



## Effect of Solid-State Fermentation on Main Nutritional Components, Some Minerals, Condensed Tannin and Phenolic Compounds of Olive Leaves

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 20/09/2018 Accepted : 16/11/2018</p> <p><b>Keywords:</b> <i>Aspergillus niger</i> Solid-state fermentation <i>Olea europea</i> Olive leaf Herb</p>	<p>This study was carried out to investigate the effects of solid-state fermentation (SSF) on main nutritional components, some minerals, condensed tannin and phenolic compounds of olive leaves. Two groups were formed as a fermented (FOL, <i>Aspergillus niger</i> ATCC 52172) and non-fermented olive leaves (OL). Suitable environmental conditions (humidity, temperature and pH) before SSF were established and fermentation lasted on day 8. After fermentation, while the crude fiber, neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of FOL compared to OL were decreased, its the crude protein, ether extract, ash and condensed tannin contents were increased. Some macro minerals (Ca, N, K, P, Mg) and micro minerals components (Fe, Mn, Zn, Cu, B) of FOL and OL were varied. Although oleuropein content of FOL was decreased, it's catechin and hydroxytyrosol contents were increased by SSF. These results showed that <i>A. niger</i> ATCC 52172 strain could be suitable inoculant to improve the nutritional content of olive leaves.</p>

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## Katı Kültür Fermantasyonunun Zeytin Yaprağı Temel Besin Madde, Bazı Mineral Madde, Kondanse Tanen ve Fenolik Bileşenlerine Etkisi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 20/09/2018 Kabul : 16/11/2018</p> <p><b>Anahtar Kelimeler:</b> <i>Aspergillus niger</i> Katı kültür fermentasyonu <i>Olea europea</i> Zeytin yaprağı Bitki</p>	<p>Bu çalışmada <i>Aspergillus niger</i> (ATCC 52172) ile katı kültür fermentasyonu (SSF)'nin zeytin yaprağının temel besin madde, bazı mineral madde, kondanse tanen ve fenolik bileşenler üzerine etkileri araştırılmıştır. Fermente edilen (FOL, <i>A. niger</i> ATCC 52172) ve fermente edilmeyen (OL) zeytin yaprağı olmak üzere iki grup oluşturulmuştur. SSF için uygun çevresel koşullar (sıcaklık, nem, pH) oluşturulmuş olup fermentasyon 8. gün sonunda sonlandırılmıştır. Fermentasyon sonrası OL ile karşılaştırıldığında FOL'ün ham selüloz, nötr deterjan lif (NDF) ve asit deterjan lif (ADF) içeriğinin azaldığı, ham protein, ham yağ, ham kül ve kondanse tanen düzeylerinin ise arttığı tespit edilmiştir. Bazı makro mineral (Ca, N, K, P, Mg) ve mikro mineral (Fe, Mn, Zn, Cu, B) düzeyleri OL ve FOL arasında değişiklik göstermiştir. Fermentasyon sonrası FOL'ün kateşin ve hidroksitirosol içerikleri artarken oleuropein miktarı azalmıştır. Elde edilen veriler <i>A. niger</i> ATCC 52172'nin zeytin yapraklarının besinsel kompozisyonunu iyileştirmede uygun bir inokulant olduğunu göstermiştir.</p>



## Introduction

Olive leaf is an agricultural by-product that emerges during pruning (10% by weight) and/or harvesting processes, approximately 25 kg olive leaf from each tree (Molina-Alcaide and Yáñez-Ruiz, 2008). It is thought that olive leaves can be harmful to the environment due to containing lignocellulosic components (Varmaghany et al., 2013).

