Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 76, Αρ. 3 (2025)

The Effects of Solid State Fermentation on Nutrient Composition of Olive Cake with Aspergillus Niger and Bacillus Subtilis

AH Ataei, F Kirkpinar

doi: 10.12681/jhvms.40350

Βιβλιογραφική αναφορά:

Ataei, A., & Kirkpinar, F. (2025). The Effects of Solid State Fermentation on Nutrient Composition of Olive Cake with Aspergillus Niger and Bacillus Subtilis. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, *76*(3), 9797–9806. https://doi.org/10.12681/jhvms.40350

J HELLENIC VET MED SOC 2025, 76 (3): 9797-9806
ΠΕΚΕ 2025, 76 (3): 9797-9806

Research article
Ερευνητικό άρθρο

The Effects of Solid State Fermentation on Nutrient Composition of Olive Cake with *Aspergillus Niger* and *Bacillus Subtilis*

A.H. Ataei,[1] F. Kırkpınar[2*]

[1]*Department of Animal Production, Faculty of Veterinary Sciences, Kabul University, Kabul 1004, Afghanistan*

[2]*Ege University, Faculty of Agriculture, Department of Animal Science, İzmir, Türkiye*

ABSTRACT: Olive cake is a cost-effective feed ingredient with potential in animal nutrition; however, its application is limited due to low protein content, high fiber levels, and anti-nutritional factors. This study aimed to enhance the nutritional value of olive cake through solid-state fermentation using *Aspergillus niger* and *Bacillus subtilis*. *Aspergillus niger* and *Bacillus subtilis* were applied to the olive cake, and samples were incubated at 30°C for 24, 48, and 72 hours. Samples were divided into control and treatment groups, each containing 200 g of olive cake mixed with 100 mL of distilled water. The results showed that solid-state fermentation generally increased crude protein content and decreased crude fiber content ($P<0.05$). A significant increase in crude protein content was observed only with *Bacillus subtilis*, while a significant decrease in crude fiber content was noted only with *Aspergillus niger* ($P<0.05$). Neutral detergent fiber content remained unchanged across all groups ($P>0.05$). Although acid detergent fiber and acid detergent lignin contents generally decreased in all fermented groups, this reduction was statistically significant only in the groups treated with *Aspergillus niger* ($P<0.05$). Moreover, a significant decrease in nitrogen-free extract, metabolizable energy, sugar, total phenolic content, total flavonoid content, and antioxidant activity was observed in all fermented olive cake groups ($P<0.05$). The findings suggest that olive cake, an inexpensive and underutilized by-product, can be effectively transformed into a valuable feed ingredient and/or feed additive for animal nutrition through solid-state fermentation.

Keyword: By-products; nutritional characteristics; solid state fermentation; poultry.

Correspondence author:
F. Kırkpınar,
Department of Animal Science, Faculty of Agriculture, Ege University-İzmir- Türkiye
E-mail address: figen.kirkpinar@ege.edu.tr

Date of initial submission: **3-2-2025**
Date of acceptance: **2-3-2025**

INTRODUCTION

Due to the increasing global population, the demand for animal products such as meat, eggs, and milk is increasing to meet rising food needs. This urge in demand for animal products consequently elevates the need for animal feed, potentially causing competition between resources allocated for animal feed and those intended for human consumption. Traditional protein sources for poultry are anticipated to become increasingly scarce and costly in the near future (Iji et al., 2017). Given the rising prices of traditional feed ingredients and the shortage of cereals, lignocellulosic materials present significant potential as alternative animal feed sources (Graminha et al., 2008). By-products and residues from various industries contain valuable compounds, including carbohydrates, proteins, lipids, and bioactive molecules (Ravindran and Jaiswal, 2016). However, it is estimated that approximately 50% of food is wasted along the 'farm-to-table' chain (Vuong, 2017). Therefore, to maximize the utilization of existing feed resources, the adoption of advanced technological processes is essential (Makkar et al., 1995).

Olive cake (OC) is an industrial by-product that contains a significant amount of solid residues. Only small quantities are utilized as fuel, natural fertilizer, or cattle feed (Pagnanelli et al., 2003). Recently it has been widely studied as a potential feed ingredient for livestock, though its high fiber content, low protein, and anti-nutritional factors limit its direct use. Additionally, OC has a high fiber content comprising cellulose, hemicellulose, lignin, and pectins and low protein content, with 80-90% of proteins bound to the lignocellulosic fraction (Fathy et al., 2018). Notably, the phenolic content of OC is 20 times higher than that of olive oil (Fernández Gutiérrez et al., 2013). Phenolic compounds can either stimulate or inhibit digestive enzyme activity, thereby affecting nutrient digestibility in poultry and also inhibiting the growth of beneficial microorganisms in ruminants (Brenes and Roura, 2010; Leskovec et al., 2018; Medouni-Haroune et al., 2018).

Various treatments have been explored to enhance its nutritional value and digestibility. Chemical treatments, such as sodium hydroxide and ammonia, improve fiber breakdown and increase crude protein content (Al Jassim et al., 1997; Yañez-Ruiz et al., 2004). Ensiling, particularly with alkalis or poultry manure, enhances preservation and nitrogen content (Molina-Alcaide & Yañez-Ruiz, 2008). Mechanical treatments like removing stones from exhausted olive cake increases (Tufarelli et al., 2013). Dietary inclusion studies show that up to 25% olive cake in goat and pig diets does not negatively affect growth or performance, while improving meat fatty acid profiles (Chiofalo et al., 2004; Nieto et al., 2002).

Biological treatments, including microbial fermentation, increase protein content and reduce fiber, enhancing palatability (Filya et al., 2000). Combined treatments, such as alkali treatment with fermentation, further enhance digestibility and nutrient content (Chammem et al., 2005).

In recent years, solid-state fermentation (SSF) has gained increased attention in biotechnology due to its low cost, high efficiency, reduced energy consumption, minimal moisture requirements, and low residual water production (Singhania et al., 2009; Mussatto et al., 2012).

SSF is a process used to cultivate beneficial microorganisms on solid substrates or to extract various active compounds from them. Unlike liquid-state fermentation, which occurs in a liquid medium, SSF takes place in a solid medium with only a small amount of moisture provided by the substrate, supplemented with additional moisture if necessary (Pandey, 2003).

