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PHYSICAL AND CHEMICAL CHARACTERISTICS OF CROSSED OLIVES AND THEIR CONVENIENCE TO GREEN TABLE OLIVE FERMENTATION BY USING *Lactobacillus plantarum* AS A STARTER CULTURE

Yasin OZDEMIR[1], Sefik KURULTAY[2]

[1]Ataturk Central Horticultural Research Institute, Department of Food Technology, Yalova, Turkey
[2]Namik Kemal University, Agriculture Faculty Food Engineering Department, Tekirdag, Turkey

Corresponding author email: yasin.ozdemir@gthb.gov.tr

Abstract
Genetic variation was reported as an important factor effects quality of table olive. So that researchers aimed to develop new cultivar which had high table olive characteristics than that's of standard cultivar. This research was aimed to determine characters of raw and processed fruits of 4crossedolive genotypes which had been reported by previous studies as promising cultivar for registration according to agronomic characteristics. Fruits of Manzanilla cultivar which is the most important green table olive cultivar in Spain 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 green table olive production; olives were debittered by 2% NaOH and then put in brine which contained 5% salt at pH4,5. At 4th day of keeping the olives in brine, 10^{7}CFU/mL Lactobacillus plantarum were inoculated to the brine for fermentation until pH fall to 3,8.All the olives of genotypes had enough reduced sugar content (>2 %) for fermentative microorganisms and higher olive weight than Manzanilla but only olive of BK013 had higher flesh to seed ratio than Manzanilla. After processing hydroxytyrosol losses were determined in the range of 30,25-88,88 % and processed olives of MT038 had higher hydroxytyrosol content this is precious for nutrition physiology of consumer. Olives of BK013 and GK131had bettertable olive and sensory characteristics so that they have potential for registration as new table olive cultivar.

Key words: *olive crossing, olive genotype, table olive selection.*

INTRODUCTION

Olive industry is seeking new cultivars better suited to modern cultivation techniques and with high quality olive oil and table olive (Bellini et al., 2008). So that generally olive cross breeding studies are aimed to obtain new olive genotypes resistance against diseases and pests, appropriate to machinery harvest, have high olive fruit and oil yield with high quality prosperitiesand less periodicity (León et al., 2008).Genetic variation was reported as one of the important factor effects final quality of table olive (Menz and Vriesekoop, 2010; Ahmed et al., 2007). So that researchers aimed to develop new cultivar which had high table olive charcteristics than that's of standart cultivar (Bellini et al., 2008; Ozdemir et al., 2011).

Classic breeding programs by crossing and selection in the progenies are reported in Turkey (Ozdemir et al., 2011; Arsel and Cirik, 1994), 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 (Lavee et al., 2014; Roca et al., 2011; Ozdemir et al., 2011;Bellini et al., 2008). Olive crossbreeding studies have been carried out since 1990 at Atatürk Central Horticultural Research Institute (Yalova, Turkey). The objective of the study is to obtain new olive cultivars which have and superior table olive characteristics. First stage of the study mainly 10 native and foreign were used as parents and hybridisation studies were realized (Yalçinkaya et al 2002). According to their agronomic characteristics previous studies had indicated that some of these crossed olive genotypes had potential for registration (Ozdemir et al., 2011; Aktepe Tangu et al., 2008). So that this research was aimed to determine the raw and table olive characteristics of these crossed olive genotypes to prepare data and define the suitability to table olive fermentation by using starter culture.

MATERIALS AND METHODS

In this study, 4 olive genotypes were evaluated which were given in Table1. They come from the crosses of foreign Belle d'Espagne (Italian cultivar) and Manzanilla (Spanish cultivar) and Karamürselsu, Tavşanyüreği and Gemlik (Turkish cultivar).

These trees were planted at in 1,5 m x 3 m distance in olive genotype observation orchard of Ataturk Central Horticultural Research Institute in Yalova city of Turkey (40°39'42.1"N 29°17'24.5"E).

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

Genotype code	Parents
BK013	Belle d'Espagne X Karamürselsu
MT038	Manzanilla X Tavşanyüreği
GK131	Gemlik X Karamürselsu
GK132	Gemlik X Karamürselsu
Manzanilla	-

Olives were randomly handpicked at 1 maturation index according to Guide for The Determination of The Characteristics of Oil-Olives (International Olive Council, 2011) from this observation orchard.