Olive leaves (OL) have been used in animal feeding traditionally in Mediterranean countries (Yanez Ruiz et al., 2004). Previous studies showed that OL could improve fatty acid profile of sheep and goat milk (Abbeddou et al., 2011a; Tsiplakou and Zervas, 2008), enhance egg yolk color (Cayan and Erener, 2015), improve immunity (Varmaghany et al., 2013) and reduce abdominal fat (Shafey et al., 2013) in broiler. However, it was reported that OL could not affect egg production in quails (Christaki et al., 2011) and milk yield of sheep (Abbeddou et al., 2011b). It also was affected negatively ruminal fermentation in goat and ram (Yanez Ruiz et al., 2004), reduced feed intake and body weight in pigs (Paiva-Martins et al., 2009). It has been suggested that these negative results are due to the low nutritional composition of olive leaf and its antinutritional components (Martín-García et al., 2003). Some methods such as chemical process, drying, fermentation etc. have been used to improve nutritional components of feedstuffs for years (Aro, 2008). In recent years, fermentation has increased popularity in animal nutrition due to the positive changes that have been introduced in the feedstuffs or agricultural by-products. However, there is a little study which was reported that the effects of fermented olive leaves with *A. niger* on main nutritional composition and tannic acid content (Xie et al., 2016). Moreover, there is no information about the effect of *A. niger* on phenolic compounds, mineral and condensed tannin content. For this reason, this study was conducted to investigate the effects of *A. niger* on main nutritional composition, phenolic compounds, mineral and condensed tannin of OL.

## Material and Method

### *Microorganisms and Substrate*

*A. niger* ATCC 52172 was obtained from the American Type Culture Collection (ATCC). OL (*Olea europaea* L. cv. Gemlik) were collected from an olive garden in Aydin province of Turkey (37°45'32" K, 27°45'11" D). Leaves were dried in the shade at approximately 30 °C on 15-20% humidity for three days and were stored at room temperature on a bench until fermentation.

### *Solid-state Fermentation*

Olive leaves were milled to a size of 2 mm before being sterilized by autoclaving at 121°C for 15 min. The nutritional salt (glucose: urea: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>: peptone: KH<sub>2</sub>PO<sub>4</sub>: MgSO<sub>4</sub>.7H<sub>2</sub>O=4:2:6:1:4:1) were mixed with the substrate to encourage microorganism to grow after sterilizing phase. Each *A. niger* strain cultured in Potato-Dextrose-Agar (PDA) was added to olive leaf substrate at 10<sup>6</sup> spore count, and uninoculated OL were assigned as

control. Samples were incubated at 60°C for 48 hours. Afterward, samples were dried at room temperature for six days in which samples reached approximately 90% dry matter. Three replicate were prepared for each treatment.

### *Main Nutritional Components Analysis*

Ash (method, 942.05, AOAC, 1990), crude protein (CP, method, 976.06, AOAC, 1990), ether extract (EE, method, 920.29, AOAC, 1990), crude fiber (CF, method, 973.18, AOAC, 1990) analyses of OL before and after SSF were conducted. Nitrogen-free extract (NFE) is determined by subtracting water, ash, CP, CF, and EE found in the feed from 100. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest (1991) using the ANKOM<sup>200/220</sup> fiber analyzer (ANKOM corporation® Technology Fairport, NY). Measurement of each sample was conducted three times, and the average was taken.

### *Condensed Tannin Analysis*

Condensed tannin was determined by Butanol-HCl method (Makkar et al., 1995). Samples (0.01 g) were mixed with 6 ml solution (n-butanol, HCl and FeSO<sub>4</sub>, 95:5:0.05) and boiled in a condenser unit for an hour. Samples were quickly cooled in iced water after boiling and analysed with a spectrometer (Genesys 10S UV-Vis, USA) at 550 nm wavelength.

### *Mineral Analysis*

Macro (Ca, N, K, P, Mg) and micro mineral (Fe, Mn, Zn, Cu, B) analysis were performed according to the method described by AOAC (1990). Briefly, samples were dried at 65°C for 48 hours. Five g samples were taken from dried samples and mixed with 6 ml 65% nitric HClO<sub>4</sub> and kept at room temperature for 24 hours. Samples were heated in the hot plate on 100-150 °C until they turn to yellow. Samples were cooled and diluted to 50 ml with distilled water. Diluted samples were analysed by inductively coupled plasma spectrometry on PerkinElmer Elan DRCe ICP-MS (PerkinElmer SCIEX, Shelton, CT).

### *Determination of Phenolic Compounds*

Olive leaves analysed for phenolic compounds according to Benavente-Garcia et al. (2000) before and after fermentation. Olive leaf extract was dissolved in dimethylsulfoxide in the ratio of 5 mg/ml and filtered through a 0.45 µm nylon membrane. HPLC used in the analyse was Hewlett Packard Series HP 1050 with LiChrospher RP18-5 analytical column (250x4 mm i.d., HICHRON, UK) and Waters 486 absorbance detector (Waters Corporation, USA).

