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Chemical Composition of *Amurca* Generated from Jordanian Olive Oil

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Abstract

Amurca (olive oil lees) is one of olive oil byproducts which is a watery bitter tasting and dark colored sediment that settles at the bottom of crude olive oil containers over time. In this study, gross composition, total phenolic compounds, antioxidant activity, HPLC profile of phenolic compounds, lipid peroxidation inhibitory activity, free fatty acid and peroxide values of *amurca* were determined. The gross composition of Jordanian *amurca* was as follows: carbohydrates 0.74 ± 0.02%, proteins 0.7 ± 0.02%, fats 49.43 ± 0.29%, moisture 47.33 ± 0.30%, ash 0.89 ± 0.05% and fiber 0. 92 ± 0.03%. Total phenolic compounds content was 289 mg GAE/100 g of *amurca* and antioxidant activity was 22.3 ± 0.21 mg vitamin E equivalent/100 g. Peroxide value was 1.78 ± 0.03 meqO_2/kg *amurca*, free fatty acid value was 1.62 ± 0.029 (oleic acid%) and LPO inhibition was 95.7%. The most abundant phenolic compounds detected by HPLC were oleuropen, gallic acid, 3-hydroxyphenol, sinapic acid, kaempherol, isopropyl-5-methyl phenol and luteolin.

Keywords: Jordanian *amurca*; Gross composition; Total phenolic compounds; Antioxidant activity; HPLC of phenolic compounds; Lipid peroxidation; Free fatty acid value; Peroxide value

Introduction

Olive oil is the major product of pressed olive fruits. Mechanical extraction of oil involves crushing whole olive fruits, kneading the resulting paste, pressing, separating, collecting free flow oil and finally separating *amurca* from olive oil via centrifugation and filtration [1].

Amurca is known as olive oil lees in english [2] and Turtub in Jordan. Although some inconsistency is found in the literature concerning the use of the term *amurca*; in this article the term *amurca* refers to watery bitter tasting and dark colored sediment that settles at the bottom of crude olive oil container over time [1,2]. Olive oil content of *amurca* varies from 12-460 mg/kg oil depending on the type of mills [3]. Jordan institution for Standards and Metrology did not specify *amurca* content in Jordanian olive oil and since the Jordanian consumer prefers the flavor and aroma of *amurca*, then Jordanian olive oil produced for local consumption contains variable amounts of *amurca* and the final step of filtration is skipped [4].

Historically, *amurca* had several uses such as: herbicide, pesticide and for oiling leather [1,2,5]. Bitler et al. (2005) [6] reported that vegetation water decreased tumor necrosis factor (α-TNF) production and anti-inflammatory activity in the mouse. Furthermore, Bitler et al. (2007) [7] reported a decrease in pain and inflammation in patients with osteoarthritis and rheumatoid arthritis supplemented with vegetation water; this has been explained by the presence of strong antioxidant activity in *amurca* [7].

The composition of Jordanian amurca was not studied earlier. So, this study was designed to study gross composition, total phenolic compounds; antioxidant activity, HPLC profile of phenolic compounds, lipid peroxidation inhibitory activity, free fatty acid and peroxide values of *amurca*.

Materials and Methods

Amurca samples

A*murca* samples were obtained from olive oil bought from an olive oil mill in the northern Jordan. *Amurca* was extracted from olive oil after 12 months of storage by centrifugation at 4000 rpm (1252xg) for 30 minutes and stored at -18°C until use.

Gross chemical analysis

Amurca gross composition was determined according to the approved Association of Official Analytical Chemists [8].

Preparation of *amurca* extracts: Fifty grams of *amurca* sample were diluted in 50 mL of hexane and the mixture was washed three times with 30 mL of methanol/water mixture (60:40). The mixture was shaken for 2 min before allowing the two phases to separate in a separator funnel. The methanolic extracts were then washed with 50 mL of hexane and finally brought up to 100 mL in a volumetric flask and stored at -18°C until use [9].

