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EFFECT OF FILTRATION OF OLIVE MILL WASTEWATER ON THE PHENOLIC COMPOSITION AND ITS INFLUENCE ON ANTIOXIDANT ACTIVITY

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Abstract

Olive Mill Wastewaters (OMW) are obtained during the extraction of olive oil. OMWs are characterized by a high concentration of sugars, lipids, proteins, acids, polyphenols and organic matter. This makes the elimination of OMW a problem to which Morocco, as well as other countries around the Mediterranean, are confronted. Due to their high concentration in polyphenols, these waters are a source of environmental hazards. The polyphenols, which are strong antioxidant, compounds are causing deoxygenation of the waters in which they are discharged.

This research work aims to study the treatment of OMW by activated carbon (AC) filtration in order to achieve an effective treatment in conformity with the requirements of the national environmental legislation. A physico-chemical characterization of OMW was performed in order to assess their pollutant load by determining the following parameters: pH, dry matter, chemical oxygen demand, biological oxygen demand and total polyphenol content. Then, several activated carbon and calcined sand filtration tests are conducted to determine their effects on the elements responsible for OMW pollution. Then, the extraction of the monomeric and bound polyphenols presents in the raw OMW and filtrated OMW, in order to compare the extracts of the two OMW. To make this comparison, an infrared analysis of the extracts was performed, followed a phytochemical study and finally an evaluation the antioxidant activity with respect to the free radicals of types 2,2-diphenyl-1-picrylhydrazyl (DPPH), ammonium salt of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) method.

Keywords: *activated carbon, antioxidant activity, chemical oxygen demand, polyphenols, Olive mill wastewaters.*

Introduction

The OMW are obtained during the extraction of olive oil, their quality and quantity depend on several criteria: the variety of olive, the season of harvest, the maturation rate of the fruits and climatic conditions [1]. It is generally estimated that one kg of olive generates 1 to 1.5 liters of OMW, according to the used extraction system [2]. OMW are characterized by a high concentration of sugars, lipids, proteins, acids, phenolics compounds, and organic matter [3]. So, once rejected without having undergone prior treatments. These OMW will have a negative impact on the environment, due to their ability to inhibit the development of plants and certain microorganisms [4].

The phytotoxicity of OMW is attributed to the presence of lipids and polyphenols [5]. These polyphenols are toxic to certain bacteria [1]. They represent an inhibitory effect of anaerobic digestion [6] hence the need for pretreatment reducing their concentration in wastewater or OMW. To overcome these problems, several solutions have been suggested such as advanced oxidation processes (AOP) [7], electrochemical methods [8; 9; 10; 11] and sonolysis [12].

The objective of this work is to study the possibility of OMW treatment by a simple filtration process, effective and conform the requirements of the national environment law. So we will be interested in looking for an alternative treatment for the purification of effluents loaded with polyphenols, it is about the filtration on an Activated Carbon (AC) and the Calcined Sand (CS) , then we conducted a phytochemical study on a raw and filtered OMW, in order to determine the polyphenolic families present in OMW and their filtrate and finally we evaluated the antioxidant activity with three methods (DPPH, ABTS and FRAP).

Methods

Olive mill wastewater sample

The sample used in our study was obtained from the olives of the Moroccan Picholine variety, pressed in a semi-automatic oil mill located in GUELMIM in 2017. OMW samples are collected in glass vials and stored at 4 ° C until the analysis.

The filtration of OMW on AC

Due to its adsorbent effect and its use for the wastewater treatment, AC appears as a good filtration material to remove the phenolic compounds responsible for staining OMW (Figure 1).

We used for filtration a volume of 100mL to filter it on a column containing two layers of CS of 20 g each and 10 g of AC.

The evaluation of the OMW filtration treatment is carried out by measuring the physicochemical characteristics in the OMWs and their filtrates.

Physico-chemical characteristics of raw and filtered OMW

The parameters determined on the raw and the filtered OMW are:

The pH, chloride ions (Cl-), dry matter (DM), Suspended Solids (SS), mineral matter (MM), Volatile Solids (VS), Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD5).

We proceeded to the filtration of the raw OMW on Wattman filter paper before the determination of the chlorides, COD and BOD5, while the other parameters are determined on the raw OMW.

