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CHANGES IN THE CHEMICAL COMPOSITION, POLYPHENOL OXIDASE AND PEROXIDASE ACTIVITIES DURING DEVELOPMENT AND RIPENING OF MEDLAR FRUITS (*Mespilus germanica* L.)

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Summary. In this study, changes in the activities of polyphenol oxidase (PPO) and peroxidase (POD) as well as in the levels of soluble protein, soluble sugar, and ascorbic acid during development and ripening stages of medlar fruits were investigated. During the early stages of medlar fruits development, PPO activity and the level of ascorbic acid gradually decreased, whereas in the post-ripening stage PPO activity increased. Ascorbic acid level increased in the pre-ripening stage followed by a decrease in the post-ripening period. Similarly, POD activity decreased during development of the fruits and increased in the pre-ripening and post-ripening stages. The level of glucose gradually increased during fruit development and ripening. Contents of pentoses, hexoses and soluble proteins decreased during fruit development, but increased in the stage of ripening. These observations suggest that the increase in PPO and POD activities as well as in sugar and protein contents has an important role in reducing the astringent taste of the medlar fruits.

Key Words: Chemical composition, development, medlar, peroxidase, polyphenol oxidase.

Abbreviation: PPO – Polyphenol oxidase, POD – Peroxidase, PEG – Polyethylene glycol, *L*-Dopa – Dihydroxyphenylalanine, BSA – Bovine Serum Albumin, Tris – Tris(hydroximethyl) aminomethan, TEMED – N,N,N′,N′-tetramethyl-ethylenediamin, EDTA – Ethylenediamintetraacetikacit, PMSF – Phenylmethylsulphonylfluorid, CBB – CoomassieBrilliant Blue G 250, TX-114 – Tritone-X-114

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Introduction

Medlar (*Mespilus germanica* L.) belongs to Rosaceae family. Spiny shrub of the medlar is 2–3 m high (in cultivation a spineless tree up to 6 m). The fruits of medlar are subglobose, 1.5–3 cm. Medlar plants have been long-cultivated for their edible fruits. The cultivated plants have larger and sweeter fruits compared to the wild forms. The native form of medlar, at least in North Anatolia, open forest, on rocks and in macchie, is also widely cultivated (Davis, 1972).

There are not enough studies on the chemical composition of the medlar fruits. The level of ascorbic acid has only been investigated in the fruits of medlar. However, no information is available on the polyphenol oxidase (PPO) and peroxidase (POD) activities and the content of some chemical compounds during the development and ripening of the fruits. One of the major medlar fruit quality problem is flesh browning associated with the enzyme polyphenol oxidase.

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 of raw fruits and vegetables (Mathew, Parpia 1971). 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 flavour. Fruits and vegetables may also contain peroxidases (EC 1.11.1.7) which can contribute to or generate browning-like reactions (Vamos-Vigyazo 1981, Mayer, Harel 1991). The activities of peroxidase have been reported to increase with senescence advancement (Grover, Sinha 1985). Ascorbic acid is a natural inhibitor of PPO (Weaver, Charley 1974). On the other hand, biochemical studies of fruits indicate that levels of acids, sugars, soluble solids are the primary quantitative parameters (Senter, Callahan 1990).

The study was conducted to examine the changes in the contents of soluble proteins, soluble sugars, ascorbic acid and in the activities of polyphenol oxidase and peroxidase during the development and ripening of the medlar fruits.

Materials and Methods

Fruit samples

Medlar (*Mespilus germanica* L.) fruits were harvested from the spiny shrub grown in the vicinity of Karadeniz Technical University in Trabzon, Turkey from the mid of July to mid of November, 1999 (two months after anthesis) and immediately brought to the laboratory in boxes cooled up to –12 °C and stored at –20 °C. Half kg of fruits was collected in every harvesting period. Collection of the fruits was based on their colour and diameter changes. The exo-(skin) and mesocarps (flesh) were used for the analyses.

Enzyme extraction

Extraction of the enzymes from the fresh medlar fruits was carried out using the method of Sanches-Ferrer et al. (1990) with some modifications. Samples were powdered with liquid nitrogen. A 100 g sample of the medlar fruits was homogenized in 100 ml of 50 mM cold acetate buffer (pH 5.5) containing 6% (w/v) Triton X-114, 2 mM EDTA, 1 mM $MgCl_2$ and 1 mM PMSF as a protease inhibitor in a blender for 10 min and then filtered. The homogenate was kept at 4 °C for 20 min and then centrifuged at 14 000 × g for 20 min at 4 °C. The supernatant was collected and stored at –20 °C until use. It was used as a crude enzyme extract for the PPO, POD and protein analyses. Protein content was determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

PPO activity assay

PPO activity was assayed spectrophotometrically as described by Leonard (1971) using DL-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate. The reaction mixture containing 1.3 ml of 0.03 M potassium phosphate (pH 6.5) buffer and 1.2 ml L-DOPA was heated to 30 °C for 2 min and finally 0.5 ml of the enzyme extract was added to the cuvette. Changes in the absorbance at 475 nm were measured for 3 min using a Schimadzu UV-120-01 spectrophotometer. Enzyme activity was expressed as "ΔA_{475}/min/g fresh weight ".

