

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SOME CROSSED OLIVES AND THEIR CONVENIENCE TO BLACK TABLE OLIVE FERMENTATION BY USING *LACTOBACILLUS PLANTARUM* AS A STARTER CULTURE

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Abstract

*This research was aimed to determine characters of raw and processed fruits of 5 crossed olive types which had potential for registration according to agronomic characteristics. Fruits of Gemlik which is the Turkey's most important black table olive cultivar were used for comparison. Number of olives per kilogram, flesh to seed ratio, water, oil, total and reducing sugar, and phenolic compounds were analyzed. Sensory and salt analyses also were applied to processed olives. For processing, olives were debittered by 0,5% NaOH and then put in brine which contained 5% salt at pH 4,5. At 4th day of keeping the olives in brine, 10⁷ cfu/mL *L. plantarum* were inoculated to the brine for fermentation. Fruit weight was increased after table olive production. GE015 and LU001 had better fruit and MU008 had better sensory characteristics so that they have potential for registration as a new table olive cultivar.*

Keywords: *table olive crossing, table olive genotype, table olive selection*

1. INTRODUCTION

The olive's early domestication and the use of vegetative propagation have resulted in the development of a huge number of varieties. Olive farmer is seeking new cultivars better suited to modern cultivation techniques (Bellini et al 2008). Classical olive breeding programs are based in intraspecific cross breeding between cultivars of known merit. (Leo'n and de la Rosa, 2007; Arcas et al., 2013). Recent technological advances to improve the quality of virgin olive oil and table olive have been focused on olive breeding programs by selecting outstanding cultivars and target progenies (Bellini et al., 2008; Sánchez de Medina et al., 2014). Classic breeding programs by crossing and selection in the progenies are reported in Turkey (Arsel and Cirik, 1994; Ozdemir et al., 2011), Tunisia (Trigui, 1996), Greece (Pritsa et al., 2003), Israel (Lavee et al., 2003), and Italy (Bellini et al., 2002). A few novel cultivars have been released in olive producer countries in recent years (Bellini et al., 2008; Roca et al., 2011; Ozdemir et al., 2011; Lavee et al., 2014). In the coming years, the continuation of breeding works with new crosses and continuous evaluation of the generated progenies will provide new olive cultivars obtained by genetic improvement that may change the current cultivar distribution in many countries (Arcas et al., 2013).

In 1990 a table olive breeding program was begun at the Ataturk Central Hrtiultural Research Institute. As a result of this breeding project, 390 trees of olive genotypes which had olives coming from different crosses of national and foreign olive cultivars (Aktepe Tangu et al., 2008; Ozdemir et al., 2013). Previous study had indicated that according to agronomic characteristics, crossed olive genotypes generated with a cross breeding project by Ataturk Central Horticultural Research Institute (Yalova, Turkey) had potential for registration (Aktepe Tangu et al., 2008; Ozdemir et al., 2011). So that this research was aimed to determine the fruit properties and table olive characteristics of these crossed olive types to prepare data and define the suitability to table olive fermentation by using starter culture for next step of variety registration procedure.

2. MATERIALS AND METHODS

2.1. Samples

In this study, 5 olive genotypes were evaluated which were given in Table1. They come from the crosses of foreign (Lucques and Manzanilla) and national olive cultivars (Gemlik, Edinciksu and Uslu). These trees were planted at a 1.5 m x 3 m distance in olive genotype observation orchard of Ataturk Central Horticultural Research Institute (Yalova/Turkey). These genotypes were chosen on the basis of their high productivity and resistance to diseases and low periodicity.

Table 1. Olive genotypes and their parents

No	Genotype code	Parents
1	GE 015	Gemlik X Edinciksu
2	GE 126	Gemlik X Edinciksu
3	GU 256	Gemlik X Uslu
4	LU 001	Lucques X Uslu
5	MU 008	Manzanilla X Uslu
6	Gemlik	-

Olives were randomly handpicked at 4-5 maturation index (when skin color is black and the black color is not reached the middle of the olive flesh) according to Guide for The Determination of The Characteristics of Oil-Olives (International Olive Council, 2011).

