Cukurova University
Journal of
Natural & Applied Sciences

Original Paper

Phenolic composition, browning degree and enzyme activities of important Turkish apricot cultivars as influenced by harvesting year and ripening

Aysun Şener Gedük[1], Mustafa Ümit Ünal[2]

[1]University of Adana Alparslan Turkes Science and Technology, Faculty of Engineering, Department of Food Engineering, Adana, Turkey
[2]Çukurova University, Faculty of Agriculture, Department of Food Engineering, Adana, Turkey

ORCID IDs of the authors: A.Ş.G. 0000-0001-5306-6722; M.Ü.Ü. 0000-0002-2686-5286.

Cite this article as: Gedük, A. Ş., Ünal, M. Ü. (2022). Phenolic composition, browning degree and enzyme activities of important Turkish apricot cultivars as influenced by harvesting year and ripening, Cukurova University Journal of Natural & Applied Sciences 1(2): 26-36

Abstract

Phenolic content, browning degree, enzyme activities (polyphenol oxidase (PPO), pectin methylesterase (PME) and β-glucosidase) of three apricot varieties of Malatya region of Turkey were assessed during ripening on trees over two years. All measured variables varied across the cultivar, ripening stage and year. Both individual and total phenolic contents (TPC) decreased with ripening in all the cultivars studied. The activity of PPO, PME, and β -glucosidase continuously decreased with ripening in both harvest years. In all varieties browning degree decreased with ripening. It was observed that the greatest browning was in Hacıhaliloğlu cultivar at all ripening stages. Significant differences ($p < 0.01$) for browning degree were found among apricot variety and ripening. There was a significant positive correlation ($p < 0.01$) between browning degree and activity of PPO (R= 0.92), chlorogenic acid (R= 0.82), catechin (R=0.73,) total phenolic content (R=0.99).

Keywords: Apricot, Ripening, Enzyme activity, Phenolics, Enzymatic browning

1. Introduction

There have been various researches on fruit ripening owing to economic importance of fruit crop [1]. Fruit ripening is related to essential biochemical changes modifying color, taste, texture and other traits of quality. Ethylene level plays a role in the regulation of ripening in climacteric fruit including apricots [2]. Various changes such as physiological, biochemical, and organoleptic may occur in fruit ripening and these changes form the soft and edible ripe fruit with the expected features [3]. A study on the polyphenolic composition of fruit play a crucial role in terms of qualitative and quantitative differences seen as the species function, cultivar, degree of ripening, and growing, ripening, and storage properties. Determination of phenolic composition of fruit is significant due to the fact that the phenolic compounds are substantially convenient as markers of chemotaxonomic. In addition, the formation of stated compounds in fruit can vary in different species. Phenolic compounds also contribute to the quality attributes of fruits such as color, sourness, bitterness, and taste [4].

Latest research showed that inclusion of fruits and vegetables in diet reduces the danger of having chronic diseases [5]. There is an increasing demand in phenolic compounds due to their possible health benefits as antioxidants. Enomoto et al [6] reported that three Japanese apricots in daily diet inhibited mucosal inflammation of the stomach and progression of chronic atrophic gastritis in individuals having the infection of *Helicobacter pylori*. Vardi et al [7] examined oxidative stress in methotrexate-induced intestinal damage. Accordingly, they found the guarding impact of this fruit and/or β-carotene against this health problem. The same researchers showed this fruit and/or β-carotene treatment can prevent the oxidative stress impairment and ameliorate methotrexate-induced intestinal injury at the levels of biochemistry and histology.

Address for Correspondence:
Mustafa Ümit Ünal, e-mail: muunal@cu.edu.tr

Received: Feb 17, 2022
Accepted: Jun 15, 2022

Enzymatic browning reaction catalyzed by PPO (EC 1.14.18.1) is commonly encountered in fruits and vegetables. Brown pigments formed in this reaction usually are considered as detrimental to food quality and cause financial losses [8-10]. Therefore, PPO is considered as an important enzyme that can affect quality in fruits and vegetables. PPO levels are related to the species, cultivar, age and maturity [11].

The hydrolysis of pectin methyl ester groups is catalyzed by PME (EC 3.1.1.11), leading to de-esterification. Losing firmness during ripening is mostly related to the decline in the insoluble forms of pectic substances controlled by PME and polygalacturonase. Demethylation of pectin by PME facilitates further degradation of pectin by polygalacturonase [3, 12, 13]. b-glucosidases (EC 3.2.1.21) catalyzing the hydrolysis of the b-glycosidic bond are important class of enzymes in biology and biotechnology. In beverage industry these enzymes can be used to enhance aroma by releasing glycosidically bound volatile aroma compounds [9, 14].

Turkey ranks the top in terms of apricot production in the world and the production was 833398 tons in 2020 [15]. Most of the production of apricot is in Malatya. This region is well-known to be an ecological zone in Turkey in terms of cultivation as well as processing. Malatya region of Turkey is well-known due to the fact that half of the fresh ones and almost 100% of the dried ones of the entire country are produced here. Alyanak, Hacihaliloglu and Kabaasi are important apricot cultivars, commonly cultivated in Malatya due to their quality attributes. For this reason, they are protected as geographical indication in Turkey by means of Turkish Patent Institute. A name or a label used in specific products corresponding to a specific origin or location is the geographical indication. A geographical indication can also be used as a certificate in order to present quality, reputation and other features of a product of a specific geographical region [16].