This study aimed to improve the changes in the nutrient composition of OC through SSF using two microorganisms, *Aspergillus niger* ATCC 16404 and *Bacillus subtilis* ATCC 6633, under in vitro conditions.

MATERIALS AND METHODS

Substrate and microorganisms

Olive cake was obtained from Morova, a local olive oil mill located in Aydın, Turkey. Microorganisms used in this study were obtained from the American Type Culture Collection (ATCC), including strains of *Aspergillus niger* (ATCC 16404) and *Bacillus subtilis* (ATCC 6633). The fermentation processes were carried out under laboratory conditions at Ege University Ödemiş Vocational Training School, while the chemical analyses of the samples were conducted at the laboratories of the Department of Animal Science, Faculty of Agriculture, Ege University.

Screening medium and solid-state fermentation preparation

The microorganisms were cultured on Potato Dextrose Agar (PDA) at 24°C for 14 days. Following

incubation, the spores were harvested by tapping the top of the inverted plates. For substrate preparation, 100 g of olive cake was adjusted to an initial moisture level of 50% and inoculated with *Aspergillus niger* (1.8×10^7 CFU/mL) and *Bacillus subtilis* (1.2×10^6 CFU/mL). The substrates were placed in polyethylene bags and subjected to solid-state fermentation (SSF) at 30°C for 24, 48, and 72 hours (Fathy et al., 2018; Güngör et al., 2017). The control group (C1) received only distilled water for moistening without the addition of microorganisms.

Chemical analysis of unfermented and fermented olive cake

The crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE) contents of OC before and after SSF were determined following the procedures outlined by AOAC (1997). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) analyses were performed using the filter bag system as modified by Goering and Van Soest (1970). The total sugar content was determined using the Luff-Schoorl method, while starch content was analyzed according to AOAC (1997). The metabolizable energy (ME) for poultry was calculated using the formula provided by TSE (2004):

ME Mcal/kg = (0.03431× EE g/kg + 0.01551× CP g/kg + 0.01669 × starch g/kg + 0.01301× sugar g/kg) / 4.184

Determination of total phenolic content, total flavonoid content, and antioxidant activity

To prepare the extracts for analysis, 0.5 g of each sample was mixed with 20 mL of a 75% ethanol solution and incubated in a shaking water bath at 60 °C for 30 minutes. Following incubation, the mixture was centrifuged at 3000 rpm for 10 minutes. The resulting supernatants were filtered and subsequently used for the determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA).

Total phenolic content (TPC) was determined using the Folin–Ciocalteu (FC) method as described by Singleton and Rossi (1965). Briefly, 300 µL of the sample extract was combined with 1.5 mL of a 10-fold diluted Folin–Ciocalteu reagent and 1.2 mL of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution. The mixture was incubated in the dark at room temperature for 90 minutes. Absorbance was measured at 725 nm, and TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter (mg GAE g^{-1}), based on a calibration curve prepared with gallic acid.

Total flavonoid content (TFC) was determined according to the protocol described by Zhishen et al. (1999). The extracted samples were diluted with 2.0 mL of distilled water in test tubes, followed by the addition of 0.15 mL of a 5% sodium nitrite ($NaNO_2$) solution. After 6 minutes, 0.15 mL of 10% aluminum chloride ($AlCl_3$) solution was added. The mixtures were allowed to stand for an additional 6 minutes before 1.0 mL of 1 M sodium hydroxide (NaOH) solution was introduced. The reaction mixtures were then incubated at room temperature for 15 minutes. Absorbance was measured at 415 nm using a UV-VIS spectrophotometer, with a blank prepared by replacing the extract with distilled water. TFC was calculated using a standard calibration curve based on quercetin and expressed as milligrams of quercetin equivalents (mg QUE g^{-1}) per gram of dry matter.

Antioxidant activity (AA) was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method described by Garcia et al. (2012). Briefly, 300 µL of a 100 µM DPPH radical solution prepared in 70% ethanol was mixed with 0.5 mL of sample extracts or standards. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 517 nm using a UV-VIS spectrophotometer. A control solution containing only the DPPH radical with added distilled water was used for comparison. The percentage of DPPH radical scavenging activity was calculated using the following equation:

Radical scavenging effect (%)= [(Control absorbance - Absorbance values of samples)/ Control absorbance × 100]

Statistical analysis

Data were analysed using the General Linear Model (GLM) procedure in the SAS software package (SAS, 2002). Significant differences between the means were determined using Duncan's multiple range test, with an alpha level of 0.05.

RESULTS

The chemical composition of OC fermented with *Aspergillus niger* and *Bacillus subtilis* strains and unfermented (control) was presented in Table 1.

The results showed that EE, total sugar, and ME values of fermented OC decreased significantly *($P<0.05$)* in all fermented groups treated with *As-*

Table 1. Chemical composition variation during olive cake fermentation using *Aspergillus niger* and *Bacillus subtilis* before (control) and after 24, 48 and 72 h (%, based on DM)

Fermentation group	Chemical composition (%)										
	DM	Ash	EE	CP	CF	NDF	ADF	ADL	Sugar	NFE	ME
A. niger											
Control	93.68^{b}	4.12	10.64^{a}	7.7	46.64^{a}	74.35	61.45^{a}	31.81^{a}	0.38^{a}	31.20^{b}	1169^{a}
A-24	95.39^{a}	4.04	10.18^{b}	7.82	44.54^{b}	73.93	60.29^{ab}	30.55^{ab}	0.11^{b}	33.80^{a}	1129^{b}
A-48	95.17^{a}	4.07	10.05^{b}	7.78	43.83^{b}	73.03	59.67^{b}	30.12^{b}	0.11^{b}	33.89^{a}	1124^{b}
A-72	95.29^{a}	4.01	10.27^{b}	7.9	43.34^{b}	71.38	59.68^{b}	30.12^{b}	0.11^{b}	34.62^{a}	1130^{b}
SEM	0.175	0.063	0.084	0.098	0.674	1.005	0.414	0.500	0.039	0.777	5.810
P-value	<.0001	0.752	0.000	0.558	0.016	0.276	0.037	0.036	0.002	0.043	0.001
B. subtilis											
Control	93.68^{b}	4.12	10.64^{a}	7.70^{b}	46.64	74.36	61.45	31.81	0.38^{a}	31.2	1169^{a}
B-24	95.48^{a}	4.1	10.14^{b}	8.06^{a}	44.74	74.97	60.48	31.17	0.18^{b}	32.95	1137^{b}
B-48	95.47^{a}	4.02	10.04^{b}	8.12^{a}	44.48	75.35	60.93	31.11	0.13^{b}	33.34	1130^{b}
B-72	95.36^{a}	4.08	10.03^{b}	8.16^{a}	44.3	74.95	60.49	30.81	0.18^{b}	33.48	1127^{b}
SEM	0.178	0.070	0.084	0.098	0.824	1.005	0.463	0.500	0.047	0.757	5.810
P-value	<.0001	0.677	0.000	0.040	0.129	0.070	0.360	0.459	0.029	0.180	0.000

