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CHEMICAL CHARACTERIZATION OF OLIVE POMACE IN THE NORTHERN REGION OF JORDAN

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Abstract

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In this study, the olive pomace was considered because of its importance in the environment. So, this study carried out to estimate the total nitrogen content and the fatty acids profile of olive pomace. The olive pomace samples were collected from Irbid, Jarash, Ajloun and Mafraq during the harvesting season 2014 to study the biochemical characteristics of fatty acid composition and the total nitrogen (TN) content. The total nitrogen content was determined by standard Kjeldahl method and the fatty acid profile was detected by gas chromatography (GC). The results show that the total nitrogen content ranged from (0.39 ± 0.0) to (0.62 ± 0.02) with statistically significant difference suggesting that the composition of matrices and their percentage may be responsible for composition of amendments. The calculated total protein percentage ranged between (2.43 ± 0.00) to (3.87 ± 0.17). Also, 13 different fatty acids were quantitatively profiled and quantitated. Oleic acid (C 18:1) was found to be the highest percentage of all other fatty acids and ranged between (59.03%) and (63.81%), moreover the C 18:1/C 18:2 (oil quality) was calculated and C.V% showed variation meaning that nutritional implication could affect the oxidative stability of oils. In conclusion, OP by-product could give a sustainable and alternative-cheap source for fertilizers, pharmaceutical industries, cosmetics and other industries.

Key words: olive pomace oil; fatty acid profile; total nitrogen content; total protein; olive pomace; biochemical characterization

Introduction

For numerous purposes, plant by-products has been attracted by the use of their components and for the reduction of ecological glitches connected with by-product degradation. Previous researchers reported that the extraction of proteins from the defatted rapeseed and sunflower meals is an instance for gaining protein isolates or smooth great added-value protein hydrolysates (El Nockrashy et al., 1977; Saeed et al., 1988; Lahl and Braun, 1994; Vioque et al., 1999; Ivanova et al., 2012; Stoilova et al., 2013; Denev et al., 2013; Penov et al., 2014; Yildirim et al., 2015).

On the other hand, olive pomace is chosen attributable to its great quantity produced and little value could be a low-priced source of proteins for use in the food industry. Actually, all earlier reports have been suggested that olive pomace could be used as animal feeding and it enhance protein content to 15–18% (Fedeli, 1996). According Kumar and Tannins (1984) and Valiente et al. (1995) the pomace as by-product is described by great-fiber content (70%) and little-protein content, ≃ (6%). The data regarding the biochemical and chemical composition of olive pomace (OP) are insufficient to fulfill the need and sometimes differ because of the use of different kind of cultivar and may the climate indifference

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affect this information. In general as stated above the OP has high content of fiber and relatively low of nitrogen amounts.

Olive oil is considered as important product in Jordan and other Mediterranean countries, even though OP is the major by-product of olive industry; and it is a main environmental concern. In Jordan the main olive varieties are Nabali (baladi and mohassan) and Raise, both native, which look to have been cultivated in Jordan from long times. Also in Jordan we have another local variety named Souri, cultivated in the area of north part of Jordan. Numerous additional varieties, most of them of Italian origin, are also grown in Jordan. (http://www.zaitt.com/about-zaitt). Earlier reports demonstrate that OP can be used as feed for fish (Nasopoulou et al., 2011), source of nutrients for domestic animal feeding (Sansoucy, 1985).

This is the first time we are going to characterize the fatty acid profile of olive pomace in Jordan. So, this study aimed to, firstly, estimate the content of total nitrogen in the olive pomace collected from north Jordan, and determine the protein concentration in the olive pomace. Finally, assess the fatty acid profile in the olive pomace.

Materials and Methods

Sample collection

All samples (200 samples) of olive pomace from three phases centrifugal mills used in this study were obtained from four provinces in the region of the northern of Jordan (Irbid, Jarash, Ajloun and Al-mafraq) between November and December harvest season 2014. Several samples from each sampling sites, all samples are presented Figure 1, the pomace was collected in a closed plastic container, and all samples were immediately maintained at 4°C and prevent biodegradation.

