影响

果实贮藏期间2个处理CHT酶活性变化均呈先上升后下降的趋势，在贮藏80 d时达到最大值。SNP处理明显地提高了果实CHT酶活性，贮藏80 d时的最大值显著高于对照，比对照高16.10%，贮藏120 d时差异最大，果实CHT酶活性比对照高46.85%（图7）。

果实2个处理GLU活性在贮藏期间均呈S型上升趋势，对照果实贮藏160 d后活性略有下降。与对照相比，SNP处理果实在贮藏60~120 d期间保持了较高的酶活性，并表现出显著差异，贮藏60 d和180 d时酶活性分别比对照高27.14%和16.34%（图8）。

2.6　SNP对板栗果实贮藏期MDA含量的影响

板栗果实MDA含量随着贮藏时间的延长呈逐渐上升的趋势（图9），SNP处理与对照果实保持较为缓慢的上升趋势，差异不大，但贮藏180 d时，SNP处理果实MDA含量为对照的84.26%。

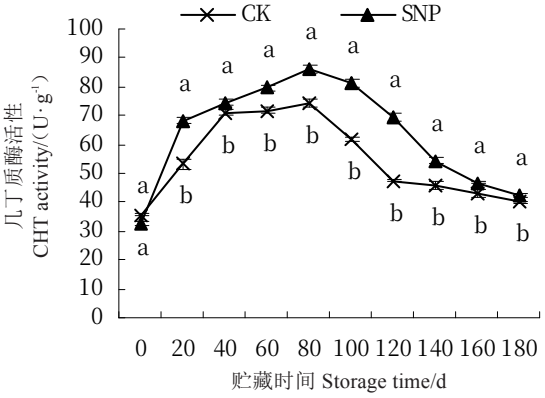


图7　SNP处理对冷藏板栗果实CHT酶活性的影响

Fig. 7　Effect of SNP treatment on chitinase activity in chestnut fruit during cold storage

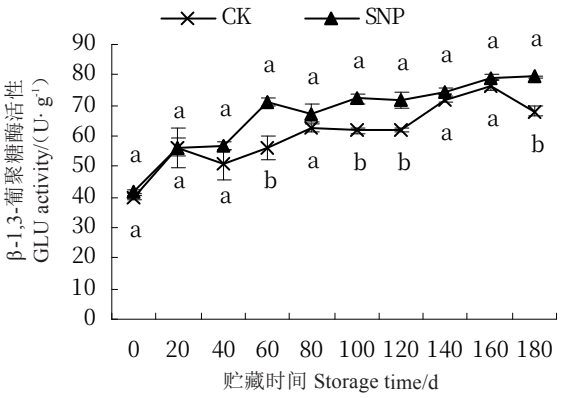


图8　SNP处理对冷藏板栗果实GLU酶活性的影响

Fig. 8　Effect of SNP treatment on β-1,3-glucanase activity in chestnut fruit during cold storage

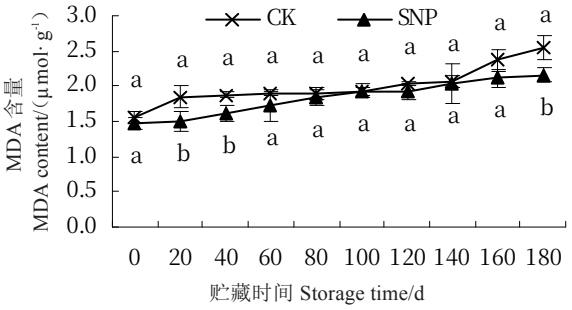


图9　SNP处理对冷藏板栗果实MDA含量的影响

Fig. 9　Effect of SNP treatment on MDA content in chestnut fruit during cold storage

3　讨　论

外源NO在植物果实采后保鲜中具有重要作用，可以有效延长果实的储存时间[15-16]，并提高果蔬采后贮藏品质[17]。本试验中，SNP处理明显地抑制

了冷藏期间板栗果实的腐烂，贮藏180 d时果实腐烂率为对照的43.60%，表明NO能应用于板栗果实的贮藏保鲜及相关研究。

POD、CAT和SOD是植物体内重要的抗氧化酶，三者协同作用以维持植物体内活性氧的正常代谢[18]，这些酶的活性变化反映了果实的活性氧清除能力。已有研究表明，NO可以提高植物抗氧化酶系统中的酶活，提高植物抗氧化能力，从而延缓衰老[19]。Wu等[20]研究了NO处理对香蕉果实采后生理的影响，结果表明，NO处理能提高香蕉果实贮藏期间SOD、CAT、POD等抗氧化酶活性及相关基因的表达，从而增强香蕉果实的抗冷性。洪克前等[21]研究了SNP处理对杧果采后生理的作用，表明SNP处理提高了杧果组织SOD、CAT和POD活性，降低了H_2O_2含量，进而降低了MDA对细胞膜的伤害。本试验中，采后SNP处理不同程度上提高了板栗果实贮藏期间SOD、CAT、POD等抗氧化酶活性，降低了板栗果实贮藏期MDA的积累；SNP对CAT酶活性影响较大，贮藏180 d时，CAT酶活性为对照的1.4倍，对POD酶活性影响较小，仅在贮藏60 d和120 d时差异显著。

PPO是在板栗仁酶促褐变中起主导作用的酶类[12]。相关研究表明，SNP处理能抑制果实采后PPO酶活性的上升[21-22]，延迟或抑制果实酶促褐变的发生[22-23]。PG被认为是在控制果实软化的过程中起关键作用的酶[24]，贮藏期间，随着PG活性逐步提高，果实硬度逐步下降[25]。NO能降低‘鸭梨’果实冷藏期PG酶活性，延缓果实PG酶活性峰值，从而延缓果实的软化和成熟[26]。本试验中，SNP处理改变了板栗果实贮藏期PPO酶活性的变化趋势，保持了较低的PPO酶活性。此外，SNP处理还明显地抑制了板栗果实贮藏0~140 d时PG酶活性的上升，从而维持了较低PG酶活性。

CHT和GLU是2类重要的病程相关蛋白，在寄主抵御病原菌侵染过程中起着重要作用[27]。Hu等[28]研究了外源NO对杧果果实炭疽病的效果和可能的作用机制，结果表明，NO处理增强了POD、GLU、CHT等果实防御相关酶的活性，延缓了果实的软化、变黄等采后生理进程，从而增强了果实的抗性，延缓果实衰老。本试验中，SNP处理明显地提高了贮藏期间板栗果实CHT和GLU活性，从而增强了果实的抗病性。

NO通过调节果实采后生理代谢、增强果实抗性，抑制或缓解果蔬贮藏过程中衰老生理进程[29-30]。板栗是典型的呼吸跃变型果实，采后生理代谢旺盛。本研究仅从SNP处理对果实抗病相关酶活性的影响进行了探索，结果表明，SNP处理提高了板栗果实采后抗病相关酶活性，抑制了果实结构软化和褐变相关酶的活性，其对板栗果实采后的其他生理代谢的影响以及相关调控机制有待进一步研究。

4 结 论

NO不同程度地提高了板栗果实贮藏期间SOD、CAT、POD、CHT和GLU等抗病相关酶的活性，同时明显抑制了贮藏期果实PG和PPO酶活性，减少了果实MDA的积累，进而延缓板栗果实的衰老，有效地抑制了冷藏期间板栗果实的腐烂。因此，采后NO处理能够应用于板栗果实采后贮藏保鲜及相关理论研究。

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