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HPAEC-PAD, biochemical characterization, and evaluation of the antioxidants activities of polysaccharides extracted from Olive Mill Wastewater of two endemic varieties of Khenchela region, Algeria

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ABSTRACT

Olive Mill Wastewater (OMWW) is considered to be one of the by-products of the extraction of olive oil that causes serious problems to the environment. This study describes a qualitative and quantitative analysis of biochemical characterizations and assessment of polysaccharides antioxidant activities from cold extraction of two endemic varieties from Khenchela region, eastern Algeria. As a result, the physicochemical characteristics of these OMWWs (Chemlal and Ferkani) were found to be acidic (pH: 4.77–5.1) and saline (1.35–1.40 mg/l). According to Chemlal and Ferkani, the dry matter (DM), total suspended solids (TSS) and mineral matter (MM) are respectively (159.3 ± 14.03 g/L, 4.13 ± 0.04 g/L, 10 ± 0.5 g/L and (117.4 ± 12.03 g/L, 1.06 ± 0.1 g/L, 9.7 ± 1 g/L). In addition, the COD and DBO5 requirements are (9625.6 ± 13.42 mg/l; 45 ± 08 mg/l for Chemlal) and (10490.6 ± 23.84 mg/l; 440 ± 3 mg/l for Ferkani) respectively. Due to their high content of organic matter and mineral salts, the OMWW studied have very little potential for biodegradation. Three extracts were prepared for each cultivar: insoluble alcohol fraction (AIR), soluble water fraction (SF) and insoluble water fraction (IF). The biochemical characterisation was carried out by colorimetric assays using an appropriate spectrophotometer for the determination of total sugars (OT), reducing sugars (OR), neutral sugars, protein, nitrogen and phenolic compounds. The qualitative and quantitative analysis of the polysaccharides was carried out using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The results obtained showed heterogeneous composition of nine monosaccharides for each fraction at different levels. The total dry matter (%DM) of the Chemlal fractions (AIRC, SFC, IFC) are respectively (14.44%, 16.93%, 15.68%) and those of the Ferkani fractions (AIRF, SFF, IFF) are respectively (10%, 15.05%, 10.52%). The antioxidant activities of two soluble fractions (SFC and SFF) were evaluated using five assays: DPPH, ABTS, CUPRAC, phenthroline and hydrogen peroxide, when the best antioxidant activity is provided by hydrogen peroxide.

1. Introduction

The olive oil industry is of great importance in Mediterranean countries, both economically and historically. (Tostiet al., 2016). The various olive oil extraction systems (either traditional or three-phase centrifugation) require large quantities of water, which are used for settling, and generating olive oil waste (Caputo et al., 2003; Tostiet al., 2013; Tostiet al., 2016). The composition of Olive Mill Waste (OMW) is highly variable and suggests that major or minor compounds may be present, depending on several factors which include: the growing region, the level of maturation of the olive, the age of the olive tree, the method of extraction of the oil, the treatment of the tree and the weather

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conditions to which the olive tree was subjected during the maturation process. In general, OMW consists of solid matter (Grignon) and liquid matter (OMWW), with a slightly acidic pH. Olive Mill Wastewater (OMWW) is composed of polyphenols, polysaccharides, fatty acids, and water (Aggoun et al., 2016; Dermacheet al., 2013).

