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Chemical changes of three native Turkish hazelnut varieties (*Corylus avellana* L.) during fruit development

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Abstract

Three native hazelnut varieties from Turkey, namely, Tombul, Palaz, and Badem, were examined for their proximate composition, minerals, and fatty acid profiles, as well as polyphenol oxidase (PPO), peroxidase (POD), and lipase activities during fruit development stages (early stage: ES, middle stage: MS, and harvest stage: HS). Proximate composition varied considerably (dry weight basis) from ES to MS. Fat was the predominant component at all stages and showed increasing trends. Six essential minerals (calcium, iron, magnesium, phosphorus, potassium, and zinc) were analysed (dry weight basis). Consuming recommended daily amount of 42.5 g hazelnut at HS supplies 23.3–25.0% of phosphorus, 11.6–18.1% of magnesium, 7.0–18.9% of iron, 4.9–8.9% of zinc, 5.1–5.7% of calcium, and 5.1–5.3% of potassium for recommended dietary allowances or adequate intake for adults. Significant ($P < 0.05$) decreasing trends were found in all mineral contents from early development to maturity, with some exceptions. Sixteen fatty acids were identified, among which 18:1ω9 was by far the most predominant one, followed by 18:2ω6, 16:0, and 18:0. As expected, total monounsaturated fatty acids constituted the main group of fatty acids ranging from 75.51 to 81.07% in Tombul, from 78.21 to 82.71% in Palaz, and from 73.69 to 81.65% in Badem through the maturation stages. In contrast, total polyunsaturated fatty acids showed decreasing trends from ES to HS. No significant changes ($P > 0.05$) were observed in total saturated fatty acids at different maturation stages. Tombul variety had the lowest PPO activity compared to those of Palaz and Badem. Badem showed highest POD activity compared to Tombul and Palaz at three stages of maturation and significant decreases ($P < 0.05$) in all hazelnut samples were observed in POD activity from ES to HS. No lipase activity was detected in any hazelnut samples at ES and MS, except in Badem at MS. In contrast, lipase activity was detected in all hazelnut samples at HS. These results suggest that some proximate compositions, minerals, and fatty acids gave good indications during fruit development stages, whereas enzymatic activities of PPO, POD, and lipase behaved differently among varieties and fruit development stages.

Keywords: Hazelnut varieties; Fruit development; Proximate composition; Minerals; Fatty acids; Peroxidase; Polyphenol oxidase; Lipase

1. Introduction

Hazelnut (*Corylus avellana* L.) belongs to the *Betulaceae* family and is a popular tree nut worldwide, mainly distributed in the coasts of the Black Sea region of Turkey, the Southern Europe (Italy, Spain, Portugal, France, and Greece), and in some areas of the US (Oregon and Washington). Besides, hazelnut can also be cultivated in some other countries such as New Zealand, China, Azerbaijan, Chile, Iran, and Georgia, among others. Turkey is the world's largest producer of hazelnuts, contributing ~74% to the total global production, followed by Italy (~16%), the US (~4%), and Spain (~3%). Other countries contribute ~3% to the total production (Turkish Hazelnut Exporter's Union, 2007).

Among tree nut species, hazelnut plays a major role in human nutrition and health, because of its special fatty acid profiles, protein, carbohydrates, vitamins, minerals, dietary fibre, phytosterols, tocols, squalene, antioxidants, phenolics, and phytochemicals, among others (Alasalvar, Amaral, & Shahidi, 2006; Alasalvar, Karamać, Amarowicz, & Shahidi, 2006; Alasalvar, Shahidi, Liyanapathirana, & Ohshima,

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2003; Alasalvar et al., 2003; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004; Shahidi, Alasalvar, & Liyana-Pathirana, 2007). Besides its nutritional characteristics, hazelnut provides a unique and distinctive flavour as an ingredient in a variety of food products (Alasalvar et al., 2004; Alasalvar, Shahidi, & Cadwallader, 2003).