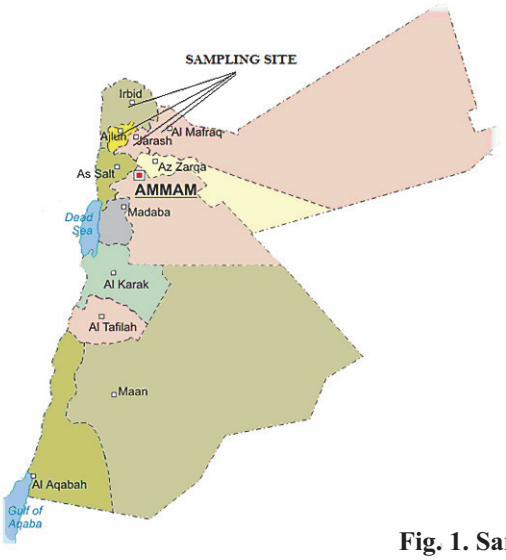

Fig. 1. Sampling site

Total nitrogen

Nitrogen content was determined by a Kjeldahl procedure (Williams, S., ed. 1984. Official methods of analysis of the Association of Official Analytical Chemists, Arlington, VA).

Protein Content

The protein content was derived from the N content found on dry biomass, using a conversion factor of 6, 25.

Lipid Extraction from Olive Pomace

Soxhlet extractions were performed: 2 g of pomace were placed in a packet made in filter paper, which was placed in a Soxhlet apparatus. The extraction was made using hexane under heating and lasted 4 hours. The hexane was then evaporated from the resulting oil using nitrogen.

Extraction and measurement of *total lipids* of OP of samples was achieved by method according to AOAC No. 905.02 (AOAC, 2000). The analyses were performed in triplicate.

TBARS content was determined spectrophotometrically at 532 nm according to the modified method of Ohkawa et al. (1979).

Methyl esters of fatty acids (FAME) were equipped by transmethylation of fat samples using a mixture of concentrated H2SO4 (95%) with methanol corresponding to AOCS (American Oil Chemists' Society) official technique Ce 2–66 (AOCS, 2000).

Fatty acids were identified on a *Shimadzu gas chromatography (GC), GC-2010, Inc., Koyoto, Japan, with improved split/splitless injector analysis with extreme repeatability, operating detectors such as. FID Rtx 2330 Restek capillary column with a stationary phase of high polarity (The characteristic of column as follow: the diameter 0.25 mm, the length 100m, the film thickness of 0.1 µm, temperature ranged from 120ºC (initial) to 210ºC (final), and time of analysis: 120 min*

The fatty acids identification was done using the standard of fat CRM 164 and and Supelco 37 No: 47885-U standard (Sigma Aldrich) as descried by Prandini et al. (2007).

Data for each trait were analyzed for using one-way analysis of variance (ANOVA) and Tukey's test The calculations were performed at a significance level of $P<0.05$.

Results and Discussion

Nitrogen and Protein

The *results* of this study *showed that* the percentage of nitrogen content and protein content of the olive pomace that collected from different locations was ranged from (0.39 ± 0.0 – 0.62 ± 0.02), (2.43% ± 0.00 – 3.87 ± 0.17) % respec-

tively. Ajloun sample shows the highest amount of nitrogen ($P \leq 0.05$) compared to other samples collected from each of Irbid, Jarash and Mafraq (Figure 2).

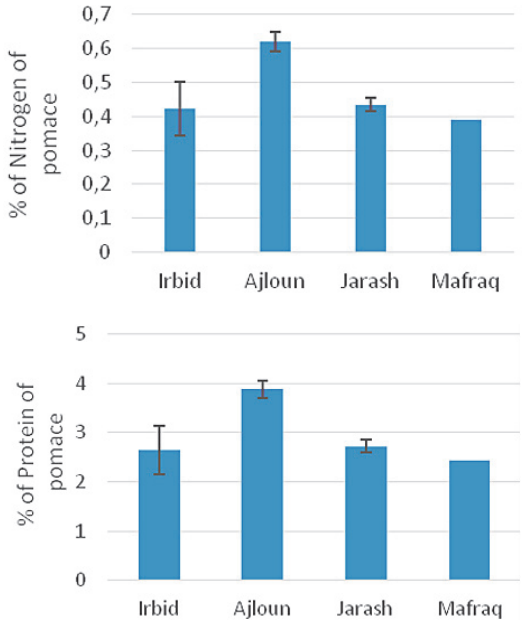

Fig. 2. The percentage of nitrogen content and protein content of the olive pomace (different location)

In the current study, the percentage of total nitrogen (TN) was calculated according to Kjeldahl method and results indicated that Ajloun olive pomace contained high amounts of TN compared to the other locations, with a value 0.62 ± 0.02, while the lowest concentration was Mafraq samples with a value 0.39 ± 0.00., these findings were similar to the previous study, on the other hand, Irbid and Jarash had a moderate amount of total nitrogen with moderate values. As mentioned before (Figure 2), the total nitrogen measurement were quiet different, suggesting that the composition of environments and their percentage may be responsible for the nutrients composition of modifications (François et al., 2008). Moreover, these differences were accounted. Interaction between TN and lignocellulos fraction represented by the fiber, may be responsible for the low amount and the significant difference between samples that collected may reflect the effect of change of temperature range between different seasons and the amount of rain in the rainy seasons.