The determination of all these parameters was done in duplicate according to the Standard Methods described below:

The pH measurement gives information on the free (H+) ions in the solution, it is carried out using a pH meter after standardization with buffer solutions (pH = 4 and pH = 9). During the measurement, the sample is kept under stirring. The technique used is the electrometric method recommended by [13]. Electrical conductivity and salinity were measured using a conductivity meter [14]. The volatile Solids is determined by differentiating between the dry matter obtained by evaporation at 105 °C. and the ash residues resulting from the calcination at 550 ° C. for 2 hours. The mineral matter was determined after mineralization at 550 ° C in an oven for 24 hours. Suspended Solids is determined by filtration on 0.45 µm diameter porosity filters and drying in an oven at 105 ° C for 24 hours [15].

Dry matter is determined by calculating the difference between the weight of the wet sample and that of the dried sample [16]. The chloride ions were determined according to the standard [17], by the method of Mohr titrimetry. The chemical oxygen demand (COD) was determined according to the standard method [18] by oxidation of the organic matter contained in the sample at 150 °C by an excess of potassium dichromate in an acidic medium and in the presence of silver sulphate. Excess of potassium dichromate was measured by colorimetry using a UV spectrophotometer at 620 nm. The chemical oxygen demand (COD) was determined by closed-loop dichromate colorimetric method. For BOD5 [19], the diluted samples were incubated in a BOD-meter which gives the amount of oxygen consumed by the bacteria for 5 days at 20 °C and in the dark.

Extraction of OMW polyphenols before and after filtration

The filtered OMW (single or on AC) is extracted three times with hexane. The hydrosoluble polyphenols are extracted from OMW delipidated three times with ethyl acetate (2v/v). The pH of the resulting aqueous phase of the hydrosoluble polyphenols is brought to 2 with HCl (6N) The acidified solution is heated at 100 ° C. for 4 hours, then extract three times with ethyl acetate (v/v) to recover the fractions of the bound polyphenols. [20, 21].

Middle Infrared spectroscopy analysis

FT-MIR spectra of duplicate samples were obtained using a FT/IR-4600 spectrometer possessing an Attenuated Total Reflectance (ATR) Type Pro One. The measurements were obtained with crude samples deposited on an attenuated reflection cell equipped with a diamond crystal. The generated spectra showed wavenumbers ranging between 400 and 4000 cm–1.

Phytochemical Screening

The detection tests for the large groups of chemical compounds have focused on the hydrosoluble and bound fractions according to the protocols described in the work of Y. A. BÉKRO et al. [22].

Phytochemical study

Determination of total phenolic content

The total phenolic contents were determined according to Folin-Ciocalteu method as described by Lister and Wilson [23]. To 0.5 mL of sample solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water 1:10, followed by the addition of 4 mL of Na2CO3 (7.5 %, w/v). The mixture is then incubated in a water bath at 45°C for 30 min and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer against blank sample. The total phenolic content was measured as Gallic acid equivalents (mg GAE/g of extract).

Determination of flavonoids content

The flavonoid assay of our extracts is carried out by the colorimetric method described by Dewanto et al. [24]. 1 mL of a precise concentration of the extract was placed in a tube containing 6.4 mL of distilled water. Then a volume of 0.3 mL of a solution of sodium nitrite (NaNO2) (5%) was added. After 5 min, 0.3 mL of 10% aluminum chloride (AlCl3) was added to the mixture. The whole thing is left for 6 min. Then 2 mL of sodium hydroxide (1M) was added to the tubes, the tubes were well mixed and allowed to rest for 30 min. Absorbances were measured at 510 nm. The total flavonoids content was measured as Quercetin equivalents (mg QE/g of extract).

Determination of condensed tannins content

The determination of condensed tannins (proanthocyanidins) was according to the method of Sun et al. [25]. 100 µL of diluted sample, 3 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 15 min, and absorbance was read at 500 nm against water/ethanol mix as a blank. The total condensed tannins is expressed as mg of catechin/g of extract. The sample was analyzed in triplicate.

Determination of total sugars content

Total sugars are determined according to the method of Dubois et al. [26]. 1 mL of sample was

added to 1 mL of phenol (5%) and 5 mL of concentrated sulfuric acid. Stir and allowed to rest for 10 min at ambient temperature. The mixture is then incubated in a water bath at 30°C for 20 min. Measuring the yellow-orange color at 488 nm, the values obtained are translated into glucose concentrations by reference to a previously established calibration curve.