Several research groups have reported the benefits of inclusion of hazelnut into human diet (Alphan, Pala, Açkurt, & Yilmaz, 1997; Durak et al., 1999; Mercanlıgil et al., 2007). This is mainly related to its fat components (around 60–65%), most of which are highly rich in monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), tocopherols, phytosterols, polyphenols, squalene, minor components, and phytochemicals (Alasalvar, Amaral, et al., 2006; Maguire et al., 2004; Miyashita, Tsubakihara, & Alasalvar, 2005; Shahidi et al., 2007).

The unsaturated fatty acid content of the hazelnut makes it a nutritional product but also makes it more susceptible to oxidation. The rates of oxidation of fatty acids are approximately 1:10:100:200 for stearic acid (18:0), oleic acid (18:1ω9), linoleic acid (18:2ω6), and α-linolenic acid (18:3ω3), respectively (Ragnarsson & Labuza, 1977). The relative content of these fatty acids greatly influences the oxidation rates, flavour deterioration, and shelf life of hazelnut.

Understanding the action and the mechanism of enzymes responsible for the rancidity in nuts is, therefore, essential for designing and developing means to control the activity of these enzymes to retain as much as possible the flavour and storage characteristics of nuts and nut products (Sanders, Vercellotti, & Grimm, 1993). Although the existence of peroxidase (POD), polyphenol oxidase (PPO), and lipase have been studied in various hazelnut varieties at post harvest stage (Bonvehí & Rosuo, 1996; Gosling & Ross, 1981; Grosch, Laskawy, & Senser, 1983; Keme & Messerli, 1976; Lopez et al., 1997), there is no information on the activities of these enzymes during the maturation stages. These cited studies have acknowledged that enzyme activity differs according to geographical areas and storage conditions. In addition, the reaction products of fatty acids due to the oxidation or the action of enzymes have a major effect on shelf life and quality of raw nuts (Ragnarsson & Labuza, 1977; Sanders et al., 1993). It is, therefore, very important to determine the changes of fatty acid composition and enzyme activity during maturation stages. The objective of this research was to compare the existing chemical changes (proximate composition, fatty acid profiles, minerals, and various enzyme activities) of three native Turkish hazelnut varieties (Tombul, Palaz, and Badem) during fruit development.

2. Materials and methods

2.1. Samples

Different hazelnut varieties such as Tombul, Palaz, and Badem were collected from the Black Sea Region of Turkey (0–250 m attitude) in consecutive years of 2002–2004. Sampling was made in each orchard according to "Z" pattern (AOAC, 1996). The same trees were marked and used for subsequent sampling throughout the three years. The sampling was done at three stages: early stage (ES), 8–15 July; middle stage (MS), 22–30 July; harvest stage (HS), 12–30 August. Hazelnut samples were placed inside a polystyrene box with cooling gel (pre-frozen to −20 °C) and transferred to the TÜBİTAK-MRC, Food Institute within the same day. Upon arriving to the laboratory, samples were stored at −20 °C until analysed.

2.2. Chemicals

All chemical reagents and standards were obtained from Sigma–Aldrich–Fluka Co. Ltd. (Istanbul, Turkey), unless otherwise stated.

2.3. Analysis of minerals

Calcium, iron, magnesium, potassium, and zinc (method 985.35) and phosphorus (method 986.24) were analysed according to the official method of the Association of Official Analytical Chemists (AOAC, 2000). A 1 g sample of finely grated hazelnut was transferred to PTFE (polytetrafluoroethylene) vessel and 8 ml of 65% nitric acid (HNO_3) were added. Digestion was performed in a microwave oven (model ETHOS Plus 900, Milestone Corp., Sorisole, Italy) using the following procedure: microwave power was increased over four steps (25, 45, 55, and 65%) at 5 min intervals. Temperatures of 120, 140, 150, and 160 °C were set for each of the four steps, respectively. Pressure limits for the four steps were 20, 80, 120, and 171 psi, respectively. Digest was diluted with 50 ml of deodorized water prior to analysis. Minerals were determined using an AAnalyst 700 AA spectrometer equipped with a FIAS 100 flame injection system, an AS 90 autosampler, and a hollow cathode lamp power supply (Perkin Elmer, Norwalk, CT). In all cases, an air acetylene flame was used. Minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with corresponding standards.

