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甜樱桃果实发育过程中细胞壁组分及其降解酶活性的变化

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摘 要:【目的】了解甜樱桃在果实发育过程中质地变化与果实细胞壁组分及其降解酶活性的关系。**【方法】**以硬肉型品种‘美早’、常规型品种‘红灯’和软肉型品种‘佳红’为试材,分别在硬核期、转白期、着色期和成熟期对果实硬度、细胞壁组分以及细胞壁降解酶活性进行了测定分析。**【结果】**‘美早’硬度降低速率较慢,成熟期硬度高于其他2个品种,WSP升高速率、纤维素降解速率低,PME、α-L-Af、Cx、β-Gal活性低。‘红灯’硬度降低速率较快,在果实发育后期硬度低于‘美早’,WSP升高速率与纤维素降解速率高,PME、α-L-Af活性高。‘佳红’在转白期硬度迅速降低且后期质地软,它的纤维素降解速率高,PME、α-L-Af、Cx、β-Gal在转白期之后活性较高。**【结论】**甜樱桃果实成熟过程中,原果胶的降解和纤维素的水解是果实软化的关键因素。果实细胞壁组分降解是多种酶协同作用的结果。PME和α-L-Af与‘红灯’和‘佳红’硬度显著负相关,并且活性在‘美早’中显著低于其他2个品种,这可能是果实硬度较高的主要原因。纤维素和原果胶降解速率低,PG活性高和β-Gal活性低可能是导致硬度高的次要原因。Cx酶活由于在‘红灯’中并没有显著影响到到果实硬度,而在‘佳红’和‘美早’中产生了不同的影响,可能是品种间的差别。

关键词:甜樱桃;硬度;细胞壁组分;细胞壁降解酶

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A study on the variation of cell wall components and activities of their degradation enzymes in sweet cherry during fruit development

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Abstract:【Objective】The study was conducted to understand the relationship between the texture changes of sweet cherry and changes in cell wall components and their degrading enzyme activities during fruit ripening.【Methods】Sweet cherry trees of three cultivars, including the hard meat- type ‘Tieton’, the conventional type ‘Hongdeng’ and the soft meat-type ‘Jiahong’, cultivated in a 12-year-old open orchard were used as the experiment materials. Samples were collected during core hardening, color- break (whitening), coloring, and full maturity to determine the fruit firmness, cell wall components, and the activities of cell wall degrading enzyme.【Results】‘Hongdeng’ fruit was harder than the other two varieties in the early stage of fruit development, but the range of decline was large during the later stages. ‘Jiahong’ had the lowest hardness after color-break. With the development of the fruit, the hardness difference between the three varieties gradually decreased, and there was no significant difference between the them at full maturity, but the fruit hardness of ‘Tieton’ was higher than the other two varieties. The WSP content of the three varieties gradually increased from core hardening to color-break, and decreased or remained stable after the coloring period. The WSP content in ‘Hongdeng’ increased from the core hardening stage to the coloring stage (189.49%), while ‘Tieton’ WSP content in-

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creased by 43.60%. The WSP content of the three varieties was negatively correlated with hardness. The ISP content in the three varieties decreased first and then increased, rapid decreases in both ISP content and hardness were found during color-break. The ISP content peaked during core-hardening, after which the content decreased significantly in the three cultivars. During the fruit development, the difference gradually decreased. The CSP content in ‘Tieton’ and ‘Hongdeng’ decreased after color-break, while that in ‘Jiahong’ declined after the coloring period. Among them, ‘Hongdeng’ had the highest rate of decline (45.08%) with an increase towards full maturity. The change pattern was opposite to that of WSP. As the fruit matured, cellulose content in ‘Hongdeng’ and ‘Jiahong’ was significantly reduced, which was significantly lower than that in ‘Tieton’ in all periods. For ‘Tieton’, there was no significant change in cellulose content from core hardening to fruit coloring. Although its cellulose content dropped significantly during maturation, it was still significantly higher than that in the other two varieties. The hemicellulose content of the three varieties showed a gradual decline during fruit development, and ‘Tieton’ and ‘Hongdeng’ had a higher content than that in ‘Jiahong’. From color-break to the coloring period, hemicellulose content gradually decreased till full maturity when it had no significant difference. The activity of PG of the three varieties increased at the beginning, then remained stable or slightly decreased. The increase rate of PG activity in ‘Tieton’ was the highest (110.56%), and it peaked during coloration in ‘Tieton’ and ‘Hongdeng’. During the coloration and maturation periods, the activity of PG in ‘Tieton’, ‘Hongdeng’ and ‘Jiahong’ decreased in turn and differed significantly. PME activity peaked during core Ihardening. ‘Hongdeng’ had a significantly higher PME activity than ‘Tieton’ and ‘Jiahong’. ‘Jiahong’ showed no significant difference in PME activity during the four stages, while in the ‘Hongdeng’, a significant decrease occurred from core hardening to color break. In ‘Tieton’, the activity of PME decreased significantly from core hardening stage to coloration stage. The PL activity in the three varieties fluctuated with time and had no correlation with fruit firmness. The α-L-Af activity in the three varieties was the lowest during core hardening, and ‘Tieton’ had a significantly higher α-L-Af activity than the other two varieties. During fruit development, the activity of α-L-Af in ‘Tieton’ was the smallest, however, the rate of rise in ‘Jiahong’ was the largest. The activity of the enzyme in ‘Hongdeng’ rose to 392 $nmol \cdot g^{-1} \cdot min^{-1}$ during the maturation period, which was significantly higher than those in the other two varieties. The β-Gal activity of the three varieties was very low in the core hardening stage, and then, there was an upward trend. Among them, ‘Tieton’ had a lowest rate of increase, ‘Hongdeng’ had a highest rate of increase. In ‘Jiahong’, a significant increase in enzyme activity was found and then the activity maintained high. The Cx activity of the three varieties showed an upward trend, but the growth rate in ‘Tieton’ and ‘Jiahong’ was higher than that in ‘Hongdeng’. The difference in fruit development gradually increased. During the maturation period, the activity of this enzyme in ‘Jiahong’ continued to rise and became significantly higher than that in ‘Tieton’ and ‘Hongdeng’. During the fruit development of ‘Tieton’, the change in hardness was significantly positively correlated with Cx. For ‘Hongdeng’, the change in hardness was positively correlated with the change in hemicellulose and cellulose contents, but the activities of α-L-Af, β-Gal and PME were significantly negatively correlated. During the development of ‘Jiahong’ fruit, fruit hardness was significantly negatively correlated with WSP content and the activities of Cx, α-L-Af, and PME. 【Conclusion】During the ripening of sweet cherry fruit, the degradation of protopectin and the hydrolysis of cellulose are the key factors for fruit softening. The degradation of fruit cell wall components is the result of a synergistic effect of multiple enzymes. The lower activities of PME and α-L-Af in ‘Tieton’ than that of the other two varieties might be the main reason for its higher fruit hardness. The low degrada-

tion rates of cellulose and protopectin, high PG activity and low β-Gal activity might be secondary causes for its high hardness. The Cx enzyme seems to have different effects in 'Jiahong' and 'Tieton' and seems not significantly affect the fruit hardness in 'Hongdeng', which might be the difference between these varieties.