Total phenolic compounds

Total phenolic compounds of *amurca* extracts were determined according to the Folin-Ciocalteu procedure adapted from Hajimahmoodi et al. [10]. Gallic acid was used as calibration standard and results were expressed as mg gallic acid equivalent (mg GAE /100 g fresh weight).

Antioxidant activity

Antioxidant activity of *amurca* extracts were determined spectrophotometrically using Fe^{3+} reducing antioxidant power assay (FRAP) [11]. For construction of the calibration curve, six concentrations of vitamin E (4, 6, 8, 10, 15 and 20 mg) were used and results were calculated as mg vitamin E equivalent (mg vitamin E/100 g fresh weight).

Free fatty acid and peroxide values

Oil extraction from *amurca*: Oil was extracted from *amurca* by

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soaking *amurca* samples in hexane (1:3) over night, the mixture was centrifuged at 3000 rpm (723xg) for 20 minutes and the supernatant was collected, and hexane was evaporated using rotary evaporator, then free fatty acid and peroxide values were determined according to the official EU method [8,12].

Determination of lipid peroxidation: Lipid peroxidation was determined by measuring the concentration of malondialdehyde in the liver homogenates according to Ohkawa et al. [13] and Lin et al. [14]. *Amurca* extracts (hexane, methanol, water) were prepared according to method described by Favati et al. [9]. The inhibition percent of nonenzymatic LPO induced by Fe^{2+}/ascorbate mixture was determined according to the following equation [15].

$$\text{LPO inhibitory percent} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Where,

Control=absorbance of MDA/TBA complex formed in the absence of *amurca* extract.

Treatment=absorbance of MDA/TBA complex formed in the presence of *amurca* extract.

Determination of phenolic compounds by HPLC

Extraction method: Phenolic compounds were isolated from *amurca* by extraction with petroleum ether. The extract was then washed with 60 mL of methanol/water mixture (60:40) 3 times. The 3 aliquots were combined and washed with 100 mL of hexane. The extract was then evaporated to dryness in a rotary evaporator, and dissolved in petroleum ether (1:3) for HPLC analysis.

HPLC analysis: Agilent series 1100 System equipped with automatic injector and a Microsorb-MV column C18 (250 mm×4.6 mm; 5µm particle size) was used.

The mobile phase (A) consisted of 2% triflouroacetic acid (2 ml TFA/1000 ml distilled water), and mobile phase (B) consisted methanol (HPLC grade) at a flow rate of 0.6 ml/min, with 30 µl injection volume. The gradient elution program used was: 100% A/0% B in 0 min; 10% A/90% B in 60 min. The fractions were detected at 280 nm.

Phenolic compounds were identified using standard substances and their UV characteristic and relative retention times.

Statistical analysis: The data was statistically analyzed by using the statistical package for social sciences (SPSS, version15.0, 2007, Chicago, IL). One way analysis of variance (ANOVA) test was performed to test difference between the samples followed by mean separation using Duncan's Analysis. Significance was declared at $p<0.05$.

Results and Discussion

Gross composition

Table 1 depicts the gross composition of *amurca*. Carbohydrates, proteins, fats, moisture, ash and fiber were 0.74% ± 0.0.411, 0.7% ± 0.023, 49.43% ± 1.08, 47.33% ± 0.447, 0.89% ± 0.292 and 0.92% ± 0.292 respectively.

Gross composition of *amurca* was not reported earlier except for nitrogen content which was found to be 0.6% [16]. The low quantity of protein and carbohydrates in *amurca* and the high quantity of fat and moisture can be attributed to components of *amurca* which are vegetation water and olive tissue [1].

Composition	Percentage (%) ± SE
Carbohydrates	0.74% ± 0.02
Protein	0.70% ± 0.02
Fat	49.43% ± 0.29
Moisture	47.33% ± 0.30
Ash	0.89% ± 0.05
Fiber	0.92% ± 0.03
Total	100.00

Table 1: Gross composition of *amurca* (%).