2.4. Proximate analysis

Percentages of moisture by vacuum oven (method 934.06), total fat by Soxtec apparatus using petroleum ether (method 960.39), protein by Kjeldahl nitrogen (method 920.152), and ash by direct analysis (method 940.26) were determined according to the Association of Official Analytical Chemists (AOAC, 2000). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 5.30 (AOAC, 2000). Total carbohydrates were calculated by subtracting the total percentage of other components from 100.

2.5. Analysis of fatty acids

Fatty acid methyl esters (FAMEs) were prepared from hazelnut oil and determined by gas chromatography (GC) according to the method described by Slover and Lanza (1979) and Alasalvar et al. (2003). FAMEs were prepared using BF_3 in methanol (20% of BF_3 in methanol) and extracted with *n*-haxene and then analyzed by GC. For this purpose, samples (1 μl) were injected into a Supelcowax 10 column (60 m × 0.25-mm i.d., 0.25-μm film thickness; Supelco, Bellefonte, PA) coated with poly-(ethylene glycol). The column was connected to a Hewlett–Packard 5890 Series II (Little Falls, Willmington, DE) GC equipped with a flame-ionization detector. The oven temperature was programmed as follows: 180 °C for 2 min, increased to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then increased to 215 °C at 2 °C/min and kept there for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. FAME identification was based on retention times compared with those of standard FAMEs.

2.6. Extraction of enzymes and protein content

The enzyme extract was obtained according to the method of Espín, Trujano, Tudela, and García-Cánovas (1997) with a slight modification. Homogenisation of hazelnut kernels, centrifugation, and dialysis were carried out at 4 °C. A 60 g sample of hazelnut kept at −20 °C was homogenised with 200 ml of cold 0.1 M phosphate buffer (pH 7.25) containing 20 mM EDTA (ethylenediaminetetraacetic acid) and 2% TX-114 for 2 min in a Waring blender (New Hartford, CT) at low speed. The homogenate was kept at 4 °C for 60 min before being centrifuged at 28 500*g* for 30 min. The supernatant was collected and used as crude enzyme extract. It was then subjected to temperature-induced phase partitioning by warming to 35 °C for 15 min. This solution was centrifuged at 7500*g* for 15 min at 25 °C. The detergent rich phase was discarded and the supernatant was acidified to pH 4.5 with acetic acid. The acidified solution was centrifuged at 28 500*g* for 20 min. The supernatant brought to 25% saturation with solid ammonium sulphate under continuous stirring at 4 °C. After 20 min, solution was centrifuged at 28 500*g* for 20 min and the pellet was discarded. Additional ammonium sulphate was added to clear supernatant to give 80% saturation, and the resultant mixture was stirred for 20 min at 4 °C. Enzymes from hazelnut were extracted by ammonium sulphate precipitation and then subjected to dialysis overnight. Ammonium sulphate precipitation offered advantage of preventing turbidity in the reaction mixture during the spectrophotometric measurements. The solution was then centrifuged at 28 500*g* for 20 min and the precipitate dissolved in a minimum volume of 0.1 M sodium phosphate buffer pH 7.25. The enzyme solution was dialysed overnight versus 4 l of distilled water. The enzyme extract was stored at −30 °C without losing its original activity over 3 months. Protein content in the extract was determined according to the method described by Bradford (1976) using bovine serum albumin solution and Pierce reagent.

2.7. Enzyme activities

The assays were recorded in an ultraviolet–visible (UV–VIS) Perkin Elmer Lambda-2 Spectrophotometer (Überlingen, Germany). The temperature during all spectrophotometric measurements was set at 37 °C and pH 4.5 (Seyhan, Tijskens, & Evranuz, 2002).