2.2. Method of Table Olive Production

Olives were processed to table olive according to method of Leal Sanchez et al. (2003). Olives were debittered by keeping in 0.5% NaOH solution until NaOH will reach 2/3 of olive. Then 4 washes performed to remove excess NaOH from olive. Olives put in brine which contained 5% salt at pH 4,5 (pH adjusted acetic acid). At 4th day of keeping the olives in brine, 10⁷ cfu/mL *Lactobacillus plantarum* (ATCC 14917) were inoculated in brine for fermentation. Olives were fermented in brine at 20°C until pH fall to 3.8.

2.3. Physical analysis

Number of olives per kilogram and flesh to seed ratio were determined according to official method TS 774 (1992). Fruit weight was calculated by weighting the 100 olive fruits. Flesh to seed ratio was calculated by using the ratio of flesh and seed weight of 100 olive fruits.

2.4. Water and oil analysis

Water content of olive samples was determined in a conventional oven at 105±2°C (ESTI et al., 1998). Before the oil analysis, seed of olives were removed and olives were crushed. After that crushed olives were dried. Oil of the dried olive paste was extracted by soxhlet apparatus for at least 8 hours with petroleum ether extraction at 50°C. Oil content of the olives was calculated at fresh weight (Cemeroglu, 2007).

2.5. Reduced sugar analysis

5 g olive paste was weighted and mixed with 5 ml potassium ferrocyanide (%15) ve 5 ml zinc sulfate (%30). This mix was completed to 250 ml with distilled water and filtered through filter paper (40 µm pore diameter). 0.5 ml of the diluted sample, 1.5 ml of distilled water and 1 ml of the dinitrophenol was added into the test tube which was held in 100°C water bath for 6 min and cooled for 3 min with

tap water. Absorbance values were determined by spectrophotometer (Shimadzu UV-2900, Japan) at 600 nm wavelength within 20 minutes (Ross, 1959).

2.6. Total sugar analysis

25 ml of the filtrate from prepared sample for reducing sugar is put into balloon flask. 5 ml of HCl was added for the inversion and placed on the 70°C water bath. Temperature of sample maintained at 67°C for 5 minutes then temperature was cooled down to 20°C. pH adjusted to 6 by using 5 N NaOH and 0.1 N NaOH. After the neutralization it is completed to 50 ml with distilled water. 0.5 ml from sample, 1.5 ml pure water and 6 ml dinitrophenol were mixed and heated on 100°C water bath for 6 minutes. Cooled at for 3 minutes under streams. 600 nm wavelength reading was taken in spectrophotometer (Ross, 1959).

2.7. Phenolic compound analysis

Hydroxytyrosol, oleuropein, luteolin and rutin content of olives analyzed according modified method of Morello et al. (2004). 5 grams of olive flesh homogenized with 50 ml of methanol and macerated in a magnetic stirrer for 2 hours. After that sample was filtered by into the evaporation flask by coarse filter paper and methanol was evaporator at 40°C. Then the residue was redissolved in 50 ml methanol and filtered into vials through 0.45 micron filters. Terms of HPLC Equipment: Injection volume: 20 µl, Flow rate: 1.2 ml / min, Column temperature: 30°C, Detectors: DAD, Stop time: 28 minutes, Mobile phase: 84.6% water - 0.4% formic acid-15% acetonitrile, Max Pressure: 400 bar, Wavelength: 240 nm, Column features: NC100-5C18-3848 Hichrom.

2.8. Salt analysis

Salt content of olives were analysed according to Mohr method (titrimetric method). Olive flesh was homogenized and weighed 10 g in flask. Hot distilled water was added and shaken vigorously for 5-10 minutes. The solution was filtered by filter paper in a 100 ml balloon flask and washed 4-5 times with hot water in to the balloon flask. After completely cooled down, balloon flask was filled to 100 ml with distilled water and 10 ml from this filtrate was added to the flask with 2-3 drops potassium chromate solution. This was titrated with AgNO₃ solution until red color was observed in flask (Cemeroglu, 2007).

2.9. Sensory analysis

Sensory analysis of table olives were carried out by participation of 18 Food and Agricultural Engineers as panelist. Appearance, color, tissue hardness, ease of seed removing, salt, sourness and eating quality of table olives were analyzed between 0-10 point score by panelist (Panago et al., 2002).

