Wageningen Academic Publishers

Effects of filtration and storage on chemical composition and sensory properties of olive oil extracted from Beylik cultivar

E. Ghanbari Shendi[1], D. Sivri Ozay[1*], M.T. Ozkaya[2] and N.F. Ustunel[3]

[1]Hacettepe University, Department of Food Engineering, 06800 Ankara, Turkey; [2]Ankara University, Department of Horticulture, 06110 Ankara, Turkey; [3]Nar Doğal Ürünler Tur. Tic. San. A.Ş., 34770 Ümraniye-Istanbul, Turkey; sivri@hacettepe.edu.tr

Received: 6 February 2018 / Accepted: 5 June 2018

RESEARCH ARTICLE

Abstract

In this study, a mobile olive oil processing unit was designed and used for cold press extra virgin olive oil (EVOO) production at optimum conditions. A local olive variety 'Beylik', from the Antalya province of Anatolia, was used. EVOO was stored prior to and after paper filtration for up to 12 months. Changes of some chemical parameters (free fatty acids, peroxide, colour, moisture and UV absorbance), as well as minor and major components were monitored during storage. All samples (filtered and unfiltered) could be categorised as EVOO as declared by trade standards of the International Olive Council based on free fatty acid, peroxide and UV absorbance (K_{232} and ΔK) values. In particular, free acidity values (<0.2%) were unusual when compared to commercial olive oils. The colour changed from green to yellow and UV absorbance values altered. No significant change was observed in fatty acid composition, with filtration having no detectable effect during the first three months. During storage, total phenol content decreased in both filtered and unfiltered samples. Luteolin was the most abundant phenolic compound and decreased in both filtered and unfiltered samples with storage. α-Tocopherol contents of filtered samples were slightly higher than unfiltered samples at the early months of storage – a significant decrease (48.3%) was observed for filtered samples at the end of storage. Sensory evaluation performed by a panel showed that fruitiness, pungency and bitterness were higher in unfiltered samples.

Keywords: olive oil, quality, Beylik, phenolic compounds, tocopherol, storage

1. Introduction

Extra virgin olive oil (EVOO) is extracted from the olive fruit by using only physical methods and it is ready to consume after production without refining. Hence, it conserves natural bioactive compounds such as vitamins, phenolics and sterols as well as providing a good balance of fatty acids. EVOO has unique aroma, taste, colour and nutritive features that identify it from other edible vegetable oils (Boskou *et al.*, 2006). EVOO is the main fatty component of the Mediterranean diet, which is characterised by a long and healthy life. Upper Mesopotamia is a part of Turkey's territory and is the homeland of the olive tree, with a wide range of genetic resources (Yorulmaz, 2009).

The number of olive trees of Beylik cvs. is approximately 100,000. Most of the trees are older than 50 years. Total yield is about 5,000 tons per year. The alternate bearing is medium level. The number of olive fruits per kg is approximately 150-200. Fruit length and diameter are 29.69 and 19.18 mm, respectively. Local growers use the fruit mainly for table olives, with the rest being processed for olive oil, however mainly for local consumption. While early harvesting fruit produces 8-10% olive oil, at the end of ripening, this value increases up to 20-22%. The Beylik cultivar is one of the two olive cultivars (Tavşan Yüreği and Beylik) grown in Antalya. The Beylik variety originates from mainly the Manavgat district of Antalya province (Figure 1), located in the West Mediterranean Region.

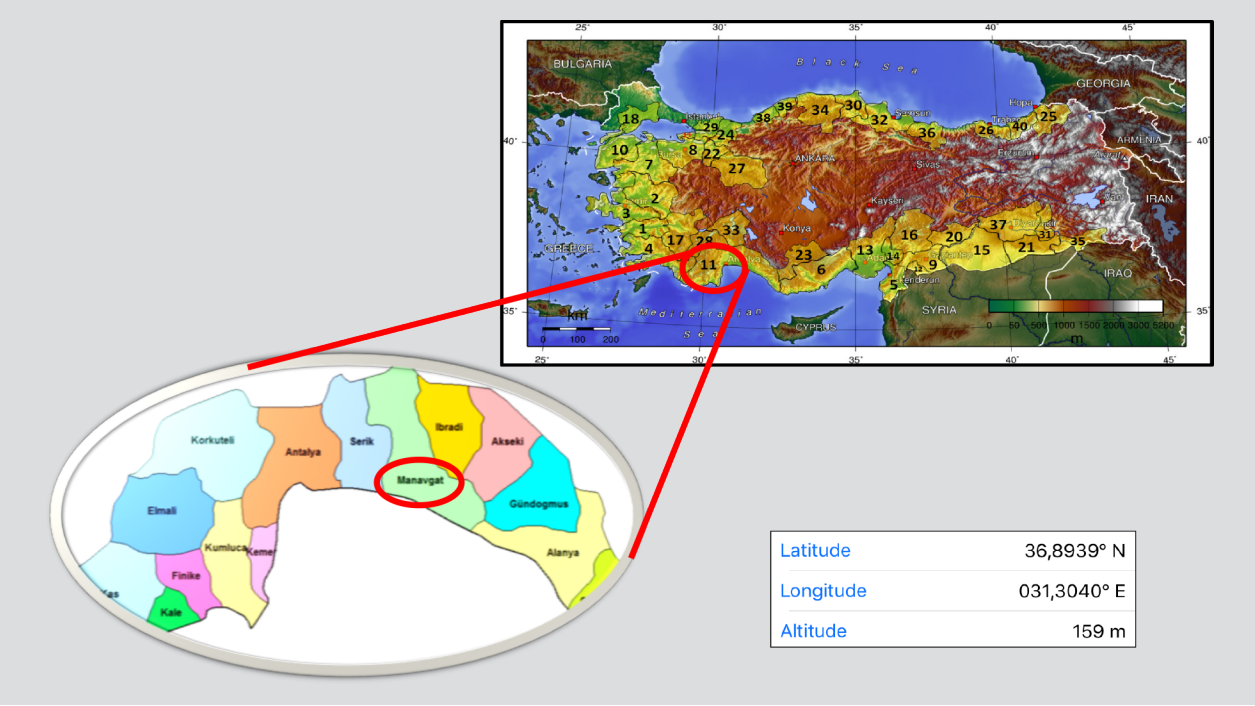

Figure 1. Beylik variety grown location in Turkey.

The chemical composition of olive oil varies depending on genetic, geographic and agronomic factors, extraction methods, processing conditions and storage. Shelf life of virgin olive oil is longer than other edible oils because of the presence of natural antioxidants (mainly polar phenols and α-tocopherol). Other factors such as free fatty acids, unsaturated hydrocarbons, enzymes and trace metals affect oxidative stability negatively. Pigments also have a negative effect on oxidative stability. Storage of olive oil under nitrogen pressure in a dark location at room temperature (25-30 °C or lower) increases shelf life (Boskou *et al.*, 2006). The amount of major and minor components as well as oxidation indices of virgin olive oil are affected during storage. Significant decreases in antioxidant contents and major increases in peroxide value (PV) and UV absorption values (K_{232} and K_{270}) were observed with increasing storage time. The reduction of total phenolic compounds ranged from 43 to 73%, and it was remarkable that the decrease was higher in samples the initial phenol contents of which were greater. Rastrelli *et al.* (2002) reported that at room temperature, no significant changes were observed in the unsaturated fatty acid composition of virgin olive oil samples filtered and stored both in colourless glass bottles and dark ones with 3% and 50% headspace, respectively.