Polysaccharides have long been known and exploited because of their abundance, renewable nature, non-toxicity, and biodegradability (Chouana, 2017, p. 203; Salama, 2020). They are natural organic biopolymers widely present in animals, plants, and microorganisms. OMWW's polysaccharides have great health benefits through their use in agri-food field and they are considered as natural antioxidant. Recently, polysaccharides have received considerable attention due to their widespread use in industrial sectors such as food and pharmaceuticals (Mirzadeh et al., 2019; Song et al., 2019; Yu et al., 2017). The interest in polysaccharides is not limited to their rheological properties. Some of them are identified as molecules that can alter the biological processes of specific species based on their biological activity. The industrial application of polysaccharides as biologically active components is still rather small and marginal, despite the relatively recent emergence of glycobiotechnologies and glycomics. Thus, if we exclude the commercial success of some polysaccharides as therapeutic agents, as dermo-cosmetic active ingredients, as nutraceutical compounds, or as elicitors of plant defense reactions, only few of them have been subject to real industrial development for uses other than those related to their texturing properties. One of the reasons associated with this poor commercial success in the field of biological assets is most probably the complexity of polysaccharide structures whose inventory is still very incomplete. Its regular evolution is often associated with commercial successes due to the identification of new structure/function relationships (Chouana, 2017, p. 203; Lukovaet al., 2017; Salama, 2020). Furthermore, plant polysaccharides have become an important class of bioactive natural products and are widely used in pharmaceuticals and biomaterials. They have a variety of biological activities, such as immunostimulatory, antioxidant, anti-inflammatory, antiviral, antitumor, radioprotector, hepatoprotective and anti-fatigue effects (Cui et al., 2018; Lukovaet al., 2017; Wang et al., 2019).

In this context and due to the increasing industrial demand, it is essential to identify new sources of polysaccharides from original plants such as those growing in extreme and unfavorable climatic conditions. Indeed, this type of investigation has two major interests,

The first is that thisparticular plant species is used as a source to discover and classify novel polysaccharide structures. The second is the potential for these plants to be used in a variety of industrial applications, including the pharmaceutical and cosmetics sectors; (Chouana, 2017, p. 203).

We focused in this study on extractions, purification, and biochemical characterization of polysaccharides from the OMWW of only 2 varieties (North East of Algeria), with a specific qualitative and quantitative analysis performed by Anion Exchange Chromatography, coupled to a Pulsed Amperometric Detection (HPAEC-PAD), and the evaluation of the antioxidant activities of the water-soluble polysaccharides of these OMWW.

2. Materials and methods

2.1. Nature of the sample

The OMWW samples were collected in January 2019 from a modern oil mill under the name *Al HadjaYamina* in the Baghai Khenchela region, located in northeast Algeria. This mill used a three-phase cold press system (temperature 16 °C). The OMWW are taken from two varieties of olive: Chemlal and Ferquani and were stored at −20 °C until use in the evaluation process.

2.2. Physico-chemical parameters of OMWW

The physico-chemical characterization was based on the study of the following parameters: Acidity pH, Electrical Conductivity (EC), Salinity (S), Dry Matter (DM), Suspended Matter (SM), Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Biodegradability Index (BI).

- **The hydrogen potential (**pH**)** was measured by a HANNA instruments France type HI 2211 pH-meter by dipping the measuring electrode into a beaker holding 30 ml of OMWW.
- **The electrical conductivity (EC)** was measured directly on the fresh OMWW by a conductivity meter of type cond7310 InoLab Germany; it is expressed in mS/cm (Rodier, 1984).
- **The salinity (S)** was measured by a multi-parameter of type EcoScan 104732 EYTECH instruments Singapore.
- **The dry matter (DM)** consists of all the organic and inorganic substances in solution or in suspension, contained in OMWW. The DM was determined by evaporation of a 10 ml sample in a porcelain crucible at 105 °C in etuve Memmert western Germany for 24 h. The dry matter content is expressed in g/l (Rodier, 1984).
- **Suspended matter (SM)** was determined by centrifugation of 20 ml of OMWW at 8000 rpm (centrifugeThermo Medifuge USA) for 20 min. The solid residue was placed in a weighed glass dish and dried overnight at 105 °C etuve Memmert western Germany. The difference between the weight of the dried sample and that of the dish determines the SM rate (Afnor T 90–105).
- **Biological oxygen demand (BOD_5)** is determined using the respirometric method in a chamber thermostated DBO OxiTop® IS 6, IS 12, WTW, France at 20 °C (AfnorT 90–103), in the dark for 5 days.
- **Chemical oxygen demand (COD)** was carried out by the potassium dichromate method (Apha, 1992). The principle of this method is based on boiling oxidation (148 °C for 2 h HEATING BLOCK CR 4200 WTW France) of the reducing materials by excess of potassium dichromate in acid medium (H_2SO_4), and in the presence of silver sulphate as a catalyst and mercury sulphate as chloride complexing agent. The optical density of the sample is obtained from spectrophotometry measurements carried out at of 440 nm (**Spectrophotomètre UV–visible** 4255/50, Auxilab S.L.Espagne).
- **Biodegradability index (BI)**: the COD/BOD_5ratiois used to determine the BI index, an indicator of the importance of polluting materials with little or no biodegradability (Rodier, 1996).

