Valorization of Margins: Physico-Chemical Characterization and Bactericidal Effect of Vegetable Waters

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Valorization of margins: Physico-chemical characterization and bactericidal effect of vegetable waters

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Abstract

Mediterranean countries are known for their olive oil production, which accounts for 97% of global production. Morocco is one of the largest producers of olive oil, and this industry is beneficial for the national economy. In particular, the Beni Mellal-Khenifra region is renowned for this industry, which not only produces olive oil but also generates vegetable water that have a detrimental effect on the environment, including surface and groundwater pollution, as well as emitting unpleasant odors. This study focuses on the valorization of these waste products, whether they come from pressing (traditional) or centrifugation (modern) processes, by characterizing their physicochemical composition and measuring the polyphenols. The obtained values range from 0.8 g/l to 1.17 g/l, highlighting that margines from the centrifugation (modern) process are richer in polyphenols than those from pressing (traditional). Using the solid diffusion method, we determined the sensitivity of four bacterial species to the vegetable water, namely Bacillus megaterium, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. These bacteria showed sensitivity to the margines, with Gram-positive bacteria being more sensitive. This effect is linked to the bioactive compounds present in the margines, such as polyphenols.

Keywords: vegetable waters*, antibacterial effect, polyphenols, bacteria*

1. Introduction

Among the primordial activities of Man, the olive industry occupies an important place due to its large production of olive oil. Mediterranean countries are particularly concerned by this activity, which represents 97% of world production [1]. In addition to olive oil, this industry also generates residues such as pomace (solid) and vegetable oil (liquid) [1].

Morocco is one of the largest producers of olive oil, and this industry is beneficial to the national economy, generating more than 15 million workdays per year, the equivalent of 70,000 full-time jobs full [2]. This activity allows the Béni Mellal-Khénifra region to contribute 17% to the national production of olive oil . In addition to olive oil, this activity generates vegetable water, also called olive oil mill waste water.

These vegetable waters are characterized by a brown-black color, a strong odor and are considered a residue posing serious environmental problems. They cause pollution of surfaces and soils, as well as emissions of unpleasant odors due to high concentrations of organic matter and polyphenols. Due to their polluting properties, much research focuses on the valorization of vegetable waters in various fields such as composting, agriculture and even the pharmaceutical industry [3].

The natural bio-transformation of vegetable waters can produce high-value molecules added; the identification of these metabolites is essential in the strategy of choice of biologically active molecules. By applying bioconversion technologies to egetable waters in a bioreactor, the bioconversion of the substrate (oleuropein, hydroxytyrosol glucosides, ...) into these degradation metabolites, was carried out on a large scale [4].

In addition, research is focused on the valorization of these effluents in various areas: composting, agriculture and even in the pharmaceutical industry.

Until today, the treatment of vegetable waters constitutes a complex problem given the quality and quantity of the chemical substances they contain. In fact, the application of a simple treatment turns out to be insufficient and incomplete [5]. Even though he does not yet exist a perfect solution allowing the treatment of vegetable waters, some processes appear to be more effective than others.

In this study, our objectives are to valorize the vegetable waters of Béni Mellal-Khénifra by characterizing them; physicochemically and by evaluating their antibacterial power on four species of bacteria.

2. Material and Methods

2.1. Sampling

The vegetable water samples were taken mainly at 4 stations (2 traditional crushing units, and 2 modern) in the town of Zaouiat cheikh in the region of Béni Mellal Khénifra.

The stations of the study samples:

Sample	Extraction process	Place	Name of station	Volume
Sample 1	pressing	Zaouiat cheikh	Bilal	2 L
Sample 2	pressing	Zaouiat cheikh	Tighboula	2L
Sample 3	centrifugation	Zaouiat cheikh	Boujdour	2L
Sample 4	centrifugation	Zaouiat cheikh	Ikour	2L

The experimental work of this study was carried out in the laboratory of the polydisciplinary faculty of Béni Mellal during the period MARCH-JUNE 2023.