Criteria	*Amurca*	Olive oil
Total phenolic compounds (mg GAE/100 g)	289.6 ± 0.402	31.62 ± 0.37
Antioxidant activity (mg vitamin E equivalent/100 g)	22.3 ± 0.53	1.29 ± 0.057
Free fatty acid value (Oleic acid%)	1.62 ± 0.029	1.39 ± 0.034
Peroxide value (meqO_2/kg)	1.78 ± 0.030	11.69 ± 0.0007

Table 2: Total phenolic compounds, antioxidant activity, free fatty acid and peroxide values of *amurca* samples extracted from olive oil after 12 months of storage in comparison with freshly pressed olive oil.

Total phenolic compounds

Table 2 shows total phenolic compounds of *amurca* extracted from freshly pressed olive oil in comparison to total phenolic compounds of freshly pressed olive oil. Total phenolic compounds of *amurca* 289.6 ± 0.402 mg GAE/100 g, which is 9.1 times higher than that of olive oil (31.62 ± 0.37 mg GAE/100 g) comes in agreement with Lozano-Sanchez and others who reported that extra virgin olive oil byproducts which settles over time at the bottom of the container is consider a natural source of phenolic compounds [17].

Antioxidant activity

Table 2 also depicts antioxidant activity of *amurca* in comparison to olive oil. Antioxidant activity of *amurca* (22.3 ± 0.53 mg vitamin E equivalent/100 g) was 17 folds higher than that of freshly pressed olive oil (1.29 ± 0.057 mg vitamin E equivalent /100g) ($p<0.05$). Frega et al. [17] also suggested that *amurca* dispersed in olive oil might have some antioxidant activity.

Free fatty acid and peroxide values

Table 2 depicts free fatty acid and peroxide values of *amurca* in comparison with those of freshly pressed olive oil. Olive oil standard according to the Jordanian Institution for Standards and Metrology free fatty acid value must be ≤ 3.3 as oleic acid percent and peroxide value must be ≤ 20 meqO_2/kg olive oil.

Free fatty acid value for *amurca* was 1.62 % ± 0.029 ($p<0.05$) which is statistically insignificant from the free fatty acid value of freshly pressed olive oil samples (1.39% ± 0.034), which can be explained by stabilizing role of suspended *amurca* against hydrolytic degradation of triglycerides [18,19]. On the contrary, it was reported freshly pressed olive oil that have a cloudy appearance had higher free fatty acid value and that filtration of cloudy olive oil decreases the rate of hydrolysis of triglycerides [20].

Furthermore, peroxide value of *amurca* samples was 1.78 ± 0.030 meqO_2/kg oil ($p<0.05$) which is significantly lower than that of freshly pressed olive oil sample 11.69 ± 0.00, which can explained by the presence of high antioxidant activity in *amurca* that inhibits the initiation stage of auto-oxidation of free fatty acids [21-23].

Lipid peroxidation

Figure 1 shows lipid peroxidation inhibition ratio of *amurca* extract in comparison with freshly pressed olive oil. Methanolic extract of *amurca* caused significant LPO inhibition (96.1%), while freshly pressed olive oil caused 53.1% LPO inhibition. This can be attributed to high total phenolic compounds and antioxidant activity that inhibits MDA formation [24,25].