Free fatty acidy and oxidative rancidity values increase during storage (Méndez and Falqué, 2006). Although fatty acid composition remained stable for up to three months, unsaturation degree appeared to decrease as the expiry date became closer and a decrease in the oleic acid content was also observed. After four months of storage, 79% of α-tocopherol decomposed, whereas <45% of the phenols disappeared under diffused light during storage (Okogeri and Tasioula-Margari, 2002). A positive correlation was found between the age of the oils and the tyrosol to total phenol ratio (Cinquanta *et al.*, 1997). EVOOs with high antioxidant contents were still 'excellent' after 240 days of storage at 40 °C (Lavelli *et al.*, 2006).

Important losses of chlorophyll, carotenoids and total phenol contents of a commercial virgin olive oil extracted from an Arbequina cultivar were reported after 12 months of storage. Increasing oleic acid percentage was observed in the fatty acid composition (Morelló *et al.*, 2004). Tyrosol and hydroxytyrosol in EVOO increased at room temperature and no change was observed in aromatic hydrocarbons of frozen samples during up to 12 months' storage (Mulinacci *et al.*, 2013). Psomiadou *et al.* (2000) suggested that appropriate handling is important for retaining high α-tocopherol levels of Greek virgin olive oil under domestic conditions for up to two years.

Filtration causes a gradual loss in stability during storage due to a lower total phenolic content (Tsimidou, 2006). Gomez-Caravaca *et al.* (2007) reported a significant loss of hydroxytyrosol content for eight virgin olive oils after filtration through a cotton filter. On the other hand, Fregapane *et al.* (2006) noted that filtration and particularly dehydration could help prolong the shelf life of some high-quality but less-stable virgin olive oils.

In addition to major olive varieties (Ayvalik, Memecik, Gemlik and Domat), there are some minor varieties (Halhalı, Saurani, Sarı Ulak, Tavşan Yüreği) and a dozen lesser-known varieties cultivated in Anatolia locally. Olive oil quality and economic potential of some of these local varieties have not been explored to date due to mishandling during processes from garden to table.

In this research, a mobile olive oil processing unit (MOOPU) was designed to produce 'monovarietal virgin olive oil' in order to explore oil quality of some local olive varieties grown in Anatolia, Turkey. 'Beylik' is one of the local varieties located in West Mediterranean (Antalya) province in Anatolia and is mostly used for table olive production. MOOPU was transferred near to the orchard, and it was therefore possible to process the olives at optimum conditions within two hours after harvest. This is the first report on the Beylik olive variety indicating olive oil quality. Effects of filtration and storage time on Beylik olive oil quality were also investigated.

2. Materials and methods

Production of extra virgin olive oil

A MOOPU with state-of-the art Oliomio (http://tem.it/en) equipment was designed in order to produce premium quality EVOO at optimum conditions. A special container was constructed and equipped with a knife crusher and a 2-phase horizontal decanter (Oliomio D500; Oliomio, Florence, Italy). The mobile unit is an articulated lorry with a special semi-trailer which is divided into three sections (2.438×12.192×2.896 mm). The first section is an olive accepting unit including bunker, leaf removers, washer and crusher units of the system. The second section is the processing unit including malaxer, decanter, filter and a bag-in-box filling machine. The third section is the support unit comprising a power plant and water supply tank. The processing unit is an isolated area and therefore provides protection from temperature changes, dust and odour. This area was equipped with an air conditioner and filter ventilation systems. MOOPU was carried by a trailer truck to orchards during the 2014-2015 season. Olive fruits were harvested by hand picking in the early harvest period and processed to 'cold press' EVOO in a few hours. Olive paste was prepared after crushing using a hammer mill and the paste was mixed in the malaxer at 27 °C for 15 min (cold press). EVOOs were packaged prior to (unfiltered) and after filtration (filtered). A filter press (Oliomio Jolly 40; Oliomio) with paper (E2; Gruppo Cordenons, Milan, Italy; paper weight: 350 g/m^2, thickness: 0.81 mm, apparent density: 0.43 g/cm^3, water absorption: 8 g/dm^2) was used for filtration. Olive oil samples were filled in 250 ml amber glass bottles with the headspace (4 cm) filled with nitrogen gas. The bottles were stored at room temperature (18-24 °C) for up to 12 months.

Chemical analyses

Free fatty acid content (%) and peroxide values were determined according to EC 2598/9 (OJECL, 1991) and AOCS Cd 8-53 methods, respectively (AOCS, 2003). Colour values (L, a, b values) were measured using a spectrophotometer (Minolta, CM-3600d, Tokyo, Japan). The maximum value for L is 100, indicating a perfectly reflecting diffuser. The minimum measurement for L is zero, indicative of a black diffuser. The a and b have no specific numerical limits. Positive a is red and negative a is green, while positive b is yellow and negative b is blue. UV absorbance was performed according International Olive Council (IOC) method COI/T.20/Doc. No 19/Rev. 3. (IOC, 2015a). UV absorbance was measured at 232, 266, 270 and 274 nm using a UV spectrophotometer (Agilent 8453; Agilent, Santa Clara, CA, USA). ΔK values were calculated using the following formula:

$$\Delta K = K_{270} - \frac{K_{266} + K_{274}}{2}$$

Moisture content of the olive oils was determined according to ISO 662 (ISO, 2016). Fatty acid composition was calculated according to the method of IOC (2001). Analysis was carried out using TRACE™ ultra gas chromatograph equipment (Thermo Fisher Scientific, Waltham, MA, USA) with the following operation conditions. The chromatograph was equipped with a flame ionisation detector. A split injector was employed (40:1). An HP-88 column (100-meter length, 0.25 mm I.D, 0.20 μm film thickness) was used for separation. The carrier gas was helium with 1 ml/min flow rate, and initial temperature was 100 °C. The temperature ramping rate was 4 °C/min. Injection temperature and detector temperature were 240 and 250 °C, respectively. Final temperature was 240 °C and analysis was completed in 12 min.