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 2% 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 pH4,5 (pH adjusted acetic acid). At 4th day of keeping the olives in brine, 10^7cfu/ml *Lactobacilus plantarum* (ATCC 14917) were inoculated in brine for fermentation. Olives were fermented in brine at 20°C until pH fall to 3,8.

Physical analysis

Number of olives per kilogram and flesh to seed ratio were determined according to official method TS 774. 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.

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).

Reduced sugar analysis

5 g olive paste was weighted and mixed with 5 mlpotassium ferrocyanide (%15) ve 5 ml zincsulfate (%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 wave length within 20 minutes (Ross, 1959).

Total sugar analysis

25 ml ofthe filtrate from prepared sample dor reducing sugar is put intoballoonflask. 5 mlof HCl was added for the inversion and placed on the 70°C water bath. Temperature of sample maintained at 67°C for 5 minutes than temperature was cooled down to 20°C. pH adjusted to 6 by using 5 NNaOH and 0.1N NaOH. After the neutralization it is completed to 50 ml with distilled water. 0,5 mlfrom 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).

Phenolic compound analysis

Hydroxytrosol, 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 µm filters. Terms of HPLC Equipment: injection volume: 20 µl, flow rate: 1,2 ml/min, column temperature: 30°C, detectors: DAD, stoptime: 28 min, mobile phase: 84,6% water –0,4% formic acid – 15% acetonitrile, max pressure: 400 bar, wave length: 240 nm, column features: NC100-5C18-3848 Hichrom.

Salt analysis

Salt content of olives were analysed according to Mohrmethod (titrimetric method). Olive flesh was homogenized and weigthed 10 gin 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, baloon 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).

Sensory analysis

Sensory analysis of table olives were carried out by participation of 18 experienced and trained 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 (Panagoet al., 2002).

Statistical analysis

Research plan was performed according to the randomized experimental design (single factor 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). Different letters indicate significant difference in same colon of tables.

RESULTS AND DISCUSSIONS

Fruit weigth and flesh to seed ratio are important criterias for table olive cultivar from commercial point of view. 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. Number of olive per kilogram and flesh to seed ratio of 48 new table olive genotypes coming from a cross-breeding programme were reported between 103,1- 909,1 and 1,7–10,0 (Medina et al., 2012). Chiquitita was a new registered olive cultivar obtained in a crossbreeding program (Picual x Arbequina) in Córdoba, Spain (Rallo, 1995). Number of olives per kilogram of Chiquitita was reported as 370,37 by Rallo et al. (2008). In this study all genotypes had higher olive weight than Chiquitita and olive weight and flesh to seed ratio values were determined in value range of results of Medina et al. (2012). All olive genotypes had higher fruit weight (lower numer of olives per kilogram) than fruits of Manzanilla. But only olives of BK013 had higher flesh to seed ratio than fruits of Manzanillain this research.

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

Olives	Number of olives per kilogram	Flesh to seed ratio
BK013	127±16,49 e	5,87±0,18a
GK131	159±18,03 c	5,04±0,20 b
GK132	152±13,11 d	5,10±0,17b
MT038	167±16,34 b	5,14±0,21b
Manzanilla	223±14,21 a	4,97±0,23b

Different letters refer statistically significant differences in same colon

Water and oil content was affect sonsory quality and hardness of table olives and sugar content was important for succes of fermentation (Tseng and Montville, 1992, Kalis and Harris 2007). Water, oil, reduced sugar and total sugar content of olives were given in Table 3. Water and oil content of Chiquitita was reported as 19.1% and 60.8 % (Rallo et al., 2008). In this research all olives had higher

water content but only GK131 had higher oil content than Chiquitita were determined. Only olives of GK132 had lower water content then olive of Manzamilla. All the olive of genotypes had higher oil content than olive of Manzanilla. GK131 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 (Menz and Vriesekoop, 2010; Nergiz and Engez, 2000).

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 flavor of the commodities (Marsilio et al., 2001).

2% sugar content of olive is reported as enough carbon sources 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). In this study, all of the olive genotypes had higher reduced sugar than 2% so that there is no need addition of sugar to brine for fermentation. Similar results were found in literature for different olives cultivars (Menz and Vriesekoop, 2010;Kailis and Harris, 2007; Marsilio et al., 2001).