### *Statistical Analysis*

Since mineral analysis was performed on a single sample per time point, no statistical analysis was possible. The other experiments were carried out triplicate, and results were expressed as means with pooled standard error of means (SEM). Differences between treatments were tested using ANOVA and Duncan's multiple range test (SPSS 21.0 Statistics).

**Results**

Nutritional composition of OL was varied with SSF (Table 1). After fermentation, although CF, NDF, ADF of FOL were decreased ( $P<0.05$ ) its CP, EE, ash ( $P<0.05$ ) and condensed tannin content ( $P<0.001$ ) were increased as compared to OL (Figure 1).

Olive leaves some macro minerals (Ca, N, K, F and Mg) and micro minerals (Fe, Mn, Zn, Cu and Br) were increased after fermentation (Table 2). Phenolic compounds of olive leaf were varied between OL and FOL. While oleuropein was decreased, catechin and hydroxytyrosol were increased (Table 3).

Mineral analysis was performed on a single sample each time. Therefore, no statistical analysis was possible.

Phenolic compound analysis was performed on a single sample each time. Therefore, no statistical analysis was possible. ND: not detected.

**Discussion**

This study shows that nutritional composition of FOL can be enhanced by SSF. These results have been similar to previous studies which were reported that CP, EE, and ash content of cassava starch residues (Aro, 2008), cassava root and peels (Aderemi and Nworgu, 2007), cassava pulp (Iyayi and Losel, 2001), cassava bagasse (Vandenberghe et al., 2000), *Ginkgo biloba* leaves (Cao et al., 2012; Zhang et al., 2013), olive leaf (Xie et al., 2016) and sour cherry kernel (Güngör et al., 2017) could be increased by SSF.

Crude protein level of FOL was increased by SSF in this study. Increase in CP content of OL could be explained with protease produced by *A. niger* as reported previous studies (Raimbault, 1998; Vandenberghe et al., 2000; Iyayi and Losel, 2001; Aderemi and Nworgu, 2007; Aro, 2008; Dei et al., 2008a; Okpako et al., 2008; Cao et al., 2012; Zhang et al., 2013; Wu et al., 2015; Xie et al., 2016; Güngör et al., 2017). Cellulose is a carbohydrate found in the cell walls of plants and is generally at a high level in agricultural wastes (Graminha et al., 2008). High content of cellulose lowers feed's digestibility (JøRrgensen et al., 1996). In this study, CF, NDF and ADF contents of FOL were decreased by fermentation. Xie et al. (2016) reported that *A. niger* can produce cellulase in olive leaf. It is thought that decrease in CF in this study may be due to this cellulase production. This idea were supported by previous studies (on cassava starch residues, cassava pulp and peels, and shea nut (Aderemi and Nworgu, 2007; Aro, 2008; Dei et al., 2008a). On the other hands, Okpako et al. (2008) suggested that *A. niger* increased CF in SSF as reported by Güngör et al., (2017). Discrepancy among the studies can be explained with used different inoculant (*A. niger*, *A. flavus*, *A. nidulans* etc.), fermentation conditions (temperature, pH, humidity etc.) or substrate (cassava, sour cherry kernel, olive leaf etc). In the study, ash content of FOL was increased after fermentation. This can be attributed to increase in mineral content. As a matter of fact, mineral content of the olive leaf was increased after fermentation (Table 2). Solid-state fermentation can not be affected NFE content of the OL. This result is consistent with previous studies which were reported that SSF cannot varied NFE content (Vandenberghe et al., 2000; Aro, 2008; Okpako et al., 2008; Güngör et al., 2017).

Table 1 Effect of *A. niger* on main nutritional components and condensed tannin content of olive leaves in solid-state fermentation

Composition (%)	OL	FOL	SEM	P
CP	12.20	14.14	0.439	***
EE	2.34	3.15	0.185	***
Ash	6.27	6.74	0.111	*
NFE	53.58	53.13	0.182	NS
CF	25.61	22.84	0.631	***
NDF	39.48	45.47	1.348	***
ADF	26.96	33.39	1.441	***

NS: Not Significant ( $P>0.05$ ), \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$

Table 2 Effects of *A. niger* on macro and micro mineral contents of olive leaves in solid-state fermentation