2.2. Analysis methods

All samples were homogenized before analysis. The analyzes carried out are as follows:

* pH: Measurements were taken at room temperature using a pH meter fitted with an electrode of glass and a combined reference electrode.

* Electrical Conductivity (EC): This electrochemical property is based on the fact that the conductance of a solution increases as the concentrations of cations and anions, carriers of charges electrical, are increasing. The measurement is done in mS/cm using a conductivity meter.

*Nitrogen (N): Determined by the Kjeldahl method which consists of mineralization of the organic nitrogen of the samples in sulfuric acid in the presence of a catalyst ($K2SO4$, selenium). The ammoniacal nitrogen thus formed is then determined by acidimetry, after distillation, in the presence of a colored indicator (methyl red and methylene blue), according to the standard (AFNOR T90-110).

*Chemical Oxygen Demand (COD): COD applies equally to mineral substances and organic. It allows the measurement of all oxidizable substances, which includes those which are biodegradable. It is expressed in mg of O2/L using oxidation reactions, while measuring the reagent residue after 2 hours. Oxidation takes place hot, in an acidic environment, in the presence of a excess oxidant.

*Biological Oxygen Demand (BOD): It is measured after 5 days (BOD5), at 20°C (temperature favorable to the activity of O2-consuming microorganisms) and darkness (in order to avoid any parasitic photosynthesis). The BOD5 constitutes a good indicator of the biodegradable OM content. The results are expressed in mg O2/L.

*biodegradability index (Ib): The COD/BOD5 ratio makes it possible to determine Ib, an indicator of the importance of polluting materials with little or no biodegradability (Rodier. 1996). If :

Ib> 6 Hardly biodegradable substrate

3 <Ib> 6 Partially (or less easily) biodegradable substrate

Ib< 3 Very easily biodegradable substrate

*Dry matter is presented by all the organic and inorganic compounds found in the vegetable waters in solution or suspension.To measure the dry matter present in our vegetable waters, we used 10 g of vegetable water for each sample placed in Petri dishes. Then, the boxes were placed in an oven at 105°C for 48 hours. Subsequently, we measured the difference in weight for each sample, divided by 10 and multiplied by 100.

dosage of polyphenols is as follows:

-Dilution of vegetable water by adding 1 ml to 9 ml of distilled water

- adding 100 µl of the final dilution to 500 µl Folins reagent in a cuvette

-After 10 min, 400 µl of sodium carbonate are added.

-At the end the cuvettes were incubated for 2 hours at room temperature and in the dark (to inhibit the photodegradation of polyphenols). This process was repeated three times for each sample (3 replicates). Finally, their optical densities were measured by Spectrophotometry at Anda = 760. The results are expressed in gallic acid equivalents.

2.3. Antibacterial activity of vegetable water

Four reference bacterial species are chosen for the study of antibacterial activity. These strains were provided to us by the laboratory of the polydisciplinary faculty of Béni Mellal, which are Pseudomonas aeroginosa, Klebsiella pneumoniae, Escherichia coli, and Bacillus megaterium

The culture media used during this study are:

-Mueller-Hinton (MH) agar: is a standardized medium recommended for studying the sensitivity of bacteria to antibiotics.

-LB culture medium (for lysogeny broth): is a non-selective nutrient medium originally used for the cultivation of bacteria.

3. Results and discussion

3.1. Physico-chemical characterization of vegetable waters

The pH values of the raw vegetable waters are presented in the table below (Table 1). The results indicate the acidic character of the vegetable waters, whose acidity varies between 4.18 as a minimum value and 4.91 as a maximum value for our samples. These values are comparable to those obtained by Abdelkhalek and Stout [6], and to those recorded by Zaier [7]. Additionally, Esmail et al [8] found values varying between 4.65 and 5.16 in their studies.

The acidity of the vegetable waters is linked to their storage duration in the storage basins. This can be explained by auto-oxidation and polymerization reactions which transform phenolic alcohols into phenolic acids. These reactions are manifested by a change in the initial coloring of the vegetable waters towards a very dark black. Indeed, our vegetable waters are characterized by a very dark coloring [8]. It should be noted that in our results, the centrifugation system gives more acidic vegetable juices (between 4.18 and 4.49) than those of the press system (between 4.84 and 4.91).