$^{a,b:}$ Means in the same column with different superscripts are significantly different ($P<0.05$); SEM: Standard error of means; A1-24, A1-48 and A1-72: Fermentation with *A. niger* for 24, 48 and 72 hours respectively; B1-24, B1-48 and B1-72: Fermentation with *B. subtilis* for 24, 48 and 72 hours respectively; DM: Dry matter; EE: Ether extract; CP: Crude protein; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; NFE: Nitrogen-free extract; ME: Metabolizable energy; *A. niger: Aspergillus niger*; *B. subtilis: Bacillus subtilis.*

pergillus niger and *Bacillus subtilis*. In contrast, DM and NOM values significantly increased *(P<0.05)* in all fermented groups. Ash content, however, did not show any statistically significant difference (*P>0.05*). Although crude protein (CP) content did not exhibit a statistically significant difference in the groups fermented with *Aspergillus niger*, it increased numerically from 7.70% in the control group to 7.90% in the A-72 group (*P>0.05*). In contrast, a significant increase in CP content was observed in all groups fermented with *Bacillus subtilis* (B-24, B-48, and B-72) compared to the unfermented control group (*P<0.05*). The starch content of OC was below the measurement limit and could not be determined, both before and after fermentation with *Aspergillus niger* and *Bacillus subtilis*.

The changes in CF, NDF, ADF, and ADL of unfermented and fermented OC with *Aspergillus niger* and *Bacillus subtilis* during different fermentation periods (24, 48, and 72 hours) was shown in Table 1. With increasing fermentation time using *Aspergillus niger*, the CF content of samples decreased significantly by 4.50%, 6.03%, and 7.07% at 24, 48, and 72 hours, respectively (*P<0.05*). In contrast, no significant changes were observed in the CF content of OC fermented with *Bacillus subtilis* (*P>0.05*). Similarly, no significant differences (*P>0.05*) were observed in the NDF content of OC fermented with either *Aspergillus niger* or *Bacillus subtilis* (Table 1). While the ADF and ADL contents did not show significant differences in the groups fermented with *Bacillus subtilis* at any time point (24, 48, and 72 hours), a numerical decrease was observed across all fermented groups.

The changes in TPC, TFC, and AA of unfermented and fermented OC with *Aspergillus niger* and *Bacillus subtilis* during different fermentation periods (24, 48, and 72 hours) was exhibited in Table 2.

There was a significant decrease in TPC between the control and the treatment groups (*P<0.05*). **Total phenolic content** of unfermented samples was 3.70 mg GAE/g. In samples fermented with *Aspergillus niger*, TPC decreased to 2.29 mg GAE/g, 2.24 mg GAE/g, and 2.20 mg GAE/g at 24, 48, and 72 hours, respectively. Similarly, fermentation with *Bacillus subtilis* resulted in a reduction in TPC to 2.30 mg GAE/g, 2.25 mg GAE/g, and 2.22 mg GAE/g at 24, 48, and 72 hours, respectively.

Table 2. Total flavonoid content and antioxidant activity variation during olive cake fermentation using *Aspergillus niger* and *Bacillus subtilis* before (control) and after 24, 48 and 72 h (%, based on DM).

Parameter	*Aspergilus niger*								
	Control	A1-24	Change (%)	A1-48	Change (%)	A1-72	Change (%)	SEM	P-value
TPC (mg GAE/g)	3.70[a]	2.29[b]	-38.22	2.33[b]	-37.07	2.20[b]	-40.45	0.017	0.004
TFC (mg QE/g)	2.03[a]	1.19[b]	-41.29	1.18[b]	-41.84	1.18[b]	-41.97	0.051	<.0001
AA (%)	75.54[a]	56.01[b]	-25.85	57.71[b]	-23.60	59.97[b]	-20.60	0.024	0.010
Parameter	***Bacillus subtilis***								
	Control	B1-24	Change (%)	B1-48	Change (%)	B1-72	Change (%)	SEM	P-value
TPC (mg GAE/)	3.70[a]	2.30[b]	-37.99	2.28[b]	-38.25	2.22[b]	-40.11	0.169	0.002
TFC (mg QE/g)	2.03[a]	1.17[b]	-42.38	1.15[b]	-43.38	1.09[b]	-46.35	0.051	<.0001
AA (%)	75.54[a]	59.99[b]	-20.59	58.46[b]	-22.61	57.71[b]	-23.60	0.024	0.005

[a,b:] Means in the same column with different superscripts are significantly different ($P<0.05$); SEM: Standard error of means; A1-24, A1-48 and A1-72: Fermentation with *A. niger* for 24, 48 and 72 hours respectively; B1-24, B1-48 and B1-72: Fermentation with *B. subtilis* for 24, 48 and 72 hours respectively; TPC: Total phenolic content; TFC: Total flavonoid content; AA: Antioxidant Activity; *A. niger: Aspergillus niger*; *B. subtilis: Bacillus subtilis.*

The changes in TFC of OC fermented with *Aspergillus niger* and *Bacillus subtilis* was displayed in Table 2. Regardless of the microorganism used, TFC significantly decreased in all fermented groups compared to the control ($P<0.05$). The reduction in TFC ranged from 41.29% to 46.35%.