Minerals	OL	FOL
Macro (%)		
Calcium	12.12	14.41
Nitrogen	1.95	2.26
Potassium	1.06	1.26
Phosphorus	0.36	0.44
Magnesium	0.22	0.25
Micro (ppm)		
Iron	65.28	99.00
Manganese	37.44	42.20
Zinc	17.18	21.50
Copper	2.40	3.40
Boron	1.98	2.01
Total (%)	15.72	18.64

Table 3 Effects of *A. niger* on phenolic compounds of olive leaves in solid-state fermentation

Phenolic compounds (1 g CE/1 g leaf)	OL	FOL
Oleuropein	23.24	2.49
Rutin	ND	ND
Catechin	0.51	2.50
Epicatechin	ND	ND
Hydroxytyrosol	0.42	2.48
Kaempferol	ND	ND
Quercetin	ND	ND
Gallic acid	ND	ND
Tannic acid	ND	ND
Caffeic acid	ND	ND
Vanillic acid	ND	ND
Vanillin	ND	ND

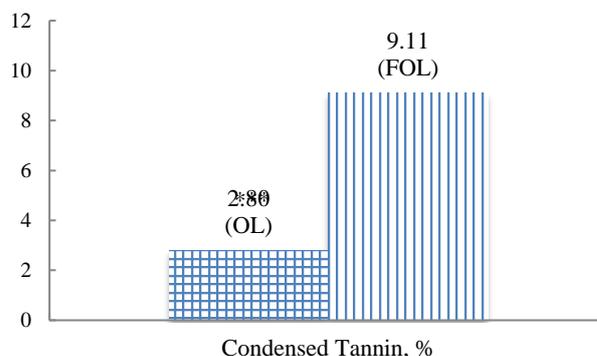


Figure 1 Effects of *Aspergillus niger* on condensed tannin content of olive leaves in solid-state fermentation (\*\*\*:  $P<0.001$ ; OL: unfermented olive leaf; FOL: fermented olive leaf)

Fungi have ability to produce microbial lipids in the substrate during fermentation (Hui et al., 2010). As a matter of fact, olive leaf's EE content was increased by fermentation in this study. Similar results obtained from the studies on cassava starch residues (Aro, 2008) and shea nut (Dei et al., 2008a). However, in some studies, ether extract content either remained the same (Aro, 2008; Güngör et al., 2017; Okpako et al., 2008) or decreased (Güngör et al., 2017) by fermentation.

Tannins are water-soluble polyphenols found in plants (Scalbert, 1991). It is divided into hydrolyzable and non-hydrolyzed (condensed) tannin (Akiyama et al., 2001). Olive leaf's condensed tannin was increased in this study. Xie et al. (2016) reported that olive leaf's tannic acid which is a type of hydrolyzable tannin was decreased by fermentation. Dei et al. (2008a) and Dei et al. (2008b) also reported a decrease in hydrolyzable tannin of shea nut. Condensed tannin in olive leaf was reported to be free (3.53%), bound to proteins (1.25%) and largely bound to cellulose (6.35%; Yanez Ruiz et al., 2004). Increase in condensed tannin content may be attributed to the fact that the cellulose and protein were broken down by fermentation and as a result, the bound tannins were free. Akiyama et al. (2001) reported that condensed tannins were more difficult to break down than hydrolyzable tannins. This may be attribute that condensed tannin in OL could not broke down by *A. niger*.

Major phenolic compounds in OL are oleuropein, hydroxytyrosol, luteolin, apigenin and verbascoside (Benavente-Garcia et al., 2000). In this study, phenolic compounds were varied between OL and FOL. While catechin and hydroxytyrosol of FOL were increased, oleuropein of OL was decreased after fermentation. This can be explained fermentation process because it can alter the amount and proportion of secondary metabolites derived from medicinal plants, resulting from smaller particles created by microbial digestion, and enhance the original treatment efficacy of active ingredients (Dei et al., 2008a) by the action of enzymes produced by bacteria, yeast and molds.

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## Conclusion

In conclusion, SSF increased CP, EE, ash, minerals, and condensed tannin, and decreased CF, NDF, ADF contents of OL. Phenolic components were varied by increasing of catechin and hydroxytyrosol and decreasing of oleuropein. These results showed that *A. niger* ATCC 52172 strain could be suitable inoculant to improve the nutritional content of OL in SSF.

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