If:
BI > 6 hardly biodegradable substrate.
3 < BI < 6 partially (or less easily) biodegradable substrates.
BI < 3 Very easily biodegradable substrate.

2.3. Extraction of polysaccharides

According to the protocol written by Galanakis *et al.* (2010a) and Nadour *et al.* (2015) the extraction of alcohol insoluble residue (AIR) is started by a delipidation of the OMWW to eliminate the oily part, by a centrifugation of 500 ml of OMWW at 20 °C with at 3000 rpm(centrifugeThermo Medifuge USA) for 30 min. The residue is suspended in the supernatant by vortex stirring, and then the delipidated OMWW are concentrated in a rotavapor (BUCHI R-100Suisse) at 70 °C until the initial volume is reduced by a factor of 3.5. A quantity of 100 ml of delipidated OMWW is mixed with 5 ml of absolute ethanol and 1g of citric acid in water bath Kalstein france at 80 °C with continuous stirring for 25 min. After cooling to room temperature, the mixture is filtered through a G3 fritted glass (15–40 μm). The precipitate is washed twice with chloroform (3 times the initial volume) for 30 min under stirring then filtered, and with the same volume of acetone then filtered and left to dry at room temperature. The obtained powder is AIR.

2g of AIR are dispersed in 100 ml of distilled water, with stirring for

18 h at 50 °C. The solutions are centrifuged at 20 °C with 3000 rpm (centrifugeThermo Medifuge USA) for 20 min in order to separate supernatants (soluble fraction) and pellets (insoluble fraction) which are freeze-dried (freeze dryer YR05186 Kalstein France). The soluble fraction is filtered through a G3 fritted glass before freeze-drying.

2.4. Biochemical characterization

Different assays were performed to make the biochemical characterization of the polysaccharide extracts of our study. The assay of total sugars is carried out by the method of phenol/sulfuric acid (Dubois *et al.*, 1956). Neutral oses are obtained according to Monsigny *et al.* (1988), which use glucose as the standard.

The content of reducing sugars is measured by the method of Miller (1956). The reducing function complexes under certain conditions with the reagent DNS (Di-Nitro-3, 5 Salicylic acid), which results in an orange coloration, the intensity of this coloration is proportional to the content of reducing sugars.

The method used for the determination of total proteins is that of Bradford (1976), which uses BSA (Bovine Serum Albumin).

The quantity of polyphenols is estimated according to Swain and Hillis (1959). The determination is based on the use of the Folin-ciocalteu reagent. The polyphenol content was expressed in microgram of gallic acid equivalent per milliliter (μg GAE/mL). All samples were analyzed in triplicate.

The Kjeldahl nitrogen is determined according to the protocol written by Nancy J-Thiex*et al.* (2002). The nitrogen was determined using the Kjeldahl technique, with slight modifications. In a matrass, combine 5 mL of raw OMWW with 7.5 g catalyst ($CuSO_4$+ K_2SO_4) and a trace of selenium. As an anti-foaming agent, it is combined 10mL of H_2SO_4 with 10 mL of 30% oxygenated water (H_2O_2). As a shock absorber, it is placed some glass balls. After that, it is heated to 100 °C for a few minutes to prevent the foam from spilling, and then continue heating until the foam evaporates and the contents are burned. Then, it is raised the mineralization temperature to 400 °C until a clear and limpid green coloring appears, and it is continued heating for 30 min before allowing it cool. In an automated distillation device, soda (32%) and distilled water are distilled. The distilled ammonia was trapped in an Erlenmeyer flask containing 20 mL of boric acid 4 percent, and the pH of the boric acid was immediately titrated with sulfuric acid (H_2SO_4) (N/50) up to the original pH of the boric acid. The nitrogen level was determined using the following formula:

$$\%N_2\ (g) = (V1 \times 0.014 \times 100 \times N) / V0$$

N: normality of the sulfuric acid N/50.
V0: sample volume in mL (5 mL).
V1: volume in mL of the sulfuric acid solution used for the titration.

3. Chromatographic analyses

3.1. Sugar hydrolysis(adapted from TAPPI T222-cm11)

0.175 g of biomass wasmixedwith about 1.5 mL of sulfuric acid (H_2SO_4 - 72%; w/v) and then incubated in a rotary water bath for at 30 °C for 1 h. Thesamplesweretaken out and 42 mL of ultrapure water were added. They were then autoclavedfor 1 h. The solid, composed of insoluble lignin – also known as Klason lignin. The liquid phase, containing monomeric sugars from cellulose, pectines and hemicelluloses, was completed with ultrapure water to 100 mL andfrozeforfurther analyses. Monomeric sugars were quantified by High-PerformanceAnion-ExchangeChromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD) (Simangunsong *et al.*, 2018).

3.2. HPAEC-PAD analysis

Monosaccharide contents of soluble fractions were analyzed by HPAEC-PAD (ICS-3000 Dionex) equipped with a Dionex CarboPac^{TM}PA-20 (3 × 150 mm) analytical column(Thermo scientific, USA). Filtered samples (20 μL) were eluted at 35 °C and at 0.4 mL/min with the following composition:ultrapure water(UPW) 99.2%/250 mM NaOH 0.8%: 0 → 20 min; (UPW) 75%/250 mM NaOH 20%/NaOAc (1M)-NaOH (20 mM) 5% 20 → 37min; UPW 40%/250 mM NaOH 20%/NaOAc (1M)-NaOH (20 mM) 40% 37 → 41min. Each elution was followed by a wash and subsequent equilibration time. External sugar and uronic acids standards were used for calibration (7 points per curve): fucose, glucose, xylose, galactose, mannose, rhamnose, arabinose and galacturonic acide, glucuronic acid (all provided by Sigma-Aldrich) (Simangunsong et al., 2018).

3.3. Evaluation of antioxidant activities

The extracts used in the evaluation of antioxidant activities are soluble fractions (SFC, SFF).

3.3.1. DPPH test

The anti-radical activity of DPPH was determined by the tests described from Blois in 1958. In a volume of 1 ml, different concentrations of the extracts to be tested are prepared (0–4 g/l). 40 μL (extract or standard) with 160 μl DPPH solution with a concentration of 0.1 mM in methanol. The mixture is incubatedat room temperaturefor 30 min in the dark and the absorbance is measured at 517 nm.

The estimation of the antiradical activity is expressed by the value of the percentage inhibition (%I) according to the equation:

$$I\ \% = (AC - AS/AC)\ X\ 100$$

AC: control absorbance.
AS: sample absorbance.

The study of the variation of the anti-radical activity as a function of the concentration of the extracts makes it possible to determine the concentration that corresponds to 50% inhibition (IC50). The lower the value of IC50, which also corresponds to a low absorbance, indicates that the extract is powerful vis-à-vis free radicals.

3.3.2. ABTS test

ABTS also forms a relatively stable free radical, which discolors in its free radical form. In this method, an antioxidant is added to a preformed ABTS radical solution and after a fixed period, the remaining $ABTS^+$ is quantified by spectrophotometry at 734 nm (Ak et al., 2008). The spectrophotometric analysis of the ABTS $^+$ trapping activity was determined according to the method of Re *et al.* (1999). 160 μl of the solution ($ABTS^+$) prepared from ABTS and potassium persulfate ($K_2S_2O_8$), 40 μl of each extract at different concentrations is added then incubated at room temperaturefor 10 min in the dark, and the measurements are performed at 734 nm.