The AA of unfermented and fermented OC with *Aspergillus niger* and *Bacillus subtilis*, as measured using the DPPH scavenging assay, was demonstrated in Table 2. The AA of unfermented OC was 73.78%. In samples fermented with *Aspergillus niger*, AA significantly decreased to 56.20%, 57.34%, and 55.44% at 24, 48, and 72 hours, respectively ($P<0.05$). Similarly, in samples fermented with *Bacillus subtilis*, AA decreased significantly to 56.96%, 54.68%, and 54.68% at 24, 48, and 72 hours, respectively ($P<0.05$).

DISCUSSION

In the present study, dry matter (DM) content significantly increased in all treatment groups after fermentation. The optimum moisture level SSF has been reported as 50% (Chutmanop et al., 2008), which was used to initiate this experiment. Typically, fungi require moisture levels between 40% and 60% for growth under SSF conditions (Singhania et al., 2009), while bacteria often require moisture levels exceeding 70% (Pandey et al., 2001). Lower moisture content facilitates direct fermentation of the substrate without the need for sterilization. Similar findings were reported by Moftah et al. (2012) for OC using SSF. In contrast, Nazem et al. (2008) observed a reduction in DM content during SSF of citrus pulp. According to Shojaosadati (1999), this decrease in DM in fermented substrates is attributed to fungal respiration, nutrient consumption, and gas production. Although there was a slight numerical change in the ash content of OC fermented with *Aspergillus niger* and *Bacillus subtilis*, it was not statistically significant ($P>0.05$). This result is consistent with previous studies on cassava peels (Aro, 2008), rapeseed pulp (Shi et al., 2016), and palm kernel (Lawal et al., 2010), which also reported no significant changes in ash content using SSF. However, several studies have reported an increase in ash content in substrates after fermentation (Assi and King, 2008; Okpako et al., 2008; Altop et al., 2018). Rajesh et al. (2010) suggested that this increase might result from the depletion of organic matter during SSF. In contrast, a decrease in ash content has been reported during the SSF of olive pomace (Oliveira et al., 2017) and OC (Moftah et al., 2012).

As shown in Table 1, the EE content significantly decreased after 24, 48, and 72 hours of fermentation in all treatment groups, regardless of the microorganism used ($P<0.05$). These findings are consistent with previous studies on mango kernel (Kayode and Sani, 2008), grape seeds (Altop et al., 2018), and *Terminalia catappa* fruit meal fermented with *Aspergillus niger* (Apata, 2011). Similarly, Oliveira et al. (2018) reported a reduction in EE content in palm kernel cake fermented with *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus awamori* compared to unfermented samples. The decrease in EE content observed in this study may be attributed to the lipase enzyme activity of fungal microorganisms, which hydrolyzes fats into fatty acids and glycerol (Kumar and Kanwar, 2012). However, several studies have reported an increase in fat content during the SSF of olive pomace (Moftah et al., 2012; Fadel and El-Ghonemy, 2015; Fathy et al., 2018) and olive leaves (Altop et al., 2019).

Although fermentation with *Aspergillus niger* resulted in a slight increase in the CP content of OC, the differences were not statistically significant ($P>0.05$). In contrast, a significant increase in CP content was observed in OC fermented with *Bacillus subtilis* (Table 1). Previous studies have reported notable increases in CP content following SSF. For instance, Altop (2019) demonstrated an increase in the CP level of olive leaves fermented with *Aspergillus niger*, while Vahidi et al. (2017) observed enhanced CP content in OC fermented with *Lentinus edodes*. Similarly, Mathot et al. (1992) reported up to a 22% increase in CP content during the fermentation of barley using *Aspergillus niger*. Several studies have suggested that microorganisms secrete enzymes such as protease, amylase, cellulase, hemicellulase, hydrolase, and pectinase during fermentation, which can utilize protein, starch, sugar, or cellulose in the plant material as a carbon source, thereby enhancing the protein content (Raimbault, 1998; Mathivanan et al., 2006). Additionally, the protein content of fungal mycelium increases during the fermentation process due to microbial growth and reproduction, which further contributes to the elevated protein levels in the final product (Belewu and Musa, 2003; Oboh, 2006). Therefore, the increase in protein content of fermented OC, particularly in samples treated with *Bacillus subtilis* in the present study, can be attributed to these factors. It has also been reported that microorganisms such as *Aspergillus niger* and *Bacillus subtilis* can secrete enzymes like protease, tanninase, and amylase, which can contribute to this process (Owens et al., 1997; Okpako et al., 2008). However, studies by Alhamad et al. (2012) on OC and Tosun and Yaşar (2021) on tomato pulp have reported no significant change in CP content. In our study, the degree of CP increase varied depending on the type of microorganism used, suggesting that the specific microorganism employed in fermentation plays a critical role in determining the extent of CP enhancement. Overall, fermentation with *Bacillus subtilis* proved to be particularly effective in increasing the CP content of OC.

In the present study, a significant decrease in sugar content was observed in all treatment groups after fermentation with *Aspergillus niger* and *Bacillus subtilis* ($P<0.05$). These findings are in line with previous studies on olive leaves (Altop, 2019) and olive cake (Oliveira et al., 2017) subjected to SSF. In contrast, Neifar et al. (2013) reported an increase in the sugar content of OC by SSF. The reduction in sugar content observed in this study may be attributed to the enzymes secreted by the microbial inoculants, their capacity to hydrolyze sugar in the OC, and the subsequent utilization of the resulting glucose by microorganisms as a carbon source (Rai et al., 1988; Oboh, 2006).

The NFE content of fermented OC increased significantly in the groups treated with *Aspergillus niger*. Although a numerical increase in NFE was observed in the groups fermented with *Bacillus subtilis*, the difference was not statistically significant ($P>0.05$). These findings are consistent with studies conducted on tomato pomace (Tosun, 2017), *Terminalia catappa* fruit meal (Apata, 2011), and cottonseed meal (Altop et al., 2019). The observed increase in NFE content may be attributed to the enzymatic activity of microorganisms during fermentation or to specific effects exerted by the microbial strains. However, these results are not consistent with studies on olive leaves (Altop, 2019), cassava peels (Aro, 2008; Okpako et al., 2008), palm kernel cake (Lawal et al., 2010), and canola meal (Safari et al., 2012), where no significant changes or a decrease in NFE content were reported. This variation in findings may be due to differences in substrate composition, fermentation conditions, or the type of microorganisms used in the fermentation process.