Total phenolic content

The polar fraction was extracted and used for total phenolic and phenolic composition analyses. The olive oil sample (2.5 g) was weighed into a Falcon tube. Hexane (6 ml) was added and shaken for 1 min. This solution was filtered through a solid phase extraction (SPE) cartridge (Superclean LC-Diol; Merck KGaA, Darmstadt, Germany) and collected in a glass tube. Then, hexane (6 ml) and 4 ml hexane : ethyl acetate (85:15, v/v) were passed through the SPE cartridge, respectively. The cartridge was washed with methanol: deionised water solution (1:1 v/v) and phenolic extract was evaporated (100 ECH; UniEquip GmbH-Freital/Dresden, Germany). After addition of 2 ml methanol : deionised water solution (1:1 v /v), the tubes were vortexed for 30 seconds. For determination of total phenols, Folin & Ciocalteu's method was used and the results expressed in terms of gallic acid equivalent (mg gallic acid/kg oil) (Inarejos-Garcia *et al.*, 2009; Romani *et al.*, 2007). Prepared phenolic extract (1 ml)

was passed through a 0.45 µm microfilter (PVDF, Millex-HV; Merck, Darmstadt, Germany) and poured into an amber vial. Ultra-high performance liquid chromatography (UHPLC; Dionex Ultimate 3000; Thermo Scientific, Sunnyvale, CA, USA) and a C18 column (4.6 mm inner diameter × 250 mm length and 5 mm particle diameter; Thermo Scientific acclaim 120) were used for determination of phenolic profile. Column temperature was fixed at 30 °C and acetic acid : deionised water (1:1) (A), methanol (B), acetonitril (C) were used in a gradient flow programme as the mobile phase. In the gradient programme, eluents were 2.5% B, 2.5% C and 95% A solutions for up to 60 min. Flow rate was 1 ml/min and the diode array detector was set at 280 nm, 320 nm and 335 nm. Apigenin, caffeic acid, gallic acid, luteolin, m-cumaric acid, p-coumaric acid, oleuropein, syringic acid, *trans*-ferulic acid, vanillic acid, vanillin, tyrosol, 3-hydroxy tyrosol, 3.4-dihydroxy benzoic acid, 4-hydroxy benzoic acid and 4-hydroxy phenyl acetic acid were used as standards.

Tocopherol composition

The EVOO sample (2 g) was weighed into a 25 ml volumetric flask (AOCS, 1997). A quantity of hexane was added and shaken to dissolve the sample. The flask was made up to volume with the same solvent. The solution was passed from a syringe filter (0.45 µm) (PVDF, Millex-HV) into the HPLC vial. The samples (20 µl) were injected into the UHPLC. A LiChrosorb SI 60-5 column (4.6 mm I.D. × 250 mm length and 5 µm particle size) was used for analysis. Column temperature was fixed at 30 °C during the process. The flow rate of analysis was 1 ml/min. For the mobile phase, isopropanol : hexane (0.5:99.5, v/v) isocratic mix was used and chromatograms were collected at 292 nm wavelength. Analysis time was 30 min and injection volume was 100 µl. Amounts of α, β, γ and δ-tocopherols were determined by using tocopherol standards.

Sensory evaluation

Every month, both filtered and unfiltered olive oil samples were transferred to the Ayvalık Olive Oil Tasting Laboratory accredited by the IOC and TURKAK (Turkish accreditation agency). The method for the organoleptic assessment of virgin olive oil (COI/T.20/Doc. No. 15/Rev. 8) (IOC, 2015b) was used. Eight trained tasting panels were able to assess the oils to determine the levels of positive attributes, such as fruitiness, bitterness and pungency. Negative attributes arising due to poor quality fruit, incorrect processing or storing, such as rancidity, musty and fusty, were determined by sensory panels.

Statistical analysis

Statistical analysis was performed using SPSS 17 (SPSS Inc., Chicago, IL, USA) statistical software and the one-way ANOVA method. Differences among all groups were determined by a Duncan test. All analyses were performed at least in duplicate.

3. Results and discussion

Chemical analyses

Free acidity, peroxide and UV absorbance values of the olive oils produced in the MOOPU are shown in Table 1. Free fatty acidity (%) value was very low and a slight increase was observed during storage time. All samples could be classified as extra virgin olive oils according to IOC standards based on free fatty acidity values. Moisture content of filtered and unfiltered samples were comparable, indicating that filtration had no detectable effect on the moisture content (0.06%). A number of surveys have shown that free acidity increases with storage depending on the packaging material, storage conditions and time (Clodoveo *et al.*, 2007; Méndez and Falqué, 2006). On the other hand, free fatty acidity value increases slowly after eight years' storage time (Abdalla *et al.*, 2014; Baiano *et al.*, 2014; Lavelli *et al.*, 2006).

Peroxides, which are a product of the early stage of oxidation, were lower in the filtered samples than that of unfiltered samples at the beginning of storage; after the second month, an increasing trend was observed for both filtered and unfiltered samples. The PV reached maximum values and were comparable for filtered and unfiltered samples at the end of storage ($P<0.01$). Significant increases were reported for the PV of olive oil samples during short- (30 days) and long-term (sixth years) storage in different packaging materials in different conditions (Abdalla *et al.*, 2014; Clodoveo *et al.*, 2007; Lavelli *et al.*, 2006; Okogeri and Tasioula-Margari, 2002).

UV absorbance values (K_{232} and K_{270}), which indicate oxidation, changed significantly during storage. K_{232} and K_{270} values slightly increased during the early stage of storage, for both filtered and unfiltered EVOO of Beylik (Table 1). After that, K_{232} values showed a decline trend for up to seventh months. However, at the end of storage, K_{232} values started to increase again. The K_{270} value of unfiltered samples was slightly higher than for filtered ones. ΔK values of filtered and unfiltered were zero or below zero (results not shown). These results are in agreement with the related literature (Baiano *et al.*, 2014; Caponio *et al.*, 2005; Del Caro *et al.*, 2006; Gómez-Alonso *et al.*, 2007; Lavelli *et al.*, 2006; Méndez and Falqué, 2006; Okogeri and Tasioula-Margari, 2002). Baiano *et al.* (2014) reported that the K_{232} value of Coratina olive oil increased up to the sixth year and then decreased; at the end of final storage, an increase was

Table 1. Oxidative stability parameters of extra virgin olive oils extracted from Beylik variety during 12 months' storage.[1]

Storage period (month)	Free fatty acid content (%)		Peroxide value ($meqO_2$/kg oil)		K_{232}		K_{270}	
	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered
0	0.1±0.00^{b}	0.1±0.00^{b}	8.94±0.003^{e}	11.89±0.011^{g}	2.1±0.00^{d}	1.8±0.00^{f}	0.17±0.00^{d}	0.18±0.00^{d}
1	0.1±0.00^{b}	0.2±0.00^{a}	8.97±0.038^{e}	11.93±0.016^{f}	2.6±0.00^{a}	2.6±0.00^{a}	0.20±0.00^{b}	0.21±0.00^{a}
2	0.2±0.00^{a}	0.2±0.00^{a}	11.80±0.143^{d}	11.97±0.000^{e}	2.2±0.00^{c}	2.3±0.00^{b}	0.20±0.00^{b}	0.19±0.00^{c}
3	0.2±0.00^{a}	0.2±0.00^{a}	11.94±0.025^{c}	12.00±0.011^{d}	2.1±0.00^{d}	2.2±0.00^{c}	0.17±0.00^{d}	0.21±0.00^{a}
4	0.2±0.00^{a}	0.2±0.00^{a}	14.88±0.116^{b}	14.91±0.010^{c}	2.0±0.00^{e}	1.9±0.00^{c}	0.14±0.00^{f}	0.15±0.00^{g}
5	0.2±0.00^{a}	0.2±0.00^{a}	14.94±0.006^{b}	14.98±0.018^{b}	1.8±0.00^{g}	1.7±0.00^{h}	0.13±0.00^{g}	0.15±0.00^{g}
6	0.2±0.00^{a}	0.2±0.00^{a}	14.99±0.001^{b}	14.99±0.000^{b}	1.8±0.00^{g}	1.6±0.00^{i}	0.18±0.00^{c}	0.16±0.00^{f}
7	0.2±0.00^{a}	0.2±0.00^{a}	14.99±0.001^{b}	14.99±0.001^{b}	1.8±0.00^{g}	1.7±0.00^{h}	0.18±0.00^{c}	0.20±0.00^{b}
8	0.2±0.00^{a}	0.2±0.00^{a}	15.00±0.000^{b}	15.00±0.000^{b}	1.9±0.00^{f}	2.0±0.00^{d}	0.10±0.00^{h}	0.21±0.00^{a}
9	0.2±0.00^{a}	0.2±0.00^{a}	17.96±0.005^{a}	17.97±0.012^{a}	1.5±0.00^{i}	1.3±0.00^{j}	0.15±0.00^{e}	0.17±0.00^{e}
10	0.2±0.00^{a}	0.2±0.00^{a}	17.99±0.002^{a}	17.98±0.016^{a}	1.8±0.00^{g}	1.6±0.00^{i}	0.18±0.00^{c}	0.14±0.00^{h}
11	0.2±0.00^{a}	0.2±0.00^{a}	17.99±0.000^{a}	17.99±0.000^{a}	1.7±0.00^{h}	1.7±0.00^{h}	0.14±0.00^{f}	0.20±0.00^{b}
12	0.2±0.00^{a}	0.2±0.00^{a}	18.01±0.025^{a}	17.99±0.001^{a}	2.4±0.00^{b}	1.7±0.00^{g}	0.21±0.00^{a}	0.18±0.00^{d}