Also, the processes of extraction may have an effect on the protein percentage because of the biological processes during extraction, but not the extraction process itself (Diacono et al., 2012).

Fatty acid

Table 1 shows the fatty acids profile distribution that shows a clear bulk for C 18:1 (oleic acid) residue in all locations and the values with wide range between 59.03% from Jarash to 63.81% Mafraq. C 16:0 (palmitic acid) values were widely ranged from 15.84% in Ajloun to 20.80% in Mafraq. And very little amounts of C 14:0 (Myristic acid) in all locations with the highest percentage (0.15%) in Jarash olive press and the lowest was 0.03 % Mafraq. other fatty acids percentage was quantified also.

Table 1
The means values of fatty acid profile in olive pomace samples

	Irbid	Ajloun	Jarash	Mafraq
C 14:0	0.04	0.14	0.15	0.03
C 16:0	19.07	15.84	20.02	20.80
C 16:1	0.71	0.77	0.78	0.81
C 17:0	0.14	2.47	0.19	0.06
C 17:1	0.12	0.11	0.18	0.06
C 18:0	3.74	3.70	3.70	2.69
C 18:1	62.64	62.99	59.03	63.81
C 18:2	11.52	11.52	12.33	10.43
C 18:3	0.69	0.87	0.67	0.67
C 20:0	0.49	0.57	0.46	0.21
C 20:1	0.26	0.27	0.25	0.22
C 22:0	0.16	0.15	0.11	0.14
C 24:0	0.40	0.63	2.14	0.06

The results revealed that 3 fatty acids had the highest percentage, one saturated C 16:0 (Palmitic acid) and one Monounsaturated C 18:1 (oleic acid) and the other one Polyunsaturated fatty acid C 18:2 (Linoleic acid), Irbid had values for previous fatty acids (19.07 ± 2.55), (62.63 ±2.39), (11.51 ±1.55) respectively, Ajloun values was (15.84 ±3.37), (62.99 ±2.12), (11.52 ±2.57) respectively, and for Jarash it was (20.02 ±2.85) (59.03 ±5.61), (12.32 ±1.19) respectively and Mafraq was (20.8 ± 0.0),(63.81 ± 0.0), (10.43 ± 0.0) respectively (Figure 3a,b). C 18:0 had low percentage ranged between 0.21 for Mafraq to 0.57 ± 0.19 for Ajloun and Irbid, Jarash were 0.49 ± 0.07, 0.45 ± 0.17 respectively, while the remaining percentage in all locations of fatty acids was very low. Monounsaturated fatty acids have huge reputation due to their nutritional insinuation and result on the oxidative stability of oils. This study found that the pomace oleic acid monounsaturated (C 18:1) is an important constituent part of the pomace content with percentage between (59.03 ±5.61) to (63.81± 0.00) (Table 1). This result agrees with the other findings studies (Clemente et al., 1997).