There are various reports on phenolic content of apricots in literature [4, 17-19]. However, changes in browning degree and PME and β-glucosidase activity during ripening of apricot or seasonal variation in the activities of these enzymes and browning degree have not been yet investigated. Three cultivars of economically important apricot varieties (Hacıhaliloğlu, Kabaaşı and Alyanak) grown in Malatya were analyzed for their phenolics contents and change in browning degrees, and enzyme activities (PPO, PME, and β-glucosidase) at three ripening stages for two consecutive years.

2. Material and Method

2.1. Plant material

The apricot varieties (Hacıhaliloğlu, Kabaaşı, and Alyanak) were kindly provided by Apricot Research Center in Malatya, Turkey. After transportation, the fruits were kept at -25 °C until they were used.

2.2. Reagents

All chemicals were purchased from Fluka (USA), Sigma-Aldrich (St. Louis, USA), and Merck (Darmstadt, Germany).

2.3 Total Phenolic Content

TPC was determined spectrophotometrically in compliance with the colorimetric method of Folin-Ciocalteu. 100 g of apricot flesh was homogenized for 1 min using Waring blender. 1 g of ascorbic acid was used to prevent oxidation. The homogenate was filtered through cheesecloth. 1 mL filtrate, 5 mL of Folin-Ciocalteu reagent, 60 mL of dH_2O and 15 mL of sodium carbonate (20%) were added in 100 mL flask. After the content was mixed, the flask volume was made up to 100 mL with dH_2O and kept for 48 minutes in the dark. Absorption at 765 nm was measured in Shimadzu 300 UV-vis spectrophotometer (Shimadzu UV-1700, Japan). Quantification was done using a standard curve of gallic acid prepared with gallic acid solutions at concentrations varying between 50 mg/L and 500 mg/L. TPC concentration was expressed as gallic acid equivalents in g/100 mL. The analyses were performed three times and the results are presented as means [20].

2.4. Analysis of phenolic compounds using HPLC

50 g of apricot flesh were homogenized in 50 mL methanol/HCl (100:1, v/v) containing 1% tert-butylhydroquinon (*t*BHQ) for one minute using a Warring blender. The homogenate was mixed under nitrogen gas at 35°C for 12 hours in water bath, followed by centrifugation at 5000 rpm. The methanol content of the supernatant was removed in a vacuum evaporator at a temperature from 35 to 40°C. The remaining residue was dissolved one more time in 25 mL of /water/ethanol (4:1, v/v) solution and extraction

was performed four times using 25 mL of ethyl acetate. The organic fractions were combined and dried for 30 to 40 minutes with anhydrous sodium sulfate, filtered and evaporated to dryness under vacuum (35–40°C). The remaining residue was dissolved in 2 mL of methanol/water (1:1, v/v) solution again and filtered through 0.45 µm filter before injected (20 µL) into the HPLC. The analyses were carried out using Agilent 1100 HPLC and separation was performed on an ACE C18 column (250 mm x 4.6 mm, 5 µm), using water and acetic acid (97/3:v/v) and acetic acid, acetonitrile and water (3/25/72:v/v) as elution solvents. Calibration curves were obtained by injecting known amounts of individual phenolic compounds (50 mg/L to 200 mg/L) [21, 22].

2.5. *β*-glucosidase and polyphenol oxidase extraction and assay of activity

Unless otherwise stated all procedures for extraction were performed at a temperature of 4 °C. The frozen apricots were deseeded and then 300 g of flesh were homogenized for 2 minutes in 400 mL of cooled acetone (-25 °C) containing 3.33 g of polyethylene glycol using a previously cooled blender. Then, the mixture was filtered by means of filter paper. Extraction of the retentate was performed again as described above using 200 mL of cooled acetone. The final powder was dried at room temperature and kept in an air-tight jar -25 °C. 5 g of powder was dissolved in 150 mL phosphate-citrate buffer (0.1 M, pH 6.8) containing 10 mM ascorbic acid, 0.1% polyvinylpolypyrrolidone, 0.5 % Triton X-100 and 1mM PMSF. The mixture was stirred with a magnetic stirrer for 30 minutes and centrifugation was performed at 10000 g for 45 minutes. Ammonium sulphate precipitation was carried out at 85 % saturation for β-glucosidase and 90% saturation for PPO. The precipitate was centrifuged at 10000 g for 30 minutes β-glucosidase and for 45 minutes for PPO. The precipitates were dissolved in phosphate-citrate buffer (0.01 M, pH 6.8) and they were dialyzed overnight against the same buffer [23]. The amount of phosphate-citrate buffer was kept at minimum. Activity of β-glucosidase was determined spectrophotometrically as described by Lecas et al [23]. PPO activity assay was conducted according to Şener et al [11]. All reactions were performed in triplicate.

2.6. PME extraction and assay of activity

The extraction of PME was carried out at 4°C in order to prevent inactivation of the enzyme. PME activity was measured titrimetrically by determining the free carboxyl groups formed by the action of PME on pectin. PME activity was calculated using the following formula: PME (units/mL) = (mL of NaOH) (molarity of NaOH) (1000) /(time) (mL of enzyme) [24].