Table 1. : Qualitative characterization of liquid olive effluents in the raw state

Paramètres	Unité	Results By centrifugation		Results By Press		Références
Sample		Ikour	Boujdour	Tighbolat	Bilal	
pH		4,18	4,49	4,84	4,91	Eroglu et al. 2008
Electrical conductivity	mS/cm	14.9	11.88	17.18	17.09	Di Serio et al. 2008
		15.5	26.8	27.7	29.8	
Polyphénols	g/L	1.17	0.95	0.95	0.85	Fenice et al. 2003
Chemical Oxygen Demand (DCO) g/L	g/L	70.12	68.98	78.12	71.56	Fenice et al. 2003
Biological Oxygen Demand(DBO5)	g/L	18	13.3	20	20	Fiestas Ros de Ursinos. 1992
Dosage de L'azote Kjeldahl (NTK)	g N/L	0.79	1.2	0.94	1.4	

Electrical conductivity is closely linked to the concentration of dissolved substances and their nature. For the conductivity of our water bottles, it is presented in Table 1 below, with a minimum value of 11.88 and a maximum value of 17.18. We note that our vegetable water samples have a conductivity higher than that found by Abdelkhalek and Stout [6] and lower than those studied by Aissam [9], while being comparable to those obtained by Esmail and al. [8]. It should be noted that in our results, the press crushing system gives high conductivity juices (between 17.09 and 17.18) than those of the centrifugation system (between 14.9 and 11.88).

The mineral composition of the different samples studied showed that these wastewaters have a high saline load, mainly due to sodium chlorides. This salt load is probably linked to the practice of salting to preserve the olives until they are crushed, in addition to the natural richness of the olives in mineral salts [10].

The dry matter content of each sample is calculated and presented in Table 1, where our results oscillate between 15.50% and 29.80%. We note that the dry matter content of our samples is lower than those studied by Zaier and al [7].

The BOD5 (biological oxygen demand) is recorded after 5 days of incubation in the dark. Our BOD5 results are collected and organized in the table below. The results obtained are between 13.3 and 20 g/l, which is lower than those cited by Zaier and al. in 2017[7], who found 86.71 g/l (Tunisian margins), but can be compared to those of Esmail and al. [8], who found values between 25 g/l and 35 g/l and also with Djeziri, and al [11] who recorded 29 g/L. This shows an average oxygen demand for the complete oxidation of the organic matter contained in these effluents, which reflects their average polluting power [7].

Our recorded results are between 68.98 g O2.l-1 as the minimum value and 78.12 g O2.l-1 as the maximum value. These results are lower than those recorded by Djeziri and al. [11], who obtained 90.5 g of O2.l-1, and is close to those of Esmail and al.[8] , who found values between 76.180 g/l and 104.31 g/l. However, they are lower than those obtained by Aissam [9], which were of the order of 154 g of O2.l-1. These results show that vegetable waters have a high oxygen demand for the complete oxidation of the organic matter present [9].

Our samples present values oscillating between 0.854 and 1.17 g/l of total polyphenols, these values being lower than those reported by Abdelkhalek and Stout [6] who found 4.3 g/l, and also lower than those of Zaier and al. [7] who found values of around 5.23 g.l-1. Aissam also recorded 9.7 g/l [9] . Our results are higher than the values found by Esmail and al.[8]. This is also explained by the nature of the vegetable waters, since the latter are not fresh vegetable waters, they come from the storage basins after the crushing process. Phenolic compounds suffered degradation during storage and consequently a reduction in their content, which explains our results. The phenolic composition of vegetable oils depends not only on the variety, fruit maturity and climatic conditions, but also on the technological processes used to separate the aqueous phase (margines) from the oily phase [8]. According to Jail et al. [12], the solubilization of polyphenols in oil is lower than that in vegetation water, which explains their high concentration in vegetable waters. It should be noted that these polyphenols confer antimicrobial power to vegetable waters [6].