HPLC profile of phenolic compounds

Figure 2 shows the chromatogram of phenolic compounds of *amurca* extract. The concentrations of the main phenolic compounds

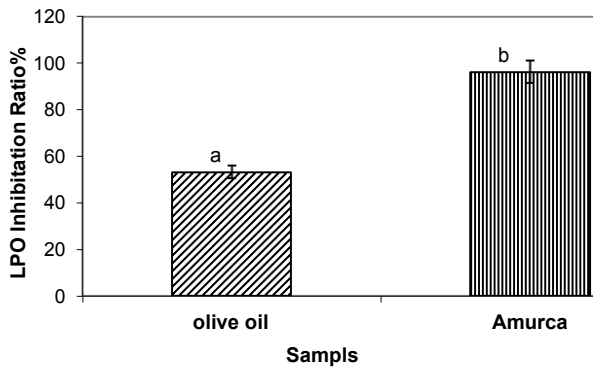

Figure 1: Effect of olive oil and *amurca* samples on lipid peroxidation (LPO) inhibition percent. Values are expressed as mean ± SEM (n=3). P-values were calculated by Students *t-test. Means* with different superscripts (a,b) differ significantly at p<0.05**.**

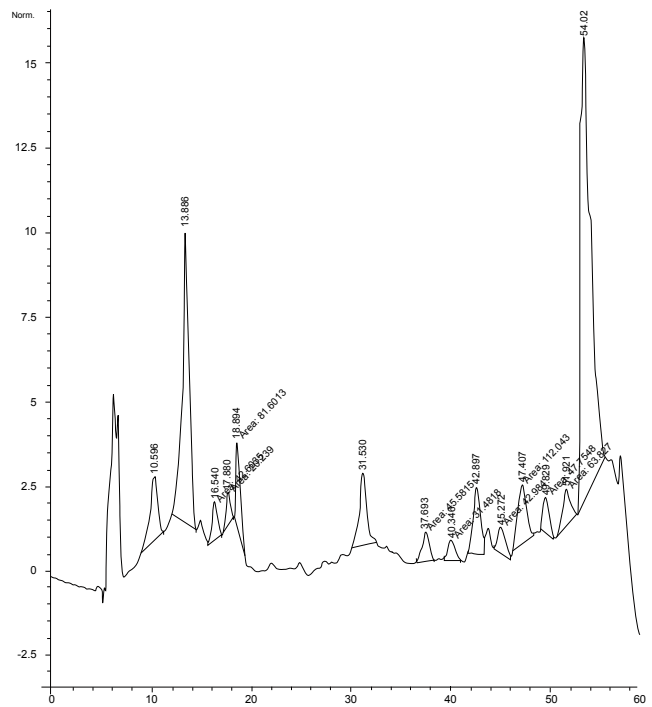

Figure 2: Separation of phenolic extracts of *amurca* by reversed phase HPLC at 280 nm. Retention time: 10.596 gallic acid; 16.540 3-hydroxy phenol; 31.530 sinapic acid; 45.272 kaempferol; 51.921 isopropyl-5-methyl phenol; 37.693 oleuropein; 42.897 luteolin.

Phenolic Compounds	Sample Area	Stander Area	mg/g *amurca*
Oleuropen	45.58152	2.00E+03	10.03
Isopropyl-5-methyl phenol	63.82699	6.07E+03	4.63
Sinapic acid	115.4224	1.38E+04	3.68
Luteolin	104.05997	1.70E+04	2.70
Gallic acid	100.08049	2.50E+04	1.76
Kaempferol	42.98399	1.27E+04	1.49
3-hydroxy phenol	42.60352	37791.7	0.494

Table 3: Phenolic compounds of *amurca* samples.

which were determined by the calibration curves obtained from their respective commercial standards are depicted in table 3. Oleuropen concentration was the highest amongst phenolic compounds in *amurca* extract (10.03 mg/g), isopropyl-5-methylphenol and sinapic acid were found to be 3.68 and 4.63 mg/g respectively, while the concentration of luteoline, gallic acid, kaempherol and 3-hydroxy phenol, were 2.7, 1.76, 1.49, and 4.94 mg/g *amurca* respectively, which comes in agreement with findings of Montedoro et al. [25], Murkovic et al. [26], Servili et al. [27], Tuck and Hayball [28], Cardoso et al. [29] and Fu et al. [30] who reported that these phenolic compounds were the most prominent in olive oil or olive fruits.

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