The ME levels in all fermentation groups, whether treated with *Aspergillus niger* or *Bacillus subtilis*, significantly decreased compared to the control group ($P<0.05$). This reduction in ME may be attributed to the observed decrease in EE and sugar

content of OC ofter SSF. The results of this study are consistent with those reported by Fadel and El-Ghonemy (2015), who investigated the effects of SSF on OC. However, in contrast to our findings, studies on apple and tomato pomace (Tosun, 2017) and olive pomace (Fathy et al., 2018) have shown an increase in ME levels treated by SSF.

The present study demonstrated that *Aspergillus niger* was more effective than *Bacillus subtilis* in significantly reducing the CF content of OC ($P<0.05$). These findings suggest that both fungi and bacteria can reduce CF content during SSF, with *Aspergillus niger* exhibiting superior effectiveness. This may be attributed to the fact that cellulase enzymes are predominantly synthesized by cellulolytic fungi such as *Chaetomium spp., Fusarium myrothecium, Trichoderma penicillium, and Aspergillus spp.* (Aguilar et al., 2008). However, it has been reported that certain bacteria, such as Streptomyces spp., cannot grow on OC during SSF due to their mycelial morphology, growth cycle characteristics and cannot produce lignocellulolytic enzymes effectively (Medouni-Haroune et al., 2017). Neifar et al. (2013) reported that the CF content of OC decreased through SSF with white rot fungi (*Fomes fomentarius*). Similarly, Fadel and El-Ghonemy (2015) reported that *Aspergillus oryzae* was effective in producing lignocellulolytic enzymes and enhancing the in vitro digestibility of OC. The hydrolytic nature of *Aspergillus niger* is likely responsible for these effects. This hypothesis is supported by Chesson (1993), who explained that fungi secrete hydrolytic enzymes during fermentation, enabling the breakdown of cell wall components. Although some previous studies have reported a decrease in NDF content through SSF of various agricultural by-products, including sour cherry (*Prunus cerasus*) kernels (Güngör et al., 2017), olive leaves (Altop, 2019), and canola meal (Safari et al., 2012), the present study found no significant effect ($P>0.05$) on the NDF content of OC fermented with *Aspergillus niger* and *Bacillus subtilis* (Table 1). While Arda (2000) reported that fermentation could increase NDF content due to the presence of cellulose and chitin in the fungal cell wall. Additionally, the effectiveness of the inoculant strain in degrading hemicellulose and lignin may vary.

As shown in Table 1, the ADF and ADL contents of OC fermented with *Bacillus subtilis* showed no significant changes ($P>0.05$). However, a significant decrease in ADF and ADL contents was observed in OC fermented with *Aspergillus niger* ($P<0.05$). Similarly, Neifar et al. (2013) reported decreases of 13% and 10% in ADF and ADL contents on fermented OC through SSF respectively, while Fathy et al. (2018) observed a 16.16% decrease in ADF content. In this study, SSF effectively reduced the ADF content of OC, with fungi (*Aspergillus niger*) exhibiting a greater impact compared to bacteria. This reduction can be attributed to the secretion of enzymes, such as cellulase, during microbial fermentation. These enzymes utilize structural carbohydrates as a carbon source, leading to a decrease in ADF, NDF, and lignin contents (Krishna, 2005). However, some studies have reported increases in ADF and ADL contents in fermented by-products, including cottonseed meal (Altop et al., 2019), cassava peels (Okpako et al., 2008), and sour cherry kernels (Güngör et al., 2017). Oliveira et al. (2017) also reported an increase in ADL content in a product obtained by fermenting OC and wheat bran with *Aspergillus niger*, *Aspergillus ibericus*, and *Aspergillus tubingensis*. The presence of lignin in lignocellulosic materials forms a protective barrier that prevents plant cell walls from being degraded by fungi and bacteria. To utilize as a carbon source, cellulose and hemicellulose must first be broken down into their corresponding monomers (sugars) (Kumar and Kanwar, 2012). Fadel and El-Ghonemy (2015) reported that *Aspergillus oryzae* is an effective microorganism for producing lignocellulolytic enzymes, which significantly increased the in vitro digestibility of OC.

As shown in Table 2, a significant decrease in the TPC of fermented OC was observed across all treatment groups ($P<0.05$). Similarly, Leite (2015) reported a reduction in TPC from 8.9 mg GAE/g to 1.5 mg GAE/g after fermentation of OC with *Aspergillus niger* and *Trichoderma reesei*. Chebaibi et al. (2019) also observed a decrease in TPC after two weeks of fermentation with *Aspergillus niger*. In contrast, Madeira et al. (2012) reported a statistically significant increase in TPC in orange pomace fermented with *Paecilomyces variotii*. The decrease in total tannins, phytic acid, and phenolic compounds during fermentation may be attributed to the enzymatic activity of tannin hydrolase and hemicellulase secreted by *Aspergillus niger*, as well as phytase, xylanase, and hydrolase produced by *Bacillus subtilis* (Sun et al., 2012).

The TFC of fermented OC also demonstrated a significant decrease ($P<0.05$), with a decrease ranging from 35% to 46% in all treatment groups

(Table 2). Chebaibi et al. (2019) similarly reported a decrease in TFC from 3 mg QE/g to 1.07 mg QE/g during the fermentation of OC with *Aspergillus niger*. In this line, Zhang et al. (2012) and Cao et al. (2012) reported a decrease in the TFC of Ginkgo leaves (Ginkgo biloba L.). The decrease in TFC during fermentation with *Aspergillus niger* is due to the conversion of flavonoids into aglycones, facilitated by the production of β-glucosidase enzymes (Hsu and Chiang, 2009). However, Altop (2019) reported no significant changes in flavonoid content or AA in olive leaves using SSF.