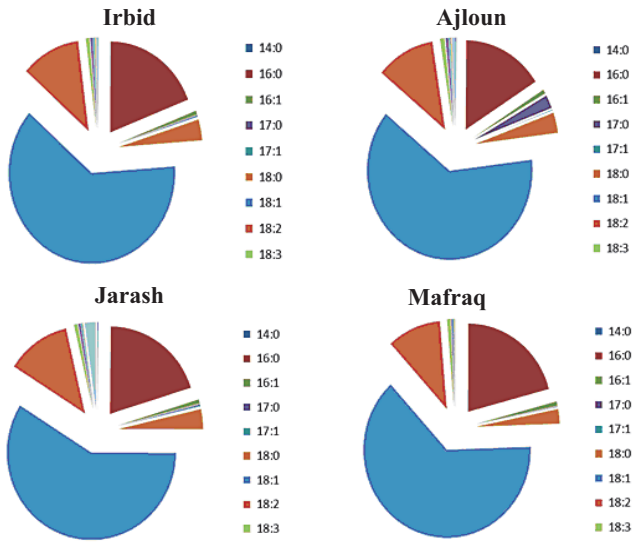

Fig. 3a. Percentage of all fatty acids in each area

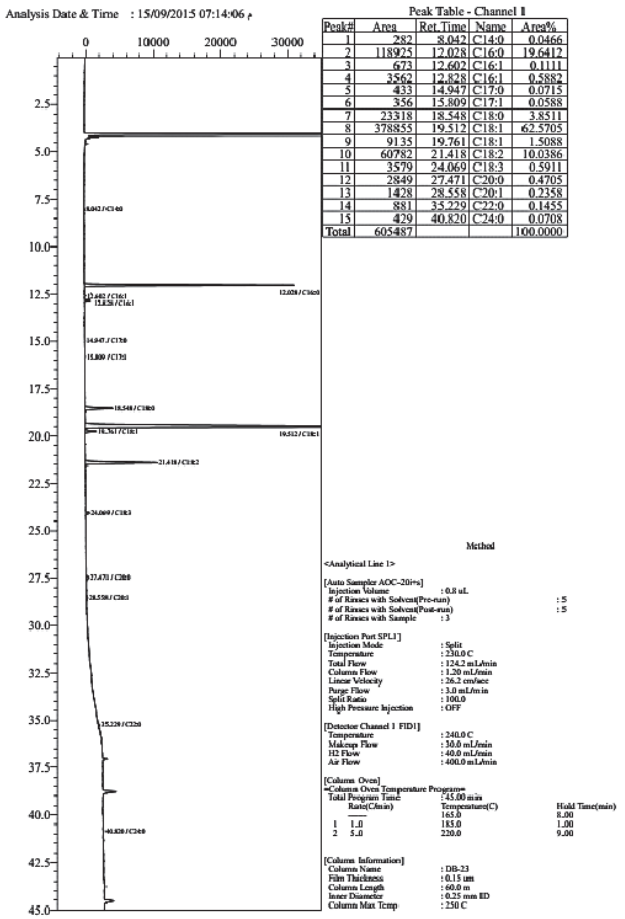

Fig. 3b. Chromatography of fatty acid analysis for Kfarat-Press (Irbid)

The distribution of relative amounts of saturated vs unsaturated fatty acids shown in Figure 4. The overall unsaturated fatty acid/saturated fatty acid for all locations was (76%: 24%), (77%: 23%), (73%: 27%) and (73%: 24%) for Irbid, Ajloun, Jarash, Mafraq respectively.

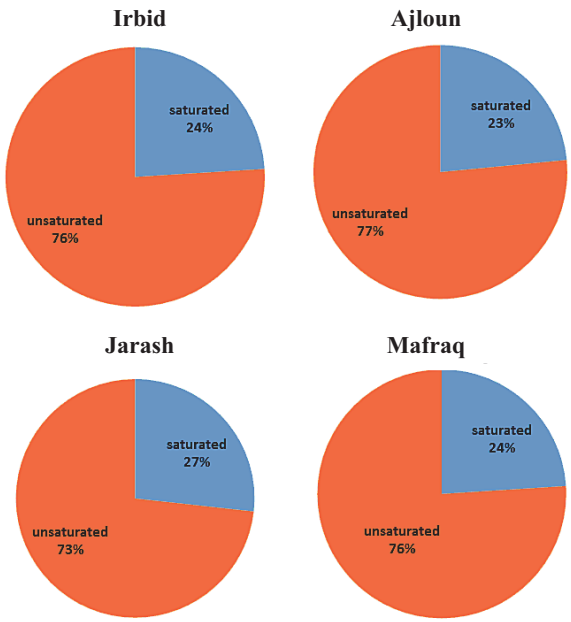

Fig. 4. Saturated and unsaturated percentage of fatty acids in different area

Strong linear positive correlation coefficients ($P \leq 0.05$) in Table 2 were found between C14:0–C18:2 (r = 0.781), C14:0–C24:0 (r =0.776), C17:0–C18:3 (r = 0.994), C17:1–C18:0 (r = 0.756), and C17:1–C18:2 (r = 0.973), and C17:1–C24:0 (r = 0.922), and C18:0–C18:2 (r = 0.86), and C18:0–C20:0 (r = 0.717), and C18:1–C22:0 (r = 0.815), and C18:2–C20:0 (r = 0.717), and C18:2–C24:0 (r = 0.881), and C18:2–C20:0 (r = 0.717), and C20:0–C20:1 (r = 0.997).

Conversely, strong negative correlation coefficients were found between C16:0–C17:0 (r = -0.953), C16:0–C18:3 (r = -0.971), and C16:0–C20:0 (r = -0.794), and C16:0–C20:1 (r = -0.785), and C16:1–C18:0 (r = -0.721), and C17:1–C18:1 (r = -0.964), and C18:1–C18:2 (r = -0.887), and C18:1–C24:0 (r = -0.986), and C22:0–C24:0 (r = -0.843) Table 2.