2.7.1. POD activity

POD activity was recorded spectrophotometrically at 414 nm using ABTS (2,2-azino-bis-(3-ethylbenz-tiazoline-6-sulphonic acid)), as described by Arnao, Cano, Hernández-Ruiz, García-Cánovas, and Acosta (1996) and Seyhan et al. (2002). The assay medium was 50 mM buffer, 10 mM ABTS, and 2 mM H_2O_2 solutions in 1 ml of total volume. ABTS and H_2O_2 solutions were prepared daily with oxygen free distilled water, kept under nitrogen gas, and covered with aluminium foil to avoid light effect during analysis. The oxidation of ABTS was followed by observing the increase in absorbance at 414 nm ($\varepsilon_{414} = 3.11 \times 10^4$/M cm). The activity of the enzyme was defined as ABS/mg protein, where the ABS is defined by Beer's law:

ABS	$\varepsilon \cdot c \cdot l$
ABS	absorbance of the sample at the wavelength
ε	an intrinsic constant of the molecule, known as the extinction coefficient or molar absorptivity ($\varepsilon_{414} = 3.11 \times 10^4$/M cm)
c	molar concentration
l	path length (1 cm) of sample the light beam traverses

2.7.2. PPO activity

PPO activity was assayed spectrophotometrically at 380 nm at 37 °C as described by Espín et al. (1997). In brief, the assay medium was 1 ml of 0.01 M catechol in 0.1 M acetate buffer (pH 4.5), 0.1 ml of 0.1 M H_2O_2, and 0.05 ml enzyme extract. Catechol and H_2O_2 solutions were prepared daily with oxygen free distilled water, kept under nitrogen gas and covered with aluminium foil to avoid light effect during analysis. The activity was calculated using the same procedure as followed for peroxidase. The hydrolysis of catechol was followed by observing the increase in absorbance at 380 nm. The activity of PPO was defined as ABS/mg protein.

2.7.3. Lipase activity

Lipase activity was measured according to a colorimetric method by recording the change in absorbance at 350 nm with DNPB (2,4-dinitrophenyl butyrate) as substrate (Mosmuller, van Heemst, van Delden, Franssen, &

Engbersen, 1992). Assay mixtures of DNPB were prepared by adding 50 μl of a 50 mM stock solution of DNPB in acetonitrile (1 mM final concentration) into 2.45 ml of 50 mM buffer (pH 4.5) kept at room temperature. The mixture was sonified for 30 s using the standard 13-mm tip of a VC-300 Sonics (Sonics and Materials, Danbury, CT) set at level 6 and pulser on 50%, to ensure complete emulsification of the substrate. A 2.5 ml of the sonified substrate mixture was mixed with 0.1 ml of enzyme extract. The activity was calculated using the same procedure as followed for POD. The hydrolysis of DNPB was followed by observing the increase in absorbance at 360 nm ($\varepsilon_{360} = 1.48 \times 10^4$/M cm). The activity of lipase was defined as ABS/mg protein.

2.8. Statistical analysis

Results were expressed as means ± standard deviation (SD) ($n = 9$) for each determination. The statistical significance (t test: two-sample equal variance, using two-tailed distribution) was determined using Microsoft Excel statistical software (Microsoft Office Excel, 2003; Microsoft Corp., Redmond, WA). Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Proximate composition

The proximate composition of three native Turkish hazelnut varieties during three maturation stages are summarised in Table 1. As the maturation proceeded, protein and ash contents showed decreasing trends, whereas fat increased. Fat was the predominant compound ranging from 48.10 to 59.83 g/100 g in Tombul, from 52.58 to 62.84 g/100 g in Palaz, and from 47.09 to 65.08 g/100 g in Badem through the maturation stages. Despite being generally insignificant ($P > 0.05$) for all hazelnut varieties, there was a increasing trend in fat content from ES to HS. The main reason for this could be explained that there was a large variation in each consecutive year [data are expressed as means ($n = 9$) of three consecutive-year] due to the environmental condition, fruit development, composition of soil, and use of fertiliser, among others. No significant ($P > 0.05$) differences existed in carbohydrate content during maturation stages.