Key words: Sweet cherry; Hardness; Cell wall components; Cell wall degradation enzymes

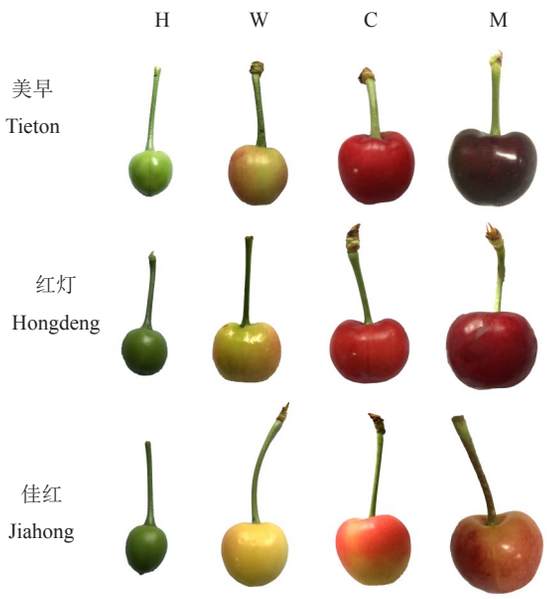
甜樱桃(*Prunus avium* L.)在果实成熟过程中会伴随着质地软化、硬度降低，直接影响果实的品质及其商品性，甚至关系到整个产业的发展[1]。与甜樱桃果实质地相关的研究主要集中在采后保鲜贮藏方面，并且在近几年的研究中发现，果实前期发育过程中细胞壁组分以及果胶含量与果实的质地有着密切的关系[2-3]，而果实质地软化会影响果实的运输、贮藏、销售等诸多方面。随着经济全球化的发展，甜樱桃的大量进口影响着我国甜樱桃产业的发展，尤其对我国甜樱桃果实品质、冷链运输以及贮藏保鲜提出了更高要求[4]。如需解决上述问题，既要选育耐贮品种，还需对现有主栽品种果实质地形成与变化规律等诸多方面进行深入、系统的研究。

随着对果实发育机制研究的逐渐深入，发现果实成熟过程中质地的变化是细胞壁结构、组分改变以及内部多糖降解的结果[5]。细胞壁的降解过程有多种酶的参与，研究其与果实质地软化的互作关系，有助于弄清果实质地形成的本质，为果实品质改良和品质调控提供理论依据，对培育耐贮新品种、提高果实品质和经济价值具有重要的意义。近年，国内外学者从细胞壁结构、组分及细胞壁降解相关酶活性等方面入手，对果实成熟过程中的质地变化机理进行了大量研究，并且在苹果[6]、桃[7-8]、杏[9]、李[10]等一些经济树木果实的研究上取得了一部分研究成果，但对甜樱桃果实发育过程中的质地形成机理研究甚少。本文通过对3种不同质地的甜樱桃在发育过程中的果实硬度、细胞壁组分以及细胞壁降解酶活性变化的分析，旨在探究甜樱桃果实成熟过程中的软化机理，为甜樱桃优质品种的选育以及进一步提高果实的商品性提供更多的理论依据。

1　材料和方法

1.1　试验材料

供试甜樱桃果实于2018年5月取于烟台市农业科学研究院，选择12 a(年)生露地栽培的甜樱桃果树，以硬肉型品种'美早'、常规型品种'红灯'和软肉型品种'佳红'为试材，根据不同品种甜樱桃果实成熟特性适时采收，在硬核期、转白期、着色期、成熟期分别采大小均匀一致、无病虫伤的果实2 kg，如图1所示，放置在冰盒中，迅速带回实验室，测定果实硬度以及细胞壁组分。同时将一部分样品去核切块，放入液氮罐中冷冻，在-40 ℃冰箱内保存，用于细胞壁降解相关酶活性的测定。



H. 硬核期；W. 转白期；C. 着色期；M. 成熟期。下同。

H. Core hardening period; W. Whitening period; C. Coloring period; M. Maturation stage. The same below.

图1　甜樱桃果实发育过程中外观的变化

Fig. 1　Changes in appearance of sweet cherry during fruit devetopmect

1.2　试验方法

1.2.1　果实硬度测定　采收当天，每个品种、每个时期随机选取20个无损伤的果实，擦除表面灰尘后，用质构仪测定每个甜樱桃硬度，每个果实测定2次，单位为N。

1.2.2　果实细胞壁物质的提取、分离及含量测定　细胞壁物质的提取和分离参考Brummell[11]的方法略加改进。称量0.3 g果肉样本，加1 mL 80%乙醇，室温快速匀浆，95 ℃水浴20 min，冷却至室温，

4 000 *g* 25 ℃离心10 min，弃上清，沉淀加入1.5 mL 80%乙醇和丙酮各洗2遍（漩涡振荡2 min左右，4 000 *g* 25 ℃离心10 min，弃上清），沉淀为粗细胞壁，后用1 mL 90%的二甲亚砜（去除淀粉）浸泡15 h，4 000 *g* 25 ℃离心10 min，弃上清，将沉淀物放于烘箱干燥，称重即得细胞壁物质（Cell wall material，CWM）。称取烘干的CWM 3 mg，按以下步骤依次提取不同成分：用1 mL 50 mmol·L^{-1}的乙酸钠（pH 6.5）提取得到水溶性果胶（Water soluble pectin，WSP）；用1 mL 50 mmol·L^{-1} CDTA和乙酸钠（pH 6.5）提取离心得到离子型果胶（Ionic pectin，ISP）；用1 mL 50 mmol·L^{-1}的Na_2CO_3（含2 mmol·L^{-1} CDTA）提取得到共价结合果胶（Covalent pectin，CSP）。果胶含量采用咔唑法测定[12]，半纤维素和纤维素含量采用蒽酮法测定[13]。