2.10. Statistical analysis

Research plan was performed according to the randomized experimental design. Three replicates were tested for each parameter. Analysis of variance was applied with the Duncan multiple comparison test of the means ($p < 0.01$) 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.). The physicochemical characteristics of genotypes were used to perform principal component analysis (PCA) with the PNTSYS statistical package program (Applied Biostatistics Inc., New York, USA).

3. RESULTS AND DISCUSSION

Fruit weight and flesh to seed ratio is one of the most important criteria for table olive variety. Statistically significant differences were observed according to number of olive per kilogram and flesh to seed ratio values of olives. Number of olives per kilogram and flesh to seed ratio of olives were given in Table 2. Only GU256 had smaller fruit weight than fruits of Gemlik variety other crossed types had higher fruit weight. All of the crossed fruits had higher flesh to seed ratio than fruits of Gemlik variety.

Table 2. Number of olives per kilogram and flesh to seed ratio of olives

Olives	Number of olives per kilogram	Flesh to seed ratio
GE 015	202±15.97 c	5.13±0.28 b
GE 126	194±13.43 d	5.14±0.23 b
GU 256	217±12.84 a	5.58±0.19 a
LU 001	183±15.42 e	5.42±0.17 a
MU 008	163±19.13f	5.24±0.26 a
Gemlik	211±20.74 b	4.72±0.18 c

Water, oil, reduced sugar and total sugar content of olives were given in Table 3. Lower water content in olives is favorable for table olive producer. Because low water content reduce the weight loss of olives during table olive processes. Fruits of GE015 and GU256 have lower water content than Gemlik cultivars. All the fruit of olive genotypes had lower oil content than Gemlik. Gemlik variety is double purpose olive which means that it has good table olive and olive oil characteristics. GE015 and GE126 can also be registered as double purpose olive variety because of their table olive characteristics and high oil content. Similar result has been reported in literature for different olive cultivars (Nergiz and Engez, 2000; Menz and Vriesekoop, 2010).

Sugars are the main soluble components in olive tissues and play an important role, providing energy for metabolic changes (Marsilio et al., 2001). In table olive processing sugars act as carbon source for microorganisms (Tseng and Montville, 1992) for producing secondary metabolites responsible for good characteristics and the distinctive flavour of the commodities (Marsilio et al., 2001). 2% sugar content of olive is reported as enough carbon source for fermentative microorganisms. If sugar content is not to 2% olive flesh, reduced sugar should be added to brine for successful fermentation (Kailis and Harris, 2007). All of the olive genotypes had higher reduced sugar than 2% so that there is no need sugar addition to brine for table olive production. Similar results were found in literature at similar ripeness stages of different olives cultivars (Marsilio et al., 2001; Kailis and Harris, 2007; Menz and Vriesekoop, 2010).

Table 3. Water, oil, reduced sugar and total sugar content of olives (%)

Olives	Water	Oil	Reduced sugar	Total sugar
GE 015	58.74±2.85 f	20.10±1.06 b	3.67±0.17 a	3.78±0.16a
GE 126	67.12±2.13 a-c	17.85±0.92 d	2.15±0.11 e	2.77±0.14 de
GU 256	58.1±2.10 f	15.96±0.73 d	3.25±0.13 a	3.41±0.12b
LU 001	64.76±3.06 de	13.74±0.72 e	2.89±0.11 bc	3.28±0.15 bc
MU 008	67.44±2.52 ab	13.28±0.80 f	2.53±0.10 d	2.69±0.09e
Gemlik	65.13±2.74 c-e	21.20±1.04 a	2.65±0.13 c	2.71±0.14e

Hydroxytyrosol, luteolin, rutin and oleuropein are main phenolics of olives and their content in fruits of olive genotypes and Gemlik variety were given in Table 4. Phenolic compounds especially oleuropein and hydroxytyrosol have important affect on sensory characteristics of table olives.

Oleuropein is also responsible from the bitter taste of olives. GE015 is remarkable characteristics according to its low oleuropein and high hydroxytyrosol content. Hydroxytyrosol, rutin and oleuropein content of fruits of Intosso, Arabequina Hojiblanca and Duro varieties were reported between 349-1160 mg/kg, 80-500 mg/kg and 63- 16500 mg/kg respectively (Bianco and Ucella, 2000; Marsilio et al., 2001; Gomez Rico et al., 2008). There are some differences between literature and our results of phenolic compound analysis. Genetic factor were thought as main reason for this difference.

