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# Effect of Plant Growth Regulators on Bitterness, Physical and Chemical Characters of Black Olives in Water

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## *Abstract*

*The main goal of this study was to characterize bitterness reduction of black olives by keeping in water which contained plant growth regulator. Main idea of this study was that plant growth regulator accelerates the ripening of olives, ripening enzymes break up oleuropein which was responsible from bitterness and bitterness of olives is able to reduced. Indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, gibberellic acid, ethephon and Signum (containing 267 g/kg boscalid and 60 g/kg pyraclostrobin) used individually. sensory characters (appearance, color, odor, taste and bitterness), oleuropein absorbance, hardness, color, pH, water content and titratable acid content were determined. When compared to raw olive color value and flesh hardness value were similar with treated samples but as a main result sensory bitterness of olives did not reduced enough to advise this method to table olive industry. Future studies maybe useful to develop new production technology of table olives with high nutritive value.*

**Keywords:** postharvest, ethephon, ripening, oleuropein

## Introduction

Their enhanced nutritional value due to the presence of phenolic compounds and monounsaturated fatty acids makes olives an important food commodity of the Mediterranean diet [1]. The content of polyphenols in table olives is highly influenced by the olive variety and the debittering process applied on olives [2]. Directly-brined olives, without prior debittering with NaOH solutions, are gaining more acceptance in the market because of the current customer preference for natural and characteristics of this products [3]. Additionally, assuming a usual consumption of 20 olive fruits per day, an approximate quantity of 25 mg of oleuropein per day can be considered as safe for human use, since it can be found in the usual diet [2]. The introduction of a new food additive requires the prior approval of the Food and Drug Administration (FDA). This approval can be granted only when the FDA concludes that the manufacturer has submitted sufficient toxicological data to demonstrate the safety of the additive [4].

Fruit growth stage and maturation was strongly affecting the contents of oleuropein in olive drupes. Green olives had much higher concentrations of this biofunctional glucoside, which decreased regularly during fruit growth and ripeness [5]. β-glucosidase activity plays a relevant role in the transformation of the phenolic glycosides present in the olive fruit, generating different secoiridoid derivatives [6]. Plant enzymes of olives not only play an important role on table olive processing but also important for olive oil processing [7]. The phenolic profile of virgin olive oil is mainly derived from the number of phenolic glycosides originally found in the tissues of olive fruit and the activity of oxidative and hydrolytic enzymes operating on these glycosides during oil processing [8,9]. Although secoiridoids biosynthesis and degradation pathways are still not fully understood [10], hydrolysis by highly specific β-glucosidases seems to be critical for the diverse roles attributed to secoiridoid derivatives [6].

Lalas et al. [11] have evaluated the possibility of increasing the nutritional value of table olives, placing debittered table olives in a plastic container with the olive leaf extract. Also, [12] reported that olive leaf extract combination with the commercial starter *L. plantarum* during fermentation, made it possible to obtain an increase in the nutritional value of fermented table olives, which were richer in antioxidant, anti-inflammatory, and antimicrobial substances than the control treatment. But there are no

publications on new debittering method for table olive which enhance increase nutritional value or with minimal loss of nutrients. This study was aimed to determine the effects of postharvest application of diluted Indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, gibberellic acid, ethephon and Signum (containing 267 g/kg boscalid and 67 g/kg pyraclostrobin) on sensory characters (appearance, color, odor, taste and bitterness), oleuropein absorbance, hardness, color and pH values and water and titratable acid content.

## Materials and Methods

### Material

Gemlik olive fruits were used as material and its maturation was followed according [13]. Olives were hand-picked at 5 maturation indexes [14] when olives skin was black and less than half of olive flesh was purple in harvest season of 2014/2015 from olive orchard of Ataturk Central Horticultural Research Institute (Yalova/Turkey). Olives were sorted according to size and 3.7-4 g of olives were used for this trial.

### Debittering Experiment

Injured and unhealthy olives were removed and olives were washed. 1 kg olives were put 3 L of water which contain plant growth regulators which are; 0.2 ppm indole-3-acetic acid, $10^{-3}$ M 2,4-dichlorophenoxyacetic acid, 50 ppm gibberellic acid, 4.8 ppm ethephon and 0.2 ppm Signum (containing 267 g/kg boscalid and 67 g/kg pyraclostrobin) individually at 20±2°C. Olives were kept in water without growth regulators for control. During debittering experiment, olives were analyzed with sensorial odor, appearance and bitterness by panelists at 2-day intervals. The limit for odor and appearance tolerance <5.5 and for bitterness was set at 7.5< because slight bitter taste in table olive is desirable by consumers [15,16]. At the end of 7 days experiment was stopped due to unfavorable and undefined taste of olives. All olive samples were analyzed immediately after debittering experiment.