1.2.3 *果实细胞壁降解酶活性测定* 细胞壁代谢相关酶的提取参照曹建康等[14]的方法进行。DNS比色法测定多聚半乳糖醛酶（Polygalactosidase，PG）、纤维素酶（Cellulase，Cx）、果胶甲酯酶（Pectin methylesterase，PME）的活性；对硝基半乳糖苷水解法测定*α*-阿拉伯呋喃糖苷酶（Alpha-arabinofuranosidase，*α*-L-Af）、*β*-半乳糖苷酶（*β*-Galactosidase，*β*-Gal）的活性。PL酶活性测定参照Pitt[15]的方法，略有修改。

1.3 结果计算

试验数据采用Excel 2010软件进行统计，采用SPSS 22.0软件进行相关性分析，Duncan新复极差法检验差异显著性。

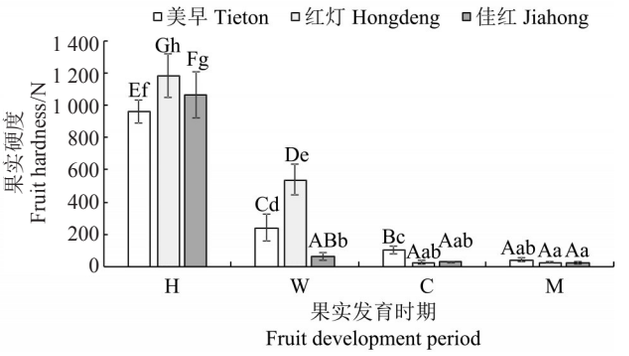
2 结果与分析

2.1 果实硬度的变化

果实硬度是果实质地的重要表现。由图2可知，在甜樱桃果实发育过程中，3个甜樱桃品种的果实硬度均呈逐渐降低趋势。转白期与硬核期相比，‘红灯’‘美早’和‘佳红’的果实硬度均显著降低。随着果实的发育，硬度差异逐渐减小。在着色期，‘美早’极显著高于其他2个品种，到成熟期3个品种间无显著差异，但‘美早’果实硬度高于其他2个品种。

2.2 果实细胞壁组分的变化

2.2.1 *果胶含量* 果实发育过程中，细胞壁中果胶由不溶的原果胶状态降解变为WSP。WSP含量的升高和CSP、ISP含量的降低直接引起质地变化[16]。



柱上不同的小写字母表示处理之间在 $p < 0.05$ 水平上存在显著差异；不同的大写字母表示处理之间在 $p < 0.01$ 水平上存在极显著差异。下同。

The different lowercase letters above the column indicate a significant difference between the treatments at the $p < 0.05$ level; different uppercase letters indicate a very significant difference between the treatments at the $p < 0.01$ level. The same below.

图2 果实发育过程中硬度的变化

Fig. 2 Changes in hardness during fruit development

图3所示，3个品种的WSP含量在硬核期到转白期均逐渐升高，到着色期后下降或保持稳定。其中，‘红灯’的WSP含量从硬核期到着色期的升高速率最大（189.49%），而‘美早’的WSP含量升高速率最小（43.60%），且3个品种的WSP含量与硬度的变化趋势负相关。图4可以看出，3个品种的ISP含量均先下降后上升，且ISP迅速降低的时期与硬度迅速降低的时期均为转白期。ISP在硬核期含量最高，此时‘美早’‘红灯’和‘佳红’含量依次极显著降低。在果实发育过程中，差异逐渐减小，到成熟期时，3个品种的含量再次升高，并与硬核时期表现出相同的显著性差异。由图5可知，3个品种的CSP含量在转白期之前变化不大，‘美早’和‘红灯’在转白期之后下降，‘佳红’在着色期后下降。其中‘红灯’的下

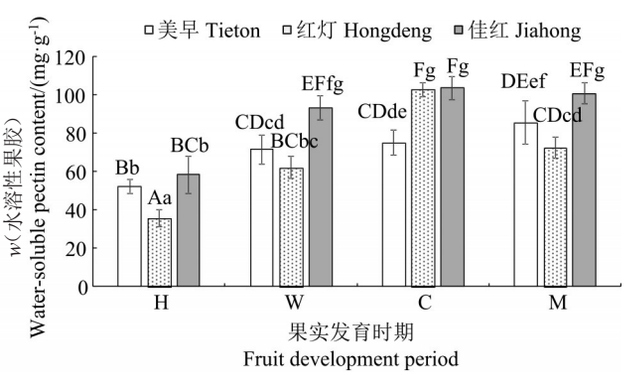


图3 果实发育过程中水溶性果胶含量的变化

Fig. 3 Changes in water-soluble pectin during fruit development

降速率最大（45.08%），且成熟期有升高趋势，与其WSP变化趋势相反。

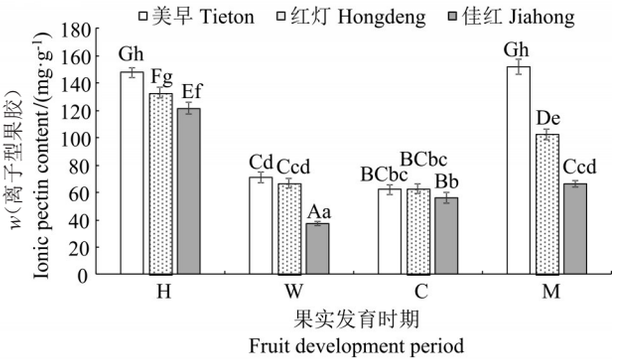


图4　果实发育过程中离子型果胶含量的变化

Fig. 4　Changes in ionic pectin content during fruit development

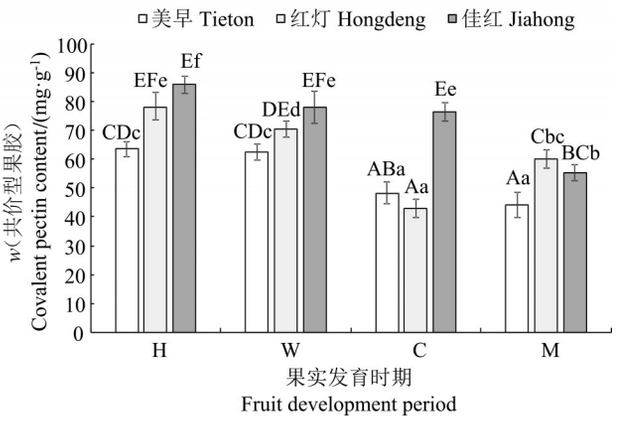


图5　果实发育过程中共价型果胶含量的变化

Fig. 5　Changes in covalent pectin content during fruit development

2.2.2　纤维素与半纤维素含量　如图6所示，在硬核期3个品种间的纤维素含量无显著性差异。随着果实的成熟，‘红灯’和‘佳红’含量极显著降低，且在各时期均极显著低于‘美早’。‘美早’从硬核期到着色期无显著性变化，到成熟期显著下降，但仍显著高于其他两个品种。而3个品种的半纤维素含量