2.7. Browning degree

Apricot juice was by obtained homogenizing and filtering 200 g of deseeded apricots. The juice was then incubated at room temperature for 24 hours. After incubation the juice was centrifuged at 10000 x *g* for 10 minutes at room temperature. Optical density of the clarified juice was taken at 420 nm. The juice which was prepared with the addition of ascorbic acid (1%) presented as the control. Degree of browning (OD_{420}) was calculated by subtracting the optical density of the control from that of the sample [25].

2.8. Protein determination

The enzyme extract's protein content was found in accordance with Bradford method which was used bovine serum albumin as a standard [9].

2.9. Statistical analysis

ANOVA dual variance analysis was used for the data analysis. The difference of means was compared by means of the multiple range test of Duncan. In addition, Pearson correlation test was performed. Data processing was carried out using SPSS, version 10.0 for Windows (SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1. Changes in phenolics content

Phenolic compounds have health promoting effect. It was found that polyphenols have strong antioxidant activity due to their ability to donate an electron or hydrogen atom, thereby neutralizing free radicals formed during cellular metabolism that are related to the development of chronic diseases [26]. On the other hand, phenolic compounds are considered as significant PPO substrates in enzymatic browning in fruits and vegetables [8].

Phenolic contents of three important Turkish apricot cultivars were monitored for two consecutive years at immature, semimature and mature stages by measuring both TPC and individual phenolic compounds. The results are tabulated in Tables 1a, 1b and 1c. The highest level of phenolics in the three investigated varieties of apricots was observed in the immature stage. Both individual and total phenolic contents (TPC) decreased with ripening in all the cultivars studied. Changes due to ripening in each phenolic compound were found to be variety-dependent. The drop in TPC during ripening was lower in Hacıhaliloğlu cultivar than that in Alyanak and Kabaaşı cultivars. According to statistical analysis phenolic compounds showed significant differences ($p < 0.01$) among apricot variety, harvest year and ripening (Table 2).

Table 1. Change in phenolic compounds of apricots during ripening

(a) **Alyanak apricot**

Phenolic Compound (mg/Kg)	Alyanak					
	Immature		Semimature		Mature	
	2008	2009	2008	2009	2008	2009
Chlorogenic Acid	84.8±0.78	132.1±0.07	38.6±0.21	63.4±0.07	24.1±0.14	33.0±0.57
Gallic acid	0.41±0.01	0.83±0.01	0.32±0.00	0.65±0.00	0.27±0.00	0.59±0.01
Coumaric acid	3.90±0.00	1.95±0.07	0.60±0.01	0.96±0.03	0.51±0.01	0.77±0.00
Ferulic acid	0.33±0.00	3.30±0.01	0.19±0.01	1.57±0.01	0.23±0.01	0.23±0.01
Catechin	13.2±0.21	17.3±0.0	9.0±0.14	5.8±0.14	8.05±0.21	3.7±0.07
Epicatechin	2.80±0.07	5.13±0.01	3.33±0.01	2.87±0.01	0.93±0.02	1.15±0.07
Procyanidin B1	17.3±0.14	22.3±0.10	8.33±0.01	8.25±0.07	8.26±0.08	1.17±0.03
Procyanidin B2	22.3±0.14	35.3±0.14	8.25±0.07	14.3±0.14	4.45±0.07	3.96±0.04
Rutin	30.5±0.14	39.0±0.92	23.3±0.14	26.1±0.14	1.49±0.01	9.57±0.01
Kaemferol 3-rutinoside	1.20±0.00	1.64±0.01	1.15±0.01	1.33±0.01	0.19±0.00	0.74±0.01
Total phenol*	314.4±2.62	386.0±4.95	268.4±4.03	291.2±1.63	190.7±2.40	230.4±8.90

*mg/L, as gallic acid

(b) **Kabaaşı apricot**

Phenolic Compound (mg/Kg)	Kabaaşı					
	Immature		Semimature		Mature	
	2008	2009	2008	2009	2008	2009
Chlorogenic acid	41.7±1.63	34.6±2.76	24.1±0.14	19.4±1.06	27.9±0.35	8.4±0.07
Gallic acid	1.15±0.07	0.45±0.01	1.05±0.01	0.44±0.07	0.57±0.01	0.26±0.00
Coumaric acid	4.20±0.07	0.40±0.01	0.47±0.01	0.37±0.00	0.57±0.01	0.24±0.00
Ferulic acid	1.75±0.01	2.74±0.01	0.78±0.01	0.21±0.01	0.13±0.00	0.16±0.01
Catechin	11.1±0.78	8.4±0.21	9.5±0.14	6.8±0.07	1.73±0.04	1.3±0.14
Epicatechin	2.80±0.02	2.33±0.01	1.34±0.01	1.15±0.01	1.46±0.01	0.19±0.00
Procyanidin B1	29.8±0.01	31.8±0.01	6.11±0.01	9.75±0.01	2.73±0.00	3.87±0.01
Procyanidin B2	26.7±0.07	25.2±0.07	19.0±0.14	20.7±0.01	3.15±0.07	2.71±0.01
Rutin	26.2±0.07	33.0±0.14	23.1±0.14	28.0±0.14	1.47±0.01	9.36±0.01
Kaemferol 3-rutinoside	2.86±0.01	3.03±0.01	1.72±0.00	1.30±0.00	0.77±0.01	0.98±0.01
Total phenol*	294.2±6.43	260.2±0.07	199.1±0.57	171.3±1.56	160.1±4.74	133.1±0.85