Evaluation of antioxidant activity

Researchers suggested extracting the phenolic compounds from olive oil effluents in order to valorize them as natural antioxidants. The evaluation of the antioxidant activity using three methods: the 2.2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method [27], the ferric reducing antioxidant power (FRAP) method [28] and ABTS radical scavenging assay [29].

DPPH Assay

The free radical scavenging activity of the OMW extracts was measured by DPPH, with some modifications. Briefly, 0.2 mM solution of DPPH in methanol was prepared and 0.5 mL of this solution was added to 2.5 mL of extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

FRAP Assay

The FRAP method was used to measure the reducing capacity of the OMW extracts with a minor modification, which involves the presence of extracts to reduce the ferricyanide complex to the ferrous form. Various concentrations of extracts from the stock solutions and the standard (Vitamin C) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Then 2.5 mL of trichloroacetic acid (10% w/v) was added to the reaction mixture. Afterward, it was centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with deionized water (2.5 mL) and 0.5 mL of ferric chloride (0.1% w/v). The absorbance was read at 700 nm. The EC50 concentration, which is defined as the effective concentration at which the absorbance is 0.5, is an index used to compare and express the potency of the reducing capabilities of bioactive substances.

ABTS Assay

The ABTS radical was determined by following the method described by Arnao et al. [29]. The solution of 2 mM ABTS and 70 mM potassium persulphate ($K_2S_2O_8$) in equal volumes were allowed to stand in the dark for 12-16 h at room temperature. Prior to assay, ABTS solution was diluted in methanol to give an absorbance of (0.70 ± 0.02) at 734 nm. 2 mL of the resulting solutions was allowed to react with 200 µL of the extracts with different concentrations, the absorbance was measured at 734 nm after 30 min. The same was done for the Trolox standard of various concentrations (5 – 60 µg/mL).

Statistical Analyses

Data were calculated as means ± SD. Pearson's correction analysis (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA) was used to test for the significance of the relationship between the concentration and percentage inhibition.

Results and Discussion

Filtration of OMW on AC

The study of the filtration of OMW on AC allows us to know if this material has an important effect on the physicochemical parameters of the OMW. The filtered OMW on the AC + CS column gave us a filtrate of 80 mL and a clear color.

Physico-chemical characteristics of raw and filtered OMW

Table 1 shows the results from the analysis of common physicochemical parameters and chemical analysis of the wastewaters from the olive processing industry before and after filtration.

According to the results, the OMW is a slightly acidic effluent with a significant pollutant load in terms of COD 300 g O_2/L and BOD_5 35 g O_2/L. It is very rich in suspended solids 131g/L, and chlorides 17.04 g/L.

The OMW which is from a semi-modern three-phase process, shows high salinity and therefore higher electrical conductivity (EC). The high content of chloride ions is probably related to the fact that

some producers use salt for conservation of olives until milling.

The pH of OMW evolves with the increase of the amount of activated carbon which indicates that the activated carbon captures the H+ ions by allowing the pH of the filtrate to be located in the neutral pH zone. The same observation was made by Mekki et al. [30] where a pH increase of three units occurred after OMW was applied to soil.

In general, all the pH values of these tests can be accepted because the Moroccan environmental legislation requires that the pH of the rejections of the olive oil industry be between 6 and 9. the environmental legislation limit, set at 0.5 mg/L for phenolic compounds [31] and 3 mS/cm for EC [32].

While the dry matter of the OMW has undergone a significant reduction which successively reaches 81% following the use of activated carbon. This drop in dry matter is proportional to the amount of activated carbon in the filtration column. Activated carbon is, therefore, good material for the retention of the dry matter of the OMW.

The DCO and DBO5 assays of margins exhibit high values of biodegradable and non-biodegradable organic pollutants respectively [DCO 300 gO2/L et DBO5 35 gO2/L], which largely exceeds the limit values set by the VLGR. However, the filtrate has a reduction of 99.8% of DCO and 99.5% of DBO5. These results are similar to those reported by Zenjari and Nejmddine [33], using a treatment with soil microorganisms.

The Environmental legislation in Morocco requires that the discharges from the olive oil industry should have a COD demand that does not exceed 0.5 g O2 / L and a BOD5 demand that does not exceed 0.1 g O2 / L. These results explain that AC filtration treatment makes these effluents biodegradable with ratios of * COD / DBO5 of the order 2.09. Generally, the ratio obtained is between 1.25 and 2.5. When the COD / BOD5 ratio is higher, between 3 and 7, wastewaters can be difficult to biodegrade.