After the fermentation of OC, a significant decrease ($P<0.05$) in AA was observed across all treatment groups (Table 2). This result is consistent with findings by Oliveira et al. (2017), who reported a significant reduction in AA in fermented OC (10 mmol Trolox kg^{-1}) compared to unfermented OC (30 mmol Trolox kg^{-1}). Similarly, Alpar (2018) reported a decrease in AA in olive leaves through SSF with *Bacillus subtilis*. In contrast, Alhamad et al. (2012) found no statistically significant changes in AA in OC after fermentation, while Tosun (2017) reported an increase in AA using both the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH methods for AA determination. The reduction in AA observed in the present study may be attributed to the concurrent decrease in TPC and TFC across all treatment groups. A positive correlation between TPC and AA has been reported previously by Alu'datt et al. (2010). It is important to note that different methods for determining AA can yield varying results. While the DPPH assay is the most commonly used method, it may not necessarily be the most accurate or definitive (Atli, 2020). Moreover, Saci et al. (2015) reported a significant decrease in AA with increasing storage temperature and duration ($P<0.05$).

Differences between the findings of this study and those of others may be due to various factors, including plant variety (Tura et al., 2007), regional differences (Vinha et al., 2005), harvesting conditions (Gomez-Alonso et al., 2002), and variations in processing methods (Ranalli et al., 2001). Zhang et al. (2021) also highlighted the influence of storage time on polyphenol content in wheat flour. Furthermore, changes in phytochemical components after fermentation may depend on the specific microbial inoculants used.

CONCLUSION

In conclusion, solid-state fermentation with both *Aspergillus niger* and *Bacillus subtilis* led to a decrease in ether extract, total sugar, total phenolic content, total flavonoid content, and antioxidant activity of fermented olive cake, while dry matter and neutral organic matter values increased. Crude protein content showed a significant increase with *Bacillus subtilis*. Additionally, changes in cell wall components were also observed, with *Aspergillus niger* significantly decreasing crude fiber content, while no significant changes were detected during fermentation with *Bacillus subtilis*.

This study highlights the potential of solid-state fermentation as an effective approach for modifying the nutritional composition of olive cake, an inexpensive and underutilized agro-industrial by-product. These modifications could enhance its value as animal feed or feed additive, particularly for use in poultry nutrition.

ACKNOWLEDGMENTS

This research was supported by the Scientific Research Projects Coordination of Ege University. Project number FDK-2020-21929. The authors would like to thank.

Funding source

This paper did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict-of-interest statement

The authors have no conflicts of interest to declare.

REFERENCES

Aguilar CN, Aguilera-Carbo A, Robledo A, Ventura J, Belmares R, Martinez D, ... and Contreras J (2008) Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. Food Technology and Biotechnology, 46, 218-222.

Al Jassim RAM, Stiles D, Hogan JP (1997) Effect of sodium hydroxide treatment on the digestibility of olive cake for ruminants. Animal Feed Science and Technology, 68(1-2), 189-195.

Alhamad M, Rababah T, Al-u'datt M, Ereifej K, Esoh R, Feng H, Yang W (2012) The physicochemical properties, total phenolic, antioxidant activities, and phenolic profile of fermanted olive cake. Arabian Journal of Chemistry, 10, 136-140.

Altop A (2019) Effect of solid-state fermentation on main nutritional components, some minerals, condensed tannin and phenolic compounds of olive leaves. Turkish Journal of Agriculture-Food Science and Technology 7, 115-119.

Altop A, Güngör E, Erener G (2019) Improvement of nutritional quality of some oilseed meals through solid-state fermentation using *Aspergillus niger*. Turkish Journal of Agriculture-Food Science and Technology, 7, 1411-1414 .

Altop A, Güngör E, Erener G (2018) *Aspergillus niger* may improve nutritional quality of grape seed and its usability in animal nutrition through solid-state fermentation. International Advanced Researches and Engineering Journal, 2, 273-277.

Alu'datt MH, Alli I, Ereifej K, Alhamad M, Al-Tawaha AR, Rababah T (2010) Optimisation, characterisation and quantification of phenolic compounds in olive cake. Food Chemistry, 123, 117-122.

Apata DF (2011) Effect of Terminalia catappa fruit meal fermanted by *Aspergillus niger* as replacement of maize on growth performance, nutrient digestibility, and serum biochemical profile of broiler chickens. Biotechnol. Res. Int., 2011:907546.

AOAC (1997) Official Methods of Analysis of the Association of Official Analytical Chemists, 16th Edition, Washington, DC, USA.

Arda M (2000) Basic Microbiology. Chapter 28, Medisan Publication, 46, Ankara, Turkiye, 268-272.

Aro SO (2008) Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. African Journal of Biotechnology, 7(25).

Assi JA, King AJ (2008) Manganese amendment and Pleurotus ostreatus treatment to convert tomato pomace for inclusion in poultry feed. Poultry Science, 87, 1889-1896.

Belewu MA, Musa AK (2003) Effect of selected azotobacter bacterial strains on the enrichment of cassava waste during sold state fermentation. Nigerian Journal of Animal Science, 6, 1-7.

Brenes A, Roura E (2010) Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology 158, 1-14.

Cao FL, Zhang XH, Yu WW, Zhao LG, Wang T (2012) Effect of feeding fermanted Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers. Poultry Science, 91, 1210-1221.

Chammem N, Kachouri M, Mejri M, Peres C, Boudabous A, Hamdi M (2005) Combined effect of alkali pretreatment and sodium chloride addition on the olive fermentation process. Bioresource Technology, 96(11), 1311-1316.

Chebaibi S, Grandchamp ML, Burgé G, Clément T, Allais F, Laziri F (2019) Improvement of protein content and decrease of anti-nutritional factors in olive cake by solid-state fermentation: A way to valorize this industrial by-product in animal feed. Journal of Bioscience and Bioengineering, 128, 384-390.

Chesson A (1993) Mechanistic models of forage cell wall degradation. Forage Cell Wall Structure and Digestibility, 347-376.

Chiofalo B, Liotta L, Chiofalo V (2004) Effects of the inclusion of olive cake in the diet of lambs on meat quality. Meat Science, 67(2), 289-294.

Chutmanop J, Chuichulcherm S, Chisti Y, Srinophakun P (2008) Protease production by *Aspergillusoryzae* in solid-state fermentation using agroindustrial substrates. Chem.Technol. 1018: 1012-1018.

Fadel M, El-Ghonemy DH (2015) Biological fungal treatment of olive cake for better utilization in ruminants nutrition in Egypt. International Journal of Recycling of Organic Waste in Agriculture 4, 261-271.

