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Prulaurasin content of leaves, kernels and pulps of *Prunus lauracerasus* L. (Cherry Laurel) during ripening

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ABSTRACT: Cyanogenic glycosides are highly toxic compounds distributed over 2500 taxa as secondary metabolites which release cyanide and benzaldehyde when degraded by endogenous enzymes. In this study, prulaurasin - a cyanogenic glycoside - content of *Prunus lauracerasus* L. (cherry laurel) leaves, kernels and pulps were investigated during the ripening period with a high-performance liquid chromatographic (HPLC) method. In leaves, prulaurasin pursues a relatively linear path while in kernels, it rapidly increases during fruit development. The higher amount of prulaurasin was quantified in the early stages of pulps and decreased over time to an undetectable level in mature form to form an edible fruit that promotes propagation.

KEYWORDS: *Prunus lauracerasus* L; cherry laurel; high-performance liquid chromatography (HPLC); cyanogenic glycosides; prunasin; prulaurasin.

1. INTRODUCTION

Prunus laurocerasus L. (cherry laurel) a member of Rosaceae family, is an evergreen small shrub up to 6 m growing in Eastern Europe and Western Asia. In Turkey, plant is distributed mainly in northern region 20-1700 m above sea level and known as "Taflan" or "Karayemiş". Cherry laurel fruits are black to dark purple in colour and up to 12 mm in diameter [1-3, 7]. Chemical composition of cherry laurel have been widely studied for its fatty acids in seeds [4], essential oil constituent in the leaves and fruits [5], sugar and low molecular weight carbonhydrate composition in fruits [1,2], and phenolic composition-antioxidant properties of fruits [3, 6].

Cherry laurel fruits are consumed as fresh or dried, pickled, juice, jam or marmalade. It is also a well-known drug used for many years in traditional medicine in Turkey for stomach ulcers, digestive system complaints, bronchitis, eczemas, haemorrhoids, and as diuretic, antipyretic, analgesic agents [8-10].

Cyanogenesis is a biological process to produce hydrogen cyanide by living organisms. Cyanide releasing compounds in plants are divided into two categories, cyanogenic glycosides (CG) and cyanogenic lipids. CGs are toxic compounds found in more than 2500 taxa as secondary metabolites. All known CGs are α-hydroxynitriles β-linked with a sugar moiety which is mostly D-glucose [11-13].

Generation of HCN from a cyanogenic compound is a two-step process which contains an enzymatic deglycosylation and a nitrile cleavage with a nitrilase. Because of the compartmentalization of CGs and their hydrolysing enzymes, enzymatic hydrolysis occurs when the plant tissue is damaged or disrupted. It also could occur by the digestive enzymes of the animal or the micro-organism in the digestive tract. CGs are also important components of plant defence. If a tissue damage occurs by an herbivore or a pathogen, CGs and hydrolysing enzymes react for an immediate HCN release [11-13].

Cherry laurel leaves, fruits and seeds all contain α-hydroxymandelonitrile derivatives of cyanogenic glycosides such as prunasin, sambunigrin and amygdalin. Prunasin and sambunigrin (Figure 1) are optical mono glycoside isomers of each other and amygdalin is a gentiobioside. The leaves contain 1% to 2.5% prulaurasin (racemic mixture of both prunasin and sambunigrin) as the major components of CGs [14].

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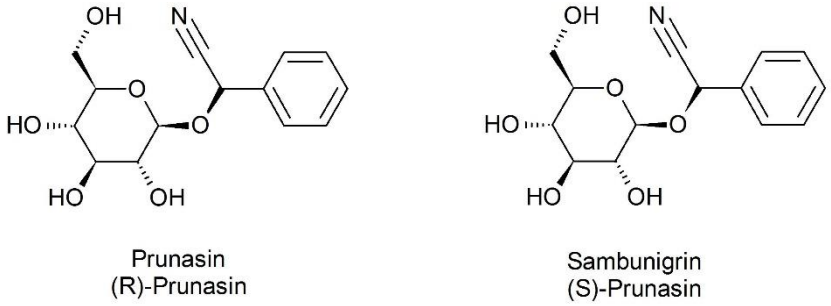

Figure 1. Structures of prunasin and sambunigrin.

Hydrolysis of prulaurasin produces mandelonitrile and a sugar moiety. A further hydrolysis step transforms mandelonitrile to HCN and benzaldehyde (Figure 2). The bitter taste of released benzaldehyde might be considered as a warning message to the herbivores about not to eat more.