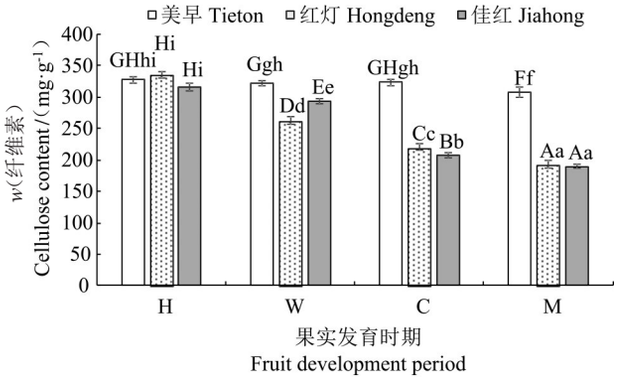


图6　果实发育过程中纤维素含量的变化

Fig. 6　Changes in cellulose content during fruit development

在果实发育过程中呈现逐渐下降的趋势，‘美早’和‘红灯’降幅大于‘佳红’（图7）。而从转白期到着色期均表现出较大降幅，随着果实成熟，含量逐渐降低，成熟期3个品种含量基本持平，无显著性差异。

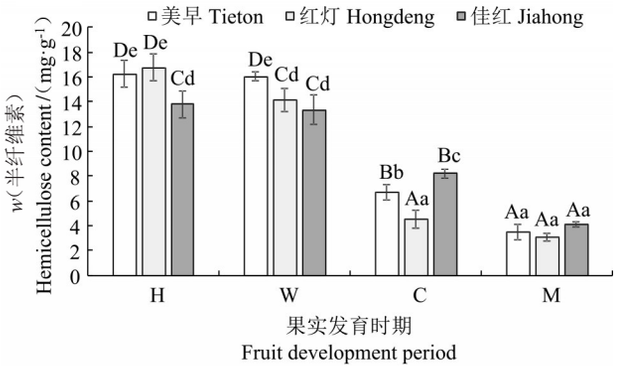


图7　果实发育过程中半纤维素含量的变化

Fig. 7　Changes in hemicellulose content during fruit development

2.3　细胞壁降解酶含量的变化

PG可以促进果胶物质的降解，导致果实质地变软[17]。图8所示，3个品种的PG活性呈现先升高，后保持稳定或降低的变化趋势，‘美早’和‘红灯’在着色期达到峰值。在硬核期，‘红灯’和‘美早’极显著高于‘佳红’。‘美早’PG活性上升速率最大（110.56%），‘佳红’从转白期到成熟期PG活性保持稳定。在着色期和成熟期，‘美早’‘红灯’和‘佳红’PG活性依次降低，并且达到极显著水平。

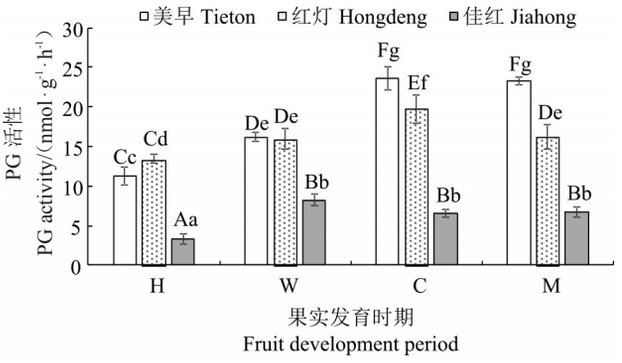


图8　果实发育过程中PG活性的变化

Fig. 8　Changes in PG activity during fruit development

PME可以催化果胶酯酸转化为果胶酸，为PG的作用提供更多的底物[17]。在甜樱桃果实发育过程中，PME的催化活性表现出了不同的变化模式（图9）。‘佳红’在果实发育4个阶段中PME活性均未出现显著差异，而在‘红灯’中，仅硬核期到转白期出现了明显的活性降低。对于‘美早’，PME的活性从硬核期到着色期显著降低，而成熟期与着色期活性无显著差异。3个试验材料中，PME均在硬核期活

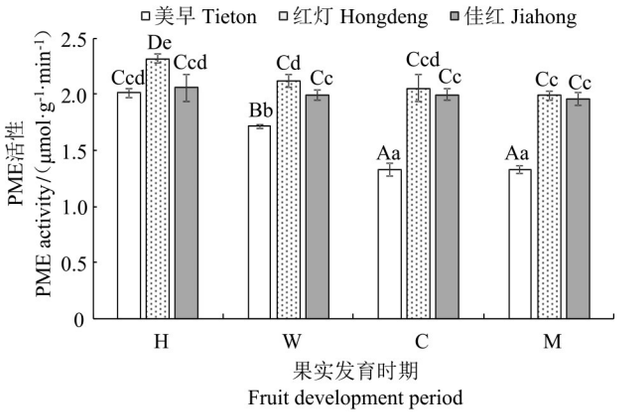


图9 果实发育过程中PME活性的变化

Fig. 9 Changes in PME activity during fruit development

性最高，且‘红灯’显著高于‘美早’和‘佳红’。

PL通过β-消除裂解去酯化果胶能降解植物细胞壁[18]。由图10可知，3个品种的PL活性表现出降低、升高再降低的变化趋势。在硬核期，‘美早’和‘红灯’极显著高于‘佳红’。转白期均无显著性差异。着色期‘美早’和‘佳红’均极显著高于‘红灯’。成熟期‘红灯’和‘佳红’均极显著低于‘美早’。