3.2. Minerals

Although there are other important minerals reported in hazelnut, only six essential minerals (calcium, iron, magnesium, phosphorus, potassium, and zinc) were selected to see the trends in three native hazelnut varieties (namely Tombul, Palaz, and Badem) through the maturation stages (Table 2). The minerals examined were thought to be important ones due to their abundance and perceived health effects. With regard to nutritional aspects, percentage of recommended dietary allowances (RDA) or adequate intake (AI) for those minerals for adult males and females (aged 19–50 years) were also calculated. Consuming recommended daily amount of 42.5 g hazelnut (FDA, 2003) at HS supplies 23.3–25.0% of phosphorus, 11.6–18.1% of magnesium, 7.0–18.9% of iron, 4.9–8.9% of zinc, 5.1–5.7% of calcium, and 5.1–5.3% of potassium for RDA or AI for adults (Dietary Reference Intakes, 1997; Dietary Reference Intakes, 2001; Dietary Reference Intakes, 2004). Significant ($P < 0.05$) decreasing trends were found in all mineral contents from early development to maturity, with some exceptions. Although no data is available on mineral content of hazelnut varieties during fruit development, mineral contents at HS are within the rage of previously reported values for Tombul, Palaz, and Badem varieties (Açkurt et al., 1999; Alasalvar, Shahidi, Liyanapathirana et al., 2003; Köksal et al., 2006; Özdemir et al., 2001). Several studies have indicated that mineral composition of hazelnut is affected by variety, geographical origin, harvest year, climate, composition of soil, irrigation, use of fertiliser, and the method of cultivation (Açkurt et al., 1999; Bonvehí & Coll, 1997; Köksal et al., 2006; Özdemir et al., 2001; Parcerisa et al., 1995).

3.3. Fatty acids

Table 3 reports the results obtained for major fatty acids, total saturated fatty acids (SFA), MUFA, and PUFA of three native hazelnut varieties through the maturation stages. Sixteen fatty acids were identified in all samples (only major ones are shown), among which oleic acid (18:1ω9) was by far the predominant one, followed by linoleic acid (18:2ω6), palmitic acid (16:0), and stearic acid (18:0). The remaining 12 fatty acids contributed less than 1% to the total fatty acids present. As expected, total MUFA was the main group of fatty acids ranging from 75.51 to 81.07% in Tombul, from 78.21 to 82.71% in Palaz, and from 73.69 to 81.65% in Badem through the maturation stages. In contrast, total PUFA showed decreasing trends from ES to HS. No significant changes ($P > 0.05$) were observed in total SFA through the maturation stages (Table 3). The ratio of oleic acid (18:1ω9) to linoleic acid (18:2ω6) was inversely related in all hazelnut varieties from ES to HS. Although no data is available on fatty acid composition of hazelnut varieties during fruit development, the major fatty acids found at HS of present study are, in general, comparable to those reported in the literature on different hazelnut varieties (Alasalvar, Amaral, et al., 2006; Amaral et al., 2006; Savage, McNeil, & Dutta, 1997).

3.4. Enzymes

The activities of PPO, POD, and lipase, and of three native Turkish hazelnut varieties (Tombul, Palaz, and Badem) through the maturation stages are given in Fig. 1. The enzymatic activities of PPO, POD, and lipase differed among varieties and harvesting stages. Tombul

variety had the lowest PPO activity compared to those of Palaz and Badem. Badem showed a higher POD activity than Tombul and Palaz at three stages and significant decreases ($P < 0.05$) in all hazelnut samples were observed in POD activity from ES to HS. No lipase activity was detected in any hazelnut samples at ES and MS, except in Badem at MS. But, lipase activity was detected in all hazelnut samples at HS. This observation is similar to the conclusion of Henderson and Osborne (1991) which reported that in fruits of the oil palm, the lipase activity increases as ripening proceeds. In contrast, Keme and Messerli (1976) have reported that lipase content of a seed depends on the degree of maturity and the age of the seed: the higher the maturity the lower the activity. Moreover, Sanders et al. (1993) reported that although lipases are not usually active in the resting and intact seeds, their action would pose some potential problems in damaged or infected cotyledons. Therefore, hazelnut kernels should not be damaged during harvesting, since any damage on the surface of the kernels during harvesting may cause oil to be squeezed out of the kernels and provide a substrate for lipase.