[1] Different superscript letters in the same column indicate significant differences between mean values (P<0.01).

observed. Gutiérrez and Fernandez (2002) showed that only two quality indices (K_{270} and sensory evaluation) of Picual and Hojiblanca olive oils decreased during storage at 2 °C in darkness and 30 °C in illumination. Quality deterioration resulted in downgraded olive oils so that they could no longer be considered extra virgin olive oils during storage, with there being an excellent correlation between initial stability and time to reach the limit of K_{270}>0.25.

Colour analysis

Although colour is not considered to be an important quality characteristic for olive oils, it has a significant influence on consumer acceptance. Virgin olive oil colour is related to olive maturity and process conditions. Analysis of colour (L, a and b values) demonstrates that olive oil sample colour alters significantly during storage (Table 2). This change has been attributed to decomposition of colour pigments such as chlorophylls, pheophytins, xanthophylls and carotenes (Boskou, 1996). The lowest L values (lightness) were seen in the seventh and twelfth months for filtered and unfiltered samples, respectively. The highest L values were observed in the eighth month for filtered and unfiltered samples. Generally, unfiltered samples had lower L and b values, indicating that they are dark green. Fluctuations were observed in a (redness) and b (yellowness) values of all samples during storage. The highest b value was obtained for the eighth month. After this month, there was a decreasing trend in b values of both filtered and unfiltered samples.

Fatty acid composition

The fatty acid composition is an important quality parameter and authenticity indicator of virgin olive oils. Results related to this variable are shown in Table 3. No change in fatty acid composition of the samples was revealed during the first three months and the analysis was not carried out any further. Filtration had no detectable effect on fatty acid composition. As expected, oleic acid (C18:1) was the most abundant (70.56%) fatty acid, followed by palmitic acid (C16:0) and linoleic acid (C18:1). Oleic acid (C18:1) contents of early harvest monocultivar olive oils produced in Turkey were between 62.41-80.26% (Boskou, 1996; Dıraman and Dibeklioğlu, 2009). Linoleic and linolenic acids, which are much more susceptible to oxidation than monosaturated fatty acids, were 9.12 and 0.5%, respectively. These results are in agreement with the results of olive oils produced in Mediterranean countries. Virgin olive oils are classified into two types based on their fatty acid compositions. Turkish, Spanish, Italian and Greek virgin olive oils are characterised by low linoleic and palmitic, and high oleic acid contents are the first type, while Tunisian oils are the second type, characterised by high linoleic and palmitic and low oleic acid contents (Yavuz, 2008). The linolenic acid levels of Turkish virgin olive oil samples were below the maximum (0.9%) value regulated by the Turkish Codex (Anonymous, 2010) and the EU (EC, 2002).

Table 2. Colour values (L, a, b values) of filtered and unfiltered extra virgin olive oils extracted from Beylik variety during a 12-month storage period (n=48).[1]

Storage period (month)	L-value		a-value		b-value	
	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered
0	37.26±0.148^{c}	34.57±0.049^{cd}	-0.32±0.014^{f}	0.01±0.007^{ab}	13.80±0.197^{bc}	9.56±0.169^{a}
1	37.12±0.021^{c}	36.82±0.028^{b}	-0.08±0.000^{cde}	0.07±0.007^{abc}	13.62±0.127^{bc}	12.86±0.183^{a}
2	39.39±0.098^{b}	31.80±0.735^{f}	-0.55±0.003^{g}	0.30±0.130^{a}	13.61±0.622^{bc}	11.85±1.131^{a}
3	34.19±1.428^{de}	35.61±0.622^{c}	0.38±0.102^{a}	0.27±0.077^{a}	15.59±1.011^{b}	15.53±3.153^{a}
4	36.36±0.056^{c}	37.05±0.162^{b}	0.10±0.035^{bcd}	-0.08±0.003^{bcde}	14.48±0.438^{bc}	13.23±0.049^{a}
5	36.31±0.226^{c}	35.52±0.502^{c}	-0.09±0.045^{de}	-0.17±0.067^{cde}	13.22±1.088^{cd}	13.77±2.651^{a}
6	37.15±0.014^{c}	36.71±0.070^{b}	-0.11±0.024^{e}	-0.06±0.038^{bcd}	13.74±0.190^{bc}	12.74±0.678^{a}
7	31.18±1.173^{f}	33.97±1.088^{de}	0.20±0.063^{ab}	0.02±0.031^{bc}	10.52±0.558^{e}	13.57±5.762^{a}
8	47.47±0.028^{a}	47.68±0.063^{a}	0.01±0.010^{bcde}	-0.40±0.007^{f}	19.92±0.042^{a}	19.19±0.056^{a}
9	32.65±0.558^{ef}	33.31±0.862^{e}	0.12±0.070^{bc}	-0.14±0.031^{bcde}	13.69±2.786^{bc}	12.00±4.426^{a}
10	35.46±0.035^{cd}	35.20±0.091^{c}	-0.10±0.007^{de}	-0.26±0.010^{def}	11.17±0.106^{de}	10.41±0.077^{a}
11	36.97±0.212^{c}	37.38±0.148^{b}	-0.14±0.003^{ef}	-0.31±0.003^{ef}	13.08±0.240^{cd}	13.13±0.162^{a}
12	34.10±2.609^{de}	31.51±0.091^{f}	-0.19±0.035^{ef}	0.00±0.001^{f}	13.23±0.968^{cd}	11.99±0.452^{a}

[1] Different superscript letters in the same column indicate significant differences between mean values (P<0.01).

Table 3. Fatty acid profile of extra virgin olive oil extracted from Beylik variety (%).

