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Oil Characteristics of High Yield Olive Genotypes Obtained by Crossing of Belle D'espagne with Uslu and Karamurselsu Cultivars

Yasin Ozdemir*, Seda Kayahan, Aysun Ozturk, Nesrin Aktepe Tangu and Ozge Keskinel

Ataturk Central Horticultural Research Institute, Yalova, Turkey

***Corresponding Author:** Yasin Ozdemir, Ataturk Central Horticultural Research Institute, Yalova, Turkey.

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Abstract

This research is aimed to determine olive oil characteristic of 4 cultivar candidates which were obtained by cross breeding program of Ataturk Horticultural Central Research Institute Yalova/Turkey. "Belle d'Espagne X Karamurselsu" was parents of BK022 and BK024 and "Belle d'Espagne X Uslu" was parents of BU015 and BU016. Gemlik cultivar was used for compare. Olives were harvested at 4,5 maturation index and oil content of their fruits and free acidity, peroxide value, K_{232} and K_{270} indices, fatty acid composition, total phenol content, total antioxidant activity and sensory profile of their oils were evaluated. BU015 had high oil yield with highest total antioxidant activity and sensory characteristic. BU016, BK022 and BK024 had lower oil content than Gemlik cultivar. This research could represent a helpful tool for breeding researcher to select cultivar candidates for registration with high quality indices and sensory profile. According to evaluated characters, registration of BU015 as oil purpose cultivar has the potential to be beneficial for producers and consumers.

Keywords: *Olive oil; Olive crossing; Olive genotype*

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Introduction

Olive cultivation is increasing around the world, expanding from its native area of cultivation and to meet this new demand, breeding is playing an important role to introduce new cultivars with desirable attributes of olive oil quality (Breidi., *et al*. 2016). From the quality point of view, olive oil is highly appreciated due to its fatty acid (high monounsaturated oleic acid content) and other compounds composition. These components are responsible from health benefits associated to the long term consumption of olive oil (León., *et al*. 2018, García-Rodríguez., *et al*. 2017). Fatty acid composition (Sánchez de Medina., *et al*. 2015a), phenolic profiles (Pérez., *et al*. 2014, El Riachy., *et al*. 2012), α-tocopherol, pigments (León., *et al*. 2011) and volatile compounds (García-Gonzalez., *et al*. 2010) of olive oil have been studied in olive breeding programs. High variability for most olive oil quality components has been reported in progenies from breeding programs (Sánchez de Medina., *et al*. 2015b; De la Rosa., *et al*. 2016). Most of the olive breeding programs were based in specific crosses between cultivars of well-known merit (De la Rosa., *et al*. 2013). One of these breeding program carried out in Ataturk Central Horticultural Research Institute, Yalova/Turkey and 393 olive genotypes were produced. The main objective of this research was to determine the usefulness of cross breeding of "Belle d'Espagne X Karamurselsu" (BK013 and BK022) and "Belle d'Espagne X Uslu (BU015 and BU016)

to obtain high oil content and quality. They produced by previously mentioned cross breeding project and selected according to high fruit yield per tree and low periodicity. In this study, oil content of olives and variability for oil quality components including free acidity, peroxide value, specific absorbance (K_{232} and K_{270}), fatty acid composition, total phenol content, total antioxidant activity and sensory profile of their oils was determined for fruit of BK013, BK022, BU015 and BU016.

Material and Method

In this study, olive of 4 cultivar candidates which were chosen by breeding researcher on the basis of their high productivity and low periodicity according to results of national cross breeding project (Obtaining New Olive Varieties by Crossing, 1990-2018) and Gemlik cultivar were evaluated to compare. All those trees were planted at in 1.5m x 3m distance in olive genotype observation orchard of Ataturk Central Horticultural Research Institute (Yalova/Turkey) in 2001. Maturity index of olives were followed and determined according [10] and olives were randomly handpicked in 2015-2016 and 2016-2017 nearly 4,5 maturity index for optimum balance between oil yield and quality (Boskou, 2006). Code of olives and their maturity index were given in Table 1.

Code	Crossing combination	Muturity index
BK 022	Belle D'Espagne X Karamurselsu	4.2 ± 0.2
BK 024	Belle D'Espagne X Karamurselsu	4.7 ± 0.4
BU 015	B. D'espagne X Uslu	4.4 ± 0.3
BU 016	B. D'espagne X Uslu	4.6 ± 0.2

Table 1: *Crossing combination and maturity index of olives.*

Oil content analysis

Then olives were turned into paste by laboratory scale hammer (100 rev/min) and kneader. After that olive paste was dried. Oil of the dried olive paste was extracted by soxhlet apparatus, for at least 8 hours, with petroleum ether extraction at 60°C (Cemeroglu 2007).