Yields extracting of OMW before and after filtration

From the results shown in the table, it is noted that the yields of hydrosoluble and bound fractions are totally reduced with a decrease of 69% for the HF and 95% for the BF.

These results confirm that the filtration OMW on activated carbon is more efficient in removing these effluents from their organic loads.

Infrared Spectra analysis

The IR spectra of four OMW extracts show similar characteristic features with some differences in bands intensity (Figure 2 and 3). The OH stretching at 3360 cm–1 from many sources (including water) is very similar and shows higher intensity [34]. The band at 2940 cm–1 from aliphatic C-H stretching (with the band at 1450 cm–1 from single bond vibrations) shows important intensity and points to important aliphatic moieties. OMW extract shows a distinct band at 1700 cm-1 attributed to the vibration of the ketone (C=O) with a large intensity. The band 1625 cm–1, which is attributed to C-C bonds conjugated with C-O and COO– groups. The OMW extracts show smaller intensity of the peak at 1400 cm–1 corresponding to COO– vibrations [35]. The detected functions probably suggest the presence of the main constituents of OMW: fatty acids and phenolic compounds.

Phytochemical Screening

The results of the phytochemical screening of the species are summarized in the table 3.

According to the table, we notice that catechic Tannins, saponosides, sterols and polyterpenes and proteins are absent in all extracts

On the other hand, with the exception of the coumarins that are present in the HF extract of the raw OMW, the flavonoids, gallic tannins are widely present in all the extracts. the HF extract of the raw OMW also contains alkaloids.

The presence of these phenolic compounds (total phenolics, tannins, and flavonoids) provides pharmacological activities such as anti-cancer activity [36,37], antioxidant [37-39], antimicrobial [40,41], anti-inflammatory [42,43] as well as the healing from wounds [44].

Phytochemical study

The values of phenolic content are given in the table 4.

The Polyphenol concentration was determined using gallic acid as standard from the fitting curve (y = 0.0074 x + 0.0465; R2 = 0.9968); flavonoid concentration was determined using quercetin as standard from the fitting curve (y = 0.0077 x + 0.028; R2 = 0.9988); tannin concentration was determined using catechin as standard from the fitting curve (y = 0.0023 x + 0.0817; R2 = 0.9881), and sugar concentration was determined using D-glucose as standard from the fitting curve (y = 0.009 x + 0.0988; R2 = 0.9926).

The variation of the OMW phenolic content and its antioxidant properties are affected by many factors such as olive cultivar, the olive oil extraction process, the physicochemical characteristics of OMW samples, the fungal and bacterial flora existing in OMW, and finally the storage conditions.

This high content of phenolic compounds gives the OMW, on the one hand, an antimicrobial property and, on the other hand, a phytotoxic effect [45,46], which hinders their purification by biological mean.

Given the antioxidant power of polyphenols, they can be used as natural antioxidants instead of synthetic antioxidants to protect food oils against oxidation [47], and give them a pronounced organoleptic quality [48].

Previous studies on the degradation of polyphenols in its original OMW matrix during the extraction process and upon storage revealed their poor stability due to the complex and reactive nature of OMW, where oxidation, condensation, polymerization, and enzymatic hydrolysis can all potentially take place [49,50]. It has been reported previously that OMW from semi-modern three-phase process has higher phenolic content than OMW from modern three- phase process [51].

Flavonoids, the most diverse and the largest group of natural phenolic compounds, are known to have antioxidant, antiallergic, antimicrobial, anti-inflammatory, and anticarcinogenic properties [52].

According to these results, we observe that the phenolic and flavonoid contents of hydrosoluble are very important that those of bound fractions. There are slight decreases in the levels after filtering OMW on activated carbon. While the bound fraction of the raw OMW is very rich in tannins, this is due to the acidification step which totally hydrolyzed the condensed tannins, on the other hand, the bound fraction of AC filtrate represents a low content, which explains why activated carbon has captured the condensed tannin molecules. The sugar content has been slightly increased during AC filtration.

Evaluation of antioxidant activity

The values of IC50 of (DPPH, ABTS), and l'EC50 of FRAP are given in the table 5.