Our results (Table 1) show that the NTK content varies between 0.79 (minimum value) and 1.4 (maximum value). These values are almost comparable to those recorded by Mebirouk et al. [12], and remain higher than those cited by Esmail and al. [8], which are of the order of 0.0476, 0.0812 and 0.0756 g N/L.

These results can be explained by several factors. First of all, the chemical composition of vegetable oils can vary depending on the variety of olives, their maturity and climatic conditions. In addition, the technological processes used to separate the aqueous phase (margines) from the oily phase [13].

3.2. Antibacterial activity of vegetable oils

3.2.1 Antibacterial activity of vegetable oils against the bacterium pseudomonas aeroginosa

The results of the inhibition of bacterial growth of Pseudomonas aeruginosa by vegetable waters are presented in the Fig1 below.

Our results show that P. aeruginosa is sensitive only to the Ikour (10.87 mm) and Tighbolat (9.75 mm) samples. The inhibition zones of our samples are lower than those found by Esmail and al. [8], who found 19 mm. However, these results remain interesting and indicate the presence of an antibacterial effect of vegetable waters on P. aeruginosa, a bacterium multi-resistant to antibiotics. This difference between our results and those of Esmail and al. [8] may be due to factors such as climate, soil or the treatment system used.

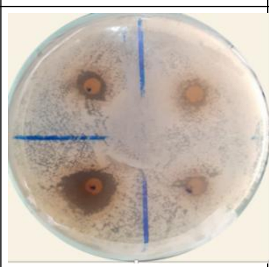
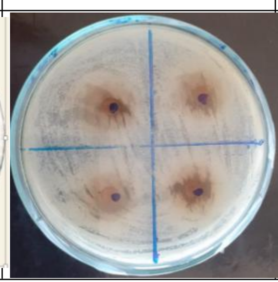
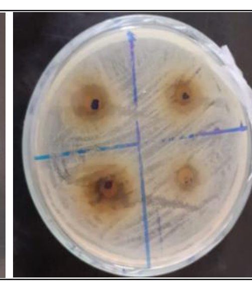
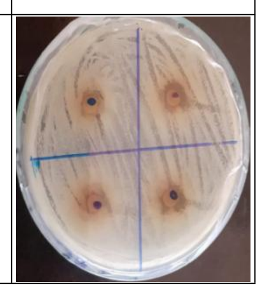
Sample	**Ikour**	**Boujdour**	**Tighbolat**	**Bilal**
Diametre in mm	10, 87	3,35	9,75	6,8
Sensitivity	Sensitive	No Sensitive	Sensitive	No Sensitive
Results				

Fig 1. : Antibacterial activity of vegetable oils against the bacterium pseudomonas aeroginosa

3.2.2 Antibacterial activity of vegetable oils against the bacterium klebsiella pneumoniae

For Klebsiella pneumoniae, the zones of inhibition are represented by the table below (Fig 2). Our results show that Klebsiella is sensitive to all samples, with a diameter varying between 11.87 mm (Boujdour) as a minimum value and 14.62 mm (Ikour) as a maximum value. It should be noted that the inhibition values of this bacteria are higher than those of Pseudomonas. Our results are in agreement with those of Esmail et al.[8], who found an inhibition diameter of around 20 mm.

Sample	Ikour	Boujdour	Tighbolat	Bilal
Diametre in mm	14,62	11,87	12,87	12,75
Sensibility	Sensitive	Sensitive	Sensitive	Sensitive
Results				

Fig 2. : Antibacterial activity of vegetable oils against the bacterium klebsiella pneumoniae

3.2.3 Antibacterial activity of vegetable oils against the bacterium Escherichia coli

For Escherichia coli, the zones of inhibition are represented by the table below (Fig 3). Our results show that Escherichia is sensitive to all samples, with a diameter varying between 12.37 mm (Tighbolat) as a minimum value and 17.85 mm (Ikour) as a maximum value. Our results show that this bacterium (Gram negative) is more sensitive than Pseudomonas (Gram negative). Our results remain comparable to those obtained by Esmail et al. [8] who found an inhibition diameter of around 15 mm, which validates the sensitivity of the E.coli bacteria towards vegetable waters.