Table 4. Hydroxytyrosol, luteolin, rutin and oleuropein content of olives (mg/kg)

Olives	Hydroxytyrosol	Luteolin	Rutin	Oleuropein
GE 015	2982.73±125.0 a	13.43±1.5 f	387.38±23.2 c	603.76±30.7 e
GE 126	1027.21±116.3 d	97.62±5.2 a	391.51±20.1 bc	443.86±26.8 f
GU 256	1371.42±110.1 b	43.71±2.1 c	120.24±9.8 f	2262.69±132.3 a
LU 001	794.60±32.7 e	86.90±3.4 b	207.16±11.0 d	817.782±42.1 d
MU 008	599.81±39.9 f	27.47±2.1 d	595.03±14.6 a	1237.15±63.2 c
Gemlik	1214.07±72.2 c	21.0± 1.4 e	113.94±9.3 g	1396.31±72.0 b

There were statistically significant differences on fruit number per kilogram, flesh to seed ratio, water, oil and sugar contents of raw and table olives. Number of olives per kilogram and flesh to seed ratio of table olives were given in Table 5. Number of olive per kilogram and flesh to seed ratio of NaOH debittered table olives were determined as 174 and 6.39 by Kailis and Harris (2004).

Table 5. Number of olives per kilogram and flesh to seed ratio of table olives

Olives	Number of olives per kilogram	Flesh to seed ratio
GE 015	192±13.86 b	4.92±0.23 b
GE 126	185±12.71 c	4.90±0.23 b
GU 256	206±10.14 a	5.34±0.22 a
LU 001	177±14.28 d	5.26±0.25 a
MU 008	157±13.90 e	5.32±0.23 a
Gemlik	202±12.16 a	4.98±0.22 b

Comparison of number of olive per kilogram before and after the processing were given in Figure 1. In this research, debittering process with NaOH did not lead to the loss of water from olives on the contrary it allows to enter small amounts of water into the olive.

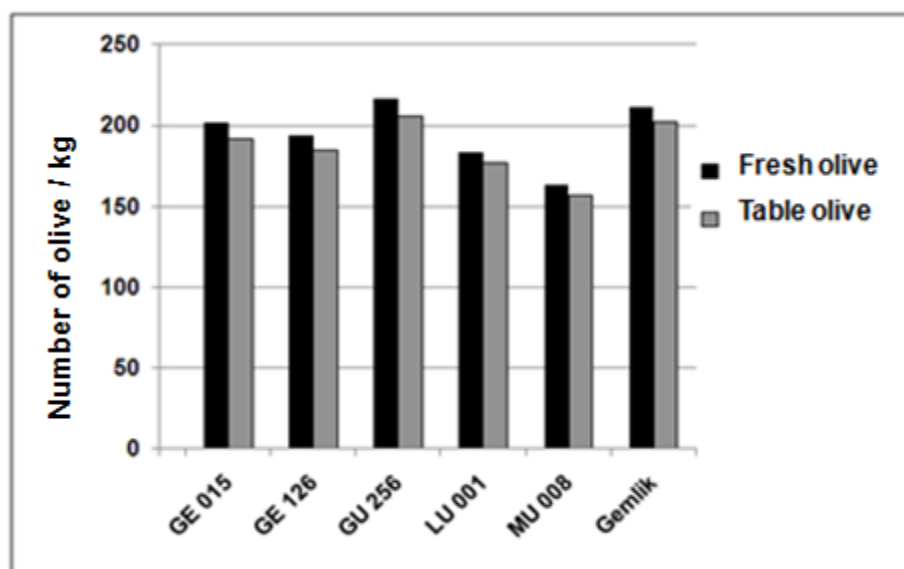


Figure 1. Number of olive per kligram before and after the processing.

Water, oil, salt and hydroxytyrosol content of table olives were shown in Table 6. Garrido Fernandez et al. (1997) and Romero et al. (2004) determined the water content of table olives processed by Spanish method between 70.34-73.40 %. Kailis and Harris (2004) reported water and oil content of table olives which debittered by NaOH between 67-79% and 11-17%. In same report reducing sugar could not be detected in any sample.