Fatty acids	(%)
Myristic acid	0.01
Palmitic acid	15.51
Palmitoleic acid	1.05
Heptadecanoic acid	0.02
Cis-10-heptadecenoic acid	0.03
Stearic acid	2.60
Oleic acid	70.56
Elaidic acid	0.00
Linoleic acid	9.12
Linolenic acid	0.50
Arachidic acid	0.36
Cis-11-eicosenoic acid	0.10
Behenic acid	0.11
Lignoceric acid	0.02

Tocopherol profile

The tocopherol (α, β, γ) profile of Beylik EVOOs was determined every two months during storage (Table 4). The results showed that tocopherol contents (α, β, γ) decreased with increasing storage time, as expected. The lowest tocopherol contents were obtained after one year of storage. Thus, 48.3% of α-tocopherol, 68.0% of β-tocopherol and 84.8% of γ-tocopherol contents were decomposed during storage in filtered EVOO samples and 24.4% of α-tocopherol, 74.8% of β-tocopherol and 85.1% of γ-tocopherol in unfiltered samples. Filtration had an important effect on tocopherol content. The amount of α-tocopherol was higher in filtered samples. However, β-tocopherol and γ-tocopherol contents were higher in unfiltered ones. These results are in agreement with other studies (Baiano *et al.*, 2014; Okogeri and Tasioula-Margari, 2002; Psomiadou *et al.*, 2000; Rastrelli *et al.*, 2002).

Total polyphenol content

Total polyphenol contents of the samples are presented in Table 5. The highest total polyphenol values were determined in fresh oils and its amount decreased with time. However, the decreases were not as dramatic as they were for tocopherols – after one year, 16.7 and 12.2% of total polyphenols were decomposed in filtered and unfiltered samples, respectively. Unfiltered samples had a higher total polyphenol content, indicating that filtration had a significant effect. After short- or long-term storage, significant decreases in total polyphenols were reported for monocultivar and commercial olive oils by Clodoveo *et al.* (2007), Morelló *et al.* (2004), Abdalla *et al.* (2014) and Baiano *et al.* (2014).

Phenolic profiles

Phenolic profiles of the samples were determined monthly, with the results being shown in Tables 6 and 7, respectively. A typical chromatogram is presented in Figure 2. Luteolin was the most abundant phenolic compound among other

Table 4. Changes of tocopherol isomer content (mg/kg) in extra virgin olive oils extracted from Beylik variety during storage (n=48).[1]

Storage period (month)	α-tocopherol		β-tocopherol		γ-tocopherol	
	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered
0	252.50±0.902^{a}	239.250±2.276^{a}	0.97±0.001^{a}	2.78±0.073^{a}	1.77±0.018^{a}	1.74±0.009^{a}
2	246.02±4.761^{a}	233.780±1.519^{b}	0.87±0.007^{b}	2.54±0.023^{b}	1.53±0.015^{b}	1.53±0.007^{b}
4	230.83±4.790^{b}	226.571±4.220^{c}	0.64±0.002^{c}	1.97±0.004^{c}	1.25±0.008^{c}	1.32±0.008^{c}
6	216.10±5.139^{c}	215.753±2.140^{d}	0.61±0.005^{d}	1.52±0.002^{d}	0.99±0.009^{d}	1.03±0.008^{d}
8	181.73±0.224^{d}	210.237±0.0537^{e}	0.57±0.011^{e}	1.30±0.006^{e}	0.79±0.008^{e}	0.95±0.009^{e}
10	148.23±0.383^{e}	182.571±0.620^{f}	0.54±0.001^{f}	1.11±0.001^{f}	0.47±0.010^{f}	0.40±0.002^{f}
12	130.61±0.052^{f}	180.894±1.402^{f}	0.31±0.002^{g}	0.70±0.013^{g}	0.27±0.003^{g}	0.26±0.004^{g}

[1] Different superscript letters in the same column indicate significant differences between mean values (P<0.01).

Table 5. Changes in total phenol content (mg/kg) of extra virgin olive oil extracted from variety Beylik during 12-month storage (n=48).[1]

Storage period (month)	Sample name	
	Filtered	Unfiltered
0	530.06±0.721^{a}	696.47±0.750^{a}
1	524.23±0.643^{b}	691.74±1.223^{b}
2	511.72±0.721^{c}	688.36±0.212^{b}
3	496.77±3.245^{d}	681.75±0.806^{c}
4	483.15±0.495^{e}	679.97±1.371^{c}
5	476.67±1.719^{f}	661.79±1.470^{d}
6	474.86±0.841^{f}	653.06±1.336^{e}
7	470.24±0.056^{g}	642.53±3.422^{f}
8	466.05±1.160^{h}	635.47±1.202^{g}
9	461.00±1.060^{i}	626.64±2.142^{h}
10	455.43±0.311^{j}	621.28±1.357^{i}
11	450.72±0.714^{k}	616.67±2.043^{j}
12	441.51±1.732^{l}	611.04±1.923^{k}

[1] Different superscript letters in the same column indicate significant differences between mean values (P<0.01).

phenolics detected in this study for filtered samples and its content ranged from 360.73 to 501.11 mg/kg during storage (Table 6). 3,4-Dihydroxy benzoic acid content appeared to increase during storage. Although its content was lower than the detection limit in the eighth, ninth and tenth months, a dramatic increase was observed at the end of storage. Tyrosol was only detected in fresh samples as well as those stored for five, nine and ten months. It was apparent that tyrosol content increased with storage. 4-Hydroxy benzoic acid content increased after the sixth month and was stable until the eleventh month. At the end of storage, it reached its maximum value (12.17 mg/kg). Although 4-hydroxy phenyl acetic acid was only detected in trace amounts (0.67 to 0.85 mg/kg) up to the fifth month, it had its maximum value (33.22 mg/kg) at the end of storage. Vanillic acid was detected in the fifth month (0.17 mg/kg). *Trans*-ferulic acid was detected in the fifth, seventh and twelfth months. m-coumaric content fluctuated during storage. Its amount increased from 0.82 to 1.93 mg/kg at the eighth month of storage. After this point, it decreased to 0.84 mg/kg. Although o-coumaric acid was quantified in the early months, its content reached its maximum at seventh months. Another important phenolic compound is oleuropein, which was higher in fresh olive oil and decreased from 5.54 mg/kg to 1.34 mg/kg with storage. Apigenin content decreased from 2.38 mg/kg to 0.34 mg/kg during the early months of storage. In the fourth month, a sharp increase (2.36 mg/kg) was observed, with a subsequent decrease to 1.94 mg/kg at the end of storage.

Unfiltered EVOO samples had the higher values of phenolic compounds compared to filtered samples. However, a similar alteration trend was observed in the unfiltered type. According to the results of phenolic compounds in the unfiltered type (Table 7), tyrosol was detected only in the fourth (6.61 mg/kg), ninth (19.62 mg/kg) and tenth (20.44 mg/kg) months and 3-hydroxytyrosol was under the detection limit until the end of storage. Their content increased during storage. 3,4-Hydroxy benzoic acid levels also increased but it was not detected during late storage. 4-Hydroxybenzoic acid contents decreased during early months of storage and an increasing trend was revealed up to the last month of storage. 4-hydroxy phenyl acetic acid content changed during the first six months and was not detected after the seventh month. Contents of m-coumaric acid ranged between 1.27-3.56 mg/kg and the highest amount was obtained in the seventh month, after which it decreased. The lowest amount of m-coumaric acid was observed in the last month (1.27 mg/kg). o-coumaric acid was detected only in the third, fourth and fifth months.