Olive oil Production

Damaged and unhealthy olives were removed and olives were washed without delay. Then olives were turned into paste by laboratory scale hammer (100 rev/min) and kneader (45 minutes) after which 0.4 kg batches of olive paste was put into press cloth and pressed (250-300 kg/cm^2) with a hydraulic press. Liquid phase from press was separated into water and oil phase by using separatory funnel. The obtained oil was centrifuged (8000 rev/min) and filtered through a coarse filter (20 µm). Finally, oil was filled into dark glass bottles without any air space and stored at 4°C until analyze. Olive oil samples were analyzed within 3 days after production.

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Oil analysis

Free acid content and peroxide value were determined by titrimetric methods according to the official methodologies of Turkish Food Codex-Communiqué of Analysis Methods of Olive Oil and Pomace Oil (Anonymous. 2015). For determination of specific absorbance value, 0.5 g of oil were weighted (with 0.0001 accuracy) and dissolved into 50 mL cyclohexane 50 mL. Mixture was put into 1 cm quartz

cuvette and its absorbance was measured at 232 and 270 nm with spectrophotometer (Anonymous, 2015). Total phenol content of these samples was determined by Folin-Ciocalteu method according to Gutfinger (1981) and antioxidant activity was detected by DPPH method according to (Usenik., *et al*. 2007).

Fatty acid methyl ester composition was determined by gas chromatography according to Anonymous (2015). 0.2g oil and 10 ml of hexane were put into a vial and shaken. After that, 0.5 mL of a methanolic KOH solution (2N) was added and stirred. 0.5 µl were taken from the upper phase and injected into the gas chromatography (Hewlett-Packard, USA). Saturated fatty acids (SFA), monounsaturated fatty acids, (MUFA) and polyunsaturated fatty acids (PUFA), MUFA/PUFA, linoleic acid/linolenic acid and iodine number (IN) of olive oil samples were determined by using their fatty acid composition (Kyriakidis and Katsiloulis, 2000). The formulas used in the calculation are given below:

SFA (%) = palmitic acid + stearic acid + arashidic acid + behenic acid
MUFA (%) = palmitoleic acid + oleic acid + eicosenoic acid
PUFA (%) = linoleic acid + linolenic acid
IN= 0.93 × (oleic acid + eicosenoic acid) + 1.35 × (linoleic acid) + 2.62 × (linolenic acid)

Negative (fusty/muddy sediment, musty, winey-vinegary acid sour, rancid etc.) and positive (bitter, pungency and fruity) sensory characteristics of olive oils were evaluated by 10 trained and experienced panelists according to official method of International Olive Council-Sensory Analysis of Olive Oil (Anonymous. 2015b). The official evaluation sensory analysis of virgin olive oils sheet of Anonymous (2015b) was used. The values, expressed as centimeters, were statistically processed to calculate the median of each characteristic.

Statistical Analysis

Randomized experimental design was used and analysis of variance was applied with the Duncan multiple comparison test of the means ($p < 0.05$) to determine the presence of significant differences among the samples. Statistical analysis was performed by using the JMP v. 5.0 statistical package program (SAS Institute, Cary, N.C., U.S.A.). Different letters indicate significant difference in same colon of tables.

Results and Discussion

The main objectives of the olive cross breeding program were reported as: earliness of bearing, high oil yield, oil quality, suitability to different plantation systems and disease resistance (Rallo., *et al*. 2016). Oil content of olives and free fatty acid content, peroxide value and specific absorption at ultraviolet light of olive oils were given in Table 2.

Sample	Oil content (%)	Free fatty acid cotent (oleic acid %)	Peroxide value ($meqO_2/kg$)	*Specific absorption at ultraviolet light*	
				K_{232}	K_{270}
BK022	16.62 ± 0.47b	0.36 ± 0.094	6.83 ± 0.53c	1.73 ± 0.17	0.15 ± 0.029
BK024	17.05 ± 1.60b	0.53 ± 0.094	10.43 ±0.49ab	1.80 ± 0.28	0.12 ± 0.018
BU015	20.67 ± 0.47a	0.50 ± 0.14	12.33 ± 1.44a	1.64 ± 0.25	0.17 ± 0.020
BU016	18.6 ± 1.24ab	0.53 ± 0.05	9.52 ±0.68bc	2.06 ± 0.16	0.14 ± 0.033
Gemlik	20.12 ± 1.63a	0.67 ± 0.05	11.97 ± 2.16ab	1.87 ± 0.21	0.18 ± 0.016
p	0.028	0.09	0.0083	0.26	0.18

