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Changes in the Chemical Content and Polyphenol Oxidase Activity during Development and Ripening of Cherry Laurel Fruits

By

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With 6 Figures

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Summary

KADIOGLU A. & YAVRU I. 1998. Changes in the chemical content and polyphenol oxidase activity during development and ripening of cherry laurel. – Phyton (Horn, Austria) 37 (2): 241–251, with 6 figures. – English with German summary.

Changes in the activity of polyphenol oxidase (PPO) and in the levels of soluble sugars, soluble protein, total phenolic substances and ascorbic acid during the development and ripening of cherry laurel fruits were investigated. PPO activity and the other chemical constituents in the fruits harvested every week from the beginning of June to the mid July were spectrophotometrically analysed. PPO activity and phenolic content gradually increased during the development of the fruits, but decreased in the stage of ripening. Soluble sugar and soluble protein levels gradually increased, while the level of ascorbic acid decreased during fruit development and ripening. Polyacrylamide gel electrophoresis revealed quantitative changes in two PPO bands in the fruits.

Zusammenfassung

KADIOGLU A. & YAVRU I. 1998. Änderungen in der chemischen Zusammensetzung und der Aktivität der Polyphenoloxidase während der Entwicklung und Reifung von Früchten des Kirschlorbeers. – Phyton (Horn, Austria) 37 (2): 241–251, 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Änderungen in der Aktivität der Polyphenoloxidase (PPO) und in den Gehalten an löslichen Zuckern, löslichen Proteinen, Gesamtphenolgehalt und Ascorbinsäure wurden während der Entwicklung und in reifenden Früchten vom Kirschlorbeer untersucht. Die PPO-Aktivität und die chemische Zusammensetzung der Früchte, welche wöchentlich von Anfang Juni bis Mitte Juli geerntet wurden, wurden spektralanalytisch untersucht. Die PPO-Aktivität und der Gehalt an phenolischen Substanzen stieg gleichmäßig während der Entwicklung der Früchte an, aber sank wieder während der Reifung. Lösliche Zucker und der Gehalt an löslichen Proteinen nahm zu, während die Ascorbinsäuremengen mit der Fruchtentwicklung und der Reifung abnahmen. Die Polyacrylamidgel-Elektrophorese zeigte quantitative Änderungen zweier PPO-Banden in den Früchten.

Introduction

Cherry laurel (*Laurocerasus officinalis* Roem, syn. *Prunus laurocerasus* L.) is an evergreen plant up to 6 m in height. Many cultivars of the plant have been described in different countries (DIRR 1990, MILAN 1984) The fruits of cherry laurel are ovoid, 8 mm in diameter (12 mm in some cultivars) and dark purple or black in mature form (DAVIS 1972). The cultivated plants have larger and sweeteer fruits than wild forms. The fruits of both the wild and cultivated plants are very poisonous in their early developmental stages; However, the fruit when ripe can be used to prepare various alcoholic drinks which taste pleasantly of almonds (MILAN 1984). In recent years fresh and dried ripe fruits have been used for making jam, dried fruit pulp, marmalade, and adorning alcoholic drinks.

There are some studies on the chemical content in the fruits of cherry laurel. For example, glucose, fructose, some unidentified phenolics, peonidin and cyanidin derivatives amounts (GOGOLISHVILI 1971), protein and some organic acid levels (ROMEO-RODRIGUEZ & al. 1992) have been investigated. However, no information is available on the polyphenol oxidase (PPO) activity and its isoenzymes of cherry laurel, as well as on the changes of PPO activity and some chemical compounds during the development and ripening of the fruits. One of the major cherry laurel fruit quality problems is flesh browning associated with enzyme PPO.