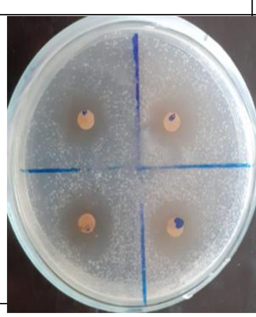
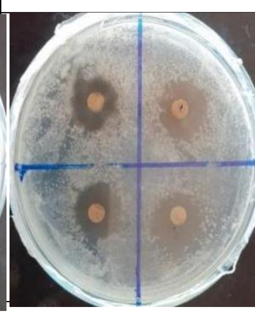
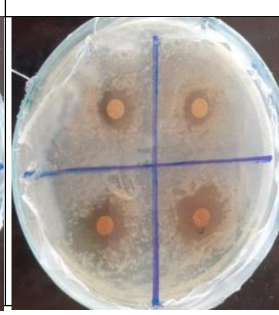
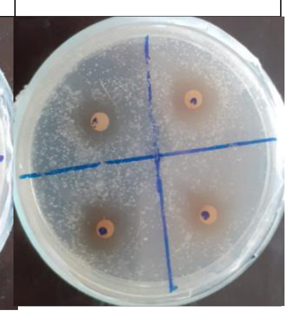
Sample	Ikour	Boujdour	Tighbolat	Bilal
Diametre in mm	17,85	12,7	12,37	13,75
Sensibility	Sensitive	Sensitive	Sensitive	Sensitive
Results				

Fig 3. : Antibacterial activity of vegetable oils against the bacterium Escherichia coli

3.2.4 Antibacterial activity of vegetable oils against the bacterium Bacillus megaterium

The results of the inhibition of bacterial growth of Bacillus megaterium by vegetable waters are presented in the table below (Fig 4). Our results show that Bacillus megaterium is very sensitive to all samples, where the minimum value is 12.87 mm (Boujdour) and 21 mm for Ikour as the maximum value. The inhibition zones of this bacteria are greater than those found for the other bacteria in our study and this is due to the nature of its wall (Gram positive).

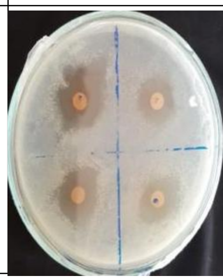
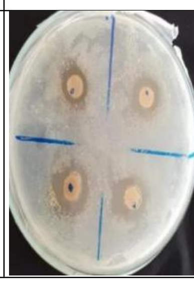
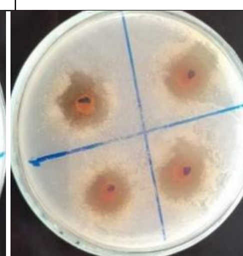
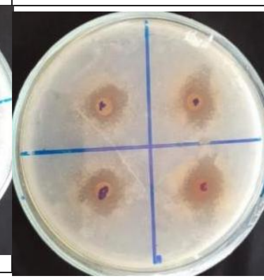
Sample	**Ikour**	**Boujdour**	**Tighbolat**	**Bilal**
Diametre in mm	21	12,87	19,37	18,25
Sensibility	Extremely sensitive	Sensitive	Very sensitive	Very sensitive
Results				

Fig 4. : Antibacterial activity of vegetable oils against the bacterium Bacillus megaterium

