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Research Paper

Oleuropein concentration in some Syrian olive oil mill wastewater

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ABSTRACT

Olive mills wastewater (OMWW) is a mixture of vegetation water, soft tissues of the olive fruit and the water used in the various stages of the oil extraction process. It's rich in water-soluble polyphenolic compounds. In Syria, the large quantities of OMWW produce due the seasonality of olive oil production. The aim of this study is to determine the concentration of oleuropein in some OMWW collected from 16 different olive mills located in Lattakia area in Syria. The oleuropein was determined by reversed phase HPLC using C_{18} column and Water-acetonitrile-formic acid (84.6: 15: 0.4) (v/v/v) as a mobile phase. The results showed that, oleuropein concentrations ranged between 2.4-132.3mg/L with P^H ranged between (4.6-6.5) in OMWW samples. The highest concentration of oleuropein was (132.3mg/L) in OMWW sample obtained from the traditional batch press process, and the lower concentration (2.4mg/L) was in the OMWW sample obtained from three-phase centrifugal system. A significant difference in oleuropein concentrations was found between samples by LSD test at $P< 0.05$.

Key words: Oleuropein; Olive Mill Waste Water; HPLC.

INTRODUCTION

Olive mill wastewater (OMWW) is produced seasonally in large quantities of small olive mills scattered in Mediterranean countries. OMWW is formed from the water content of the fruit with water used in washing and processing of olive oil extraction. (Sorlini et al., 1986; Borja et al., 1993). The characteristics of olive mill waste are variable, depending on many factors such as method of extraction, variety and maturity of olives, region of origin, climatic conditions and associated cultivation/processing methods.

It has been estimated that $30\times10^6 m^3$ of waste water are produced per year. In the olive growing countries of the Mediterranean area (Greece, Italy, Lebanon, Portugal, Spain, Syria, Tunisia and Turkey) (Beccari et al., 1996; Merchichi and Sayadi, 2005). The difficulties treatment of olive mill effluents are mainly related to its high organic load, seasonal operation, high territorial scattering, and the presence of organic compounds which are hard to biodegrade such as long-chain fatty acids and phenolic compounds. Earlier studies reported the physicochemical and microbiological changes that take place in the soil after OMWW contamination (Paredes et al., 1986; Moreno et al., 1987; Capasso et al., 1995).

In general, olive mill wastewater is characterized by intensive violet-dark brown up to black color, strong specific olive oil smell, high degree of organic pollution (chemical oxygen demand; 40-220g/L and biochemical oxygen demand; 35-110g/L), P^H between 3 and 6, total organic compound of 25-45g/L, high electrical conductivity, reducing sugars up to 60% of the dry substance, LD_{50} toxicity for fish: 8.7%; in addition, they contain large amounts of suspended solids and high concentrations of polyphenols (from 0.5-24g/L) (Ramos-Cormenzana, 1986; Paredes et al., 1987; Niaounakis and Halvadakis, 2006; Roig et al., 2006). Moreover, OMWW possesses considerable amounts of mineral nutrients such as potassium, phosphorus, and a wide-range of micronutrients (Tomati and Galli, 1992; Roig et al., 2006; Mekki et al., 2007).

More than 30 different phenolic compounds have been identified in OMWW and the types and concentrations of phenolics reported in OMWW vary tremendously. Among them biologically active compounds simple such as tyrosol, hydroxytyrosol and more complex biophenols such as secoiridoids (oleuropein and ligstroside) (Bianco and Uccella, 2000). In fact, phenolic compounds are responsible for several biological effects, including antibiotic and phytotoxicity (Dalis et al., 1996).

Oleuropein which is a secoiridoid, is the most abundant phenolic compound in olive leaves and fruits and is responsible for the characteristic bitterness of olive fruit (Soler-Rivas et al., 2000). It has several pharmacological properties including antioxidant (Visioli et al., 2002), anti-inflammatory (Visioli et al., 1998), anti-atherogenic (Carluccio et al., 2003), anti-cancer (Owen et al., 2000), antimicrobial (Tripoli et al., 2005), and antiviral (Fredrickson and Group, 2000), and for these reasons, it is commercially available as food supplement in Mediterranean countries. In addition, oleuropein has been shown to be cardio protective against acute adriamycin cardiotoxicity (Andreadou et al., 2007) and has been shown to exhibit anti-ischemic and hypolipidemic activities (Andreadou et al., 2006).