$ABTS^+$ activity was expressed as a percentage and calculated using the following formula:

$$ABTS^+\ \text{scavenging effect}\ (\%) = (AC - AS/\ AC)\ X\ 100$$

AC: control absorbance.
AS: sample absorbance.

3.3.3. Cupric reduction (CUPRAC)

The cupric reduction of the extracts is evaluated by the method of Apak*et al.* (2004). This method is based on the formation of a complex between neocuproine and copper (I) in the presence of antioxidants and consequently the reduction of Cu^{2+} to Cu $^+$ Tel et al. (2012). 40 μl of extract at different concentrations (12.5–800 μg/ml), 50 μl of Cu(II) (10 mM), 50 μl of neocuproine (7.5 mM) and 60 μl of $ACNH_4$ buffer (1 M, pH

= 7). Samples were incubated in the dark for 60 min at 25 °C, and absorbance was measured at 450 nm. The results are expressed in absorbance and A0.5 (µg/ml) corresponding to the concentration indicating the absorbance of 0.50.

3.3.4. Phenanthroline assay

Phenanthroline activity was estimated by the method described by Szydłowska-Czerniaket al. (2008). Ten µl of the extract was mixed with 50 µl of iron chloride anhydrous (0.2%), 30 µl of phenanthroline (0.5%), and 110 µl of methanol. The mixture obtained was incubated in the oven at 30 °C for 20 min and the absorbance was measured at 510 nm. The BHA and BHT were used as standards. Results were calculated as A0.50 (µg/ml).

3.3.5. H_2O_2 scavenging activity

To study the H_2O_2 scavenging activity, we used the method described by Ruchet al. (1989) and Bozinet al. (2008). 300 µl of extract for each variety is mixed with 300 µL of a 40 mM hydrogen peroxide solution prepared in 0.1 M phosphate buffer (pH 7.4) and incubated for 10min. The reaction of the samples with hydrogen peroxide is monitored using a spectrophotometer at 230 nm. Ascorbic acid is used as a positive control.

The results are expressed as percentage inhibition according to the following formula:

$$\% \text{ of } H_2O_2 \text{ trapping} = [(AC - AT) / AC] \times 100$$

AC: Absorbance of the control.
AT: Absorbance of the test.

3.4. The statistical analysis

To promote the work carried out, all obtained results are subjected to an analysis of variance followed by an analysis of the averages based on the LSD (5%) in order to highlight the homogeneous groups. The statistical analyzes of the physicochemical and biochemical characteristics and those of the qualitative and quantitative characterizations as well as the biological activities were carried out by using the SAS 9.1.3 software. The results are expressed as the mean ± standard deviation (SD). The IC50 values (50% inhibitory concentration) are calculated by the linear regression method from the curve [% inhibition = f (concentration)]. The difference between the control and the various tests is determined by the Student test for single comparisons or ANOVA followed by the Dunnett/Tukey test for multiple comparisons and the determination of the significance rates. The values of $p \leq 0.05$ are considered statistically significant. The principal component analysis of soluble fraction is carried out using the EXCEL STAT (version 2014) software.

4. Results and discussion

4.1. Study of the variety effect on the physicochemical parameters of OMWW

Table 1 describes the means of the physicochemical parameters of each variety (chemlal and farkani):pH, EC,S,DM,TSS,MM,DBO_5,DCO (see Table 2 describes the ANOVA test of the variety effect on the physicochemical parameters of OMWW.).

The means comparison by the small significant difference shows that the OMWW of the Chemlal variety are characterized by the best levels of DM and TSS, compared to the Ferkani variety OMWW, which are characterized by the highest COD.

The ANOVA of the variety effect shows that there are no significant differences between the OMWW from the studied varieties regarding the following parameters: pH, EC, S, MM, BOD5, and BI. Conversely, there are significant differences in DM and COD and a very highly significant difference in TSS between the Chemlal and Ferkani varieties.