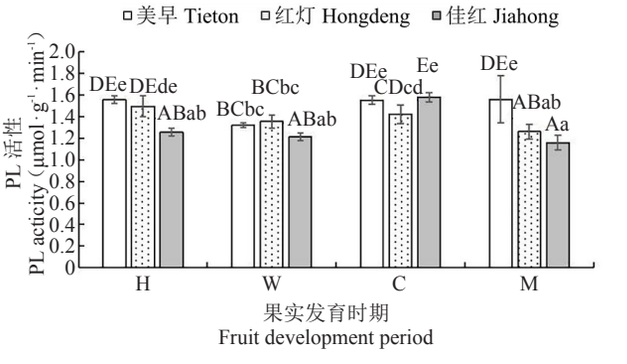


图10 果实发育过程中PL活性的变化

Fig. 10 Changes in PL activity during fruit development

α-L-Af能够促进细胞壁多糖降解，导致细胞壁结构松弛，引起果实软化[6]。图11所示的酶活变化

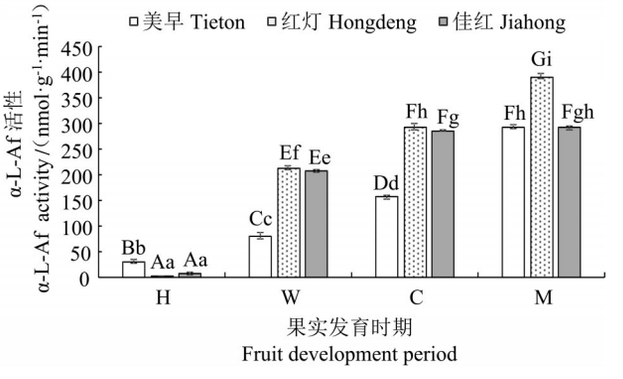


图11 果实发育过程中α-L-Af活性的变化

Fig. 11 Changes in α-L-Af activity during fruit development

中，3个品种的α-L-Af活性均在硬核期活性最低，并且‘美早’极显著高于另外2个品种。在果实发育过程中，‘美早’活性上升速率最小，‘佳红’上升速率最大。在成熟期，‘红灯’上升至392 nmol·min^{-1}·g^{-1}，极显著高于其他2个品种。

β-Gal主要通过降解果胶多聚醛酸，切除半乳糖苷键，从而促使果胶物质降解，果胶-纤维素网络松动，最终导致果实软化[19]。由图12可知，3个品种的β-Gal活性均在硬核期活性很低，之后有上升趋势。其中‘美早’上升速率较低，‘红灯’上升速率较高，‘佳红’也表现出显著上升且一直保持高活性。在转白期和着色期，‘美早’‘红灯’和‘佳红’依次极显著升高。到成熟期，‘红灯’和‘佳红’活性很高，极显著高于‘美早’。

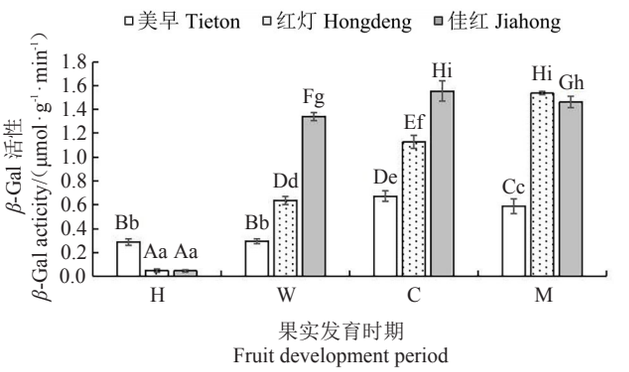


图12 果实发育过程中β-Gal活性的变化

Fig. 12 Changes in β-Gal activity during fruit development

Cx能够降解纤维素，导致细胞壁中结构松散，促进果胶质的分解，加速果实的成熟与软化[18]。由图13可知，3个品种的Cx活性基本呈现上升趋势，但‘美早’和‘佳红’上升速率高于‘红灯’，果实发育过程中差异逐渐加大。硬核期3个品种无显著性差

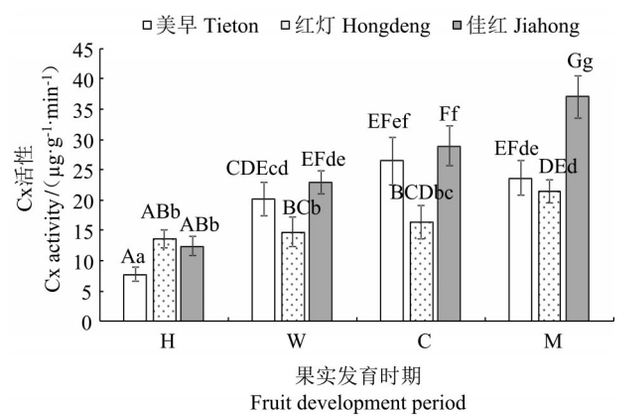


图13 果实发育过程中Cx活性的变化

Fig. 13 Changes in Cx activity during fruit development

异。转白期和着色期‘美早’和‘佳红’极显著高于‘红灯’。成熟期，‘佳红’活性继续上升，极显著高于‘美早’和‘红灯’。

2.4　甜樱桃果实发育过程中细胞壁组分及其降解酶活性的相关性分析

表1、2和3的研究结果表明，‘美早’果实发育期间，硬度的变化与Cx活性显著正相关。‘红灯’果实发育期间，硬度的变化与半纤维素、纤维素的含量变化显著正相关，与α-L-Af、β-Gal、PME的活性显著负相关。‘佳红’果实发育期间，硬度的变化与WSP含量，Cx、α-L-Af和PME活性显著负相关。

表1　‘佳红’果实发育过程中细胞壁组分及其降解酶活性的相关性

Table 1　Correlations between cell wall components and degradation enzyme activities during fruit development in ‘Jiahong’

指标 Index	硬度 Hardness	PG	Cx	α-L-Af	β-Gal	PL	PME
硬度 Hardness	-	0.603	-0.988*	-0.959*	-0.540	-0.183	-0.975*
半纤维素 Hemicellulose	0.594	-0.267	-0.927	-0.765	-0.171	0.049	0.746
纤维素 Cellulose	0.708	-0.375	-0.935	-0.866	-0.401	-0.288	0.760
WSP	-0.984*	0.834	0.885	0.993**	0.817	0.290	0.908
ISP	0.931	0.989*	0.600	-0.808	0.897	-0.133	-0.794
CSP	0.621	-0.417	-0.914	-0.718	0.089	0.334	-0.851

注：*显著线性相关，**极显著线性相关。下同。

Note: * Significant linear correlation, ** extremely significant linear correlation. The same below.