Table 2: *Oil content of olives and free fatty acid content, peroxide value and specific absorption at ultraviolet light of olive oils.*

Zegane., *et al*. (2015) reported that free fatty acid cotent and peroxide value (meqO_2/kg) olive oils from different cultivars and geographical origins between 0.14-0.42% oleic acid and 3.04-10.00 meq O_2/kg. Free fatty acid cotent (0.36-0.67%) and peroxide value (6.83-12.33 meq O_2/kg) determined higher than result of Zegane., *et al*. (2015). Specific absorption of ultraviolet light at K_{232} and K_{270} of ninety olive oil samples were reported between 1.4-3.18 and 0.10-0.84 respectively (Guzmán., *et al*. 2015). In this study specific absorption at ultraviolet light determined between 1.64-2.06 (K_{232}) and 0.12-0.18 (K_{270}).

Phenolic compounds have been widely studied because of their nutraceutical effects, relevant contribution to the sensory properties of olive oil, with special emphasis on bitterness and pungency, and stabilizing role to ensure the long shelf-life of olive oil as compared to other vegetable oils (Segura-Carretero., *et al*. 2010). Total phenolic content, antioxidant activity and sensory characteristics of olive oil samples were given in Table 3.

Sample	Total phenolic content (mg gallic acid/kg)	Antioxidant activity (µmol trolox/kg)	Sensory characteristics		
			Fruity	Pungent	Bitter
BK022	218.35 ± 11.02bc	613.17 ± 98.38b	3.57 ± 0.29	2.8 ± 0.53ab	3.63 ±0.30a
BK024	306.82 ± 29.86a	710.23 ± 81.50b	2.77 ± 0.13	2.93 ± 0.20ab	3.17 ± 0.49ab
BU015	246.83 ± 24.71b	938.07 ± 24.51a	3.17 ± 0.20	3.43 ± 0.30a	3.87 ± 0.54a
BU016	176.56 ± 7.18c	629.57 ± 90.91b	2.7 ± 0.51	2.2 ± 0.25bc	3.03 ± 0.37ab
Gemlik	184.85 ± 14.66c	568.83 ± 47.41b	3.17 ± 0.25	2.0 ± 0.25c	2.4 ±0.21b
p	0.0003	0.004	0.09	0.0092	0.04

Table 3: *Total phenolic content, antioxidant activity and sensory characteristics of olive oils.*

High variability was reported within and between the different cross progenies, with considerable deviation from their parents and highest total phenol content of oil (700 mg gallic acid/kg) in 'Leccino' × 'Ascolana Tenera' seedlings were reported by Breidi., *et al*. (2016). In this study total phenol content and antiozidant activity of cultivar candidates were between 176.56-306.82 mg gallic acid/kg and 613.17-938.07 µmol trolox/kg. Our total phenol resulr were lower than result of Breidi., *et al*. (2016). Among of 136 olive genotypes from a Picual × Arbequina crosses, UCI-41, UCI-36, UCI-39, UCI-68, UCI-133 and UCI-63 were reported, reported as outstanding genotypes because of their remarkable sensory properties. In this study statistical different not detected for fruity characteristic but BK015 and BK024 were determined as featured olive oils according to pungent and bitter characteristics. Negative (fusty/muddy sediment, musty, winey-vinegary acid sour, rancid etc.) characteristic was not detected in sensory panel. Median of positive characters were higher than 2 for all of the samples.

Fatty acid composition, with special emphasis on oleic acid and palmitic acid, is one of the most critical quality factors to be evaluated in olive oil. For this reason, the profile of fatty acids is frequently used as a decision tool in olive breeding programs (Breidi., *et al*. 2016, Sánchez de Medina., *et al*. 2015a). In this study Major and minor fatty acids of olive oil samples were given in Table 4 and Table 5 respectively.

Unfavorable composition of fatty acids has been reported as one of the main shortcomings, lowering the quality of olive oils due to the essential role of this fraction in oils stability with direct responsibility for undesired odors and flavors (Sánchez de Medina., *et al*. 2015a, León., *et al*. 2004). It is worth mentioning that qualitative restrictions according to the fatty acids composition are imposed by the International Olive Oil Council (IOOC) regulations. Thus, the allowed ranges for the two most concentrated FAs, oleic acid and palmitic acid are 55.0–83.0% and 7.5–20.0%, respectively (both expressed as w/w) (Anonymous, 2012). In this study oleic acid and palmitic acid resu lts within the appropriate limits of Anonymous (2012).