We notice that the two extracts of the hydrosoluble fraction represent a very strong reducing power with IC50 values comparable to that of Vitamin C. Furthermore, extracts of the bound fraction gave lower antioxidant capacity with high IC50. For the FRAP test, it is observed that the two extracts of the bound fraction have very high EC50 values. We noticed that for the antioxidant activity, either by the DPPH, ABTS or FRAP reduction method, that the two extracts of the hydrosoluble fraction of raw OMW or AC-filtered OMW represent the highest antiradical activity. On the other hand, the two extracts of the bound fraction represent the weakest antiradical activity.

The phenolic extracts of OMW exhibited a good antioxidant potential (Table 5), this is mainly due to the presence of polyphenols, that are known to be powerful sensors or "scavengers" of free radicals [53, 54]. The results obtained from our free radical scavenging assays using the OMW phenolic extracts is further confirmed by the previously reported studies, where the powerful antioxidant effect of phenolic extracts is demonstrated [53,55-59].

The antioxidant potency of the phenolic extracts can be explained by the content of simple phenols, phenolic acids and in particular o-diphenols (hydroxytyrosol, 3,4-dihydroxyphenylacetic acid, caffeic acid, etc.).

Bouaziz et al. [55], have reported an IC50 value 0.65 mg / L of for phenolics extracted from hydrolysed olive leaves (acidic) which was due to its high hydroxytyrosol content (hydroxytyrosol: IC50 = 0.58 mg / L), while being more important than that of oleuropein (Oleuropein: IC50 = 1.19 mg / L).

The IC50 values of these four extracts suggest that they contain concentrations of hydroxytyrosol, which comes from the hydration of oleuropein.

The phenolic acid extracts with ethyl acetate exhibit a potential for the elimination of the DPPH radicals similar to that of synthetic antioxidants. Nevertheless, the extracts of the leafs obtained with aqueous alkaline solutions show a high effect of trapping the free radicals [55] cited by Condes et al. [58], probably because of a higher concentration of oleuropein and hydroxytyrosol in these extracts.

Statistical analysis

Two-tailed Pearson's correlation was determined to establish the relationship between the antioxidant activity and the contents of tannins, Sugars, polyphenolic and flavonoids compounds.

The results revealed excellent negative correlations between the antioxidant activity (IC50) of OMW and its content of polyphenolic ($p = 0.003$) and flavonoids compounds ($p = 0.002$) in DPPH scavenging assays.

The antioxidant activity and phenolic and flavonoids compounds are inversely proportional.

A strong significant correlation between the content of tannins compounds and the antioxidant activity displayed by the OMW has been observed ($p = 0.001$).

Conclusion

During this study, the filtration tests of the OMW on activated carbon gave us good results to exploit for the purification of OMW. The use of 10 g of activated carbon in the form of a layer allows the purification of 80 ml of OMW and the recovery of 50 ml of the filtrate whose physicochemical characteristics are as follows: neutral pH, very low dry matter content, zero total polyphenols content and zero chemical and biological oxygen demand. For the extracts we identified the chemical families, and we could not detect catechin tannins, sterols, saponosides, and proteins in all extracts of raw OMW and filtered OMW. The spectrophotometric assays reveal an appreciable amount of polyphenols and flavonoids especially in the extracts of the hydrosoluble fraction. The results of the DPPH, ABTS and FRAP tests showed that the two extracts of the hydrosoluble fraction have greater anti-radical power than those of related fractional extracts. For these reasons, OMW deserve further study.

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Table 1. Physico-chemical characteristics of raw and filtered OMW

Parameters	OMW before filtration	OMW after filtration
pH	5.18 ± 0.05	7.6 ± 0.05
EC (mS/cm)	16.36 ± 0.05	2.9 ± 0.05
DM (%)	24.3 ± 0.5	6.3 ± 0.5
Mineral matter (g/L)	39 ± 0.7	11 ± 0.2
Humidity (%)	75.7 ± 0.5	94.7 ± 0.5
SS (g/L)	131 ± 8	0.27 ± 0.01
Volatile Solids (g/L)	217.6 ± 10.9	55± 4
Chlorides (Cl-) (g/L)	17.04 ± 0.06	4.26 ± 0.02
Nitrogen (N)	>1	< 1
COD (mgO2/L)	300000	335
BOD5 (mgO2/L)	35000	160
COD/ BOD5	8.57	2.09

Table 2. Yields extracting of OMW before and after filtration

Fraction	Yield (%) of OMW before filtration	Yield (%) of OMW after filtration
Fats	0,010 ± 0,001	-
Hydrosoluble fraction (HF)	1,79 ± 0,21	0,56 ± 0,05
Bound fraction (BF)	8,49 ± 0,62	0.44± 0,03