表2　‘红灯’果实发育过程中细胞壁组分及其降解酶活性的相关性

Table 2　Correlations between cell wall components and their degrading enzyme activities during fruit development in ‘Hongdeng’

指标 Index	硬度 Hardness	PG	Cx	α-L-Af	β-Gal	PL	PME
硬度 Hardness	-	-0.825	-0.778	-0.958*	-0.966*	0.678	-0.961*
半纤维素 Hemicellulose	0.966*	-0.729	-0.847	-0.908	-0.956*	0.626	0.895
纤维素 Cellulose	0.979*	-0.717	-0.849	-0.997**	-0.991**	0.815	0.995**
WSP	-0.893	0.990**	0.419	0.755	0.747	-0.328	0.783
ISP	0.598	-0.827	-0.048	-0.551	-0.446	0.295	0.607
CSP	0.876	-0.966*	-0.434	-0.707	-0.729	0.246	-0.727

表3　‘美早’果实发育过程中细胞壁组分及其降解酶活性的相关性

Table 3　Correlations between cell wall components and their degrading enzyme activities during the fruit development period in ‘Tieton’

指标 Index	硬度 Hardness	PG	Cx	α-L-Af	β-Gal	PL	PME
硬度 Hardness	-	-0.909	0.971*	-0.756	-0.707	0.147	0.922
半纤维素 Hemicellulose	0.725	-0.921	0.832	-0.938	-0.933	-0.561	0.916
纤维素 Cellulose	0.659	-0.640	0.809	-0.931	0.477	0.157	0.568
WSP	-0.642	0.672	-0.803	0.956*	0.546	0.268	0.686
ISP	0.427	-0.266	0.202	0.231	-0.162	0.529	0.266
CSP	0.743	-0.934	0.842	-0.932	-0.943	-0.545	0.929

3　讨　论

3.1　细胞壁组分分析

细胞壁结构和成分的改变是引起果实质地变化的主要原因[20]。果实成熟软化期间，原果胶的降解，以及纤维素的水解是导致果实质地变化的关键因素[21]。原果胶向WSP的转变，可促使初生壁溶解、胞间层降解、细胞壁结构破坏、引起果实软化[22]。胡留申等[7]研究表明硬度大、贮藏性好的桃品种果实，通常原果胶降解速率低，WSP含量上升慢。阚娟等[23]也发现，桃果实成熟中WSP含量越高，软化速度越快。本试验研究发现，果实成熟过程中，‘美早’与‘红灯’果实的WSP含量持续低于‘佳红’，且‘美早’的WSP上升速率较低，导致了三者之间质地差异，进一步的验证了果胶成分在果实软化中的作用。有研究表示纤维素的变化并不是果实质地的变化的关键因素[24]。但李萍[9]对新疆杏的研究发现，杏成熟过程中，纤维素含量逐渐降低，并且易软化的果实降解速率更高。焦云等[25]研究发现，硬度大的杨梅纤维素含量更高。本试验中3个品种的纤维素含量在硬核期无显著性差异，到成熟期，‘红灯’和‘佳红’含量持平，均极显著低于‘美早’。易软化的品种‘佳红’和常规性品种‘红灯’纤维素降解速率很高，而硬肉型品种‘美早’的纤维素含量一直保持在较高水平，由此得出纤维素的水解可以促进微纤丝的降解，从而导致细胞壁结构瓦解。纤维素降解速率不同是导致果实质地差异的另一大因素，进一步的验证了纤维素成分在果实软化中的作用。研究

表明，果实成熟过程中半纤维素的含量变化不大，但分子量明显变小[26]。本试验中3个甜樱桃品种在果实发育过程中半纤维素含量逐渐下降，但其含量高低与降解速率与三者质地差异之间无明显规律。由此推测半纤维素结构的改变可能导致果实质地变化。

3.2 细胞壁降解酶分析

果实细胞壁组分降解是多种酶共同作用的结果[27]。PG通过催化果胶分子的多聚半乳糖醛酸裂解而参与果胶的降解，进而促进果实软化[17]。本试验结果表明，PG在不同品种间的活性存在很大差异，但均可以在果实发育前期启动果实成熟，促进果实硬度降低。赵胜锦[28]研究发现PG酶可能对促进樱桃成熟中后期细胞壁多糖水解、降低果实硬度起到关键作用。但本试验相关性表明，果实硬度的变化与PG无显著相关性，可能是由于细胞壁半乳糖醛酸聚糖必须先经PME去酯化才能被PG所酶解，PG的作用受PME影响很大。

PME可以催化果胶酯酸转化为果胶酸，与PG协同发生作用[17]。周厚成[29]的研究表明在草莓成熟软化过程中，软质品种‘丰香’果实PME酶活性始终高于硬质品种‘甜查理’，PME能提高果实软化速率，间接促进果实软化。本试验表明，PME的产生导致果实在成熟软化阶段酸性果胶含量增加，从而进一步促进其他果胶酶水解去酯化果胶，加速果实软化，从而导致‘红灯’‘佳红’与‘美早’的果实软化速率依次降低，两试验研究结果一致。

α-L-Af能够促进细胞壁结构松散，引起果实质地变化[6]。金昌海等[30]对桃果实的研究发现，α-L-Af活性增加时期与果实硬度下降时期都集中在果实成熟的中后期。本试验研究表明α-L-Af与乙烯的积累时间一致，作用于果实发育中后期，并且活性升高时期与果实硬度降低时期相同。‘红灯’的α-L-Af在各时期的的活性很高，可能导致乙烯积累与果实软化速率高于其他两个品种。活性最低的‘美早’，硬度降低最慢。进一步验证了α-L-Af的活性是导致质地变化的一个因素。

β-Gal溶解细胞壁多糖，从而使细胞组分变得不稳定，促使细胞壁膨胀而软化[19]。本试验研究表明，硬核期β-Gal活性很低，转白期活性迅速升高，果实硬度迅速下降，‘美早’中β-Gal活性最低，降解速率也最低，导致果实硬度降低慢，而‘红灯’和‘佳红’的β-Gal活性高，可以加快果胶物质的降解，导致果实硬度降低较快，证实了β-Gal的作用。

Cx降低细胞壁黏性，从而降低果实硬度，在不同树种甚至不同组织上的软化机理都有明显差别[18]。已有研究证明，几乎所有的果实种类在成熟进程纤维素酶活性都表现增加[31]。薛炳烨等[32]的研究表明随着果实成熟，纤维素酶活性不断升高，软质品种纤维素酶活性明显高于硬质品种。在本试验中，3个甜樱桃品种Cx活性随着果实成熟，基本呈现上升趋势，但与果实硬度变化趋势不完全对应，因此推测纤维素酶对核果类果实软化有一定影响。但受品种间的差别、发挥作用的时期以及其他降解酶的影响，未来可做深入研究。