### Analysis

pH value and titratable acidity content of olives were determined according to official method TS 774 [17]. Absorbance value of oleuropein was determined at 345 nm according to spectrophotometric method which was described by Mastorakis et al. [18]. Color of olive skin were determined with a color meter (Konica Minolta, Japan) at 23 ± 1°C. Water content of olive samples was determined by using drier at 75±2°C [19]. Flesh hardness of olive was determined with fruit harness tester (W.O.W FRH-5, Japan). Color and flesh hardness analyses were done in 15 and other analysis were done in 3 replicates. The sensory profiles of olives were evaluated with sensory profile sheet used by the trained panelist according to [20]. Sensory characteristics were evaluated with 9-point scale (9: like extremely, 8: like very much, 7: like moderately, 6: like slightly, 5: neither like or dislike, 4: dislike slightly, 3: dislike moderately, 2: dislike very much and 1: dislike extremely) by panelists. Sample preparation, serving and tasting procedures were organized according to Galán-Soldevilla and Pérez-Cacho [21]. The appearance and color attributes were assessed by the panelists on the complete sample before tasting. Odor, taste and bitterness were evaluated respectively.

### Statistical analysis

Randomized experimental design was used and analysis of variance was applied with the Tukey 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 programs (SAS Institute, Cary, N.C., U.S.A.). Different letters indicate significant difference in same colon of tables.

## Results and Discussion

Phytohormones regulate numerous aspects of plant growth and postharvest fruit quality such as color, texture and acidity [22]. Color and hardness are the important characters which highly effect the preference of consumer for table olive marketing [23]. Color value and hardness of olive samples were given in Table 1. L value, a value, b value and hardness of black table olives were reported as 29.13-31.03, 2.05-2.30, 1.51-1.56, and 317-346 g. respectively [24]. In this study b value and hardness and L and a color values were determined lower than the results reported by Kiritsakis [14].

**Table 1:** Color value and hardness of olives

| Sample | Color values | | | Hardness (g) |
|---|---|---|---|---|
| | L | a | b | |
| 2,4 dichlorophenoxyacetic acid | 29.48±1.2 | 2.11±0.5 | 1.55±0.3 | 317±3.6 |
| Gibberellic acid | 30.74±2.7 | 2.30±0.4 | 1.51±0.3 | 322±5.8 |
| Indole-3-acetic acid | 31.03±1.2 | 2.14±0.5 | 1.53±0.2 | 324±3.6 |
| Signum | 29.44±0.6 | 2.12±0.3 | 1.56±0.2 | 346±4.2 |
| Ethephon | 29.26±0.8 | 2.05±0.5 | 1.52±0.4 | 332±3.5 |
| Control | 30.60±0.4 | 2.23±0.2 | 1.55±0.2 | 327±4.9 |
| Raw olive | 29.13±0.2 | 2.09±0.4 | 1.56±0.2 | 329±5.3 |

An acid sensation defines the taste associated with acids that are naturally present in the flesh of the olive fruit (e.g., tartaric acid, malic acid, and citric acid) or that are produced during the lactic fermentation by homofermentative and heterofermentative lactic acid bacteria [15]. Water content, oleuropein absorbance value,

pH and titratable acidity content of olive samples were given in Table 2. pH value, titratable acidity and water content of black olives were reported as between 5.08-5.14, 0.14-0.17 [24] and 61.38-59.38 % [23] Oleuropein absorbance value of raw olive, control and treated olives was reported as 0.72, 0.68 and between 0.48-0.63. On the other hand [25] reported the oleuropein absorbance value of raw and processed Gemlik olives 0.57 and between 0.06-0.16. Whose results were lower than that was found in this study. There were statistically significant differences for reduction in oleuropein absorbans value. Higher reduction was detected for ethephon treated olive samples and followed by indole-3-acetic acid and Signum treated samples.

