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Research Note

Amygdalin Contents in Peaches at Different Fruit Development Stages

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ABSTRACT: Amygdalin contents of the seeds, endocarps, and mesocarps from three peach cultivars (i.e., Stone Peach, Hikawa Hakuho, and Bakhyang) were measured at three stages of fruit development (stone-hardening, fruit enlargement, and ripening). The peach samples were dried and defatted with a Soxhlet apparatus, reflux extracted with methanol, and analyzed using reverse phase high-performance liquid chromatography. During all fruit development stages, the amygdalin contents in the seeds were higher than those in the endocarps and mesocarps. The amygdalin contents of the Stone Peach were comparatively higher than the Hikawa Hakuho and Bakhyang ($P<0.05$). Further, the amygdalin contents during ripening were very low or not detected. Overall, the amygdalin contents of the three peach cultivar samples (seed, endocarp, and mesocarp) increased until the fruit enlargement stage and either remained constant or decreased during ripening.

Keywords: amygdalin, fruit development stages, peaches, HPLC, seeds

INTRODUCTION

Due to the abundance of sugar and organic acids, peaches are primarily consumed as fresh produce in Korea. In 2015, the peach production in Korea was 238,000 M/T, and 0.4% was processed (1). Peaches are processed as jam and jelly and fermented to alcoholic drinks (2-4). Unripe peaches contain more nutrients than ripe peaches, such as organic acids, minerals, and polyphenols (5). In Korea, unripe peaches are thinned out and discarded in the orchard mostly from early April to late May. Some studies were carried out on the chemical composition (5-7) and utilization of unripe peaches as functional materials (8,9). Peach seeds were also studied in regard to their use in the prevention of atherosclerosis (10), and were also processed to functional materials (11). Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-D-glucoside) is a cyanogenic glycoside plant toxin contained in relatively high concentrations in the kernels and seeds of apples, apricots, almonds, cherries, and peaches (12,13), and it is abundant in plum seeds (14). Notably, amygdalin is hydrolyzed to HCN, benzaldehyde, and D-glucose and can cause acute intoxication and chronic human central nervous system maladies (15,16). The quantitative profile of amygdalin content during peach development should be studied in order to utilize unripe peaches as functional materials, and to enhance the quality of processed peach products. In this study, the amygdalin contents of three cultivars of peach seeds, endocarps, and mesocarps were analyzed during fruit development (stone-hardening stage, fruit enlargement stage, and ripening period).

MATERIALS AND METHODS

Sample materials and reagents

Three cultivars of peaches [Stone Peach (P1), Hikawa Hakuho (P2), and Baekhyang (P3)] were obtained from the Cheongdo Peach Experimental Station of Gyeongsangbuk-do Agricultural Research Extension Services (Cheongdo, Korea). Table 1 shows the harvest time of each cultivar. Fruits with uniform colors and sizes were selected and divided into mesocarps, endocarps, and seeds. The samples were then air-dried in the shade. Amygdalin standards were purchased from Sigma Co. (St. Louis, MO, USA), and solvents for high-performance liquid chromatography (HPLC) analysis were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals used in this study were obtained from Duksan

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Table 1. Harvest date of peaches at different fruit development stages

Cultivar[1)]		Fruit development stages		
		Stone-hardening	Fruit enlargement	Ripening
P1	Harvest date	Jun 17th	Aug 8th	Sep 16th
	Average fruit weight	18.2	49.2	84.2
P2	Harvest date	Jun 17th	Jul 2nd	Jul 8th
	Average fruit weight	61.4	139.1	193.1
P3	Harvest date	Jun 17th	Jul 29th	Aug 26th
	Average fruit weight	56.9	178.3	337.9

[1)]P1, Stone Peach; P2, Hikawa Hakuho; P3, Baekhyang.

Co. (Ansan, Korea).

Amygdalin extraction and HPLC analysis

The air-dried peach seeds, endocarps, and mesocarps were pulverized, screened with a 60-mesh sieve, and defatted with ethyl ether for 3 h using a Soxhlet apparatus. After evaporating the solvent and drying in an oven, the defatted powders were reflux extracted with methanol at 60°C for 6 h, filtered, concentrated with a rotary evaporator, filtered, and then injected into an HPLC instrument (LC-10A, Shimadzu Co., Kyoto, Japan). The HPLC column was a Supelcosil™ LC-18-S (ϕ 4.6×250 mm, Supelco, Bellefonte, PA, USA), and the samples were analyzed at a UV wavelength of 210 nm with 20% methanol (0.7 mL/min) as the mobile phase (17). The amygdalin contents were determined with linear regression methods using an amygdalin standard curve.