Table 6. Changes in phenolic compounds of filtered Beylik (Antalya) during 12-month storage time (mg/kg) (n=48).

	Months												
Phenolic Compounds	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
3,4-dihydroxy benzoic acid	0.36±0.024	0.46±0.058	0.85±0.069	0.72±0.099	0.65±0.042	0.43±0.038	7.75±0.086	10.12±0.714	nd[1]	nd	nd	15.11±0.255	26.2±0.585
Tyrosol	9.94±0.009	nd	nd	nd	nd	15.97±0.062	nd	nd	nd	16.83±0.907	18.53±0.214	nd	nd
4-hydroxy benzoic acid	6.77±0.151	8.26±0.339	6.46±0.081	5.43±0.066	4.30±0.049	4.81±0.057	10.27±0.113	10.37±0.214	10.53±0.112	10.56±0.142	10.77±0.414	11.02±0.327	12.17±0.281
4-hyroxy phenyl acetic acid	nd	0.67±0.021	0.69±0.027	0.85±0.065	0.73±0.057	nd	nd	nd	nd	nd	nd	nd	33.22±0.652
Vanillic acid	nd	nd	nd	nd	nd	0.17±0.052	nd	nd	nd	nd	nd	nd	nd
Trans-ferulic acid	nd	nd	nd	nd	nd	0.06±0.031	nd	0.13±0.027	nd	nd	nd	nd	0.04±0.021
m-coumaric acid	0.82±0.057	0.91±0.013	1.03±0.028	1.51±0.014	1.64±0.017	1.67±0.004	1.8±0.006	1.89±0.037	1.93±0.145	1.42±0.326	1.36±0.040	1.11±0.027	0.84±0.032
o-coumaric acid	0.18±0.004	nd	0.08±0.016	nd	0.06±0.021	nd	nd	2.20±0.052	nd	nd	nd	nd	nd
Oleuropein	5.54±0.235	5.31±0.914	5.22±0.014	4.99±0.007	4.77±0.042	4.62±0.037	3.61±0.178	2.87±0.257	2.45±0.013	2.14±0.021	1.87±0.028	1.68±0.014	1.34±0.114
Luteolin	501.11±2.563	493.88±3.965	480.66±1.778	474.25±1.421	450.75±0.757	423.23±10.800	417.96±2.432	405.79±6.771	396.17±2.424	391.11±1.764	375.35±4.971	363.23±10.541	360.73±1.191
Apigenin	2.38±0.099	2.07±0.056	1.99±0.038	0.34±0.048	2.36±0.014	2.49±0.027	2.40±0.025	2.38±0.019	2.35±0.089	2.01±0.022	1.98±0.010	1.96±0.027	1.94±0.030

[1] nd = not detected.

Table 7. Changes in phenolic compounds of unfiltered Beylik (Antalya) during 12-month storage time (mg/kg) (n=48).

	Months												
Phenolic Compounds	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
3-hydroxy tyrosol	nd[1]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	52.72±0.707	54.22±0.214
3,4-dihydroxy benzoic acid	1.08±0.215	nd	6.87±0.236	0.60±0.015	0.55±0.045	0.72±0.014	9.22±0.232	27.28±1.521	nd	nd	nd	nd	nd
Tyrosol	nd	nd	nd	nd	6.61±0.618	nd	nd	nd	nd	19.62±0.556	20.44±0.212	nd	nd
4-hydroxy benzoic acid	7.99±0.325	3.29±0.297	4.10±0.126	6.74±0.211	7.38±0.214	7.76±0.132	7.86±0.314	8.78±0.012	9.48±0.125	9.65±0.114	10.57±0.218	11.02±0.126	10.53±0.369
4-hyroxy phenyl acetic acid	0.42±0.038	0.56±0.067	0.77±0.027	0.72±0.021	0.68±0.022	0.66±0.021	0.65±0.013	nd	nd	nd	nd	nd	nd
m-coumaric acid	2.97±0.110	2.74±0.016	2.01±0.247	1.61±0.027	2.60±0.225	3.01±0.238	3.23±0.114	3.56±0.452	2.8±0.241	2.64±0.055	2.50±0.069	2.25±0.128	1.27±0.079
o-coumaric acid	nd	nd	nd	0.09±0.002	0.07±0.014	0.07±0.016	nd	nd	nd	nd	nd	nd	nd
Oleuropein	6.53±0.127	6.23±0.227	6.11±0.014	6.03±0.078	5.87±0.067	5.75±0.041	5.34±0.216	4.24±0.028	3.79±0.071	3.23±0.014	2.50±0.071	1.77±0.216	1.87±0.084
Luteolin	524.73±3.707	519.89±0.622	502.24±9.421	496.28±1.841	490.57±1.156	485.93±2.396	461.35±5.245	448.08±10.580	420.24±2.495	400.40±2.106	396.1±0.888	375.89±1.096	355.15±6.990
Apigenin	3.85±0.214	3.87±0.113	3.91±0.327	3.96±0.112	4.03±0.021	4.12±0.127	4.34±0.016	4.57±0.021	4.26±0.014	4.02±0.028	3.93±0.017	3.78±0.014	3.59±0.053

[1] nd = not detected.

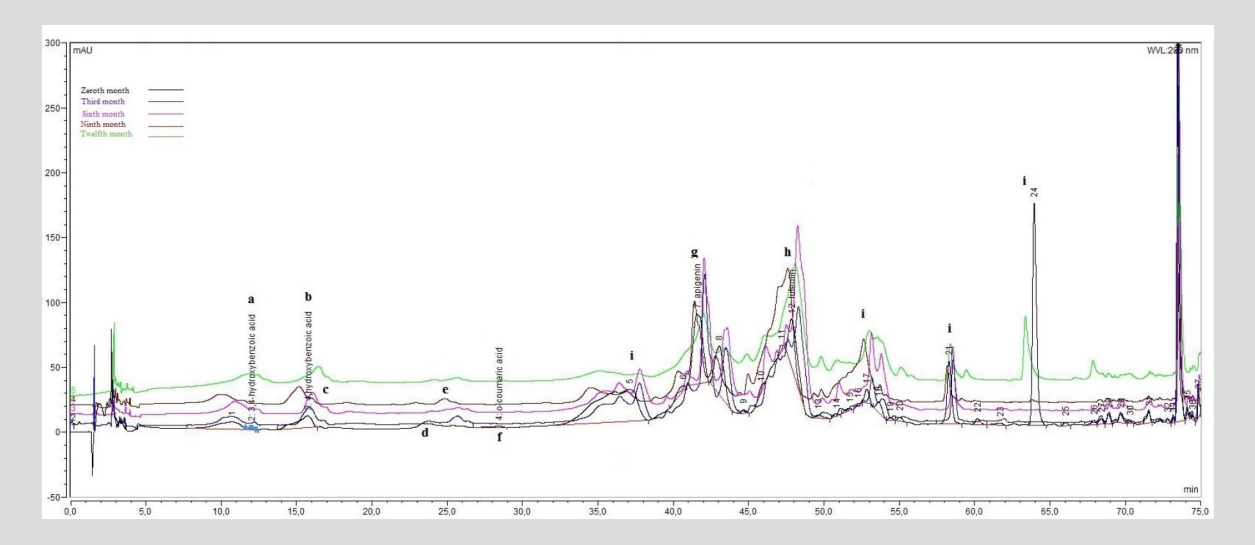

Figure 2. Chromatogram of filtered Beylik (Antalya), alteration of phenolic compounds during 12-month storage. Letters indicate some main phenolic compounds in filtered Beylik EVOO: (a) 3,4-hydroxybenzoic acid; (b) 4-hydroxybenzoic acid; (c) vanillic acid; (d) *trans*-ferulic acid; (e) m-coumaric acid; (f) o-coumaric acid; (g) apigenin; (h) luteolin; (i) other unknown derivatives.

