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ORIGINAL ARTICLE

The physicochemical properties, total phenolic, antioxidant activities, and phenolic profile of fermented olive cake



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KEYWORDS

Olive cake; Phenolics; Antioxidant; Fermentation; Feed **Abstract** Fermentation of olive cake during 60 days by evaluating the physicochemical properties, total phenolic, antioxidant activities and phenolic profile was investigated. The chemical composition showed that olive cake is rich in crude fiber and carbohydrates (Nitrogen free extract) and contains moderate amounts of crude protein, fat and ash. Total phenolics which are determined by the folin–ciocalteuic method were decreased during fermentation while no change was observed in antioxidant activities and nitrogen and carbon percentages. On the other hand olive cake samples contain little amounts of antioxidants. The HPLC profiles for olive cake extracts contain the highest amount of caffeic acid followed by vanillic acid, gallic acid, and catechin, respectively also phenolic acids and compounds decreased during fermentation. It can be concluded that olive cake fermentation is considered a good procedure to reduce phenolic compounds which could be harmful in using olive cake "as is" for animal feeding or plant fertilizers.

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1. Introduction

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A huge quantity of organic waste is produced yearly as a byproduct from the agro-food industries. The management of these residues is crucial for the preservation of the environment and the valorization of these byproducts. The most significant wastes are released by olive oil mills, where Jordan is considered the tenth largest olive oil producer in the world. There are more than 250 olive milling factories available to process olive fruits in Jordan. Three major products produced by these

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mills are olive oil (20%), solid residues (called also olive cake) (30%), and waste water (50%). The olive oil industries play an important economical role in the Mediterranean region. Unfortunately each metric ton of oil produced is associated with three or more tons of difficult to dispose byproducts that might result in environmental pollution (Vera et al., 2009). Generally, olive cake is utilized as a fuel, raw material for soap making, animal feed or high quality fertilizer (Arjona et al., 1999; Krokida et al., 2002; Gül et al., 2010).

A wide range of phenolic compounds have been identified in virgin oil, including phenolic alcoholic, secoirdoid derivatives phenolic acids and flavonoids (Suarez et al., 2010). However, only about 2% of the total phenols found in olive fruits are transferred to the extracted olive oil, while the other 98% are retained in the olive cake (Suarez et al., 2010). The analysis of these phenolic extracts has demonstrated their high antioxidant activity and suggested their potential use as additives for the food industry (Eu, 2000).

Olive cake is a ligno-cellulosic organic material (Boskou, 1996) and has low digestibility and energy content (Al-Masri and Guenther, 1999). The digestibility of the wastes is limited by the cellulose crystallinity (Sealbert et al., 1985). In addition, acetyl groups on hemicellulose can be a limiting factor for microorganisms and enzymatic hydrolysis of carbohydrates (Kong et al., 1992). Recycling of agricultural wastes and their utilization as alternative energy sources for ruminant feeding are important for economical and environmental reasons. Chemical treatments (Ballet et al., 1997) and physical treatments (Brownell and Saddler, 1987) have been tested to improve the nutritive value of agricultural wastes. However, the cost of these treatments might be a limiting factor. Therefore, the present study was conducted to study the behavior of physical properties, chemical composition, total phenols and antioxidants of olive cake material during fermentation process. Also, the HPLC analysis was used to quantitatively find out the phenolic acids' profiles for olive cake extracts as triggered by fermentation process. The experimental findings of this investigation will be crucial to evaluate the applicability of applying a simple fermentation method to enhance the nutraceutical properties of olive cake.

2. Experimental

2.1. Materials

Raw materials were obtained from olive mill (Ajloun-Jordan). Ethanol, HCl, methyl linoleate, 2,2,4-trimethylpentane and all other reagents from Segma (Agent in Amman-Jordan).

2.2. Fermentation process

Raw materials were mixed during the composting process (fermentation process) with their specific characteristics. This method of composting was constructed as developed by Bangalore (FAO, 1980) as the following pit was prepared by digging a hole of 20 m deep and 90 cm slope to prevent water logging .Olive cake was put in the pit at a height of about 1 m. The materials are allowed to remain in the pit without turning and watering for one week. Olive oil biomass volume during this period decreases. Additional water was added every 4–7 days depending on the weather condition. After the initial

aerobic composting (about 10 days), the evaluated measurements were started for two months of fermentations and every 20 days.

2.3. Proximate chemical analysis

Dry matter, ash, crude protein, ether extract and carbohydrates (Nitrogen free extract) were determined according to the procedures outlined by the AOAC (1984).