Polyphenol oxidase (o-diphenol: oxygen oxidoreductase, E.C. 1.10.3.1.) has been found in most higher plants, and is responsible for enzymatic browning in raw fruits and vegetables. This reaction is important in food preservation and processing, and is generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant development of an off-flavor (MATHEW & PARPIA 1971). Ascorbic acid is a natural inhibitor of PPO (WEAVER & CHARLEY 1974). On the other hand, biochemical studies of fruit indicate levels of acids, sugars, soluble solids and polyphenols are the primary quantity parameters (SENTER & CALLAHAN 1990). PPO catalyses the oxidation of polyphenols in plants to o-quinones,

which react with themselves or other phenolics to form a brown pigment (MAYER & HAREL 1979, VAMOS-VIGYAZO 1981, MAYER 1987).

The study was conducted to examine the changes in the content of total phenolic, soluble protein, soluble sugar and ascorbic acid and in the level of activity and isoenzymes of PPO during the development and ripening of the cherry laurel fruits. The main objective of our study was to determine some characteristics of PPO and variations in the chemical compenents at different stages of development and ripening in the fruits. A second objective was to test whether there was any correlation among the biochemical parameters in these periods.

Materials and Methods

1. Fruit samples

Cherry laurel (*Laurocerasus officinalis* cv. Oxygemmis, AYAZ & al. 1997) fruits were harvested from the trees grown in the vicinity of Karadeniz Technical University in Trabzon, Turkey. The fruits were sampled on a continuing basis at seven days intervals from 1st June (two months after anthesis). Twenty fruits in every week were randomly picked from three trees, fifteen years old, on June 1, 7, 14, 21, 28 and July 5, 12 and 19, 1996. Harvested fruits of each week were used for analysis of soluble sugar, ascorbic acid, phenolic substance, soluble protein and PPO activity. The fruits were stored at -2 °C and all analyses (except electrophoretic analysis) were made on their harvesting day. Prior to analysis the exo- and mesocarps were removed from the endocarps. The exo- (skin) and mesocarps (flesh) were used for the analysis.

2. Enzyme extraction

Extraction of the enzyme from the fresh fruits was carried out using the method of PARK & LUH 1985 with little modification. Five grams of each sample were homogenized in 20 ml cold 0.5 M phosphate buffer pH 7.3 containing 10 mM ascorbic acid and 0.5% polyethylene glycol in a blender for two min, filtered and centrifuged at 20 000 × g for 20 min at 4 °C. Temperature was maintained below 5 °C throughout the extraction process. Then the supernatant was dialysed at 4 °C in the 0.005 M phosphate buffer (pH 6.3) in a cellulose dialysis tube for 12 hr. After the dialysis, the enzyme activity was immediately measured. A part of the enzyme extract was stored at –18 °C for the isoenzyme analysis until use. The amount of soluble protein found in the enzyme extract was estimated by the procedure of BRADFORD 1976 using bovine serum albumin as standard.

3. Activity assays and electrophoresis for PPO

PPO activity was determined spectrophotometrically as described by PONTING & JOSLYN 1948. The substrates (DL-3,4-dihydroxyphenylalanine (L-DOPA), catechol, caffeic acid) were used individually to determine the PPO activity of enzymatic extracts from the fruits. 1 ml 0.05 M phosphate buffer and 1.5 ml 0.02 M substrate at 25 °C was put in a cuvette and finally 1 ml of the enzyme extract was added to the cuvette. Changes in the absorbance at 420 nm were measured for 3 min using a

Schimadzu UV-120–01 spectrophotometer. Enzyme activity was expressed as absorbance change per min in g fresh weight.

Polyacrylamide gel electrophoresis was conducted as described by CONSTANTINIDES & BEDFORD 1967. A separating gel of 7.5 % acrylamide and a stacking gel of 3 % acrylamide were used. The enzyme extract in 50 % glycerol with 0.001 % bromophenol blue was applied to gel. Electrophoresis was conducted in a cold room at 4 °C using reservoir buffer (14.4 g glycine and 3 g tris per litre, pH 8.3) at 10 mA. The enzyme staining solution was prepared with 15 mM L-DOPA. The gel was soaked in this solution at 37 °C for two hours, then incubated for 5 min in 1 mM ascorbic acid. The gel was stored in 30 % ethyl alcohol.