The initial concentration of oleuopein was 6.53 mg/kg and decreased during storage. Luteolin was the most abundant phenolic compound in unfiltered samples. Its concentration decreased from 524.73 mg/kg to 355.15 mg/kg with storage. A slight increase was monitored in apigenin content of unfiltered samples up to the sixth month, then a decrease was seen.

These results suggest that phenolic composition is qualitatively and quantitatively affected by filtration and storage. Many phenolic compounds were also observed to disappear during some months, a process which is related to conversion of phenolic compounds to other phenolic compounds or decomposition to aglycon forms (Rodríguez-Morató *et al.*, 2015). Generally, unfiltered samples had higher phenolic contents than filtered samples, as expected. Further, some phenolics such as 3-hydroxy tyrosol were detected only during late storage, while some of them decreased such as oleoropein, luteolin and apigenin (Table 7). Although filtered samples had low amount of vanillic acid and *trans*-ferulic acid, these phenolics were not detected in unfiltered samples. Syringic acid and hydroxytyrosol were measured only in unfiltered samples. In fact, it is widely recognised that the simple phenols, tyrosol and hydroxytyrosol, increase over time due to hydrolytic processes of the secoiridoidic derivatives representing their linked forms (Mulinacci *et al.*, 2013). Yorulmaz (2009) reported that luteolin was the most abundant phenolic compound following *trans*-cinnamic acid and luteolin-7-glucoside. They also quantified tyrosol, syringic acid, p-coumaric acid, luteolin-7-glucoside, *trans*-cinnamic acid, luteolin and apigenin in Turkish olive oils extracted from different olive varieties. Their results are compatible with those of this research.

Montedoro *et al.* (1992) found 3,4-DHPEA, *p*-HPEA, vanillic acid, caffeic acid, 3,4-DHPEA-EDA and 3,4-DHPEA-EA in olive oils. Morelló *et al.* (2004) suggested that although storage did not appear to have any effect on vanillic acid or vanillin, both of which were present at low concentrations, there was a significant decrease in the concentration of the rest of the quantified phenolic compounds. That reduction was more marked in the secoiridoid derivatives, such as 3,4-DHPEA-EDA, p-HPEA-EDA and 3,4-DHPEA-EA, indicating a more active participation in the oxidative processes because they were more easily oxidised. Among the most representative phenolic compounds in olive oil, lignans seem to be the most stable during oil storage. Mulinacci *et al.* (2013), Gómez-Alonso (2007) and García *et al.* (2003) demonstrated increased tyrosol and hydroxytyrosol contents over time due to hydrolytic processes of the secoiridoidic derivatives. Gómez-Alonso *et al.* (2007) stated that the main phenols were the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA; 9±7 mg/kg), oleuropein aglycon (8±6 mg/kg) and the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA; 5±8 mg/kg). Baiano *et al.* (2014) reported that there were increasing and decreasing trends in phenolic compound (3,4-DHPEA, *p*-HPEA, vanillin, *p*-coumaric acid, 3,4-DHPEA-AC, 3,4-DHPEA-EDA, *p*-HPEA-AC, *p*-HPEA-EDA, 1-acetoxipinoresinol + *trans*-cinnamic acid, *p*-HPEA-EA) content.

Sensory evaluation

According to a sensorial evaluation of the panel, generally fruitiness, pungency and bitterness were higher in unfiltered samples (Table 8). During storage of samples, fruitiness and bitterness were not reduced below 4.0 out of 10. However,

Table 8. Sensory evaluation values for Beylik EVOOs during 12 months' storage.

	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Filtered												
Fruitiness	4.0	4.0	4.0	4.0	4.2	3.8	3.8	4.2	4.1	4.0	4.0	4.0
Bitterness	4.5	4.6	4.3	4.5	4.4	4.5	4.5	4.5	4.8	3.8	4.3	4.2
Pungency	5.5	4.4	4.6	5.0	5.2	5.3	4.7	4.8	4.0	4.0	4.0	4.2
Median for fruitiness	4.0	4.0	4.0	4.0	4.2	3.8	3.8	4.2	4.1	4.0	4.0	4.0
Median for defects	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unfiltered												
Fruitiness	4.4	4.5	4.0	4.5	4.4	4.0	3.8	4.0	4.5	4.0	4.0	4.0
Bitterness	4.4	5.0	4.9	4.9	5.2	5.0	4.1	4.5	5.0	4.5	4.8	4.0
Pungency	5.3	5.4	4.5	5.1	5.0	5.6	4.7	5.0	5.0	4.5	4.5	4.4
Median for fruitiness	4.4	4.5	4.0	4.5	4.4	4.0	3.8	4.0	4.5	4.0	4.0	4.0
Median for defects	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

pungency was 5.5 out of 10 in the beginning and reduced to 4.0. The tasting panel did not detect any defects (median for defects = 0.0) in the samples during the entire 12-month storage period at room temperature. This result can be attributed to high amounts of polyphenols and tocopherols.

Conclusions

In this study, an innovative project was put into practice in Turkey, with a MOOPU being designed to yield premium olive oil that reflects the quality of the variety and region. Therefore, it was possible to process olives harvested in their own ecological environment within two hours. Although Turkey is rich in olive gene resources and there are many olive varieties locally, only a few have economic potential in terms of olive oil and/or table olive markets. The Beylik variety cultivated in the Antalya region is particularly relevant in this regard, but its olive oil quality had not previously been determined due to inappropriate processing conditions. As expected, it was possible to reach as low as 0.1% free acidity and no significant changes were seen during storage due to optimum processing and storing conditions. Olives were harvested by hand-picking and processed immediately using a specially designed two-phase horizontal decanter working without water addition. The samples were packed in amber bottles under nitrogen and stored in the dark. Samples were also found to have high amounts of phenolics and tocopherols, which both function as antioxidants. Therefore, the samples were stable for a long time. Filtration also had a significant effect on some physicochemical features of EVOOs. Lightness of filtered samples was improved in terms of appearance. Peroxide values of filtered EVOOs were lower compared to unfiltered samples at the early stage of storage.

These results demonstrate that it is possible to produce excellent olive oils from the Beylik variety. It has superior quality with low free fatty acidity and high total polyphenol content (up to 690 mg/kg), values which are relatively uncommon for commercial Turkish olive oils, with this variety also having good oxidative stability during storage. Its quality was substantiated with a first place at the Monocultivar Olive Oil Expo 2015 in Milan. It represents a highly suitable candidate to apply for geographic indication among the minor varieties locally grown in Turkey.

Acknowledgements

The authors are grateful for the financial support provided by the Republic of Turkey, Ministry of Science, Industry and Technology (project number: SANTEZ-0560-STZ-2013-2).