Sample	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
BK022	13.55 ± 0.7	1.29 ± 0.3	2.15 ± 0.3	71.88 ± 1.2a	9.01 ± 1.5	0.87 ± 0.12
BK024	12.36 ±0.1	0.64 ± 0.05	2.82 ± 0.5	65.72 ± 2.7b	8.63 ± 5.1	0.94 ±0.11
BU015	13.75 ± 0.6	1.14 ±0.3	1.93 ± 0.08	66.70 ± 7.03b	12.79 ± 7.8	0.96 ± 0.03
BU016	12.7 ± 1.6	0.88 ± 0.35	2.23 ± 0.2	70.19 ± 4.6a	12.02 ± 3.3	0.85 ± 0.14
Gemlik	14.94 ± 1.3	1.49 ± 0.50	2.04 ± 0.2	66.23 ± 4.5b	12.18 ± 1.3	0.87 ± 0.09
p	0.18	0.15	0.069	0.048	0.79	0.77

Table 4: *Major fatty acids of olive oils (% in fatty acid).*

Sample	Margaric acid	Heptadecanoic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoseric acid
BK022	0.11 ± 0.01	0.25 ± 0.07a	0.39 ± 0.02	0.31 ± 0.05	0.10 ± 0.03	0.043 ± 0.02
BK024	0.13 ± 0.08	0.22 ± 0.20a	0.45 ± 0.06	0.33 ± 0.03	0.11 ± 0.01	0.026 ± 0.004
BU015	0.067 ± 0.02	0.13 ± 0.07b	0.37 ± 0.02	0.30 ± 0.05	0.09 ± 0.01	0.043 ± 0.01
BU016	0.07 ± 0.04	0.12 ± 0.10b	0.40 ± 0.04	0.32 ± 0.07	0.12 ± 0.03	0.040 ± 0.01
Gemlik	0.05 ± 0.01	0.06 ± 0.03c	0.39 ± 0.06	0.27 ± 0.07	0.10 ± 0.02	0.045 ± 0.06
p	0.35	0.03	0.41	0.78	0.60	0.65

Table 5: *Minor fatty acids of olive oils (% in fatty acid).*

Sample	SFA	MUFA	PUFA	IN	MUFA/PUFA	Linoleik/Linolenik
BK022	16.34	73.73	9.88	83.03	7.49	10.28
BK024	15.89	66.92	9.57	76.34	6.99	9.22
BU015	16.24	68.28	13.75	83.29	4.96	13.33
BU016	15.57	71.51	12.87	84.97	5.55	14.15
Gemlik	17.60	68.05	13.04	81.99	5.21	14.05

Table 6: *Calculated important parameters of olive oils by using fatty acid composition.*

Olie oils of Belle D'Espagne X Karamürselsu (BK013) were reported as remarkable high PUFA content of 22 genotypes and Lucas x Tavsanyuregi crossing (LT017) was reported as a lanced on LA and LnA with a ratio 3.66 (Ozdemir and Kurultay, 2016). In this study BU015 had highest PUFA and BK022 highest MUFA content. BK024 had lowest IN values. MUFA/PUFA and Linoleic/Linolenic ratios were determined between 4.96-7.49 and 9.22-14.15 respectively.

Conclusion

Regarding olive oil quality, generic parameters according to the official methods described in Regulation EC 2568/91 of the Commission of the European Union such as free acidity, peroxide value, UV spectrophotometric indices (K232, K270) and sensory analysis have been taken into account (Anonymous, 1991). All of our result were within limit of Codex Standart (Anonymous, 2015c) Negative characteristic did not detected and median of positive characters were higher than 2 for all of the samples.

Evaluation of the total phenol contetn, antioxidant activity, fatty acid profile and sensory characteristics at the initial stage of selection can serve to identify potential new olive cultivars in breeding programs that produce oils with improved qualities. MUFA especially

oleic acid is favorable in diet because of its important cardiovascular benefits. In turn, SFA are nutritionally unfavorable since they increase the amount of cholesterol. From these perspective; BK022 and BU016 were determined remarkable cultivar candidate for tehir low SFA and high MUFA content oils.

BK022, BK024 and BU016 had lower oil content than Gemlik. It was a major disadvantage for selection of this cultivar candidates. BU015 had similar oil content in fruit and MUFA and PUFA content in oil with Gemlik. Additionally, BU015 had higher pungent and bitter sensory characters, total phenol content and antioxidant activity and lower SFA than that is of Gemlik. So that as a general evaluation of this study, BU015 can be selected among cultivar candidates because of its mentioned characteristics.

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