4. Determination of phenolic content

The total phenolics in the fruits were extracted using a modification of the procedure described by WALTER & al. 1979. A 2 g sample of cherry laurel was homogenized in a Waring Blender with 20 ml 95 % ethanol for 2 min. Then, 3 ml of the homogenate was evaporated and alcohol was removed. The residue was mixed with 15 ml of 0.1 M sodium phosphate buffer (pH 6.3) and passed through four layers of cheese cloth. Absorbances were measured with and without Dowex (chloride form) at 323 nm. The total phenolics were determined from a standard curve (1mg/10 ml) prepared at the same time.

5. Determination of ascorbic acid content

The determination of ascorbic acid was performed using the procedure of SHIEH & SWEET 1979 with pure ascorbic acid as the standard. The samples were homogenized with 0.01 M phosphate-citric acid buffer at pH 3.0, filtered and centrifuged at 5000 rpm, for 5 min at 25 °C. The supernatant was used to determine the ascorbic acid content. The assay mixture consisted of 0.5 ml of 0.01M the buffer at pH 3.0, 2.4 ml of 2,2′-Cu -biquinoline solution $(Cu(biq)^{2+})$ and 0.1 ml of the extract. Ascorbic acid content in the fruits was determined spectrophotometrically at 540 nm.

6. Determination of soluble sugar content

Soluble sugar content was determined by phenol-sulfuric method (DUBOIS & al. 1956). A standard curve was prepared to quantify hexose and pentose. Two gram flesh samples were extracted in distilled water and centrifuged at 3000 rpm for 5 min. The fruit extracts were treated with pure sulfuric acid and phenol (5 %) and then their absorbances were measured at 480 nm for pentose and 488 nm for hexose.

Results and Discussion

In this study, the changes in polyphenol oxidase activity, and soluble sugar, soluble protein, phenolic substance and ascorbic acid concentrations were determined during development and ripening of cherry laurel fruit. We designated fruit as immature from the first to sixth week of development. Fruit ripening started in the sixth week. We have previously

observed that fruit color might simply be an indicator of cherry laurel fruit developmental process and that the development period of fruits ends at the sixth week, then fruit ripening commences.

PPO activity gradually increased during the development, then decreased during the ripening period of the fruits.The highest and lowest activities were observed in dopa oxidase and caffeic acid oxidase, respectively, during the development and ripening of the fruits (Fig. 1). Dopa oxidase activity gradually increased up to fifth week, then abruptly decreased during the ripening. The general trend was an increase in the activities of catechol oxidase and caffeic acid oxidase from the first week to the sixth week. Their activities decreased from the sixth week to the end of the ripening. The highest activities of both enzymes were determined in the sixth week. To our knowledge, this is the first report of polyphenol oxidase in the fruits of cherry laurel. KUMAR & al. 1986 have obtained similar results in apple. Their studies showed that polyphenol oxidase activity in apple increased between the pre-ripening and ripening stages, then decreased at the post-ripening stage. Similar results were also obtained in similar studies (VAMOS-VIGYAZO 1981, KUMAR 1987, COSETENG & LEE 1987, PARK & al. 1989).