Liquid chromatography, gas chromatography, König reaction based colorimetric method and picrate tests (Guignard Method) are widely used for quantitative analysis of CGs [15-22]. Liquid chromatography needs standard materials for known CG compounds and the other methods are used to quantify CGs in total.

In this study, we report the level of CGs in leaves, kernels and pulps of *Prunus laurocerasus* L. during ripening via analysing prulaurasin content with HPLC.

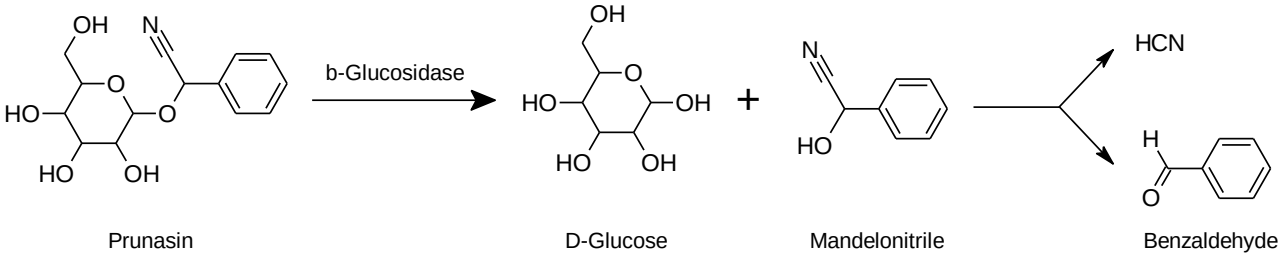

Figure 2. Hydrolysis pathway of prunasin/sambunigrin.

2. RESULTS AND DISCUSSION

Analytical method applied to quantify the CG content was linear in the chosen concentration range. Prunasin peak purity index was calculated to be 0.99 pure for all reference solutions and leaf samples via LCSolution software (Shimadzu, Japan). Also min 0.96 purity index achieved for pulp and kernel samples. A typical chromatogram of the leaf, kernel, unripe pulp and mature pulp samples is given in Figure 3.

2.1. Physical properties of the plant material

Plant material in the same size and weight were collected from the same orchard in a three days basis. The collecting date, colours, average size and weight of cherry laurel fruits are given in Table 1. Repeat number of measurements for physical properties is 50 for leaves and fruits. The average values for leaves were 18.2±0.7 cm in length and 3.3±0.4 g in weight.

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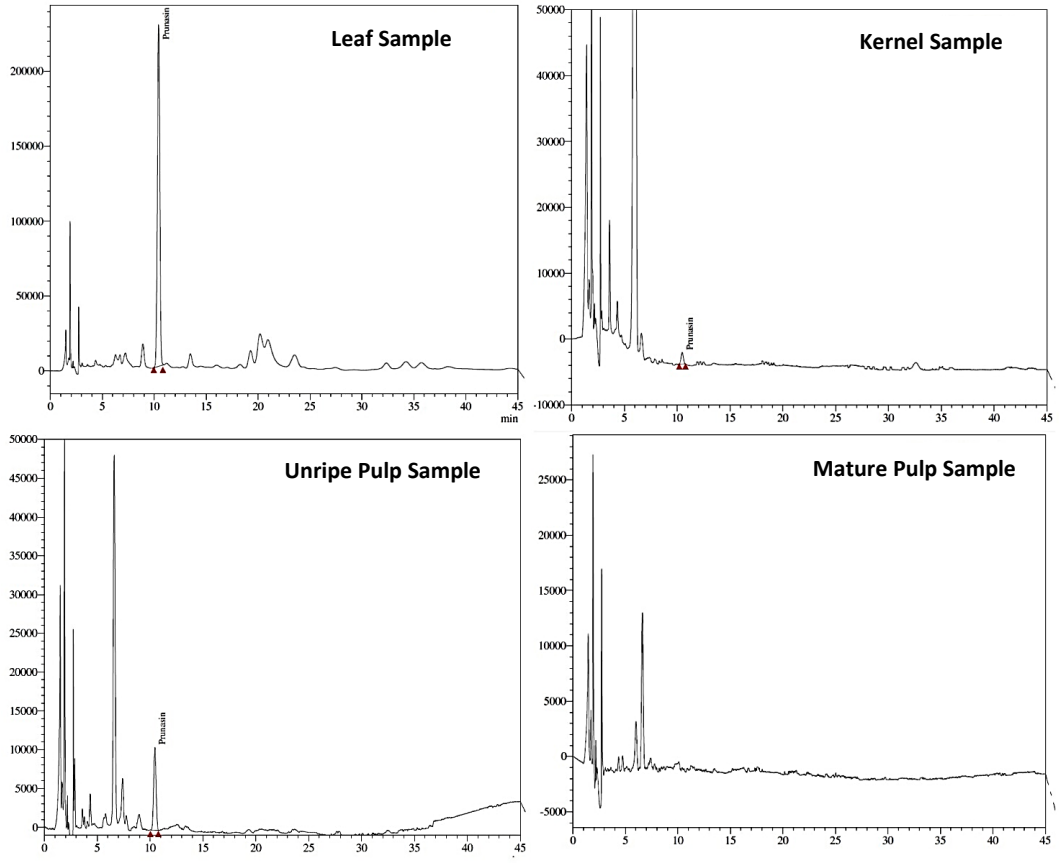