Fathy SA, Mahmoud AE, Rashad MM, Ezz MK, Mohammed AT (2018) Improving the nutritive value of olive pomace by solid state fermentation of Kluyveromyces marxianus with simultaneous production of gallic acid. International Journal of Recycling of Organic Waste in Agriculture 7, 135-141.

Fernández Gutiérrez A, Segura Carretero A, Lozano Sánchez J (2013) Procedimiento para filtrar aceite usando tortas filtrantes de almidón nativo. Patent ES-201101121, Feb. [http://hdl.handle.net/10481/24164] Accessed date: August 29, 2022.

Filya I, Ashbell G, Hen Y, Weinberg ZG (2000) The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. Animal Feed Science and Technology, 88(1-2), 39-46.

Garcia AMN, Moumen A, Ruiz DY, Alcaide EM (2003) Chemical composition and nutrients availability for goats and sheep of two-stage olive cake and olive leaves. Animal Feed Science and Technology 107, 61-74. 70.

Goering HK, Van Soest PJ (1970) Forage fiber analysis. Agricultural handbook no. 379. US Department of Agriculture, Washington, DC, 1-20.

Gomez-Alonso S, Salvador MD, Fregapane . (2002) Phenolic compounds profile of Cornicabra virgin olive oil. Journal of Agricultural and Food Chemistry, 50, 6812-6817.

Graminha EBN, Gonçalves AZL, Pirota RDPB, Balsalobre MAA, Da Silva R, Gomes E (2008). Enzyme production by solid-state fermentation: Application to animal nutrition. Animal Feed Science and Technology, 144, 1-22.

Güngör E, Altop A, Öztürk E, Erener G (2017) Nutritional changes of sour cherry (*Prunus cerasus*) kernel subjected to *Aspergillus niger* solid-state fermentation. The Special Issue of 2nd International Balkan Agriculture Congress. Ondokuz Mayis University, Samsun.

Hsu MF, Chiang BH (2009) Effect of *Bacillus subtilis* natto–fermanted Radix astragali on collagen production in human skin fibroblasts. Process Biochemistry, 44, 83-90.

Iji P, Toghyani M, Ahiwe E, Omede A (2017) Alternative sources of protein for poultry nutrition, Burleigh Dodds Science Publishing Limited Cambridge, United Kingdom, pp. 237-269.

Kayode RMO, Sani A (2008) Physicochemical and proximate composition of mango (*Mangifera indica*) kernel cake fermanted with mono-culture of fungal isolates obtained from naturally decomposed mango kernel. Life Science Journal, 5, 55-63.

Krishna C (2005) Solid-state fermentation systems an overview. Critical Reviews in Biotechnology, 25, 1-30.

Kumar A, Kanwar SS (2012) Lipase production in solid-state fermentation (SSF): recent developments and biotechnological applications. Dynamic Biochemistry, Process Biotechnology and Molecular Biology, 6, 13-27.

Lawal TE, Iyayi EA, Adeniyi BA, Adaramoye OA (2010) Biodegradation of palm kernel cake with multienzyme complexes from fungi and its feeding value for broilers. International Journal of Poultry Science, 9, 695-701.

Leskovec J, Levart A, Žgur S, Jordan D, Pirman T, Salobir J, Rezar V (2018) Effects of olive leaf and marigold extracts on the utilization of nutrients and on bone mineralization using two different oil sources in broilers. The Journal of Poultry Science, 55, 17-27.

Madeira JV, Macedo JA, Macedo GA (2012) A new process for simultaneous production of tannase and phytase by Paecilomyces variotii in solid-state fermentation of orange pomace. Bioprocess and Biosystems Engineering, 35, 477-482.

Makkar H, Blümmel M, Becker K (1995) Formation of complexes between *polyvinyl pyrrolidones* or *polyethylene glycols* and tannins, and their implication in gas production and true digestibility in in vitro techniques. British Journal of Nutrition, 73, 897-913.

Mathivanan R, Selvaraj P, Nanjappan K (2006) Feeding of fermanted soybean meal on broiler performance. International Journal of Poultry Science, 5, 868-872.

Mathot P, Debevere C, Walhain P, Baudart E, Théwis A, Brake lJ (1992) Composition and nutritive value for rats of *Aspergillus niger* so-