*mg/L, as gallic acid

(c) **Hacıhaliloğlu apricot**

Phenolic Compound (mg/Kg)	Hacıhaliloğlu					
	Immature		Semimature		Mature	
	2008	2009	2008	2009	2008	2009

Chlorogenic acid	166.7±0.35	110.7±0.49	78.6±0.0	34.7±0.14	68.7±0.0	21.5±1.48
Gallic acid	1.78±0.01	0.70±0.07	0.85±0.01	0.70±0.01	1.10±0.01	0.46±0.00
Coumaric acid	4.60±0.02	1.79±0.01	1.21±0.01	0.40±0.00	0.43±0.00	0.20±0.07
Ferulic acid	0.67±0.07	0.34±0.01	0.52±0.01	0.23±0.02	0.22±0.00	0.29±0.00
Catechin	47.4±1.63	40.3±0.28	37.6±0.0	16.7±0.0	15.5±0.14	3.7±0.0
Epicatechin	13.3±0.14	8.9±0.01	6.30±0.01	6.9±0.07	4.60±0.03	3.46±0.01
Procyanidin B1	11.2±0.07	5.62±0.03	3.81±0.01	5.09±0.01	2.87±0.00	1.54±0.00
Procyanidin B2	60.7±0.14	46.4±0.03	37.3±0.07	23.4±0.07	36.5±0.14	20.2±0.07
Rutin	39.8±0.07	36.9±0.07	24.7±0.28	32.8±0.14	20.5±0.14	5.26±0.08
Kaempherol 3-rutinoside	3.47±0.01	2.38±0.01	2.88±0.02	1.38±0.00	0.40±0.02	0.20±0.01
Total phenol*	587.7±4.31	509.0±5.10	419.6±1.34	400.6±2.76	389.6±4.10	367.3±2.26

*mg/L, as gallic acid

Table 2. Effect of apricot variety, harvest year and ripening on phenolic compounds (mg/Kg)

Phenolic compound	Harvest year			Variety				Ripening			
	2008	2009	S.	A.	H.	K.	S.	I.	S-M.	M.	S.
Chlorogenic acid	60.47^{a}	50.84^{b}	**	62.65^{b}	80.13^{a}	24.20^{c}	**	95.05^{a}	43.75^{b}	28.17^{c}	**
Gallic acid	0.82^{a}	0.55^{b}	**	0.51^{c}	0.91^{a}	0.65^{b}	**	0.88^{a}	0.66^{b}	0.53^{c}	**
Coumaric acid	1.78^{a}	0.79^{b}	**	1.44^{b}	1.44^{b}	0.97^{c}	**	2.79^{a}	0.67^{b}	0.39^{c}	**
Ferulic acid	0.53^{b}	1.00^{a}	**	0.97^{a}	0.38^{c}	0.96^{b}	**	1.52^{a}	0.58^{b}	0.21^{c}	**
Catechin	16.99^{a}	11.54^{b}	**	9.49^{b}	26.86^{a}	6.45^{c}	**	22.92^{a}	14.23^{b}	5.66^{c}	**
Epicatechin	4.08^{a}	3.56^{b}	**	2.69^{b}	7.23^{a}	1.54^{c}	**	5.86^{a}	3.63^{b}	1.97^{c}	**
Procyanidin B1	10.03^{a}	9.93^{b}	**	10.94^{b}	5.01^{c}	14.0^{a}	**	1.97^{a}	6.89^{b}	3.41^{c}	**
Procyanidin B2	24.25^{a}	21.33^{b}	**	14.76^{c}	37.39^{a}	16.23^{b}	**	36.08^{a}	20.48^{b}	11.82^{c}	**
Rutin	21.22^{b}	24.43^{a}	**	21.65^{b}	26.64^{a}	20.18^{c}	**	34.20^{a}	26.33^{b}	7.94^{c}	**
Kaempherol 3-rutinoside	1.62^{a}	1.44^{b}	**	1.04^{a}	1.77^{b}	1.78^{b}	**	2.43^{a}	1.62^{b}	0.54^{c}	**
Total phenol$^{\&}$	313.7^{a}	305.4^{b}	**	280.2^{b}	445.6^{a}	203.0^{c}	**	391.9^{a}	291.7^{b}	245.2^{c}	**

A: Alyanak, H: Hacıhaliloğlu, K: Kabaaşı, I: Immature, S-M: Semi-mature, M: Mature, S: Significant

$^{\&}$mg/L, as gallic acid

*: Means in the same line with different letters are significantly different according to Duncan test ($p < 0.05$).