POD activity assay

The POD activity was also determined spectrophotometrically according to a modification of the method described by Rodriguez and Sanchez (1982). The assay mixture contained 1 ml of 0.05 M phosphate-citrate buffer (pH 4.6), 1 ml of 40 mM guaiacol and 0.5 ml of 26 mM H_2O_2. The mixture was incubated for 15 min at 25 °C and finally 0.5 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. POD activity was expressed as "ΔA_{420}/min/g fresh weight".

Determination of ascorbic acid content

The content of ascorbic acid was analyzed using the procedure of Shieh and Sweet (1979). The samples were homogenized with 0.01 M phosphate-citric acid buffer at pH 3, filtered and centrifuged (8 000 × g 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 0.01 M phosphate-citric acid buffer at pH 3, 2.4 ml of 2,2′-Cu-biquinoline solution and 0.1 ml of the extract. The absorbance at 540 nm was recorded and ascorbic acid content was calculated on the basis of standard curves (pure ascorbic acid in the range of 6–33 µg/ml in phosphate-citric acid buffer).

Determination of soluble sugar content

Soluble sugar content was determined by the phenol-sulphuric acid method (Dubois et al., 1956). A standard curve was prepared to quantify pentose and hexose contents. Two grams of the samples were extracted in distilled water and centrifuged (6 000 × g for 5 min). The fruit extracts were treated with pure sulphuric acid and phenol (5%) and then their absorbances were measured at 480 nm, 488 nm and 490 nm for pentose, hexose and glucose, respectively. The sugar content was expressed on a dry weight basis.

Statistical analysis

All extractions and determinations were conducted 3 times at least. Analysis of variance of the data was evaluated by the Statistical Analysis System (Statgraph Version 5.0). Duncan's Multiple Range Test was employed to determine the statistical significance of the differences between the means.

Results and Discussion

In this study, we designated the fruit as immature from the first to fourth month of development. Fruit ripening started in the fourth month and ended in the fifth month. We observed that fruit colour and diameter might simply be indicators of medlar fruit developmental process and that the development period of fruit ends at the fourth harvesting period, then fruit ripening commences (Table 1).

Polyphenol oxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period, whereas the activity of PPO in the first months of development was higher than in the ripening months. The lowest activity was found at the third stage of development. To our knowledge this is the first report of PPO activity in the fruits of medlar. Similar results were also obtained in other studies (Vamos-Vigyazo, 1981; Kumar, 1987; Coseteng and Lee, 1987; Park et al., 1989; Kadioglu and Yavru, 1998). Peroxidase activity gradually decreased during the development periods, while during the periods of fruit ripening it

Table 1. Collection data and stages of development of the fruits of medlar

Harvest No.	Harvest date	Fruit Diameter	Fruit colour and state of fruit maturity
1	15 Jun 1999	1.8	Unripe, fully green
2	15 Jul 1999	1.9	Unripe, greenish
3	15 Aug 1999	2.1	Half ripe, greenish, partly brownish
4	15 Oct 1999	2.2	Pre-ripe, yellowish brown
5	15 Nov 1999	2.5	Post- ripe, yellow-brown

increased. Mukherjee and Rao (1993) have also obtained similar results with *Cajanus cajan* leaves. Their studies showed that POD activity during maturation and in the stage of senescence of the leaves continuously increased.

Protein content and activities of PPO and POD were found to be higher in the beginning of development and during the final process of ripening compared to the other periods, most probably due to higher metabolic activity during these development stages. Phenolic compounds are thought to be sequestered in cell vacuoles and include anthogenins, flavonols, cinnamic acid derivates, simple phenols, and catechin. Most of these phenolics are intermediates and derivates of the shikimate and phenylpropanoid pathways (Cheng and Breen, 1991). So, in the present study, the increases in PPO and POD activity in the beginning of fruit development may be involved in these processes. On the other hand, POD and PPO degrade fruit anthocyanin indirectly by reacting with D-catechin to form quinones that polymerise with anthocyanin pigments (Wesche-Ebeling, Montgomery 1990). The increase in both enzyme activities during ripening found in our study may depend on anthocyanin metabolism.