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

Olives	Dry matter	Oil	Reduced sugar	Total sugar
BK013	66,89±2,16 ab	17,04±0,68 b	3,96±0,18 a	4,18±0,22 a
GK131	65,37±2,81 b	20,73±1,04 a	2,81±0,16 bc	2,97±0,15 c
GK132	63,80±2,16 c	14,87±0,68 e	2,72±0,10 c	3,04±0,13 bc
MT038	68,41±2,28 a	16,02±0,83 c	2,94±0,12 b	3,1±0,15 bc
Manzanilla	67,46±2,33 ab	13,78±0,67 d	2,83±0,12 bc	3,02±0,14 bc

Different letters refer statistically significant differences in same colon

Oleuropein is responsible from bitter taste of oleuropein and its content is reduced during table olive processing (Kailis and Harris, 2007).Phenolic component also highly affect taste of table olives (Pereira et al., 2006). So that quantity of phenolic compounds particularly oleuropein was an important selection criteria for olive of new cultivar candidate. Hydroxytyrosol, luteolin, rutin and oleuropein are main phenolic of olives and their content in olives of genotypes and Manzanilla were given in Table 4. Phenolic compounds especially oleuropein and hydroxytyrosol have important effect on sensory characteristics of table olives (Morelló et al., 2004). Oleuropeinis also responsible from the bitter taste of olives (Pereira et al., 2006). GK132 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 (Gomez Rico et al.,2008; Marsilio et al., 2001; Bianco and Ucella, 2000). There were some differences between literature and our results of phenolic compound analysis. In this research all olives grown under same conditions and cultivation techniques so that genetic factor wasthought as main reason for this difference between analysis results of olives of genotypes.

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

Olives	Hydroxytyrosol	Luteolin	Rutin	Oleuropein
BK013	1864,10±87,4 c	19,01±2,2 b	84,32±6,5 d	1935,52±112,4 b
GK131	2168,39±103,9 b	13,98±2,1 d	63,20±4,1 e	1314,4± 78,3 d
GK132	2447,62±106,5 a	21,06±1,5 a	102,14±7,2 c	1095,37±82,1 e
MT038	1644,32±98,1 d	15,27±2,0 c	466,32±25,6 a	1397,85±95,8 c
Manzanilla	819,85±42,9 e	14,8±1,9 d	167,387±8,4 b	1987,58±122,1 a

Different letters refer statistically significant differences in same colon

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).

Water, oil and salt content of table olives were shown in Table 6. In this research, processing increased fruit weight of olives because NaOH allowed small amounts of water to penetrate into the olive. Garrido Fernandezet al. (1997) and Romeroet al.(2004) determined the water content of table olives processed by Spanish method (NaOH debittered) 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.

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
BK013	123±17,32e	5,69±0,25a
GK131	156±12,61c	4,94±0,26b
GK132	147±15,58d	4,93±0,21b
MT038	161±16,55b	4,97±0,28b
Manzanilla	214±14,58a	4,77±0,26b

Different letters refer statistically significant differences in same colon

Table 6. Water, oiland salt content of table olives(%)

Olives	Water	Oil	Salt
BK 013	64,88±2,56b	17,51±1,22b	2,67±0,17a
GK 131	64,08±2,39bc	20,58±1,16a	2,18±0,18b
GK 132	61,89±2,51c	14,13±0,84d	2,23±0,21b
MT 038	65,90±2,58ab	15,71±0,79c	2,19±0,14b
Manzanilla	64,76±2,36b	13,42±0,86d	2,14±0,09b

Different letters refer statistically significant differences in same colon

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. Hydroxytyrosol, rutin, luteolin and oleuropein content of table olives were given in Table 7. As a result of debittering, washing and fermentation steps of table olives processing oleuropein were not detected and except MT038 and GK132 luteolin and rutin could not be detected in table olive samples.