**: Significant at the 0.01 significance level

Of the cultivars examined, Hacıhaliloğlu had the highest TPC at all ripening stages, followed by Alyanak. TPC at all ripening stages in Alyanak apricot were found to be higher in the year 2009, whereas those in Kabaaşı and Hacıhaliloğlu apricots were higher in the year 2008. It was asserted that plants decrease their content of fruits polyphenols during maturation to make them more palatable at the stage of ripening and to spread their seeds to increase their reproductive fitness [26]. There are contradictory results reported for the change in TPC during ripening. Dragovic-Uzelac et al [4], who investigated change in TPC during ripening of three Croatian apricot cultivars found that TPC level decreased with ripening, which is also the case in our study. On the contrary, Hegedüs et al [19] stated a stable increase in TPC in two Hungarian apricots during ripening, which is contradictory to our results. They attributed the inconsistencies in TPC change during ripening to differences in genotypes, analytical methods employed and nonidentical ripening stages. It was reported that composition and concentration of apricot phytochemicals were affected by various factors such as variety, maturity, climate and the analyzed part of the fruit (peel or flesh) [18].

The most abundant phenolic compound at all maturation stages in all three cultivars was chlorogenic acid with the exception that rutin was the major phenolic compound in Kabaaşı cultivar at semimature and mature stages in 2009. The other predominant phenolics compounds were procyanidin B1, procyanidin B2, rutin, and catechin. Campbell et al [18] reported that the predominant phenolic compounds were catechin, chlorogenic acid, and neochlorogenic acid in five different American apricots. Dragovic-

Uzelac et al [4] reported that flavan-3-ols, chlorogenic acid and quercetin-3-rutinoside were major phenolic compounds in all ripening phases of three Croatian apricot cultivars.

3.2. Changes in enzyme activities

Changes in the activities of PPO, PME and β-glucosidase during ripening of the apricot cultivars were given in Table 3 and Table4. The enzyme activities varied across the cultivar, year and ripening stage. PPO, PME and β-glucosidase activities continuously decreased with ripening in both harvest years. Significant differences (p <0.01) for PME, PPO and β-glucosidase activity were observed among apricot variety, ripening. However, no statistical differences were found among harvest year. There were year-to-year variations in the enzyme activities analyzed. Moreover, a general trend was evident, whereby the activities of PPO, PME and β-glucosidase at all ripening stages in Alyanak apricot cultivar were higher in 2009 than in 2008, whereas those in Kabaaşı apricot were found to be lower in 2009. As for Hacıhaliloğlu apricot, the activity of PME was higher in 2009 than in 2008, whereas, the activities of PPO and β-glucosidase were lower in 2009. Hacıhaliloğlu apricot cultivar had the highest PPO activity throughout ripening, followed by Alyanak. Kabaaşı had the lowest PPO activity. The highest PPO activity at maturity was found in Hacıhaliloğlu cultivar, whereas PME activity at maturity was highest in Kabaaşı cultivar in both years. The highest β-glucosidase activity at maturity was observed in Hacıhaliloğlu cultivar in 2008 and in Alyanak cultivar in 2009.

Table 3. Changes in PPO, PME, b-glucosidase activities (Unit/mg protein) and browning degree (OD_{420}) during ripening

Cultivar	Parameter	Immature		Semimature		Mature	
		2008	2009	2008	2009	2008	2009
Alyanak	PME	25.3	29.7	24.5	29.4	23.4	28.8
	PPO	318685	451246	262655	422438	211376	389354
	β-glucosidase	19.3	24.1	18.1	24.0	17.6	22.6
	Browning degree	0.90	0.97	0.60	0.74	0.30	0.45
Hacıhaliloğlu	PME	39.4	51.9	36.8	41.4	34.0	37.9
	PPO	487957	467251	457302	438293	429993	403958
	β -glucosidase	21.2	19.5	19.6	18.5	19.3	17.0
	Browning degree	1.46	1.34	1.11	1.11	0.91	0.72
Kabaaşı	PME	71.1	51.3	61.1	47.6	52.6	46.7
	PPO	301106	265445	259566	228359	201463	184655
	β -glucosidase	20.3	17.3	17.7	13.8	17.4	13.4
	Browning degree	0.703	0.64	0.60	0.51	0.19	0.13

Table 4. Effect of apricot variety, harvest year and ripening on enzyme activities (Unit/mg protein) and browning degree (OD_{420})

	PME	PPO	b-glucosidase	Browning degree
Variety	**	**	**	**
Alyanak	26.85^{c}	342625.7^{b}	20.95^{a}	0.66^{b}
Hacıhaliloğlu	40.23^{b}	447459.0^{a}	19.18^{b}	1.09^{a}
Kabaaşı	55.07^{a}	240099.0^{c}	16.65^{c}	0.46^{c}
Harvest Year	NS	NS	NS	NS
2008	40.91	325567.0	18.94	0.74
2009	40.52	361222.1	18.91	0.73
Ripening	**	**	**	**
Immature	44.78^{a}	381948.33^{a}	20.28^{a}	1.00^{a}
Semimature	40.13^{b}	344768.83^{b}	18.62^{b}	0.77^{b}
Mature	37.23^{c}	303466.50^{c}	17.88^{c}	0.45^{c}

*: Means in the same column with different letters are significantly different according to Duncan test ($p < 0.05$).
**: Significant at the 0.01 significance level, NS: no significant differences.