lid fermanted barley. Animal Feed Science and Technology, 39, 227-237.
Medouni-Haroune L, Zaidi F, Medouni-Adrar S, Kecha M (2018) Olive pomace: from an olive mill waste to a resource, an overview of the new treatments. J. Crit. Rev, 5, 1-6.
Medouni-Haroune L, Zaidi F, Roussos S, Desseaux V, Medouni-Adrar S, Kecha M (2017) Solid state fermentation based olive pomace using *streptomyces* strains: a preliminary study. Asian J. Biotechnol. Bioresour. Technol., 2, 1-9.
Moftah OAS, Grbavčić S, Žuža M, Luković N, Bezbradica D, Knežević-Jugović Z (2012) Adding value to the oil cake as a waste from oil processing industry: production of lipase and protease by Candida utilis in solid state fermentation. Applied Biochemistry and Biotechnology, 166, 348-364.
Molina-Alcaide E, Yáñez-Ruiz DR (2008) Potential use of olive by-products in ruminant feeding: A review. Animal Feed Science and Technology, 147(1-3), 247-264.
Mussatto SI, Ballesteros LF, Martins S, Teixeira JA (2012) Use of agro-industrial wastes in solid-state fermentation processes. Industrial Waste 274.
Nazem K, Rozbehan Y, Shodjaosadati SA (2008) The nutritive value of citrus pulp (lemon and orange) treated with *Neurospora sitophila*. JWSS-Isfahan University of Technology, 12, 495-505.
Neifar M, Jaouani A, Ayari A, Abid O, Salem HB, Boudabous A, Najar T, Ghorbel RE (2013) Improving the nutritive value of olive cake by solid state cultivation of the medicinal mushroom *Fomes fomentarius*. Chemosphere, 91, 110-114.
Nieto R, Miranda A, García MA, Aguilera JF (2002) The effect of dietary protein content and feeding level on the rate of protein deposition and energy utilization in growing Iberian pigs from 15 to 50 kg body weight. Br. J. Nutr. 88(1):39-49.
Oboh G (2006. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus spp* solid media fermentation techniques. Electronic Journal of Biotechnology, 9(1), 0-0.
Okpako CE, Ntui VO, Osuagwu AN, Obasi FI (2008) Proximate composition and cyanide content of cassava peels fermanted with *Aspergillus niger* and *Lactobacillus rhamnosus*. Journal of Food Agriculture and Environment, 6, 251.
Oliveira AC, Amorim GM, Azevêdo JAG, Godoy MG, Freire DM (2018) Solid-state fermentation of co-products from palm oil processing: production of lipase and xylanase and effects on chemical composition. Biocatalysis and Biotransformation, 36, 381-388.
Oliveira F, Salgado JM, Abrunhosa L, Pérez-Rodríguez N, Domínguez JM, Venâncio A, Belo I (2017) Optimization of lipase production by solid-state fermentation of olive pomace: from flask to laboratory-scale packed-bed bioreactor. Bioprocess and biosystems engineering, 40, 1123-1132.
Owens FN, Secrist DS, Hill WJ, Gill DR (1997) The effect of grain source and grain processing on performance of feedlot cattle: a review. Journal of Animal Science, 75, 868-879.
Pagnanelli F, Mainelli S, Vegliò F, Toro L (2003) Heavy metal removal by olive pomace: biosorbent characterisation and equilibrium modelling. Chemical Engineering Science 58, 4709-4717.
Pandey A (2003) Solid-state fermentation. Biochemical Engineering Journal, 13, 81-84.
Pandey A, Soccol CR, Rodriguez-Leon, JA, Nigam PSN (2001) "Solid State Fermentation in Biotechnology: Fundamentals and Applications" Reference Book, 1st edition, New York, 221p.
Rai SN, Singh K, Gupta BN, Walli TK (1988) Microbial conversion of crop residues with reference to its energy utilization by ruminants an overview. Fibrous Crop Residues as Animal Feed, ICAR, New Delhi, 66-74.
Rajesh N, Joseph I, Raj RP (2010) Value addition of vegetable wastes by solid-state fermentation using *Aspergillus niger* for use in aquafeed industry. Waste Manag. 30, 2223-2227.
Raimbault M (1998) General and microbiological aspects of solid substrate fermentation. Electronic Journal of Biotechnology 1, 26-27.
Ranalli A, Cabras, P, Iannucci E, Contento S (2001) Lipochromes, vitamins, aromas and other components of virgin olive oil are affected by processing technology. Food Chemistry, 73, 445-451.
Ravindran R, Jaiswal AK (2016) Exploitation of food industry waste for high-value products. Trends in Biotechnology 34, 58-69.
Safari O, Farhangi M, Carter C, Yakhchali B, Bai S, Sangatash MM (2012) Study on the effect of solid state fermentation with *Aspergillus niger* on antinutritional factors of canola protein concentrate with aim of using in the diet of rainbow trout (*oncorhynchus mykiss*), The 1th International and the 4th National Congress on Recycling of Organic Waste in Agriculture.
Saci F, Meziant L, Louaileche H (2015) Effect of storage time and temperature on the health-promoting substances and antioxidant activity of two commercial fruit based-beverages. International Journal of Bioinformatics and Biomedical Engineering, 1, 118-122.
SAS Institute Inc (1985) SAS user's guide: Statistics, Version 4.18 Edition. Cary, NC, USA.
Shojaosadati SA, Faraidouni R, Madadi-Nouei A, Mohamadpour I (1999) Protein enrichment of lignocellulosic substrates by solid state fermentation using *Neurospora sitophila*. Resources, Conservation and Recycling, 27, 73-87.
Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid-state fermentation. Biochemical Engineering Journal 44, 13-18.
Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.
Sun H, Tang JW, Yao XH, Wu YF, Wang X, Feng J (2012) Improvement of the nutritional quality of cottonseed meal by *Bacillus subtilis* and the Addition of papain. International Journal of Agriculture and Biology, 14(4).
TSE (2004) Animal Feeds- Determintion of Metabolizable Energy (Chemical Method), TS 9610, Ankara, Türkiye.
Tosun R, Yaşar S (2021) Fungal fermantasyonu ile elma ve domates posasının besleme değerinin artırılması. Journal of the Institute of Science and Technology, 11(1), 819-830. (in Turkish)
Tufarelli V, Introna M, Cazzato E, Mazzei D, Laudadio V (2013) Suitability of partly destoned exhausted olive cake as by-product feed ingredient for lamb production. Journal of Animal Science, 91(2), 872-877.
Tura, D, Gigliotti C, Pedò S, Failla O, Bassi D, Serraiocco A (2007) Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europea L.*) and correlations with oxidative stability. Scientia Horticulturae, 112, 108-119.
Vahidi H, Reza MAS, Kobarfard F (2017) Protein enrichment of olive cake substrate by solid state fermentation of *Lentinus edodes*. Trends in Peptide and Protein Sciences, 1, 177-182.
Vinha AF, Ferreres F, Silva BM, Valentao P, Gonçalves A, Pereira JA & Andrade PB (2005) Phenolic profiles of Portuguese olive fruits (Olea europaea L.): Influences of cultivar and geographical origin. Food Chemistry, 89, 561-568.
Vuong QV (2017) Utilisation of Bioactive Compounds from Agricultural and Food Production Waste. 11, 4627-4633. CRC Press.
Yañez-Ruiz DR, Moumen A, Martín-García AI, Molina-Alcaide E (2004) Ruminal fermentation and degradation patterns, protozoa population and urinary purine derivatives excretion in goats and withers fed diets based on two-stage olive cake. Small Ruminant Research, 52(2), 253-262
Zhang Y, Truzzi F, D'Amen E, Dinelli G (2021) Effect of storage conditions and time on the polyphenol content of wheat flours. Processes, 9(2), 248.
Zhang X, Cao F, Sun Z, Yu W, Zhao L, Wang G, Wang T (2012) Effect of feeding *Aspergillus niger*-fermanted Ginkgo biloba-leaves on growth, small intestinal structure and function of broiler chicks. Livestock Science, 147, 170-180.
Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559.