2.4. Determination of pH and water activity

The regular pH meter was used to determine the pH of fermented and unfermented olive cakes. Four replicate measurements were taken for each sample. The average was calculated.

The water activity was measured in the chemical engineer faculty laboratory by the water activity apparatus that is used to determine the water activity of fermented and unfermented olive cakes. Four determinations (about 20–30 g) were taken for each measurement.

2.5. Determination of total phenolics

2.5.1. Sample preparation

Fermented and unfermented olive cakes were dried using drying oven at 105 °C then ground in grinding mill and kept in polyethylene bags until the time of analysis.

2.5.2. Sample extraction

Fifty milligrams of each extract was weighed and dissolved in 25 mL of the extraction solvent (40 mL acetone: 40 mL methanol: 20 mL water: 0.1 mL formic acid) then vortexed and heated at 60 °C (water bath) for 1 h, then allowed to cool at room temperature, and homogenized for 30 s with sonicator at setting 6. The homogenized sample was filtered through a Micracloth into a screw-capped test tube.

2.5.3. Determination of total phenolics

Phenolic compounds were determined using the Folin– Ciocalteu method (Singleton and Rossi, 1965). Filtrates from each extract (200 μ L, three replicates) were introduced into screw-capped test tubes; 1.0 mL of Folin–Ciocalteu's reagent and 1.0 mL of sodium carbonate (7.5%) were added. The tubes were vortexed and allowed to stand for 2 h. Absorption at 726 nm was measured (spectrophotometer). The total phenolic content was expressed as chlorogenic acid equivalents (CAE) in mg/g of dry material as follows.

Total Phenolics Concentration in mg/g

$$= (A/b)^*[(SW + 25)/SW]$$

where A = absorbance at 726 nm; SW = sample weight (g); b = slope of the standard curve of chlorogenic acid.

2.6. Antioxidant activity determination -methyl linoleate

Antioxidant testing was carried out by oxidizing linoleic acid methyl ester (MeLo) in the presence of antioxidants as described by Heinonen et al. (1998). Ten milligrams of each extract was dissolved in 50 mL of methanol. After that, 0.5 mL from each methanolic extract was added to MeLo (0.2 g), and methanol was evaporated under nitrogen. Sample aliquots (10 mg) were taken at zero time and after 72 h at 40 °C in the dark and dissolved in 5 mL of 2,2,4-trimethylpentane (isooctane), and the conjugated diene absorption at 234 nm was measured using a spectrophotometer. The antioxidant activity was expressed as percentage (%) inhibition of the formation of MeLo-conjugated diene hydroperoxides after 72 h of oxidation compared with blank from MeLo. Percentage inhibition of linoleic acid oxidation was calculated as follows:

 $\% \ Inhibition = [(AB72h - AB0h) - (AE72h \\ - AE0h)/(AB72h - AB0h)] \times 100$

where A: Absorbance; E: Extract; B: Blank.

2.7. Quantitative determination of phenolic acids

The HPLC analysis will be performed on a Merch-Hitachi auto sampler, (series L-7200), diode detection (L-7455) with a quaternary pump L-7150, D 7000 according to the procedure described by Rababah et al. (2004). The individual separation of the phenolic compounds in both the extracts and standard solution will be performed on an ultra base C-18 column (250 mm \times 4.6 mm) using an injection volume of 20 µL and a flow rate of 1 mL/min and the column temperature will be set at room temperature throughout the experiment.

2.8. Statistical analysis

Data were analyzed using the general linear model (GLM) procedure with SAS Version 8.2 software package (SAS, 2002 Institute Inc., Cary, NC, USA). Means were separated by LSD analysis at a least significant difference of 0.05 2p-value.

3. Results and discussion

3.1. Chemical analysis

Dry matter content from 0 to 60 days ranged from 64.8% to 66.2% with insignificant differences (Table 1). Also, the results for ash ranged from 4.1% to 4.5%, crude protein from 7.1 to 7.6, crude fiber from 43.3 to 44.6%, ether extract from 11.2% to 11.7% and carbohydrate from 31.3% to 34.9% during the fermentation periods (0–60 days). The results showed that olive cake is rich in nutrients which allow the microorganisms to grow for longer periods. Also, the results showed that fermented olive cake stays rich in carbohydrate which means fermented on the state of the

mentation needs extra time for digestion. No significant differences were found during fermentation periods (Table 1). However, longer time (i.e. one year) is expected to give a higher digested feed for animals.