In this research water content of table olives determined lesser than literature and but similar to the literature sugar could not be detected in table olives. Reduced and total sugar contents varied among olive cultivars according to processing conditions in processed olives (Kailis and Harris, 2007). In this research all of olives processed by same method so that these differences were caused by genetic difference. As a result of debittering, washing and fermentation steps, oleuropein, luteolin and rutin were not detected in this table olives.

Table 6. Water, oil, salt and hydroxytyrosol content of table olives

Olives	Water (%)	Oil (%)	Salt (%)	Hydroxytyrosol (mg/kg)
GE 015	56.19±2.51 f	20.08±1.26 b	2.97±0.14 c	466.29±38.2 b
GE 126	69.74±2.72 a	16.43±1.12 cd	3.05±0.12 ab	318.74±34.7 c
GU 256	55.19±2.34 f	15.12±1.06 de	2.94±0.09 c	128.50±28.2 f
LU 001	62.82±2.43 de	12.26±0.92 gh	3.11±0.24 a	531.16±34.6 a
MU 008	64.97±2.93 b	12.07±0.87 h	3.07±0.13 ab	141.62±27.6 e
Gemlik	64.21±2.35 b-d	20.01±1.15 b	3.10±0.13 a	209.22±51.8 d

During debittering processing of olives oleuropein was degraded to hydroxytyrosol but hydroxytyrosol was removed by washing steps. Also fermentation steps can also be reduce the content of hydroxytyrosol. Hydroxytyrosol losses were determined in the range of 33.15-83.33% after the table olive production and highest hydroxytyrosol loss (83.33%) was identified for GE015.

Result of sensory evaluation of 174 table olive samples showed that appearance, tissue hardness and salinity had low score, easy of seed removing and color had middle and sourness and bitterness had high scores (Kailis and Harris, 2004). Askolana olive was processed with three different processing method by Marsilio et al. (2005) and they were evaluated with 0-10 point sensory test by panalists. Tissue hardness of this table olives was 6.0-7.6. The results of sensory analysis of table olives were given in Table 7. Overall eating quality of the olives is 5.0-7.6 and the average of all evaluated sensory criteria was in the 6.2-7.2. As a result of the statistical evaluation of the panelists' points, it is understood that fruit of MU008 is the most popular crossed type.

Table 7. Sensory analysis results of table olives

Criteria	MU008	GE015	GU256	GE126	LU001	Gemlik
Appearance	8,3 a	8,9 a	7,3 b	9,1 a	8,3 a	6,3 b
Color	7,4 c	8,8 a	6,4 d	9,3 a	8,1 a	5,9 b
Tissue hardness	6,9 a	5,6 b	5,0 c	5,0 d	6,6 b	5,1 c
Easy of seed removing	8,8 a	6,4 d	7,5 b	6,8 b	6,3 b	7,2 a
Salt	6,1 a	6,3 a	6,1 a	5,1 cd	6,3 b	5,6 b
Sourness	5,4 b	5,8 a	5,0 c	4,6 d	5,5 c	4,3 d
General eating quality	7,6 a	6,3 b	6,2 b	5,7 c	6,2 bc	5,1 c
The average of the criteria	7,2	6,9	6,2	6,5	6,7	5,6

4. CONCLUSION

Genetic diversity of those genotypes was clearly affected physical and chemical characteristics of their olives. Fruit weight and flesh to seed ratio were important characters which determine the commercial value of table olives. Only GU256 had smaller fruit weight than fruits of Gemlik variety other crossed types had higher fruit weight. All of the crossed fruits had higher flesh to seed ratio than fruits of Gemlik variety. Reduced sugar content of raw olive is important for fermentation because fermentative microorganisms need reduced sugar as a carbon source. All the fruits of olive types had enough reduced sugar content for fermentation. During debitterng processing of olives oleuropein was degraded to hydroxytrosol but hydroxytrosol was removed by washing steps. Also fermatation steps can also be reduce the content of hydroxytrosol. Olive properties of crossed types varied in a wide range and some of them had good fruit characteristics so that they have potential for registration as a new table olive cultivar especially GE015 and LU001. Result of this research will be used for variety registration and definition of characteristics of these olives.

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