本试验结果表明，这3个甜樱桃品种的细胞壁组分降解以及相关酶活性的变化与果实质地密切相关。果实成熟软化期间，原果胶的降解，以及纤维素的水解均会导致果实质地软化。细胞壁降解酶中，PME活性越高，会使PG更好的促进果胶物质的降解。β-Gal和α-L-Af在硬核期不发挥作用，在果实发育中后期活性升高，利于多种水解酶与底物的接触，促进多糖降解，果实硬度下降。

WSP升高速率与纤维素降解速率低，PME、α-L-Af、Cx、β-Gal活性低，是导致‘美早’硬度降低速率较慢，成熟期硬度高于其他两个品种的原因。相对的，WSP升高速率与纤维素降解速率高，PME、α-L-Af活性高，是导致‘红灯’硬度降低速率较快，在果实发育后期硬度低于‘美早’的原因。而纤维素降解速率高，PME、α-L-Af、Cx、β-Gal在转白期之后活性较高，是导致‘佳红’在转白期硬度迅速降低且保持质地软的主要原因。后期可深入研究细胞壁组分和相关基因表达方面的相关关系，为甜樱桃果实发育成熟的分子机制研究提供理论依据。

4 结 论

甜樱桃果实成熟软化期间，原果胶的降解，以及纤维素的水解是导致果实质地变化的关键因素。果实细胞壁组分降解是多种酶共同作用的结果。PME、α-L-Af活性在‘美早’中显著低于其他2个品种，这可能是果实硬度较高的主要原因。至于Cx、β-Gal酶活的影响，由于在‘红灯’中并没有显著影响到过果实硬度，而在‘佳红’和‘美早’中产生了影响，这个有可能是次要原因。

参考文献 References：

[1] 焦中高, 刘杰超, 王思新 . 甜樱桃采后生理与贮藏保鲜[J]. 果树学报, 2003, 20 (6): 498-502.
JIAO Zhonggao, LIU Jiechao, WANG Sixin. Advances of research on postharvest physiology and storage technology of sweet cherry[J]. Journal of Fruit Science, 2003, 20 (6): 498-502.

[2] RAFFO M D, PONCE N M A, SOZZI G O, STORTZ C A, VICENTE A R. Changes on the cell wall composition of tree-ripened ‘Bartlett’ pears (*Pyrus communis* L.)[J]. Postharvest Biology and Technology, 2012, 73: 72-79.

[3] GWANPUA S G, VAN BUGGENHOUT S, VERLINDEN B E, CHRISTIAENS S, SHPIGELMAN A, VICENT V, KERMANI Z J, NICOLAI B M, HENDRICKX M, GEERAERD A. Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of Jonagold apples[J]. Food Chemistry, 2014, 158: 283-291.

[4] 崔建潮, 王文辉, 贾晓辉, 王志华, 佟伟 . 从国内外甜樱桃生产现状看国内甜樱桃产业存在的问题及发展对策[J]. 果树学报, 2017, 34(5): 620-631.
CUI Jianchao, WANG Wenhui, JIA Xiaohui, WANG Zhihua, TONG Wei. The domestic industry problems from the sweet cherry import situation and its development countermeasure[J]. Journal of Fruit Science, 2017, 34(5): 620-631.

[5] CORNUAULT V, POSE S, KNOX J P. Extraction, texture analysis and polysaccharide epitope mapping data of sequential extracts of strawberry, apple, tomato and aubergine fruit parenchyma[J]. Data in Brief, 2018, 17: 314-320.

[6] 魏建梅 . 苹果（*Malus domestica* Borkh.）果实质地品质发育及采后调控的生理和分子基础[D]. 杨凌: 西北农林科技大学, 2009.
WEI Jianmei. Study on physiological and molecular mechanism of fruit texture development and post-harvest regulation of apple (*Malus domestica* Borkh.) [D]. Yangling: Northwest A&F University, 2009.

[7] 胡留申, 董晓颖, 李培环, 王永章, 刘成连, 原永兵 . 桃果实成熟前后细胞壁成分和降解酶活性的变化及其与果实硬度的关系[J]. 植物生理学通讯, 2007, 43(5): 837-841.
HU Liushen, DONG Xiaoying, LI Peihuan, WANG Yongzhang, LIU Chenglian, YUAN Yongbing. Changes in cell wall components and degrading enzyme activity and their relation to hardness in fruits of peach(*Prunus pesrica* L.)before and after ripening[J]. Plant Physiology Journal, 2007, 43(5): 837-841.

[8] 阎香言, 张熠可, 李福瑞, 徐泽, 郑继成, 赵彩平 . 不同质地桃果实软化过程中细胞壁组分变化的差异 [J]. 北方园艺, 2017 (20): 60-66.
YAN Xiangyan, ZHANG Yike, LI Furui, XU Ze, ZHENG Jicheng, ZHAO Caiping. Differences in cell wall composition during softening of peach fruit with different flesh texture[J]. Northern Horticulture, 2017(20): 60-66.

[9] 李萍 . 新疆杏果实发育期及采后生理生化机理研究[D]. 乌鲁木齐: 新疆农业大学, 2013.
LI Ping. Studies on physiological and biochemical mechanism of Xinjiang apricot fruit during the period of developmental and storage[D]. Wulumuqi: Xinjiang Agricultural University, 2013.

[10] 徐晓波 . 李果实成熟过程中细胞壁多糖的降解和相关酶的研究[D]. 扬州: 扬州大学, 2008.
XU Xiaobo. Research on the degradation of wall polysaccharides and relevant enzymes during plum fruit softening[D]. Yangzhou: Yangzhou University, 2008.

[11] BRUMMELL D A. Cell wall metabolism during maturation, ripening and senescence of peach fruit[J]. Journal of Experimental Botany, 2004, 55: 2029-2039.

[12] 韩雅珊 . 食品化学实验指导[M]. 北京: 中国农业大学出版社, 1996.
HAN Yashan. Food chemistry experiment guide[M]. Beijing: China Agricultural University Press, 1996.

[13] 李合生 . 植物生理生化实验原理和技术[M]. 北京:高等教育出版社, 2000: 211-212.
LI Hesheng. Principles and techniques of plant physiological and biochemical experiments[M]. Beijing: Higher Education Press, 2000: 211-212.