3.2. Water activity and moisture contents

Results show that moisture content and water activity exhibited no significant difference during the fermentation time of 0, 20, 40 and 60 days (Table 2). Moisture content ranged from 33.8% (40 day) to 35.2% (0 day). Miranda et al. (2008) found higher values of moisture content (0 day) than our results, these variations could be related to variation in species and environmental conditions. Results of water activity values were high and ranged from 0.93 (40 day) to 0.96 (0 day) and no significant difference was found during fermentation (Table 2). The high water activity could be due to the fat contents that let the water molecules to move easily in the media (olive cake). The results showed that a high water activity will enhance the growth of microorganisms especially the bacteria, knowing that best water activity for bacteria growth is between 0.90 and 0.98.

3.3. Determinations of pH

The values of pH were between 4.6 and 4.8 during the period of storage. Condition of fermentation or storage was still acidic. No significant differences were observed during the periods of storage, this could be related to low ammonia production resulting from protein degradation. Hang et al. (2001) also reported that pH during fermentation affects the structure and stability of phenolic compounds.

3.4. Nitrogen and carbon determination

Results showed that nitrogen and carbon percentages did not change significantly during fermentation (Table 2). Our results are not in agreement with Miranda et al. (2008) who found that C/N ratio decreases during composting process. According to Van Loo and Koppejan (2003) nitrogen higher than 0.6% can cause problems like nitrogen emission into the atmosphere. Therefore, the emissions of oxides from this element will be minimized. Miranda et al. (2008) reported lower carbon (33.5%) considered to be within the normal range for biomass wastes.

3.5. Total phenolics and antioxidant activities

Total phenolics and antioxidant activities showed significant variations among fermentation periods (Table 3) and ranged

 Table 1
 Chemical composition values^a of olive cake during 60 days of fermentations.

	1		e ;					
Fermentation period	Dry matter	% Of dry matter						
		Ash	Crude protein	Crude fiber	Ether extract	Carbohydrates		
0 day	64.8 ± 4.5	4.5 ± 0.4	7.4 ± 0.6	43.3 ± 3.1	11.3 ± 0.8	34.9 ± 2.7		
20 days	65.5 ± 4.4	$4.4~\pm~0.2$	7.1 ± 0.5	$43.5~\pm~3.8$	11.2 ± 0.9	33.0 ± 2.6		
40 days	66.2 ± 4.2	$4.3~\pm~0.2$	$7.6~\pm~0.6$	44.6 ± 3.1	11.4 ± 0.8	32.8 ± 2.6		
60 days	$64.9~\pm~4.0$	$4.1~\pm~0.3$	$7.5~\pm~0.4$	$43.8~\pm~3.4$	$11.7~\pm~0.7$	$31.3~\pm~2.3$		
0.34								

^a Means \pm SD.

Fermentation period	Moisture contents%	Water activity	Nitrogen %	Carbon %
0 day	35.2 ± 2.3	0.96 ± 0.03	1.18 ± 0.08	33.50 ± 2.42
20 days	34.5 ± 2.2	0.95 ± 0.04	1.14 ± 0.09	33.94 ± 2.64
40 days	33.8 ± 2.8	0.93 ± 0.05	1.22 ± 0.07	34.39 ± 2.58
60 days	35.1 ± 2.5	$0.96~\pm~0.06$	1.20 ± 0.08	33.78 ± 2.55
^a Means \pm SD.				

Table 2 The moisture content, water activity values^a of olive cake, nirogen % and carbon % of olive cake during 60 days of fermentation.

Table 3 The total phenolics and antioxidant activities. The identified phenolic compounds of olive cake during 60 days of fermentation.