Table 3. Phytochemical screening of different OMW extracts

Chemical groups	OMW before filtration		OMW after filtration	
	HF Extract	BF Extract	HF Extract	BF Extract
Coumarins	-	+	-	+
Flavonoids	++	+	++	+
Alkaloids	+	-	-	-
Saponosides	-	-	-	-
Catechic Tannins	-	-	-	-
Gallic Tannins	+	++	+	+
Sterols and polyterpenes	-	-	-	-
Proteins	-	-	-	-
Sugars	+	+	+	+

+ +: certain presence / +: uncertain presence / -: absence

Table 4. Total phenolic, flavonoid, tannin and sugar content

Test	OMW before filtration		OMW after filtration	
	HF Extract	BF Extract	HF Extract	BF Extract
TPC (mg GAE/ 1g of Extract)	160.8 ± 5.5	56.8 ± 0.4	155.3 ± 1.1	46.5 ± 1.1
TFC (mg QE/ 1g of Extract)	187 .3± 4.9	28.1 ± 1.2	165.1 ± 3.9	15.2 ± 0.6
TTC (mg CE/ 1g of Extract)	16.0 ± 0.3	53.4 ± 0.9	8.6 ± 0.9	9.3 ± 0.6
TSC (mg D-Glu E/ 1g of Extract)	97.2 ± 4.3	75.8 ± 5.1	82.1 ±5.6	70.4 ±5.0

Values are given as means of triplicates ± SD, TPC: Total Phenolic Content, TFC: Total Flavonoid Content, TTC: Total Tannin Content, TSC: Total Sugar Content, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, CE: Catechin Equivalent, D-Glu E: D-Glucose Equivalent.

Table 5. Antioxidant activity of different OMW extracts.

Test	OMW before filtration		OMW after filtration		Standard
	HF Extract	BF Extract	HF Extract	BF Extract	
DPPH IC50 (µg/mL)	7.3 ± 0.4	71.0 ± 1.2	8.0 ± 0.4	94.7 ± 1.4	1.91 ± 0.04 (Vitamin C)
FRAP EC50 (µg/mL)	138.6 ± 3.8	339.8 ± 10.6	161.0 ± 5.1	1002.6 ± 25	45.8 ± 1.9 (Vitamin C)
ABTS IC50 (µg/mL)	36.2 ± 3.3	132.3 ± 6.7	43.3 ± 5.2	189 ± 7.4	30.85 ± 2.1 (Trolox)

Values are given as means of triplicates ± SD, DPPH, ABTS, FRAP: See Text, IC50: The inhibitory concentration of the extract needed to inhibit 50% of the DPPH radicals obtained from the standard curve was compared to that of standard/commercial antioxidants (vitamin C and trolox).

Table 6. Pearson Correlation between olive mill wastewater content and antioxidant activity

	Polyphenols	Flavonoids	Tannins	Sugars
Antioxidant activity	-0.997**	-0.998**	0.999**	-0.950

** p<0,01

Figure 1. OMW filtration assembly on AC layer.