References

Abdalla, I.I.H., Khaddor, M., Boussab, A., Garrouj, D.E. and Ayadi, M., 2014. The effect of storage time on the quality of olive oil produced by cooperatives for olive growers in the north of Morocco. Asian Journal of Agriculture and Food Science 2: 129-138.

American Oil Chemists' Society (AOCS), 2003. Determining peroxide value acetic acid-chloroform. AOCS official method Cd 8-53. AOCS, Urbana, IL, USA.

American Oil Chemists' Society (AOCS), 1997. Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. AOCS official method Ce 8-89. AOCS, Urbana, IL, USA.

Anonymous, 2010. The official gazette of Republic of Turkey, number 27665, 7th August. Gazette of Republic of Turkey, Ankara, Turkey.

Baiano, A., Terracone, C., Viggiani, I. and Del Nobile, M.A., 2014. Changes produced in extra-virgin olive oils from cv. Coratina during a prolonged storage treatment. Czech Journal of Food Science 32: 1-9.

Boskou, D., Tsimidou, D. and Blekas, M., 2006. Polar phenolic compounds in olive oil. AOCS Press, Champaign, IL, USA, pp. 73-92.

Boskou, D., 1996. Olive oil chemistry and technology. AOCS Press, Champaign, IL, USA, 162 pp.

Caponio, F., Bilancia, M.T., Pasqualone, A., Sikorska, E. and Gomes, T., 2005. Influence of the exposure to light on extra virgin olive oil quality during storage. European Food Research and Technology 221: 92.

Cinquanta, L., Esti, M. and La Notte, E., 1997. Evolution of phenolic compounds in virgin olive oil during storage. Journal of the American Oil Chemists' Society 74: 1259-1264.

Clodoveo, M.L., Delcuratolo, D., Gomes, T. and Colelli, G., 2007. Effect of different temperatures and storage atmospheres on Coratina olive oil quality. Food Chemistry 102: 571-576.

Del Caro, A., Vacca, V., Poiana, M., Fenu, P. and Piga, A., 2006. Influence of technology, storage and exposure on components of extra virgin olive oil (Bosana cv) from whole and de-stoned fruits. Food Chemistry 98: 311-316.

Dıraman, H. and Dibeklioğlu, H., 2009. Characterization of Turkish virgin olive oils produced from early harvest olives. Journal of the American Oil Chemists' Society 86: 663-674.

European Commission, 1991. Commission Regulation (EEC) No 2598/91 of 30 August 1991 fixing the export refunds on oil seeds. Official Journal of the European Communities L 243/28.

European Commission, 2002. Commission Regulation (EEC) No 796 of 6 May 2002 on change EC Regulation no. 2568/91. 2002. Official Journal of the European Communities L 128/8.

Fregapane, G., Lavelli, V., Leon, S., Kapuralin, J. and Salvador, M.D., 2006. Effect of filtration on virgin olive oil stability during storage. European Journal of Lipid Science and Technology 108: 134.

García, A., Brenes, M., García, P., Romero, C. and Garrido, A., 2003. Phenolic content of commercial olive oils. European Food Research and Technology 216: 520-525.

Gómez-Alonso, S., Mancebo-Campos, V., Salvador, M.D. and Fregapane, G., 2007. Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chemistry 100: 36-42.

Gomez-Caravaca, A.M., Cerretani, L., Bendini, A., Segura-Carretero, A., Fernandez-Gutierrez, A. and Lercker, G., 2007. Effect of filtration systems on the phenolic content in virgin olive oil by HPLCDAD-MSD. American Journal of Food Technology 2: 671.

Gutiérrez, F. and Fernández, J.L., 2002. Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of 'Extra' quality. Journal of Agricultural and Food Chemistry 50: 571-577.

Inarejos-Garcia, A.M., Androulaki, A., Salvador, M.D., Fregapane, G. and Tsimidou, M.Z., 2009. Discussion on the objective evaluation of virgin olive oil bitterness. Food Research International 42: 279-284.

International Olive Council (IOC) Regulation, 2015a. Spectrophotometric investigation in the ultraviolet. IOC, Madrid, Spain.

International Olive Council (IOC) Regulation, 2015b. Sensory analysis of olive oil, method for the organoleptic assessment of virgin olive oil. IOC, Madrid, Spain.

International Olive Council (IOC) Regulation, 2001. Preparation of fatty acid methyl esters from olive oil and olive-pomace oil. IOC, Madrid, Spain.

International Organization for Standardization (ISO), 2016. Determination of the moisture and volatile matter. No. 662. ISO, Geneva, Switzerland.

Lavelli, V., Fregapane, G. and Salvador, M.D., 2006. Effect of storage on secoiridoid and tocopherol contents and antioxidant activity of monovarietal extra virgin olive oils. Journal of Agricultural and Food Chemistry 54: 3002-3007.

Méndez, A.I. and Falqué, E., 2006. Effect of storage time and container type on the quality of extra-virgin olive oil. Food Control 18(5): 521-529.

Montedoro, G., Servili, M., Baldioli, M. and Miniati, E., 1992. Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. Journal of Agricultural and Food Chemistry 40: 1571-1576.

Morelló, J.R., Motilva, M.J., Tovar, M.J. and Romero, M.P., 2004. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry 85: 357-364.

Mulinacci, N., Ieri, F., Ignesti, G., Romani, A., Michelozzi, A., Creti, M., Innocenti, D.M. and Calamai, L., 2013. The freezing process helps to preserve the quality of extra virgin olive oil over time: a case study up to 18 months. Food Research International 54: 2008-2015.

Okogeri, O. and Tasioula-Margari, M., 2002. Changes occurring in phenolic compounds and alpha-tocopherol of virgin olive oil during storage. Journal of Agricultural and Food Chemistry 50: 1077-1080.

Psomiadou, E., Tsimidou, M. and Boskou, D., 2000. Alpha-tocopherol content of Greek virgin olive oils. Journal of Agricultural and Food Chemistry 48: 1770-1775.

Rastrelli, L., Passi, S., Ippolito, F., Vacca, G. and De Simone, F., 2002. Rate of degradation of alpha-tocopherol, squalene, phenolics, and polyunsaturated fatty acids in olive oil during different storage conditions. Journal of Agricultural and Food Chemistry 50: 5566-5570.

Rodríguez-Morató, J., Xicota, L., Fito, M., Farre, M., Dierssen, M. and La Torre, R., 2015. Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. Molecules 20: 4655-4680.

Romani, A., Lapucci, C., Cantini, C., Ieri, F., Mulinaci, N. and Visioli, F., 2007. Evolution of minor polar compounds and antioxidant capacity during storage of bottled extra virgin olive oil. Journal of Agricultural and Food Chemistry 55: 1315-1320.

Tsimidou, M.Z., 2006. Olive oil quality. In: Boskou, D. (ed.) Olive oil. Chemistry and technology (2nd Ed.). AOCS Publishing, New York, NY, USA, pp. 93-111.

Yavuz, H., 2008. Determination of some quality and genuineness of Turkish olive oils. PhD-thesis, Ankara University, Ankara, Turkey.

Yorulmaz, A., 2009. Türk Zeytinyağlarinin Fenolik, Sterol Trigliserit Yapilarini Belirlenmesi. PhD thesis, Ankara University, Ankara, Turkey.