Table 7. Hydroxytyrosol, rutin, luteolin and oleuropein content of table olives

Olives	Hydroxytyrosol	Rutin	Luteolin	Oleuropein
BK013	207,24±16,7 d	ND	ND	ND
GK131	583,77±34,0 b	ND	ND	ND
GK132	297,41±15,3 c	ND	3,06±0,2	ND
MT038	638,05±21,5 a	2,20±0,1	ND	ND
Manzanilla	571,88±27,4 b	ND	ND	ND

ND = not detectable, Different letters refer statistically significant differences in same colon

During debittering processing of olives oleuropein was degraded to hydroxytyrosol but hydroxytyrosol was removed by washing steps (Ozdemir et al., 2014; Romero et al., 2004). Also fermentation steps can also reduce the content of hydroxytyrosol (Brenes et al., 1995). Hydroxytyrosol losses were determined in the range of 30,25-88,88 % after the table olive production and lowest (30,25 %) and highest (88,88 %) hydroxytyrosol loss was identified for olive of Manzanilla and BK013 respectively.

Table olives are highly appreciated for both their sensory characteristics and nutritive value (Marsilio et al., 2005). In this study results of sensory analysis of table olives were given in Table 8. General eating quality of the olives is 6,0-8,4 and the average of all of the evaluated sensory criteria was in the 6,1-7,6. As a result of the statistical evaluation of the panelists' points, it is understood that olives of GK131 and GK132 were most popular genotypes. Table olives processed with strain OM13 as adjunct culture, showed better sensory characteristics compared to those processed without starter (Sabatini et al., 2008). 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 Marsilioet al. (2005) and they were evaluated with 0-10point sensory test by panalists. Tissue hardness of this table olives was 6,0-7,6.

Table 8. Sensory analysis results of table olives

Evaluated Criteria	BK013	MT038	GK131	GK132	Manzanilla
Appearance	6,0 c	6,3 c	6,7 b	8,5 a	6,7 b
Color	5,6 d	6,4 c	7,6 b	9,0 a	7,3 b
Tissue hardness	7,4 a	6,3 c	7,2 a	7,4 a	6,7 b
Easy of seed removing	6,4 b	6,8 ab	7,2 a	5,8 c	5,6 c
Salt	5,4 c	6,1 b	6,3 b	7,3 a	5,2 c
Sourness	5,0 c	5,3 c	6,0 b	7,1 a	5,0 c
Generaleating quality	6,8 b	6,0 c	8,4 a	8,3 a	6,6 b
The average of thecriteria	6,1	6,2	7,0	7,6	6,2

Different letters refer statistically significant differences in same colon

CONCLUSIONS

In this research raw and processed olives of 4 new table olive genotypes coming from a cross-breeding programme and grown in same condition and processed in same processng methodwere evaluated. Acording to statistical results, genetic diversity of those genotypes were determined as signifcantly effective factoron 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. All of the olives of genotypes had higher fruit weight than olives of Manzanilla and some fruit of other standard cultivar such as Ascolano and Arbequina reported by Kailis and Harris (2007). But only olives of BK013 had higher flesh to seed ratio than that's of Manzanilla. All the olives of genotypes had 2,72-3,96 % reduced sugar content which was enough for fermentative microorganisms. After processing especially debittering and washing steps caused high loss content in oleuropein, hydroxytyrosol, luteolin and rutin.Hydroxytyrosol is a valuable phenolic component in terms of nutrition physiology. After processing, 30,25-88,88 % loss determined in hydroxytyrosol content. Also oleuropein could not detected in olive samples after process. Fruit weight was increased between 1,88-4,04 % because of used NaOH de-bittering step when compared to raw and processed olives. Positive effect of lactic acid bacter starter cultures uses in green olive fermentation and sensory attributes of olive samples were reported when compared to spontonues fermantation (Aponte et al., 2012; Sabatini et al., 2008). In this research all samples were processed by using starter culture and high quality table olives were obtained according to result of chemical and sensory test. GK132 had highest scores except for easy of seed removing in sensory evaluation.GK132 had both highest reduced sugar content before process and sourness value after process. This result maybe related with conversion of sugar into lactic acid by fermentation.GK132 had lowest water and oil content among raw and table olive samples. Table olive characteristics of genotypes varied in a wide range and BK013, GK131and GK132 had good table olive characteristics so that they had potential for registration as new table olive cultivar.Result of this research will be used final selection of breeding program and definition of characteristics of these olives for new cultivar certification by breeding researcher.

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