Figure 3. Chromatograms of leaf, kernel, unripe and mature samples.

2.1. CG Content of the plant material

Prulaurasin contents of the leaf samples are shown in Figure 4. Triplicate test results indicates that during the ripening period, prulaurasin content of leaves were relatively linear from 1250 to 1650 mg/100 g and shows no significant changes. In developmental period, prulaurasin content in kernels increases drastically from 3.5 and further increases slightly to 11 mg/100 g after reddening begins (Figure 5). Unlike kernels and leaves, prulaurasin decreases over time in the pulps (Figure 6). The higher amount of prulaurasin was quantified in the early stage of fruit development as 95 mg/100 g and not detected in mature form.

Table 1. Physical properties and colours of fruits according to the collecting date.

Collecting Date	Colour of the Fruit	Size (mm) (n=50)	Weight (g) (n=50)
14-06-2016	Green	5.2± 0.12	0.9± 0.081
17-06-2016	Green	5.4± 0.14	0.9± 0.073
20-06-2016	Green	5.8± 0.21	1.1± 0.024
23-06-2016	Green	6.7± 0.17	1.5± 0.032
26-06-2016	Green (Reddening started)	7.0± 0.13	1.7± 0.021
29-06-2016	Almost pink	8.4± 0.23	2.0± 0.017
02-07-2016	Pink	9.8± 0.09	2.1± 0.018
05-07-2016	Almost red	11.9± 0.11	2.2± 0.031
08-07-2016	Red	13.2± 0.18	2.4± 0.019
11-07-2016	Red	13.9± 0.14	2.7± 0.025
14-07-2016	Reddish black	14.6± 0.12	2.9± 0.027
17-07-2016	Reddish black	14.7± 0.17	3.2± 0.027
20-07-2016	Almost black	14.4± 0.13	3.3± 0.023
23-07-2016	Black	14.9± 0.22	3.4± 0.034
26-07-2016	Black	15.1± 0.13	3.7± 0.019

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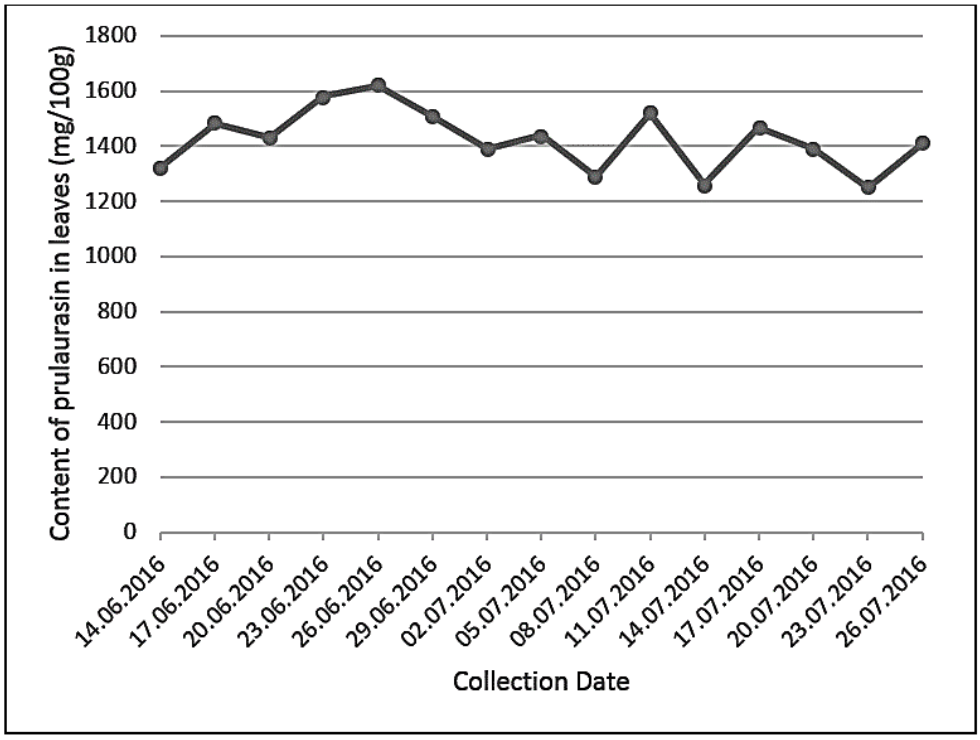