**Table 2:** Water content, oleuropein absorbance value, pH and titratable acidity content of olives

| Sample | Water (%) | Oleuropein absorbance value ($K_{345}$) | pH | Titratable acidity (% oleic acid) |
|---|---|---|---|---|
| 2,4 dichlorophenoxyacetic acid | 60.39±1.3 | 0.63±0.08b | 5.11±0.08 | 0.17±0.04 |
| Gibberellic acid | 61.07±2.1 | 0.62±0.07b | 5.08±0.04 | 0.15±0.02 |
| Indole-3-acetic acid | 59.38±1.5 | 0.54±0.02c | 5.10±0.05 | 0.17±0.03 |
| Signum | 61.38±1.3 | 0.56±0.02c | 5.14±0.03 | 0.15±0.02 |
| Ethephon | 60.65±1.2 | 0.48±0.04d | 5.11±0.05 | 0.16±0.02 |
| Control | 59.46±1.4 | 0.68±0.02b | 5.14±0.06 | 0.14±0.03 |
| Raw olive | 59.92±1.3 | 0.72±0.03a | 5.13±0.08 | 0.16±0.02 |

Hardness, bitter and acid parameters showing the most discriminant role in the explanation of the variability among the sensory data [26]. Sensory evaluation scores of appearance, color, odor, taste and bitterness were given in Table 3. López-López reported appearance, odor and bitterness taste of olives were reported between 7.2-5.9,4.6-6.1 and 1.7-2.3 by using 1-11-point sensory score scale. Hardness and bitterness sensory attributes Conservolea natural black olives at the end of the spontaneous and inoculated fermentation processes were reported between 3.2-3.85 and 4.05-4.15 [27].

**Table 3:** Sensory evaluation scores of olives (1-9)

| Sample | Sensory characters | | | | |
|---|---|---|---|---|---|
| | Appearance | Color | Odor | Taste | Bitterness |
| 2,4 dichlorophenoxyacetic acid | 7.2±0.3a | 6.6±0.3a | 5.7±0.2a | 2.2±0.4 | 2,2±0,3 |
| Gibberellic acid | 6.9±0.3a | 6.4±0.4a | 6.1±0.2a | 2.4±0.3 | 1,8±0,4 |
| Indole-3-acetic acid, | 7.0±0.1a | 6.2±0.2a | 6.0±0.4a | 2.1±0.2 | 2,3±0,5 |
| Signum | 5.9±0.2c | 5.8±0.6b | 4.6±0.3b | 2,4±0,3 | 1,7±0,4 |
| Ethephon | 6.5±0.2b | 6.4±0.4a | 5.6±0.3a | 2,2±0,6 | 2,0±0,3 |
| Control | 6.2±0.4b | 6.3±0.5a | 5.8±0.4a | 2,3±0,4 | 1,9±0,4 |

The bitter sensation of olive depends on the presence of bitter substances that come from, oleuropein and its glucoside derivatives which are important polyphenols of olives [28]. Debittering process aimed to reduce oleuropein content but unfortunately it causes a reduction on total polyphenol content of olive [29]. These polyhenols are potent antioxidant and radical scavengers with anti-tumor and anti-inflammatory properties [30]. In this study appearance and color values were determined at higher than acceptable level (5.5). Taste was determined as unacceptable at the end of 6 days for Signum treated sample. In this study bitterness score of samples was between 1.7-2.3 which indicate that olives had high bitterness value. This also effected taste value and taste value determined between 2.1-2.4. As a result, the scores of taste and bitterness were lower than acceptable levels. Also determined odor and taste scores were lower than reported by López-López et al. [31]. Tuna S. & Akpinar-Bayizit A. [32] reported that bitterness and skin separation of olives negatively influenced the sensory evaluation panel, overall acceptability was a based on the correlations of the different sensory attributes of table olives and panelists preferred olives with an acidic/bitter taste and firm appearance. In this study skin separation of olives did not detected in any sample and there was no fermentation or acidification step so that sensory evaluation result was different from result of research on fermented or acidified olives.

## Conclusion

In this study an experimental investigation evaluated the possibility of decreasing bitterness of olives by with a lesser decrease in the nutritional value of f table olives by keeping olives in water which contained plant growth regulators. Recent concepts suggest that ripening of some fruit during postharvest storages are not only deteriorative processes which was need to slow down but also useful processes in which some growth regulators play important roles such as ripening acceleration of palm, banana and olive in some extend [22,33]. The health effects of the chemicals used should be considered before applying them to foods. In this study, debittering of olives was planned to acceleration of indigenous enzymes of olives by added plant growth

regulators. At the end of 6 days there was detected unfavorable odor for Signum treated sample. Desired reduction on oleuropein absorbance value and sensory bitterness scores of samples were not reached enough at the end of 6 days. According to these result debittering of olives by exogenous growth regulator not feasible but future studies may be focused of different plant growth regulators at different doses to achieve desired reduction on bitterness of olives for table olive industry.

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