Statistical analysis

All measured values are reported as mean±standard deviation, and evaluated by the analysis of variance (ANOVA), followed by Duncan's multiple range test ($P<0.05$) using the Statistical Analysis System software package (version 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Amygdalin analysis by HPLC

As a cyanogenic glycoside, amygdalin is enzymatically hydrolyzed and ultimately transformed into benzaldehyde and HCN (Fig. 1). The total cyanide content used to be determined using the picrate and acid hydrolysis method for the quantification of cyanogenic glycosides (18). However, since the development of reverse phase HPLC (19), this newer approach has been used to quickly determine the amygdalin content in a variety of matrices (20). Fig. 2 shows the reverse phase HPLC chromatograms of the amygdalin standard and a P1 seed. Amygdalin was isolated fairly well in the reverse phase C18 column with a 20% methanol mobile phase. Amygdalin contents were obtained from the linear regression curve of the standard amygdalin contents.

Amygdalin contents of peaches during fruit development

Amygdalin is mainly contained in the seeds of Rosaceae and is catabolized upon germination as a source of nitrogen and carbon (21) for later use during flowering and

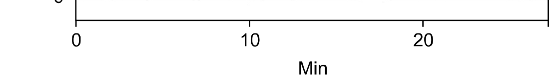

Fig. 2. HPLC chromatograms of amygdalin standard (amygdalin STD) and Stone Peach seed extract (sample; June 17th).

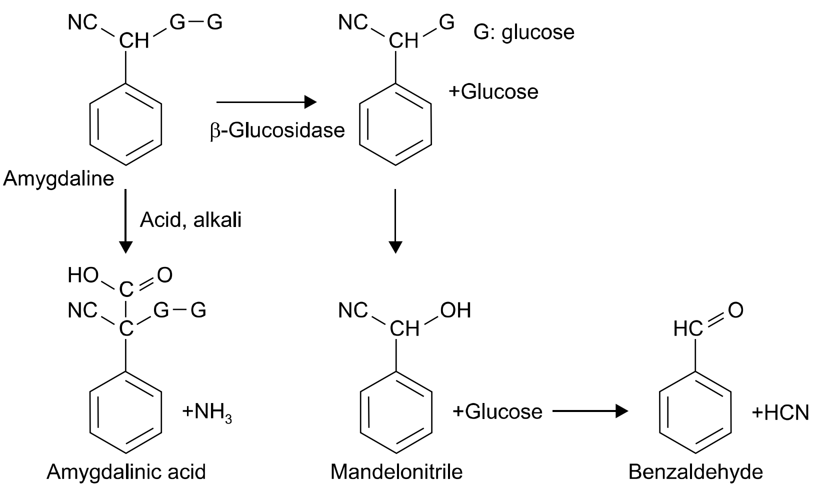

Fig. 1. Amygdalin decomposition.

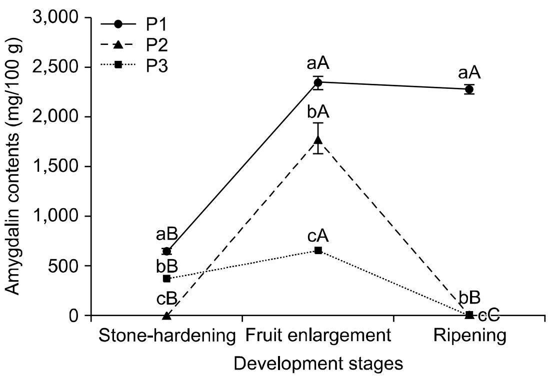

Fig. 3. Amygdalin contents in the seeds of three peach cultivars during different fruit development stages. Means followed by the same letters within the same peaches (a-c) and the same stages (A-C) are not significantly different (P<0.05). P1, Stone Peach; P2, Hikawa Hakuho; P3, Baekhyang.

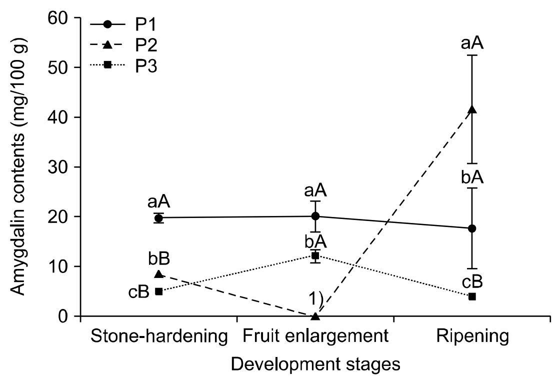

Fig. 4. Amygdalin contents in the endocarps of three peach cultivars during different fruit development stages. Means followed by the same letters within the same peaches (a-c) and the same stages (A-C) are not significantly different (P<0.05). P1, Stone Peach; P2, Hikawa Hakuho; P3, Baekhyang. [1)]Not detected.