The oil quality had well known for oleic acid C 18:1 / Linoleic acid C 18:2 Ratio, the results showed that the ratio for each location (Irbid, Ajloun, Jarash, and Mafraq) were 5.43 ± 1.53, 5.46 ± 0.82, 4.78 ± 1.3 and 6.11 ± 0.0 respectively (Table 3). There was no significant difference in olive pomace oil quality.

Table 2
Pearson correlation test for fatty acids

	C 14:0	C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 22:0	C 24:0
C 14:0	1												
C 16:0	-0.507	1											
C 16:1	0.100	0.296	1										
C 17:0	0.553	-0.953	0.006	1									
C 17:1	0.678	0.000	-0.333	-0.116	1								
C 18:0	0.617	-0.560	-0.721	0.351	*0.756	1							
C 18:1	-0.671	-0.204	0.030	0.235	-0.946	-0.517	1						
C 18:2	*0.781	-0.228	-0.382	0.107	*0.973	*0.86	-0.877	1					
C 18:3	0.482	-0.971	-0.073	*0.994	-0.157	0.363	0.302	0.070	1				
C 20:0	0.645	-0.794	-0.644	0.623	0.553	*0.947	-0.298	**0.717	0.639	1			
C 20:1	0.599	-0.785	-0.690	0.599	0.536	*0.949	-0.269	0.697	0.621	*0.997	1		
C 22:0	-0.566	-0.405	-0.550	0.259	-0.589	0.017	**0.815	-0.489	0.359	0.171	0.220	1	
C 24:0	*0.776	0.094	0.055	-0.092	*0.922	0.519	-0.986	*0.881	-0.169	0.342	0.305	-0.843	1

Strong correlation coeffecients ($P < 0.05$).

Table 3
C 18:1 / C18:2 Coefficient of variation

	C18:1 (%)	C18:2 (%)	C18:1/C18:2	STDV	C.V%
Irbid	62.64	11.52	5.44	1.54	27.77
Ajloun	62.99	11.52	5.47	0.82	14.73
Jarash	59.03	12.33	4.79	1.35	28.24
Mafraq	63.81	10.43	6.12	0	0

Oleic acid/linoleic acid ratio and monounsaturated fatty acids/polyunsaturated fatty acids (MUFAs/PUFAs) ratios showed variation (Table 3). The analysis of variance (CV%) revealed a significant differences between locations which agree with the study done by (Zarrouk et al., 2009).

This research supports that the levels of gamma linoleic acid C 18:3 was mostly below 1%, but Some *other studies* have also been carried out showed that olive oil fatty acids profile have different results (Stefanoudaki et al., 19990). On the other hand, the International Olive Council reported that the total linolenic acid is not specific for many olive varieties (IOOC, 2008).

El Antari et al. (2003) are describing that that the levels of C18:3 is more than 1% in Moroccan oils. Results of some researchers clarified that the levels up to 1.42% C18:3 in Argentinean oils (Ravetti, 2000). Similar results are also found in New Zealand (Meehan, 2001) with up to 1.5% and in Lecce (Italy), C18:3 levels in a series of olive samples ranged from 1.1% to 1.4% (Dettori and Russo, 1993).

These large *variations were attributed* to seasonal variations, mainly rainfall and temperature (Paz Romero et al., 2003). Zarrouk et al. (2009) reported that the percentage of C18:1 is negatively correlated with the relative humidity of the atmosphere.

Sioriki et al. (2016) reported that the fish nourished with OP might be proposition that these stuffs are likely to be the significant polar phospholipids that have the capacity to be in vitro Platelet Activating Factor (PAF) inhibitors, i.e. inhibit the development of atherosclerotic plaques in blood vessels.

On the other hand, Nasopoulou et al. (2011) reported that the total lipids of gilthead sea bream nourished with olive pomace diet restricted and decreased levels of fatty acids, while offered the highest biological activity against platelet aggregation induced by Platelet Activating Factor.

This study did not show significant difference in C 18:1 and that may be due to very similar geographical area with same atmosphere and humidity conditions. The results of this study prove that the fatty acid composition have a significant discriminating power even with in close geographical areas.

This work allowed to describe the main chemical characteristics of olive pomace collected from the northern part of Jordan that showed higher unsaturated fatty acids composition compared to saturated fatty acids, C 18:1 percentage had a negative correlation with humidity in atmosphere which

positively affects oil stability. Also, high concentration of total nitrogen sample was due to change in climate, those with cold and rainy climate show higher concentrations. In a future work, further exploration for olive pomace oil stability and pomace chemical characteristics. And study the all fractions of lipid content of OP (polar and neutral) and compere the values with published values to evaluate the importance of these fractions in all aspects.

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