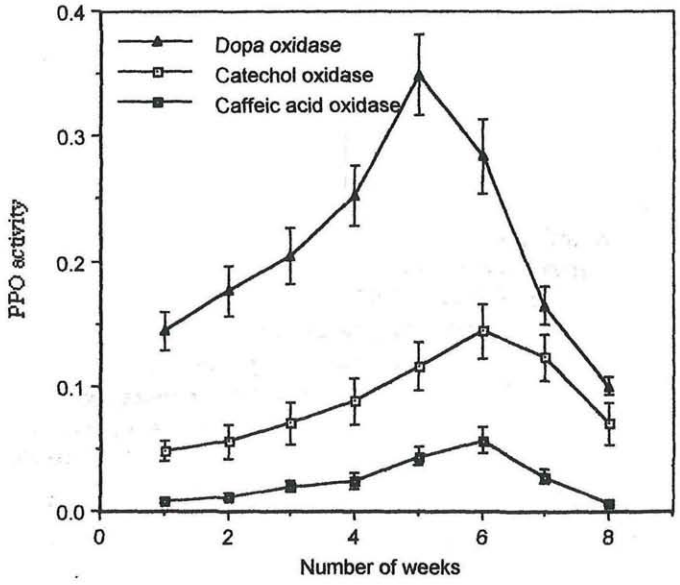

Fig. 1. Changes in the PPO activity during the development and ripening of cherry laurel. Weekly harvesting periods of the fruits; 1: 1 June; 2: 7 June; 3: 14 June; 4: 21 June; 5: 28 June; 6: 5 July; 7: 12 July; 8: 19 July. Vertical bars represent standard errors of the means of three replications.

We detected only two isoenzyme bands of PPO during the development and ripening of the fruits (Fig. 2). The highest activities in both isoenzyme band were observed at the fifth and sixth week, then they decreased in the seventh and eighth week of ripening. The number of PPO isoenzymes was not changed during the development and ripening of the fruits, but their activities at the early stages of the development and the ripening stages were lower than the other stages. However, a better correlation between PPO activity and their isoenzyme band activities was established. Our observation is consistent with a report that isoenzyme activity was affected during development of fruits by MAYER & HAREL 1979. The number of PPO isoenzymes in plants are known to vary with plant species. For example, the isoenzyme numbers were reported 3–4 for peach, 2 for dog rose, 3–8 for pear and 2–3 for apple (WONG & al. 1970, SAKIROGLU 1994, JANOVITZ-KLAPP & al. 1989, OKTAY 1992, WISSEMAN & MONTGOMERY 1985).

Fig. 3 shows total phenolic content changed significantly during the development and ripening of cherry laurel fruits. Phenolic content continued to increase up to fifth week, then decreased. Total phenolic content was 0.52 mg g^{-1} dw in the first week and 3.71 mg g^{-1} dw in the fifth week. The phenolic content decreased after the fifth week of the development. For example, the value was 0.57 mg g^{-1} dw at the end of the ripening.Total phenolic content gradually increased during the development and decreased during the ripening similar to the changes noted for polyphenol oxidase activity. We obtained a high correlation between total phenolics and PPO activity (r = 0.93). In addition, negative low correlation between PPO activity and soluble protein content was determined (r = –0.179). Many workers have reported that the changes in PPO activity depend on the content of phenolic substances as substrate. For example, in peach fruit, the phenolic content and PPO activity increased during the initial period of fruit growth, but the phenolic content decreased and PPO activity continued to increase until the fruit attained full size (KUMAR 1987). In addition, COSETENG & LEE 1987 found that PPO activity and polyphenol concentration of apple decreased during development.This decline was postulated by HAREL & al. 1966 to be due to the cessation of synthesis and conversion of other o-diphenols to other compounds. SPAYD & MORRIS 1981 found that total soluble phenols decrease as strawberries ripen, from 0.6% in green berries to 0.3% in red berries, probably from synthesis of anthocyanins.

The ascorbic acid content decreased continuously during the development and ripening of fruits. Fruit ascorbic acid content was 20.4 mg g^{-1} dw at the first week and decreased to 6.4 mg g^{-1} dw at the end of the ripening period. During this period, decreasing ratio of ascorbic acid content was 70 % (Fig. 4). Our results involved in gradually decreasing of ascorbic acid in the development and ripening stages confirm those of FUKE & MATSUOKA