fruit development (22). The amygdalin contents in the seeds, endocarps, and mesocarps from three peach cultivars during stone-hardening, fruit enlargement, and ripening stages are shown in Fig. 3, 4, and 5, respectively. The amygdalin contents in the seeds of the three cultivars increased until the fruit enlargement stage. During the ripening period, the amygdalin contents of the P1 remained constant, but decreased largely for the P2 and P3. In the endocarp of P1, the amygdalin contents showed no significant differences among the fruit development stages. In P3, the amygdalin contents increased slightly to the fruit enlargement stage. But the amygdalin contents were not detected in P2. During the ripening period, the amygdalin contents increased in P2, but were not detected in P3. The amygdalin contents of the P1 and P3 mesocarps (flesh) increased until the fruit enlargement stage, and then decreased slightly during ripening. In contrast, no significant differences between the stone-hardening and fruit enlargement stages were observed for the amygdalin contents of the P2 mesocarps, and no amygdalin was detected during the ripening period. The amygdalin contents of the three peach cultivars depend-

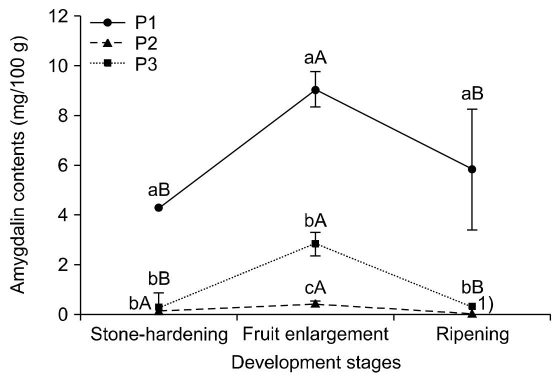

Fig. 5. Amygdalin contents in the mesocarps of three peach cultivars during different fruit development stages. Means followed by the same letters within the same peaches (a-c) and the same stages (A-C) are not significantly different (P<0.05). P1, Stone Peach; P2, Hikawa Hakuho; P3, Baekhyang. [1)]Not detected.

ing on their parts at different development stages are shown in Table 2. Overall, the amygdalin contents in the seeds were reasonably higher than those in the endocarp and mesocarp. Relative to P2 and P3, P1 had the great-

Table 2. Amygdalin contents of three peach cultivars at different fruit development stages (unit: mg/100 g, DW)

Fraction	Cultivar[1)]	Fruit development stage		
		Stone-hardening	Fruit enlargement	Ripening
Seed	P1	654.19±51.46^{aB}	2,349.16±79.45^{aA}	2,311.88±62.57^{aA}
	P2	0.20±0.02^{fB}	1,792.65±158.20^{bA}	21.16±3.00^{cB}
	P3	372.36±24.92^{bB}	669.67±66.58^{cA}	12.14±4.80^{cC}
Endocarp	P1	19.77±1.47^{c}	19.99±3.14^{d}	17.90±8.11^{c}
	P2	8.56±0.12^{dB}	ND[2)]	41.64±10.77^{bA}
	P3	5.12±0.10^{eB}	12.04±1.75^{eA}	3.96±1.05^{dB}
Mesocarp	P1	4.29±0.10^{eB}	9.09±0.71^{fA}	5.85±2.45^{dB}
	P2	0.19±0.72^{f}	0.46±0.23^{h}	ND
	P3	0.19±0.18^{fB}	2.88±0.48^{gA}	0.46±0.02^{eB}

Means followed by the same letters within the column (a-h) and the row (A-C) are not significantly different (P<0.05).
[1)]P1, Stone Peach; P2, Hikawa Hakuho; P3, Baekhyang.
[2)]Not detected.

est amygdalin content (P<0.05). Additionally, the amygdalin contents increased until the fruit enlargement stage and kept constant or decreased slightly during ripening. Likewise, the amygdalin contents of four stone fruit species (apricot, peach, plum, and bitter apricot trees) also increased during fruit development and remained stable or decreased slightly when they ripened (17,23).

In summary, the amygdalin contents in the seeds, endocarps, and mesocarps of three peach cultivars (P1, P2, and P3) were determined during fruit development by reverse phase HPLC. For every stage and cultivar, the amygdalin contents in the peach seeds were greater than those in the endocarps and mesocarps (P<0.05). The amygdalin contents of the P1 were moderately greater than those of the other cultivars (P2 and P3). Notably, amygdalin contents were very low or not detected during the ripening periods of the peaches. Further, the amygdalin contents of the peaches increased until the fruit enlargement stage and remained constant or decreased slightly during the ripening periods.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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