Several previous studies have reported oleuropein content but in the olive leaf and ethanolic methanolic extracts of the fruit (Esti et al., 1998; Benavente-Garcia et al., 2000; Savournin et al. 2001; Lee-Huang et al. 2003; Omar, 2010; Altinyay and Altun, 2006; Ansari et al., 2011; Arslan and Ozcan, 2011; Tayoub et al., 2012).

Syria is one of the countries that cultivate olive. The estimated cultured area is 684490 hectares with annual production of about 1000,000 tons of olive fruit. Olive mills belong to the foremost polluters: the volume of OMWW produced 800.000 m^3 during the production season (Ministry of agriculture and land reform, 2011data). Most frequently, OMWW is pumped and discharged into evaporation ponds or directly dumped in rivers or spread on soil (UNDP, 2006). Since there is no data on oleuropein concentrations in OMWW in Syria, and because this substance is lost during olive oil production processes, this study is the first to determine oleuropein concentrations in olive mills wastewater in Syria. The study also conducted to evaluate the possibility of using this wasted oleuropein in preparing antioxidant medicines and other beneficial medical or industrial products.

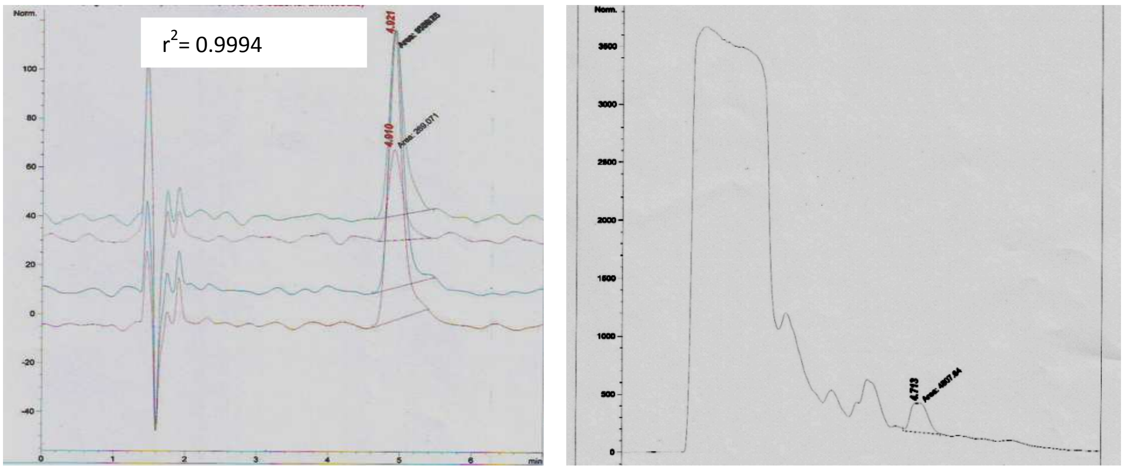
MATERIALS AND METHODS

Sampling: Raw material Samples of OMWW were collected from 16 olive oil mills located in Lattakia suburb during 2012 season, 6 samples from traditional batch press process, and 10 samples from three-phase centrifugal system. All samples were stored at-20°C until analysis.