Table 1
Proximate composition (g/100 g) of hazelnut varieties from early development to maturity

Composition	Tombul			Palaz			Badem		
	ES	MS	HS	ES	MS	HS	ES	MS	HS
Protein	24.46 ± 3.74^{a}	16.88 ± 1.96^{b}	15.01 ± 0.81^{b}	19.70 ± 2.11^{a}	13.05 ± 1.86^{b}	13.66 ± 2.14^{b}	21.56 ± 2.85^{a}	18.05 ± 0.49^{a}	15.41 ± 1.35^{b}
Fat	48.10 ± 7.09^{a}	52.07 ± 4.03^{a}	59.83 ± 5.88^{a}	52.58 ± 6.50^{a}	60.42 ± 5.05^{a}	62.84 ± 3.45^{a}	47.09 ± 7.53^{a}	58.75 ± 9.29^{ab}	65.08 ± 3.07^{b}
Carbohydrates	21.98 ± 8.62^{a}	28.30 ± 2.22^{a}	22.77 ± 5.28^{a}	22.83 ± 8.55^{a}	24.17 ± 3.29^{a}	21.32 ± 1.20^{a}	26.07 ± 4.86^{a}	20.02 ± 8.09^{a}	16.99 ± 3.03^{a}
Ash	5.46 ± 0.67^{a}	2.75 ± 0.07^{b}	2.39 ± 0.23^{b}	4.89 ± 1.02^{a}	2.36 ± 0.11^{b}	2.18 ± 0.14^{b}	5.28 ± 1.34^{a}	3.18 ± 0.85^{ab}	2.52 ± 0.24^{b}

Data (3 consecutive-year) are expressed as means ± SD ($n = 9$) on a dry weight basis. Carbohydrates were calculated by subtracting the total percent values of other measurement from 100. Means ± SD followed by the same letter, within a row (for Tombul, Palaz, and Badem, separately), are not significantly different ($P > 0.05$). ES: early stage; MS: middle stage; HS: harvest stage. Moisture content (g/100 g) of Tombul hazelnut (ES: 70.44 ± 1.21; MS: 40.23 ± 5.11; HS: 28.99 ± 3.76), Palaz hazelnut (ES: 71.85 ± 4.87; MS: 35.58 ± 4.01; HS: 30.66 ± 2.49), and Badem hazelnut (ES: 74.57 ± 8.43; MS: 49.61 ± 11.00; HS: 34.91 ± 0.20).

Table 2
Mineral content (mg/100 g) of hazelnut varieties from early development to maturity

Minerals	Tombul			Palaz			Badem		
	ES	MS	HS	ES	MS	HS	ES	MS	HS
Calcium	401 ± 125^{a}	202 ± 48^{b}	133 ± 25^{c}	249 ± 72^{a}	144 ± 48^{b}	119 ± 28^{b}	339 ± 104^{a}	180 ± 54^{b}	122 ± 29^{c}
Iron	5.22 ± 1.43^{a}	3.15 ± 0.59^{b}	3.00 ± 0.76^{b}	4.43 ± 1.74^{a}	3.71 ± 0.43^{a}	2.96 ± 0.88^{a}	5.20 ± 0.97^{a}	3.86 ± 0.42^{b}	3.55 ± 1.46^{b}
Magnesium	310 ± 64^{a}	174 ± 11^{b}	134 ± 30^{c}	221 ± 30^{a}	147 ± 27^{b}	133 ± 32^{b}	238 ± 46^{a}	159 ± 62^{b}	112 ± 40^{b}
Phosphorus	708 ± 147^{a}	508 ± 115^{b}	412 ± 15^{b}	651 ± 120^{a}	444 ± 130^{b}	396 ± 43^{b}	640 ± 102^{a}	423 ± 52^{b}	367 ± 28^{c}
Potassium	1190 ± 208^{a}	715 ± 72^{b}	566 ± 99^{c}	1204 ± 131^{a}	716 ± 45^{b}	591 ± 43^{c}	1345 ± 187^{a}	937 ± 168^{b}	604 ± 197^{c}
Zinc	1.99 ± 0.37^{a}	1.44 ± 0.29^{b}	1.27 ± 0.25^{b}	2.81 ± 0.86^{a}	1.69 ± 0.40^{b}	1.29 ± 0.23^{b}	2.29 ± 1.16^{a}	1.33 ± 0.40^{a}	1.67 ± 0.46^{a}