Soluble proteins decreased during development, then increased during ripening, due most probably to increased synthesis of enzymes involved in ripening and senescence of the fruit (Perkins-Veazie, 1995).

Pentose and hexose levels likewise soluble protein content decreased during fruit development, then continuously increased during fruit maturation. The level of glucose continuously increased during the development and ripening of medlar. Chapman and Horvat (1993) observed that glucose content of mayhaw fruit increased with maturation. Tsantili (1990) found that glucose concentration increased considerably during the rapid growth phase. The soluble sugar content gradually increased from the first week of the development to the end of the ripening periods of cherry laurel (Kadioglu and Yavru, 1998). The unripe medlar fruit has an astringent taste because of high level of proanthocyanidin and reduced sugar content. The increased sugar content probably affects the taste of the medlar fruits in the present study.

The highest ascorbic acid content was determined at the first stage of the developmental period and the lowest ascorbic acid content was determined at the post-ripening

Table 2. Changes in the some chemical composition of medlar fruits during development and ripening. The data followed by the same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

Harvest No.	Glucose	Pentose	Hexose	Ascorbic acid	Soluble protein
	(mg g^{-1} dry weight)				
1	0.55 a	333.5 c	259.7 b	7.3 c	0.61 c
2	0.47 a	189.6 a	143.1 a	4.5 b	0.17 a
3	1.07 b	263.4 b	189.6 a	3.7 ab	0.17 a
4	1.14 b	375.9 c	255.3 b	6.7 c	0.24 b
5	9.99 c	662.1 d	510.9 c	3.3 a	0.56 c

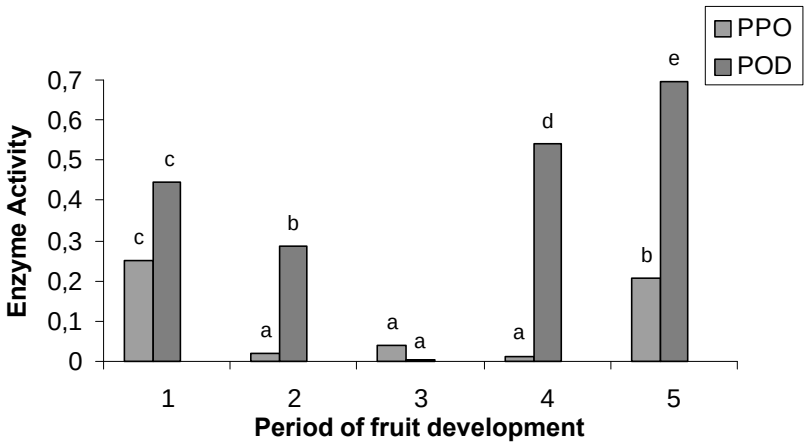

Fig. 1. Changes in the PPO "ΔA_{475}/min/g fresh weight" and POD "ΔA_{420}/min/g fresh weight" activities during the development and ripening of medlar. Monthly harvesting period of the fruits; 1 – 15 July; 2 – 15 August; 3 – 15 September; 4 – 15 October; 5 – 15 November. The mean of each enzyme activity was compared within it. The data followed by the same letter are not significantly different at 5% level (Duncan's Multiple Range Test).

stage of medlar fruits. Fuke and Matsuoka (1983) found that the total ascorbic acid content decreased with fruit enlargement. The ascorbic acid content of kaki was found as a continual decline depending on their development process (Inaba et al., 1971). Kadioglu and Yavru (1998) obtained similar results with cherry laurel. It has been reported that ascorbic acid acts as a free radical scavenger in animal and plant tissues (Foyer, 1993). The changes in ascorbic acid levels found in our study could be associated with its antioxidant properties. In addition, we observed that the decrease in ascorbic acid content was accompanied by increased PPO activity (not exactly in the first stage of the development). This interaction may be due to the inhibitor effect of ascorbic acid on PPO activity, because ascorbic acid is a natural inhibitor of PPO (Weaver and Charley, 1974).

The results presented in this work showed that there were important changes in the activities of PPO and POD as well as in the contents of protein, sugars and ascorbic acid. Especially the increase in sugar content and PPO and POD activities may reduce the astringent taste of the medlar fruits in the ripening stage. In addition, understanding of the biochemical changes and some enzyme activities in medlar, the chemistry of their transformations in the fruit and their functions in plant physiology, food science, nutrition and health should stimulate interest in maximizing beneficial sensory, nutritional effects of polyphenols in the diet. Such efforts should lead to better foods.

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