With regard to the change in PPO activity during fruit ripening, contradictory results have been reported in the literature. For instance, Barret [27] who studied characterization of apricot PPO during apricot fruit development reported that PPO activity increased with increasing fruit development to full ripeness, which stands in contrast to our results. Murata et al [28] reported that the PPO activity per milligram of protein decreased during apple ripening. Duan et al. (2014) [29] investigated changes in enzymatic activities during fruit ripening in Hawthorn. They reported that PPO activity declined significantly with fruit ripening. These were consistent with our findings.

Abu-Sarra & Abu-Gough [30] investigated changes in the activity of enzymes degrading cell wall during mango ripening. The authors reported that pectin methylesterase activity decreased continuously during the ripening of two mango cultivars and increased up to firmness 88N, subsequently decreased in another cultivar. El-Zoghbi [31] found that PME activity declined in mango, guava, and strawberry during ripening and increased in date during ripening. Jain et al [32] who studied compositional and enzymatic changes during ripening guava fruit reported that PME activity increased up to color turning stage and decreased at ripe stage. Majumder and Mazumdar [33] reported that PME activity was not directly associated with fruit ripening of cape-gooseberry. Abu-Goukh and Bashir [30] studied changes in PME activity during ripening of white and pink-fleshed guava fruits. They found PME activity increased in both guava varieties and then decreased. It appeared that there was no clear trend concerning the changes in PME activity during fruit ripening, which was also noted by Prasanna et al [3].

Fils-Lycaon and Buret [34] investigated differences in various glycosidases in mesocarp tissues of muskmelon (*Cucumis melo* L., var. Alpha) at the time of fruit ontogeny. It was reported that there was a significant increase in the activity of α-D-glucosidase from the turning to the overripe stages. Gerardi et al [35] also reported a stable rise of β-glucosidase during ripening of sweet cherry. These results stand in contrast to our results.

3.3. Changes in browning degrees

Among the essential reactions occurring in fruits and vegetables, enzymatic browning is a crucial one. It occurs as a result of oxidation of phenolic compounds to quinones catalyzed by PPO when oxygen is available. Enzymatic browning encountered during harvesting, processing and storage of fruit and vegetables impairs color, taste, flavor, and nutritional value of the product [8, 20]. Therefore, enzymatic browning of fruits and vegetables is a significant subject from the standpoint of food science and technology [28]. Susceptibility of grape varieties to browning was linked to the differences in the content of reductive species which are able to react with glutathione, ascorbic acid and cysteine [36].

Browning degrees of three Turkish apricot cultivars was monitored during fruit ripening for two years. Browning degrees varied across the cultivar, ripening stage and year (Table 3 and Table 4). In all varieties browning degree decreased with ripening, which was accompanied by a decrease in PPO activity, TPC and chlorogenic acid which is a good substrate for PPO. The highest degree of browning was found in Hacıhaliloğlu cultivar at all the stages of ripening, followed by Alyanak and Kabaaşı. When the susceptibilities to browning were compared, Hacıhaliloğlu was the most sensitive to browning and Kabaaşı was found to be the least. It would be interesting to note that Hacıhaliloğlu cultivar with the highest browning degree had also the highest PPO activity and TPC (See Tables 1c and 3). Hacıhaliloğlu cultivar also had the highest amount of chlorogenic acid which is also a good substrate for PPO. Significant differences (p <0.01) for browning degree were found among apricot variety and ripening while no statistical differences were found among harvest year. There was a significant positive relation (p < 0.01) between browning degree and PPO activity (R= 0.92), chlorogenic acid (R= 0.82), catechin (R=0.73,) total phenol (R=0.99).

Murata et al [28] reported a decrease in degree of browning of apple juice during ripening, which was also consistent with the results of our study. Holderbaum et al [8] found a high correlation between that enzymatic browning showed and polyphenol content in four apple cultivars they studied while a high correlation between browning and PPO activity in two cultivars.

Cheng and Crisosto [37] investigated browning potential and PPO activity of buffer extracts of peach and nectarine skin tissue. A significant correlation was found between chlorogenic acid and (-)-epicatechin content and browning in the first hour of incubation, while there was no significant correlation between PPO activity and browning potential of peaches and nectarines. Lee et al [38], however, reported that there was a relation between the degree of browning and PPO activity of individual peach cultivars. Moreover, they expressed browning degree was associated with total phenolics. Ünal & Şener [10] found no significant correlation (P<0.05) between browning degrees of three white grapes and PPO activity and total phenolics contents.

Walter & Purcell [38], who investigated correlation between browning degree and phenolics content, polyphenolase activity, and ascorbic acid levels in five sweet potato cultivars highlighted that browning degree was found to be significantly correlated only to phenolics content. Furthermore, they observed a considerable within-year and year-to-year cultivar variation in browning potential. Arzani et al [40] examined the correlation between PPO activity, polyphenol and ascorbic acid content and internal browning of Asian pear (*Pyrus sertina* Rehd.) during storage in association with the time of harvesting. The authors reported lower internal browning in earlier harvested Asian pear which was attributed to the presence of higher level of ascorbic acid and a lower level of PPO activity compared to the later harvested product.