Figure 4. Prulaurasin content in leaves during fruit ripening.

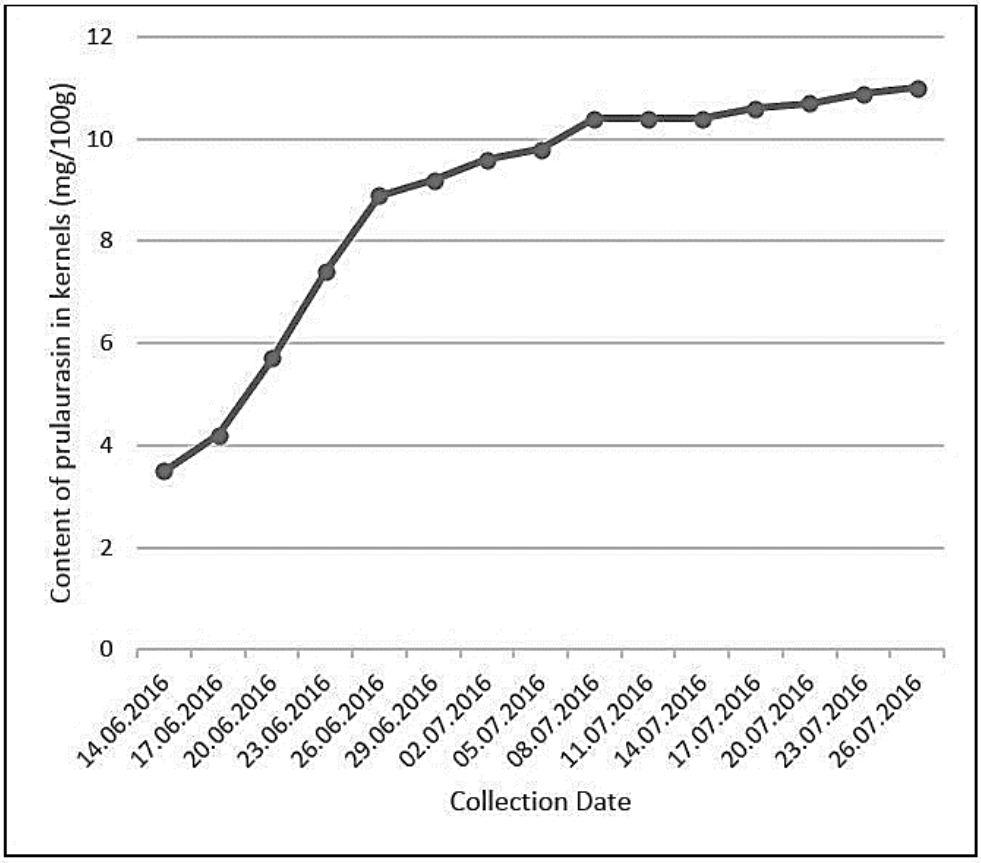

Figure 5. Prulaurasin content in kernels during fruit ripening.