Fermentation period							
0 day		20 days		40 days		60 days	
4287.3	а	4039.4	ab	3794.6	bc	2742.3	d
5.7	а	5.5	а	5.6	а	5.3	а
359.2	a*	316.2	b	284.4	с	265.3	d
44	а	31	b	26	с	18	d
261.4	а	232.4	b	211.4	с	187.4	d
14.2	а	8.1	b	7.3	b	6.5	b
83.9	а	64.9	b	54.6	с	42.6	d
1288.9	а	1130.9	b	1009.2	с	854.3	d
64.3	а	55.1	b	45.8	с	31.7	d
1732.3	а	1642.3	b	1298.2	с	1107.3	d
62.5	а	41.5	b	34.5	с	21.3	d
153.5	а	134.5	b	114.6	с	87.4	d
	0 day 4287.3 5.7 359.2 44 261.4 14.2 83.9 1288.9 64.3 1732.3 62.5 153.5	0 day 4287.3 a 5.7 a 359.2 a* 44 a 261.4 a 14.2 a 83.9 a 64.3 a 1732.3 a 62.5 a 153.5 a	0 day 20 days 4287.3 a 4039.4 5.7 a 5.5 359.2 a* 316.2 44 a 31 261.4 a 232.4 14.2 a 8.1 83.9 a 64.9 1288.9 a 1130.9 64.3 a 55.1 1732.3 a 1642.3 62.5 a 41.5 153.5 a 134.5	0 day 20 days 4287.3 a 4039.4 ab 5.7 a 5.5 a 359.2 a* 316.2 b 44 a 31 b 261.4 a 232.4 b 14.2 a 8.1 b 83.9 a 64.9 b 1288.9 a 1130.9 b 64.3 a 55.1 b 1732.3 a 1642.3 b 62.5 a 41.5 b 153.5 a 134.5 b	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Row values with the same letters were not significantly different (p < 0.05).

from 2742.3 mg/100 g (60 days) to 4287.3 mg/100 g (0 day). Results show that total phenolic content decreases gradually with increasing fermentation time, lower values of total phenolic compounds (1053 mg/100 g) were found in virgin olive oil, the lower concentration could be due to that many phenols like flavonoids are retained in the olive cake (Suarez et al., 2010). Total phenolic content found by Alu'datt et al. (2010) was lower than the values found by the investigated samples. Indeed compost stability and degree of maturity are closely related to polyphenols and lipid concentration. Similar results were reported by Alburquerque et al. (2006). Several microbial groups and processes contribute to phenol degradation and to detoxify the compositing substrate (Ait baddi et al., 2004). Also, Filippi et al. (2002) reported similar results where the phenolic compounds reduction synchronized with the reduction in toxicity during the initial phase of compositing process. Because of the high organic load and substantial amounts of plant nutrients (N, P, K, Ca and Fe) in the composite olive cake, it can be used as fertilizers, especially it showed a high degree of humification and no phyto toxic effect (Hachicha et al., 2006).

No significant difference was found during the fermentation process and antioxidant activity values ranged from 5.3 (60 days) to 5.3 (0 day) as shown in Table 3. Higher values of antioxidant activity were reported by Amro et al. (2002) and Alu'datt et al. (2010) at 0 day. As olive oil is resistant to peroxidation (Vera et al., 2009), it makes cake lipid peroxides more stable at room temperature, but breaks down during fermentation releasing aromatic compounds associated with rancidity (Halliwell, 2001). Lower values of antioxidants' activity was found by De Leonardis et al. (2007) while higher values were reported for fermentation at 0 day by Obeid et al. (2005).

3.6. Phenolic compounds profile by HPLC

The identification of phenolics of olive cake during 60 days of fermentation is shown in Table 3. In general there were quantitative differences observed during fermentation. Of special interest was the decrease in the amount of phenolic acids (gallic acid, protocatechin, catechin, gentitics acid, chlorogenic acid, vanillic acid, syringic acid, casffeic acid, epicatechin, benzoic acids and caffeic acid) the highest content was detected in caffeic acid (1732.3 mg/100 g) and vanillic acid (1288.9 mg/100 g) at 0 day. On the other hand intermediate values were found in gallic acids, catechin and benzoic acid (359.2, 261.4 and 153.5 mg/100 g respectively), while lower values were found in protcatechin, gentistic acid, cholorogenic acid, syringic acid and epicatechin (44.0, 14.2, 83.9, 64.3 and 62.5 mg/100 g, respectively). Lower values of vanillic acid and gallic acid were reported by (Alu'datt et al., 2010; Suarez et al., 2010). Also Tsimidou (1998) reported that the major phenolic compounds in olive meal are gallic acid, protocatechin acid, vanillic acid, syringic acid, cinamic acid. On the other hand, caffeic acid and vanillic acid were found by Bianco et al. (2003) to be the most abundant phenolic compound. Results show that phenolic compounds decreased considerably in olive cake thus fermentation improves the nutritional value of this by-product, but more experiments are needed in order to improve these by-products and at the same time reduce its environmental impact.

4. Conclusion

The results indicated that chemical composition of olive cake is not significantly influenced by fermentation so, it will have high nutritive value on the other hand fermentation reduces phenolic content of olive cake which means that it is recommended for other purposes of animal feeding and fertilizer, also by using olive cake in different industrial sectors we can reduce the environmental impact of olive cake (environmental pollution).

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