4. Conclusions

Changes in phenolic contents, some enzyme activities (PPO, PME, b-glucosidase) and browning degrees of Alyanak, Hacihaliloğlu and Kabaaşı apricots were monitored for two consecutive years at immature, semimature and mature stages by measuring both TPC and individual phenolic compounds. The highest level of phenolics in all varieties was observed in the immature stage. Both individual and total phenolic contents decreased with ripening. The most abundant phenolic compound at all maturation stages in all three cultivars was chlorogenic acid with the exception that rutin was the major phenolic compound in Kabaaşı cultivar at semimature and mature stages in 2009. The other predominant phenolics compounds were procyanidin B1, procyanidin B2, rutin, and catechin. PPO, PME and β-glucosidase activities varied across the cultivar, year and ripening stage. PPO, PME and β-glucosidase activities continuously decreased with ripening in both harvest years. Browning degrees varied across the cultivar, ripening stage and year. In all varieties browning degree decreased with ripening, which was accompanied by a decrease in PPO activity, TPC and chlorogenic acid which is a good substrate for PPO. The highest degree of browning was found in Hacıhaliloğlu cultivar at all the stages of ripening, followed by Alyanak and Kabaaşı.

Acknowledgements

This study (ZF2008D4) was funded by Research Fund of University of Çukurova (CUBAP), Turkey.

References

[1] Payasi, A., Sanwal, G. G. (2010). Ripening of climacteric fruits and their control. Journal of Food Biochemistry. 34, 679-710.

[2] Bureau, S., Renard, C. M. G. C., Reich, M., Ginies, C., Audergon, J-M. (2009). Change in anthocyanin concentrations in red apricot fruits during ripening. LWT - Food Science and Technology. 42, 372-377.

[3] Prasanna, V., Prabha, T. N., Tharanathan, R. N. (2007). Fruit ripening phenomena-An overview. Critical Reviews in Food Science and Nutrition. 47, 1-19.

[4] Dragovic-Uzelac, V., Levaj, B., Mrkic, V., Bursac, D., Boras, M. (2007). The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. Food Chemistry. 102, 966–975.

[5] Hooper, L., Cassidy, A. (2006). A review of the health care potential of bioactive compounds. Journal of the Science of Food and Agriculture. 86, 1805–1813.

[6] Enomoto, S., Omoto, K., Yanaoka, H., Utsunomiya, T., Niwa, K., Inada, H., Deguchi, K., Ueda, C., Mukoubayashi, I., Inoue, T., Maekita, K., Nakazawa, M., Iguchi, K., Arii, H., Tamai, N., Yoshimura, M., Fujishiro, M., Ichinose, O. M. (2010). Inhibitory effects of Japanese Apricot, (Prunus Mume Siebold ET Zucc.; Ume), on Helicobacter pylori-related chronic gastritis. Nature Reviews Gastroenterology & Hepatology. 7, 478.

[7] Vardi, N., Parlakpinar H, Ozturk, F., Ates, B., Gul, M., Cetin, A., Erdogan, A., Otlu, A., (2008). Potent protective effect of apricot and b-carotene on methotrexate-induced intestinal oxidative damage in rats. Food and Chemical Toxicology. 46, 3015-3022.

[8] Holderbaum, D. F., Kon, T., Kudo, T., Guerra, M. P. (2010). Enzymatic browning, polyphenol oxidase activity, and polyphenols in four apple cultivars: Dynamics during fruit development. American Society for Horticultural Science. 45, 1150–1154.

[9] Ünal, M.Ü., Aksoy, V. A., Şener, A. (2014). Isolation, purification and determination of some biochemical properties of β–glucosidase from Muscat of Bornova grape. European Food Research and Technology. 238(1), 9-15.

[10] Ünal, M. Ü., Şener, A. (2016). Correlation between browning degree and composition of important Turkish white wine grape varieties. Turkish Journal of Agriculture and Forestry. 40: 62-67.

[11] Şener, A., Ünal, M. Ü., Aksay, S. (2011). Purification and characterization of polyphenol oxidase from Goldnugget loquat (Eriobotrya japonica cv Goldnugget). Journal of Food Biochemistry. 35, 1568-1575.

[12] Fischer, R. L., Bennett, A. B. (1991). Role of cell wall hydrolyses in fruit ripening. Annual Review of Plant Physiology and Plant Molecular Biology. 42, 675-703.

[13] Sila, D. N., Smout, C., Satara, Y., Truong, V., Loey, A.V., Hendrickx, M. (2007). Combined thermal and high pressure effect on carrot pectinmethylesterase stability and catalytic activity. Journal of Food Engineering. 78, 755-764.

[14] Esen, A. (2003). β-Glucosidase. In: Whitakeer, J. R., Voragen, A. G. J., Wong, D. W. S. (eds). Handbook of Food Enzymology, Marcel Dekker, New York. pp 791-803.

[15] TUIK (2021). Turkish Statistical Institute. Retrieved in October 25, 2021 from https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1.

[16] Akin, E. B., Karabulut, I., Topcu, A. (2008). Some compositional properties of main Malatya apricot (*Prunus armeniaca* L.) varieties. Food Chemistry. 107, 939-948.

[17] Ali, S., Masud, T., Abbasi, K. S. (2011). Physico-chemical characteristics of apricot (Prunus armeniaca L.) grown in Northern Areas of Pakistan. Scientia Horticulturae. 130, 386-392.

[18] Campbell, O. E., Merwin, I. A., Padilla-Zakour, O. I. (2013). Characterization and the effect of maturity at harvest on the phenolic and carotenoid content of northeast USA apricot (Prunus armeniaca) varieties. Journal of Agricultural and Food Chemistry. 61, 12700-12710.