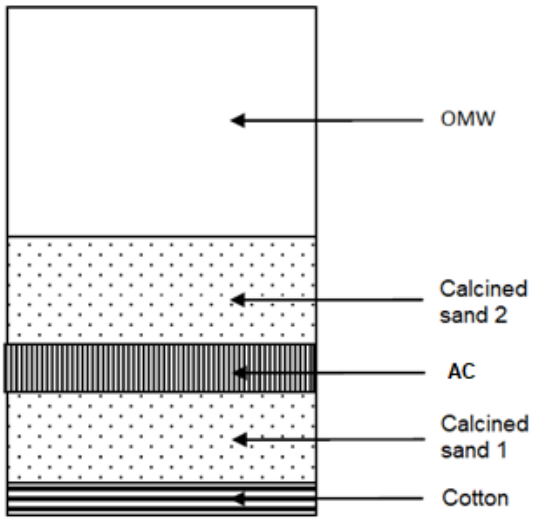


Figure 2. IR Spectra of two OMW HF extracts originating from Guelmim

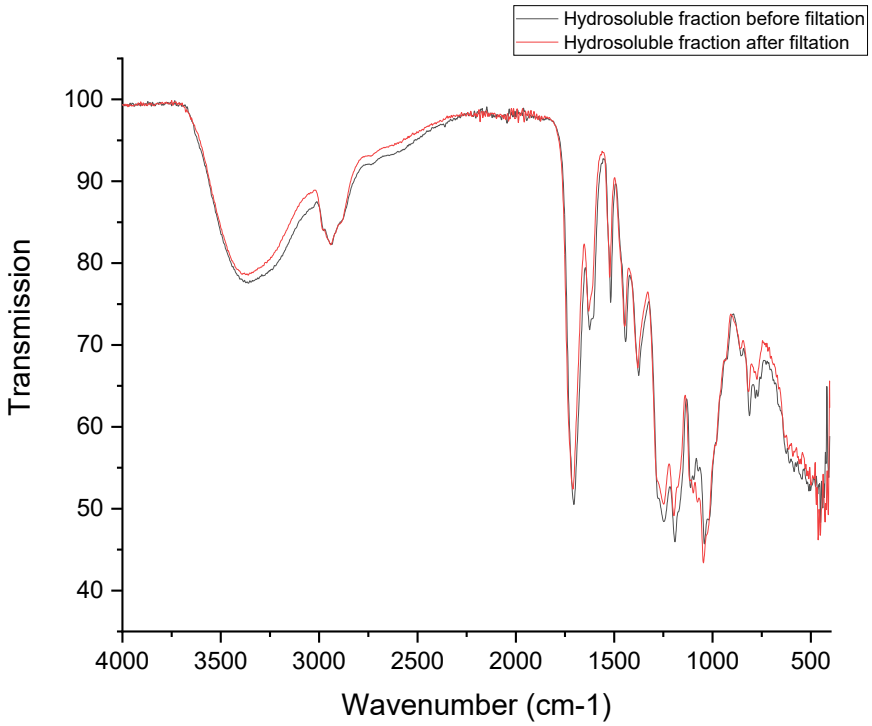
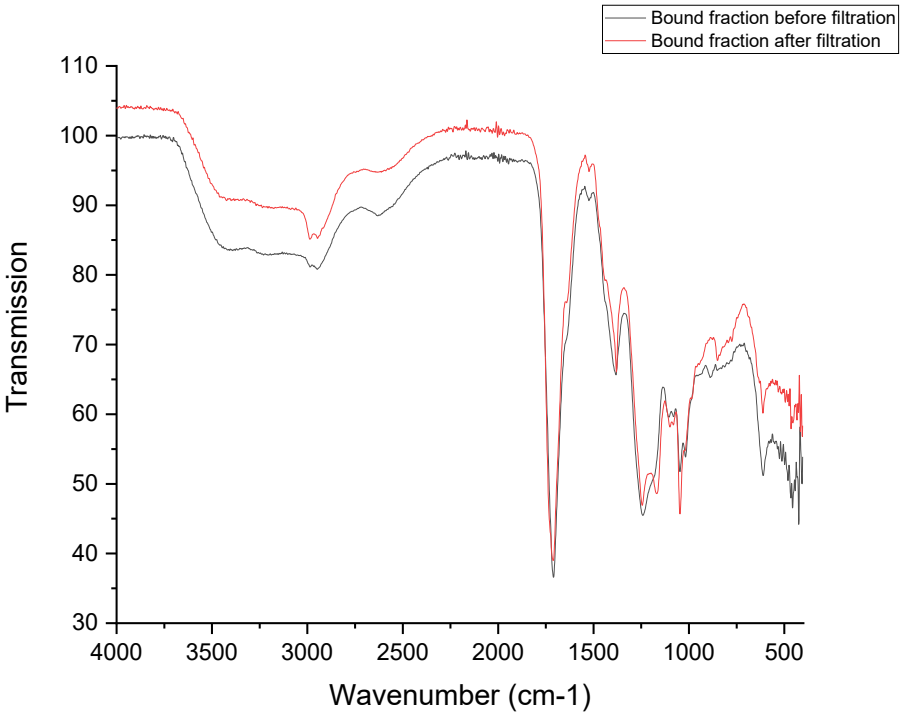


Figure 3. IR Spectra of two OMW BH extracts, the IR spectra were similar. The band identities were so: 3360 cm–1: (OH), 2940 cm–1:(C-H), 1700 cm-1: (C=O), 1625 cm–1: (C=C), and 1450 cm–1: (COO–).


Bound fraction before filtration
Bound fraction after filtration
Transmission
110
100
90
80
70
60
50
40
30
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)