Data (3 consecutive-year) are expressed as means ± SD ($n = 9$) on a dry weight basis. Means ± SD followed by the same letter, within a row (for Tombul, Palaz, and Badem, separately), are not significantly different ($P > 0.05$). ES: early stage; MS: middle stage; HS: harvest stage.

Table 3
Fatty acids composition (%) of oils extracted from hazelnut varieties from early development to maturity

	Tombul			Palaz			Badem		
	ES	MS	HS	ES	MS	HS	ES	MS	HS
16:0	6.62 ± 0.69^{a}	6.28 ± 0.67^{a}	5.91 ± 0.08^{b}	6.83 ± 0.31^{a}	6.21 ± 0.27^{b}	6.21 ± 0.26^{b}	6.17 ± 1.48^{a}	5.46 ± 0.14^{a}	5.12 ± 0.08^{a}
18:0	1.78 ± 0.24^{a}	2.36 ± 0.28^{b}	2.53 ± 0.17^{b}	2.26 ± 0.16^{a}	2.54 ± 0.11^{b}	2.53 ± 0.04^{c}	1.45 ± 0.27^{a}	1.73 ± 0.47^{b}	2.18 ± 0.18^{b}
18:1ω9	74.60 ± 1.52^{a}	78.57 ± 1.99^{b}	80.64 ± 2.97^{b}	77.92 ± 2.95^{a}	82.29 ± 2.82^{b}	82.29 ± 3.05^{b}	72.78 ± 4.97^{a}	76.69 ± 3.85^{b}	81.17 ± 3.60^{b}
18:2ω6	15.88 ± 0.63^{a}	11.95 ± 2.22^{b}	10.29 ± 2.61^{c}	11.92 ± 0.75^{a}	7.91 ± 0.52^{b}	8.10 ± 0.72^{b}	17.83 ± 1.62^{a}	15.04 ± 1.12^{b}	10.68 ± 0.73^{b}
Total SFA	8.65 ± 0.67^{a}	8.82 ± 0.67^{a}	8.65 ± 0.54^{a}	9.53 ± 0.50^{a}	8.89 ± 0.23^{a}	8.89 ± 0.34^{a}	8.11 ± 1.96^{a}	7.38 ± 0.46^{a}	7.49 ± 0.25^{a}
Total MUFA	75.51 ± 1.60^{a}	79.15 ± 1.60^{a}	81.07 ± 2.44^{b}	78.21 ± 2.92^{a}	82.89 ± 0.81^{b}	82.71 ± 3.08^{b}	73.69 ± 6.85^{a}	77.36 ± 5.88^{b}	81.65 ± 3.60^{b}
Total PUFA	15.84 ± 0.78^{a}	12.03 ± 0.78^{a}	10.28 ± 2.61^{ab}	12.26 ± 4.19^{a}	8.22 ± 0.51^{b}	8.40 ± 1.15^{b}	18.20 ± 4.83^{a}	15.26 ± 6.15^{ab}	10.86 ± 3.72^{b}

Data (3 consecutive-year) are expressed as means ± SD ($n = 9$). Means ± SD followed by the same letter, within a row (for Tombul, Palaz, and Badem, separately), are not significantly different ($P > 0.05$). ES: early stage; MS: middle stage; HS: harvest stage.