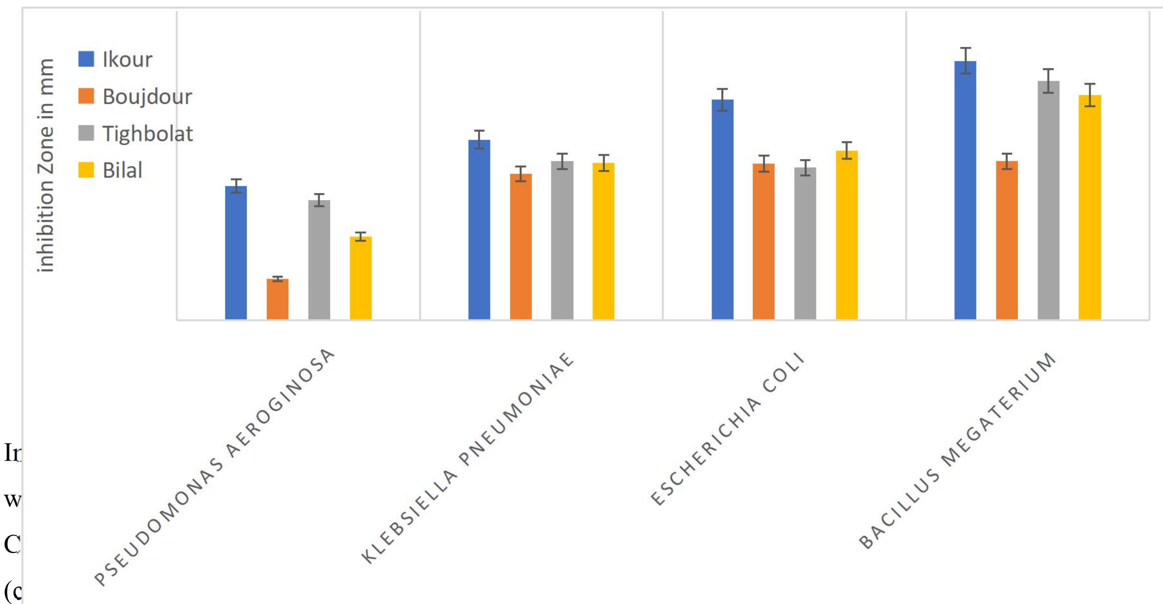
3.2.4 Sensitivity of bacteria to vegetable water oils

According to the results obtained, it is found that Gram-positive bacteria (Bacillus) are more sensitive, with an inhibition diameter reaching 21 mm, than Gram-negative bacteria. This variance can be explained by the nature of the wall, which is made up of lipopolysaccharides in Gram-negative bacteria.

For Gram-negative bacteria, Klebsiella is the most sensitive, followed by Escherichia, and finally Pseudomonas, which is known for its famous antibiotic resistance (Fig 5).

In general, all four samples (Fig 5), whether obtained by centrifugation or pressing, all have a bactericidal effect. However, the Ikour sample, from the modern system, has the highest inhibition value for all bacteria, indicating that the vegetable waters from the modern system are more effective than those from the traditional system. On the other hand, according to the pH and conductivity indices, we can say that the press system generates more polluted vegetable water than those produced by the centrifugation system.

Gram - **Gram +**



Ir getable
w Zaouiat
C system
(c demand
(BOD5), dry matter content, chemical oxygen demand (COD), and Kjeldahl nitrogen content (NTK). Phenolic compounds were also identified and measured using molecular absorption spectrophotometry, these compounds are characterized by their bactericidal power and they showed values between 0.854 g/L and 1.17 g/L.

Regarding the bactericidal effect produced by the direct diffusion method on agar, it was found that the vegetable waters are very effective against Gram-positive bacteria with a diameter varying between 12.87 mm and 21 mm. The maximum inhibition zone was observed in Bacillus megaterium (21 mm), while the minimum inhibition zone was observed in the Gram-negative bacterium Pseudomonas aeroginosa (2 mm). Furthermore, we found that the samples present variable bactericidal effects, where the sample named "Ikour" presents the highest effect values, probably due to the crushing system, the soil, the climate, and the composition of bioactive substances. These results are encouraging because they suggest that olive oil from the olive industry could be used beneficially in various fields, such as agriculture or even the pharmaceutical industry. By recovering these residues, we could not only reduce the environmental problems linked to their elimination, but also exploit their bactericidal potential to fight against bacterial infections.

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