[14] 曹建康, 姜微波, 赵玉梅 . 果蔬采后生理生化实验指导[M]. 北京: 中国轻工业版社, 2007:79-88.
CAO Jiankang, JIANG Weibo, ZHAO Yumei. Physiological and biochemical experiment guidance for postharvest fruits and vegetables[M]. Beijing: China Light Industry Press, 2007:79-88.

[15] PITT D. Biomass part B: Lignin, Pectin, and Chitin: Pectin lyase from *Phoma medicaginis* var. *pinodella*[J]. Methods in Enzymology, 1988, 161: 350-354.

[16] DONG Y, ZHANG S, WANG Y. Compositional changes in cell wall polyuronides and enzyme activities associated with melting /mealy textural property during ripening following long－term storage of ‘Comice’ and ‘d’Anjou’ pears[J]. Postharvest Biology and Technology, 2018, 135: 131-140.

[17] 王中凤 . 细胞壁分解酶与果实软化的关系研究进展[J]. 中国农学通报, 2009, 25(18): 126-130.
WANG Zhongfeng. Research advancement in relation of enzymes for cell wall metabolism with fruit softening[J]. Chinese Agricultural Science Bulletin, 2009, 25(18): 126-130.

[18] 刘慧, 陈复生, 杨宏顺 . 影响桃果实质地的细胞壁降解酶的研究进展[J]. 食品与机械, 2008(3): 136-140.
LIU Hui, CHEN Fusheng, YANG Hongshun. Research advances on cell wall-degrading enzymes in peach causing textural changes[J]. Food & Machinery, 2008(3): 136-140.

[19] NG J K, SCHRöDER R, BRUMMELL D A, SUTHERLAND P W, HALLETT IC, SMITH B G, MELTON L D, JOHNSTON J M. Lower cell wall pectin solubilisation and galactose loss during early fruit development in apple (*Malus*×*domestica*) cultivar ‘Scifresh’ areas sociated with slower softening rate [J]. Jour-

nal of Plant Physiology, 2015: 129-137.

[20] ZHOU H W, SONEGO L, KHALCHITSKI A, BEN ARIE R, LERS A, LURIE S. Cell wall enzymes and cell wall changes in ‘Flavortop’ nectarines: m RNA abundance, enzyme activity, and changes in pectic and neutral polymers during ripening and inwoolly fruit[J]. Journal of the American Society for Horticultural Science, 2000, 125(5): 630-637.

[21] NATH P, PECH J C. Fruit ripening: physiology, signalling, genomics[J]. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources, 2014, 9（14）. Doi: 10.1079/PAVSNNR20149014.

[22] 宣继萍, 王刚, 贾展慧, 郭忠仁 . 李属植物果实成熟软化研究进展[J]. 中国农学通报, 2015, 31(31): 104-118.
XUAN Jiping, WANG Gang, JIA Zhanhui, GUO Zhongren. Research advances of ripening and softening in prunus fruit[J]. Chinese Agricultural Science Bulletin, 2015, 31(31): 104-118.

[23] 阚娟, 谢海艳, 金昌海 . 桃果实成熟软化过程中生理特性及细胞壁超微结构的变化[J]. 江苏农业学报, 2012, 28(5): 1125-1129.
KAN Juan, XIE Haiyan, JIN Changhai. Physiological characteristics and cell wall ultrastructure during ripening and softening of peach fruit[J]. Jiangsu Journal of Agricultural Sciences, 2012, 28(5): 1125-1129.

[24] NEWMAN R H, REDWELL R J. Cell wall changes in ripening kiwifruit:13C solid state NMR characterization of relatively rigid cell wall polymers[J]. Carbohydrate Polymers, 2002, 49(2): 121-129.

[25] 焦云, 柴春燕, 马炜炜, 舒巧云 . 不同质地杨梅果实肉柱细微构造与细胞壁组分及相关酶活性分析[J]. 江西农业学报, 2018, 30(5): 12-17.
JIAO Yun, CHAI Chunyan, MA Weiwei, SHU Qiaoyun. Study on microstructure, cell wall components and related enzymes activity in carneous column of different texture of Chinese bayberry fruit[J]. Acta Agriculturae Jiangxi, 2018, 30(5): 12-17.

[26] 阚娟 . 不同溶质型桃果实成熟软化机理研究[D]. 扬州: 扬州大学, 2011.
KAN Juan. Research on ripening and softening mechanism of different solute peach fruits[D]. Yangzhou: Yangzhou University, 2011.

[27] NIKOLIC M V，MOJOVIC L. Hydrolysis of apple pectin by the coordinated activity of pectic enzymes[J]. Food Chemistry, 2007, 101: 1-9.

[28] 赵胜锦 . 中国樱桃的果实软化生理及贮藏性研究[D]. 杭州: 浙江大学, 2014.
ZHAO Shengjin. The research of Chinese cherry’ softening physiology and storage properties[D]. Hangzhou: Zhejiang University, 2014.

[29] 周厚成 . 草莓果实成熟软化相关基因研究[D]. 杨凌: 西北农林科技大学, 2014.
ZHOU Houcheng. Research on genes related to fruit ripening and softening of strawberry[D]. Yangling: Northwest A&F University, 2014.

[30] 金昌海, 索标, 阚娟, 王红梅, 汪志君 . 桃果实成熟软化过程中细胞壁多糖降解特性的研究[J]. 扬州大学学报, 2006, 27(3): 70-75.
JIN Changhai, SUO Biao, KAN Juan, WANG Hongmei, WANG Zhijun. Studies on degradation characteristic of cell-wall polysaccharide during peach fruit ripening[J]. Journal of Yangzhou University, 2006, 27(3): 70-75.

[31] BRUMMELL D A, HARPSTER M H, CIVELLO P M, BENNETT A B, DUNSMUIR P. Modification of expansin protein abundance in tomato fruit alters softening and cell wall metabolism during ripening[J]. The Plant Cell, 2001, 11: 2203-2216.

[32] 薛炳烨，毛志泉，束怀瑞 . 草莓果实发育成熟过程中糖苷酶和纤维素酶活性及细胞壁组成变化[J]. 植物生理与分子生物学学报, 2006, 32(3): 363-368.
XUE Bingye, MAO Zhiquan, SHU Huairui. Glycosidase and cellulase activities and cell wall composition changes during strawberry fruit ripening[J]. Chemical Journal of Plant Physiology and Molecular Biology, 2006, 32 (3): 363-368.