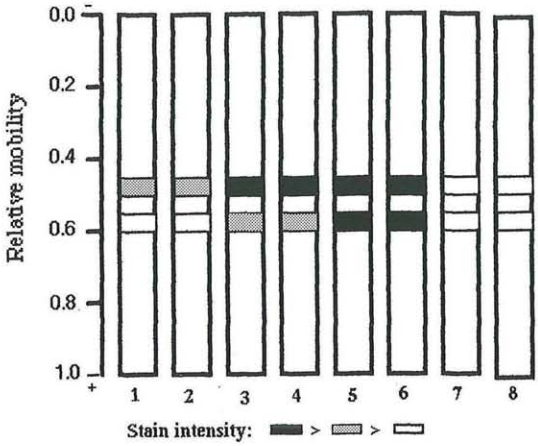

Fig. 2. Diagramatic representation of polyphenol oxidase isoenzymes in cherry laurel. Following discontinuous native polyacrylamide gel electrophoresis, isoenzymes were stained with 15 mM Dopa for 2 hrs, the mobilities of the isoenzymes recorded, and the relative intensities of the bands were rated as indicated. Each lane contained 50 mg of the protein. The direction of electrophoretic migration was from top (–) to bottom (+). Lane 1; 1st harvest week (1 June), lane 2; 2nd week (7 June), lane 3; 3rd week (14 June), lane 4; 4th week (21 June), lane 5; 5th week (28 June), lane 6; 6th week (5 July), lane 7; 7th week (12 July), lane 8: 8th week (19 July).

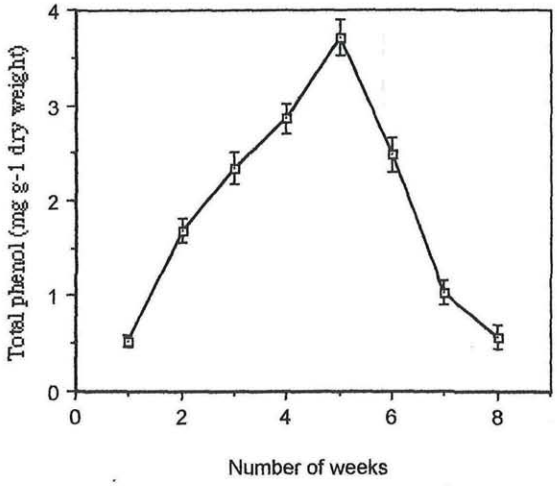

Fig. 3. Changes in total phenolic content during the development and ripening of cherry laurel. Vertical bars represent standard errors of the means of three replications.

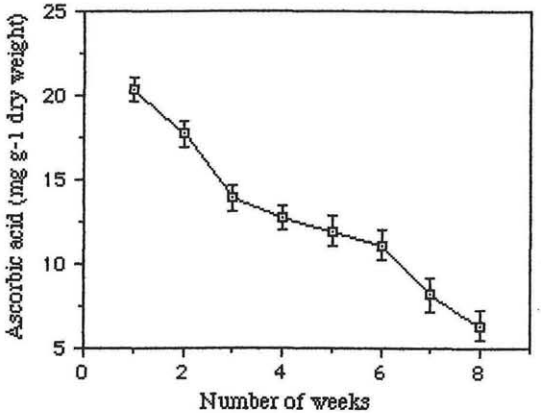

Fig. 4. Changes in the ascorbic acid during the development and ripening of cherry laurel. Vertical bars represent standard errors of the means of three replications.

1984, who reported the diminishing of ascorbic acid during the stages of development and maturation of kiwi. In addition, the ascorbic acid content of kaki was found as a continual decline depending on their development process (INABA & al. 1971). We obtained a low correlation between ascorbic acid and PPO activity (r = 0.125).

Fig. 5 shows the changes in soluble sugar content during the development and ripening of the fruits. The sugar content gradually increased

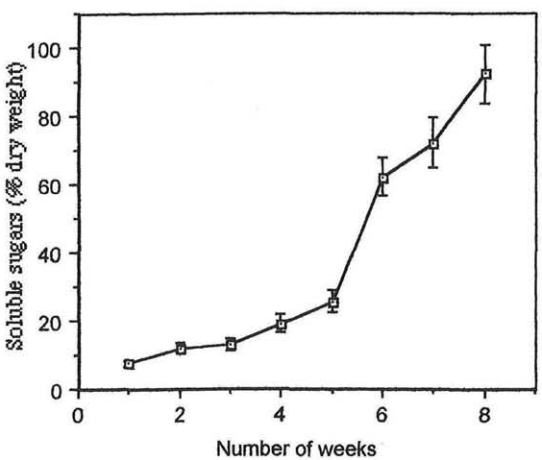

Fig. 5. Changes in soluble sugar content during the development and ripening of cherry laurel. Vertical bars represent standard errors of the means of three replications.