Extraction: The samples are extracted according to a modified method of La Cara, (La Cara et al., 2012). Briefly, aliquots of 0.5ml of each sample were diluted in 5ml of acid methanol (70:29:1; methanol: water: HCl) and incubated at 37°C for 30min in a rotary shaker. The suspension was centrifuged for 15min at 3500rpm and the supernatant was recovered and used for oleuropein assay.
Chemicals, Standards solution: A Stock solution of oleuropein (100µg/ml) was prepared in methanol using oleuropein standard obtained from sigma (sigma No. 0889). Then a serial dilution (0.1, 0.5, 1, 2µg/ml) was prepared in water: methanol (80:20, v/v) to build a standard curve.
***High performance liquid chromatography (HPLC)*:** The Agilent 1260 Infinity Isocratic LC system (HPLC), equipped with DAD detector and Chemstation software was used in this study. Oleuropein were separated on Zorbax Eclipse C_{18} column, (250 × 4.6, 3.5µm) using a mobile phase of Water: Acetonitrile: Formic Acid (84.6: 15: 0.4) with flow rate 0.5ml/min. The column temperature set at 35°C, the sample injection volume was 20µl, and detection wavelength at 280nm.
***Statistical Analysis*:** The mean ± standard deviation (SD) of oleuropein concentration in triplicate (n=3) was calculated for each sample. One way analysis of variance (ANOVA) using the least significant difference LSD test at P values less than 0.05 was used to assess the significance of differences in oleuropein concentrations among olive mills. Pearson Correlation test at 0.01 level (2-tailed) was used to assess correlation between oleuropein level and p^H degree of effluent. Statistical software used was SPSS version17**.**

RESULTS

Typical HPLC chromatograms of chemical standards and oleuropein concentrations are shown in Figure 1. The retention times of A and B were 4.910-4.920 and 4.713min, respectively. In the range of 0.1-2µg/ml, good correlation of linearity has been achieved (n=3; R^2 = 0.9994).



Retention time (min)
A

Retention time (min)
B

Figure-1: Typical HPLC chromatograms of Oleuropein in standard solutions (A), and in OMWW sample (B) (t_R= 4.713min).

Table 1 shows oleuropein concentrations and P^H degrees for analyzed OMWW samples taken from 16mills distributed along Lattakia region, North West of Syria.

Oleuropein concentrations ranged between 2.4-79.27mg/L for the three-phase centrifugal system and between 3.3-132.3mg/L for the traditional batch press process. Analysis of variance test using LSD at P values less than 0.05 showed statistically significant differences in the oleuropein concentration between the different olive presses (at $P<0.05$).

Table-1: Oleuropein concentration and P^H value in 16 OMWW samples from traditional batch press process and three-phase centrifugal system.

Sample n°.	type of olive press	Oleuropein concentration (mg/L)	P^H
1	*traditional batch press process*	12.27±0.6	5.5
2		132.3±0.95	4.8
3		37.23±0.91	5.5
4		3.3±0.62	6.5
5		13.3±0.9	6.0
6		92.18±0.8	5.0
7	*three-phase centrifugal system*	2.4±0.89	6.5
8		20.47±0.9	6.0
9		3.23±0.47	6.0
10		2.47±0.7	6.0
11		27.47±0.97	5.8
12		3.4±0.66	6.0
13		79.27±0.86	5.0
14		2.63±0.74	6.0
15		15.47±0.83	6.0
16		2.7±0.47	6.0

DISCUSSION

Studies that report oleuropein concentrations in OMWW are rare in the available literature. Oleuropein concentrations measured were lower or within the range, (100-200mg/L), reported by Benavent (2008), and those reported by La Cara (100 to 1100mg/L), (La Cara et al., 2012).

We also measured p^H values of OMWW. They ranged from 4.6 to 6.5. Those values are consistent with those reported by others (Andreozzi, 1998; Azbar et al., 2004). There was a significant strong negative correlation between P^H values and oleuropein concentrations, It was -0.87 at $P<0.01$ for the traditional batch press process, and -0.9 for the three-phase centrifugal system at $P<0.01$.

Differences seen in oleuropein concentrations and P^H values among different mills might be due to many factors such as method of extraction, variety of olive trees, olives maturity, region of origin, climatic conditions, processing methods, amount of water consumed in olive oil extraction.

Since OMWW is harmful to the environment and is a cheap and wealthy source of oleuropein we recommend the use of this source as raw material for pharmaceutical industry and fungicides or insecticides industries. This also will help in reducing contamination of the environment including groundwater.

In summary this study is the first report on oleuropein concentrations in OMWW of some olive mills from Syria, Lattakia region as an example.

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