[19] Hegedüs, A., Pfeiffer, P., Papp, N., Abranko, L., Blázovics, A., Pedryc, A., Stefanovits-Bányai, E. (2011). Accumulation of antioxidants in apricot fruit through ripening: characterization of a genotype with enhanced functional properties. Biological Research. 44, 339-344.

[20] Ünal. M. Ü., Şener, A, (2016). Two-year comparison of the biochemical properties of polyphenol oxidase from Turkish Alyanak apricot (*Prunus armenica* L.). Food Chemistry. 190, 741-747.

[21] Bengoechea, M. L., Sancho, A. I., Bartolome, B., Estrella, I., Gomes-Cordoves, C., Hernandez, M. T. (1997). Phenolic content of industrially manufactured purees and concentrates from peach and apple fruits. Journal of Agricultural and Food Chemistry. 45, 4071-4075.

[22] Dragovic-Uzelac, V., Pospisil, J., Levaj, B., Delonga, K. (2005). The study of phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. Food Chemistry. 91, 373–383.

[23] Lecas, M., Günata, Z. Y, Sapis, J. C., Bayonove, C. L. (1991). Purification and partial characterization of β-glucosidase from grape. Phytochemistry. 30, 451-454.

[24] Denes, J. M., Baron, A., Drilleau, J. F. (2000). Purification, properties and heat inactivation of pectin methylesterase from apple (cv *Golden Delicious*). Journal of the Science of Food and Agriculture. 80, 1503-1509.

[25] Lee, C. Y., Jaworski, A. W. (1988). Phenolics and browning potential of white grapes grown in New York. American Journal of Enology and Viticulture. 39, 337-340.

[26] Valle, A. Z. D., Mignani, I., Spinardi, A., Galvano, F., Ciappellano, S. (2007). The antioxidant profile of three different peaches cultivars (*Prunus persica*) and their short-term effect on antioxidant status in human. European Food Research and Technology. 225, 67-172.

[27] Barret, R. (2002). Characterization of apricot polyphenol oxidase during fruit development. MSc Dissertation. University of Adelaide: Adelaide, Australia.

[28] Murata, M., Tsurutani, I. M., Tomita, M., Homma, S., Kaneko, K. (1995). Relationship between Apple Ripening and Browning: Changes in Polyphenol Content and Polyphenol Oxidase. Journal of Agricultural and Food Chemistry. 43, 1115-1121.

[29] Duan, J-.L, Ma, X-L., Meng, G-T. & Xu, J-G. *(2014).* Changes in chemical compositions and enzymatic activities during fruit ripening in hawthorn (Crataegus Pinnatifida). Asian Journal of Agricultural Sciences. 2, 397-402.

[30] Abu-Sarra, A. F., Abu-Gough, A. A. (1992). Changes in pectinesterase, polygalacturonase and cellulose activity during mango fruit ripening. Journal of Horticultural Science and Biotechnology. 67, 561-568.

[31] El-Zoghbi, M. (1994). Biochemical changes in some tropical fruits during ripening. Food Chemistry. 49, 33-37.

[32] Jain, N., Dhawan, K., Malhotra, S., Randhir, S. (2003). Biochemistry of fruit ripening of guava (*Psidium guajava* L.): Compositional and enzymatic changes. Plant Foods for Human Nutrition. 58, 309–315.

[33] Majumder, K., Mazumdar, B. C, (2002). Changes of pectic substances in developing fruits of cape-gooseberry (*Physalis peruviana* L.) in relation to the enzyme activity and evolution of ethylene. Scientia Horticulturae. 96, 91-101.

[34] Fils-Lycaon, B., Buret, M. (1991). Changes in glycosidase activities during development and ripening of melon. Postharvest Biology and Technology. 1, 143-151.

[35] Gerardi, C., Blando, F., Santino, A., Zacheo, G. (2001). Purification and characterisation of a β-glucosidase abundantly expressed in ripe sweet cherry (*Prunus avium* L.) fruit. Plant Science. 160, 795–805.

[36] Moreno-Arribas, M. V., Polo, M. C. (2009). Wine Chemistry and Biochemistry. Springer, New York. pp: 104(207), 512.

[37] Cheng, G. W., Crisosto, C. H. (1995). Browning potential, phenolic composition, and polyphenoloxidase activity of buffer extracts of peach and nectarine skin tissue. Journal of the American Society for Horticultural Science. 120, 835-838.

[38] Lee, C. Y., Kagan, V., Jaworski, A. J., Brown, S. K. (1990). Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. Journal of Agricultural and Food Chemistry. 38, 99-101.

[39] Walter, J.. R W. M., Purcell, A. E. (1980). Effect of substrate levels and polyphenol oxidase activity on darkening in sweet potato cultivars. Journal of Agricultural and Food Chemistry. 28, 941-944.

[40] Arzani, K., Khoshghalb, H., Malakouti, J., Barzegar, M. (2009). Polyphenoloxidase activity, polyphenol and ascorbic acid concentrations and internal browning in asian pear (Pyrus serotina Rehd.) fruit during storage in relation to time of harvest. European Journal of Horticultural Science. 74, 61-65.