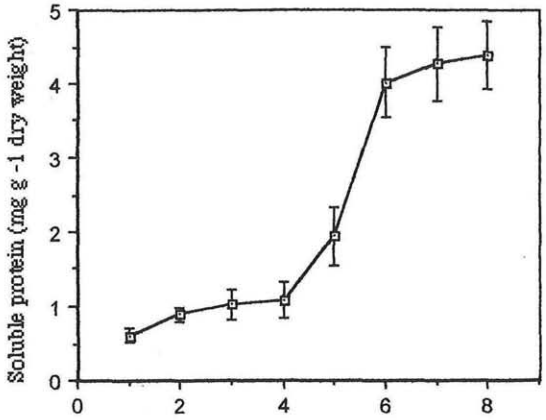

Fig. 6. Changes in soluble protein content during the development and ripening of cherry laurel. Vertical bars represent standard errors of the means of three replications.

from the first week of development to the end of the ripening periods of the fruits. For example, the sugar values were found 7.5 % for the first week and 93 % for the eighth weeks. In addition, the highest increase in the soluble sugar content was obtained after fifth week of the development. The sugar level enhanced constantly with the other parameters during the development and ripening of cherry laurel fruits. In addition, a significant negative correlation between ascorbic acid and soluble sugar contents was observed ($r = -0.83$). The soluble protein content like soluble sugar continually increased during fruit development and ripening. For example, soluble protein content was 0.6 mg g^{-1} dw at the first week and reached 4.38 mg g^{-1} dw at the end of the ripening (Fig. 6).

Acknowledgement

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Recensio

ROBBRECHT Elmar, PUFF Christian & SMETS Erik (Eds.) 1996 [eingelangt 9. 4. 1997]. **Second International *Rubiaceae* Conference. Proceedings.** – Lex. 8°, 432 Seiten, zahlreiche Abb.; kart. – Opera Botanica Belgica 7. – National Botanic Garden of Belgium, Meise. – BEF 2950,–. – ISBN 90-72619-29-3, ISSN 0775-9592.

Der Band enthält Beiträge, die auf der Zweiten Internationalen Rubiaceen-Konferenz – vom 13.–15. September 1995 am Nationalen Botanischen Garten von Belgien – präsentiert worden sind. Sie gruppieren sich um die Themen Evolution (innerhalb der Familie und der *Gentianales*-Familien; 10 Beiträge), Phytochemie (2), Ethnobotanik (1), paläotropische (2) bzw. neotropische (5) *Rubiaceae* und Biologie und Struktur (4). Die Menge fundierter neuer Informationen über die nach der Artenzahl je nach Schätzung viert- bis fünftgrößte Pflanzenfamilie ist eindrucksvoll. Dennoch können hier natürlich nur einige der Arbeiten angesprochen werden.

B. BREMER, Phylogenetic studies within *Rubiaceae* and relationships to other families based on molecular data, p. 33–50 ist eine nützliche Diskussion des gegenwärtigen Kenntnisstandes aufgrund neuer molekularer Daten und vor dem Hintergrund des Systemvorschlages von ROBBRECHT 1988 bzw. 1993 (Opera bot. belg. 1 und 6). Die Unterfamilien *Rubioideae, Cinchonoideae* und *Ixoroideae* werden im wesentlichen bestätigt, während die Unterfamilie *Antirheoideae* nicht aufrechtzuhalten ist. Einen Schwerpunkt des Beitrages bildet weiter die Gliederung innerhalb der *Rubioideae*, die auch noch in anderen Beiträgen behandelt wird; diese Unterfamilie ist unter anderem durch eine spezielle Deletion im Chloroplastengenom ausgezeichnet (J.-F. MANEN & A. NATALI, p. 51–57). M. E. ENDRESS & al., A phylogenetic