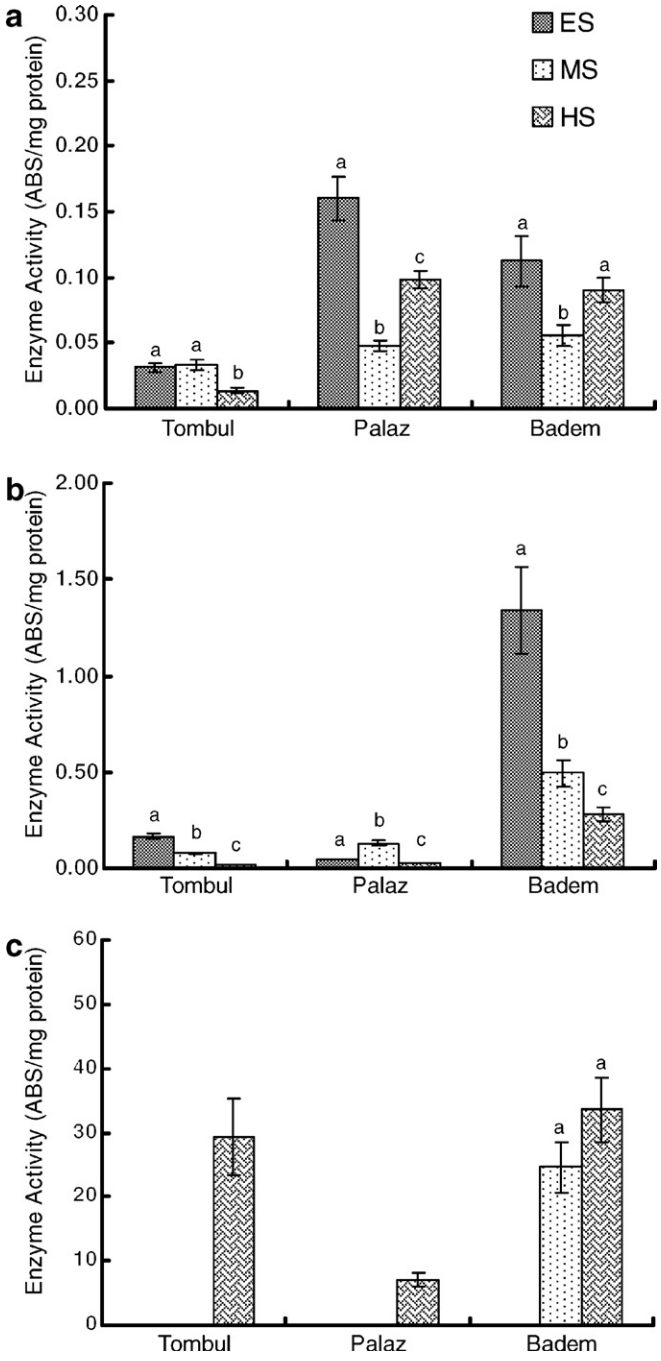

Fig. 1. Enzyme activities: (a) PPO, (b) POD, and (c) lipase of Turkish hazelnut varieties (Tombul, Palaz, and Badem) through the maturation stages (ES: early stage; MS: middle stage; HS: harvest stage). Means ± SD by the same letter, with a column for each hazelnut variety, are not significantly different ($P > 0.05$).

4. Conclusions

Proximate composition varied considerably during maturation stages. Statistical analysis showed that all minerals analysed decreased significantly ($P < 0.05$) from early development to maturity, with some exceptions. Total MUFA increased through the maturation stages, whereas total PUFA increased. The ratio of oleic acids (18:1ω9) to linoleic acid (18:2ω6) was inversely related in all hazelnut varieties from ES to HS. The activities of PPO, POD, and lipase differed among varieties and stages. To conclude, although minerals and most fatty acids showed clear trends during maturation stages, each enzyme activity behaved differently. This could be due to either the climatic effects during three consecutive sampling years or different maturation levels. Further research is needed to examine the functional lipid characteristics (lipid classes, fatty acids from crude, neutral, and polar lipids, sterol composition, tocol composition, squalene, polyphenol composition, and triacylglycerol composition, among others) of various hazelnut varieties during fruit development.

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