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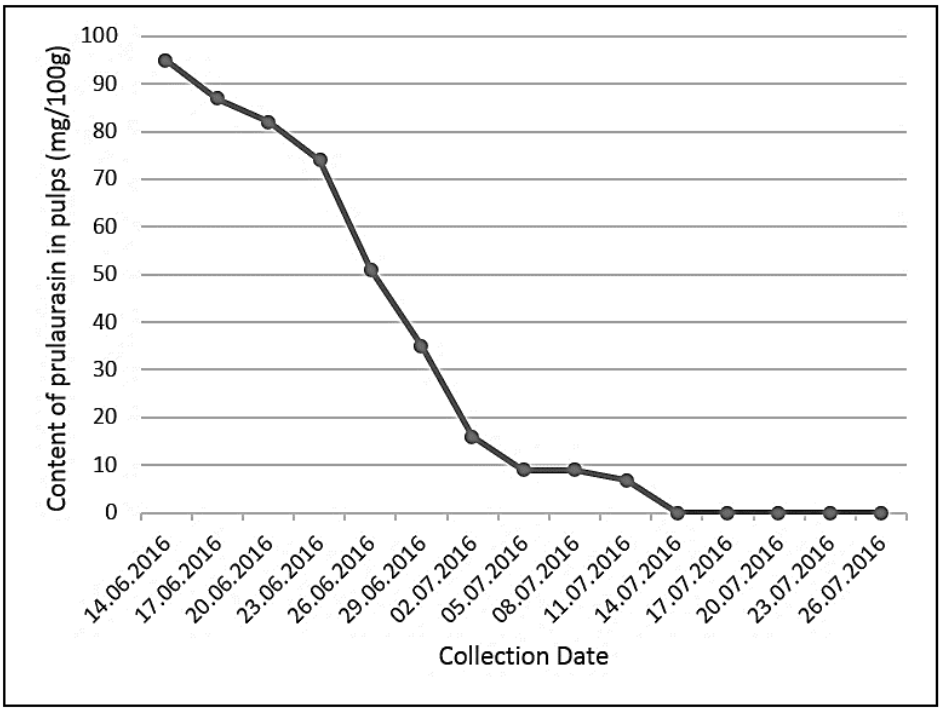

Figure 6. Prulaurasin content in the pulps during fruit ripening.

A previous study [23] also determined the prunasin levels in dried leaves, pulps and kernels of *Prunus laurocerasus*. The results between the studies are different because of the extraction techniques and locations of the plant materials. It is also known that CGs content decreases if materials are dried [24].

3. CONCLUSION

The results presented in this study clearly shows that in the early stages of fruit development, *Prunus lauracerasus* L. utilizes high prulaurasin levels of the unripe fruit pulps to protect its kernels with bitter taste of released benzaldehyde and highly poisonous cyanide. When the kernels have enough CGs to protect itself, prulaurasin decreases in the pulp to form a tasty edible fruit and thus there is a chance of kernel transportation by the herbivores to assist propagation.

4. MATERIALS AND METHODS

4.1. Chemicals

(R)-Prunasin was commercially purchased from Santa Cruz Biotechnology (Dallas, TX, USA). HPLC grade acetonitrile and methanol were commercially available from Sigma-Aldrich (St. Louis, MO, USA). Water was obtained from Sartorius Arium Pro ultrapure water system.

4.2 Collection of plant material

Cultivated *Prunus laurocerasus* L. leaves and fruits were collected from Rize province Turkey at 3-day intervals with uniform colour and size from the same orchard between 14th June and 26th July. Average size and weight of the fruits were measured then kernels were removed from the fruits. Leaves, kernels and pulps were cleaned and stored at -20 ^{0}C until analysis. The identity of plant specimen is confirmed by Prof.Dr. Murat Kartal.

4.3. Materials and sample preparation

As the current used analytical method and column couldn't separate R and S forms of prunasin, (R)-prunasin was dissolved in methanol to achieve prulaurasin reference solutions at the concentrations of 250-5 ppm.

20 gram of frozen plant materials (leaves, kernels, pulps) were extracted in the homogenizer for 1 hours with 200 mL of methanol. All samples were filtrated and diluted to 250 ml with the same solvent.

4.4. Instrumentation

HPLC-PDA analysis was performed on a Shimadzu Prominence UFLC system. The CGs were analysed by using a GLSciences ODS-3 250x4.6x5 analytical column at 40 ^{0}C. Mobile phase consisting of water and acetonitrile (85:15, v/v) was delivered isocraticaly at a flow rate of 1.5 mL/min. Injection volume was 20 µL and UV detection performed at 218 nm. After each plant sample injection, column was rinsed with acetonitrile for elution of other components and conditioned with the mobile phase for 25 minutes.

The IKA Ultra-Turrax T-50 Homogenizer coupled with IKA S 50 N - W 65 SK cutting-homogenizing head was used to mince and homogenise the plant material to extract the CGs in an organic solvent to avoid enzymatic reactions.

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Conflict of interest: Authors declare no conflict of interest.

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