The obtained results have shown that the Olive Mill Waste Water (OMWW) are acidic effluents and the Ferkani variety is more acidic than the Chemlal variety 4.77±0.18 and 5.1±0.13 respectively, the pH values are similar to those Zaier et al. (2017); Arabi et al. (2018) and Boughrara et al. (2021). According to literature, the presence of this acidity is due to their richness in organic acids phenolic acids, fatty acids (Zaier et al., 2017; Yaakoubi et al., 2021). The Electrical conductivity (EC) of the two varieties Chemlal and Ferkani is of the order of 2.83±0.06 mS/cm, and 3.15±0.2mS/cm. These values are low compared to those founded by Arabi et al. (2018), Boughrara et al. (2021) and Yaakoubi et al. (2021) which are 7.72 mS/cm, 13.73mS/cm, and 6.7 mS/cm, respectively. This parameter reflects the high concentration of dissolved mineral salts and especially the potassium, chloride, calcium, and magnesium ions present in the effluents (Magdich et al., 2015; Munir et al., 2016; Yaakoubi et al., 2021).Furthermore, the Salinity(S) levels range between 135±0.07mg/l for Chemlal and 1.40±0.13mg/l for Ferkani due to the concentration of dissolved substances and their nature (Benyahia et al., 2003). These values are low compared to the value reported by Boughrara et al. (2021) 9.611 g/l and Kadi et al. (2020) 1.5 g/l. The salinitygaps are relates to the amount of salt added for olives conservation purposes (Ouabou et al., 2014). Dry matter (DM) and mineral matter (MM) are in the order of 159.3±14.03 g/l, 10±0.5 g/l (Chemlal), and 117.4±12.03 g/l, 9.7±1g/l (Ferkani) respectively. These results show that these OMWW are characterized by a low organic matter content compared to those of previous studies (Abichou et al., 2014; Chatzistahist et al., 2017; Yaakoubi et al., 2021). This is explained by the amount of cold water added to the olive paste in the centrifugation system, for a dilution of the vegetation water produced (Abichou et al., 2014). The total suspended solids (TSS) found in the Chemlal and Ferkani OMWW are 4.13±0, 04 g/l, 1.06±0, 1 g/l, respectively, correlate with the standards (1 to 9g/l) identified in several research studies (Abichou et al., 2014; Zaier et al., 2017). The pollutant expressed in terms of chemical oxygen demand (COD) and biological oxygen demand (BOD_5) (Achak et al., 2008) ranges between 9625.6±13.42 mg O_2/l, to 450±8 mg O_2/l for Chemlal and 10490.6±23.84 mg O_2/l, 440±3 mg O_2/l for Ferkani, respectively, due to autoxidation and polymerization reactions, in, addition to the decomposition of recalcitrant molecules into biodegradable molecules or into mineral compounds such as H_2O, CO_2. These COD levels are higher than those recorded by Boughrara et al. (2021), which equaled 9099 mg O_2/l. These results demonstrate the high demand for oxygen for the complete oxidation of the organic matter contained in these effluents, reflecting their significant polluting powers (Zaier et al., 2017). It is worth noting these parameters vary according to the storage conditions of the olives, the extraction system, the degree of maturation, as well as the variety of olives, and the geographic area (Kadi et al., 2020; Mekersi et al., 2021; Yaakoubi et al., 2021; Gueboudji et al., 2022).

Table 1
Means of the physicochemical parameters according to the varieties.

Parameters varieties	pH	EC (ms/cm)	S (mg/l)	DM (g/l)	TSS (g/l)	MM (g/l)	DBO_5 (mg/l)	COD (mg/l)
Chemlal	5.1 ± 0.13	2.83 ± 0.06	1.35 ± 0.07	159.3 ± 14.03 a	4.13 ± 0.04 a	10 ± 0.5	450 ± 8	9625.6 ± 13.42 b
Ferkani	4.77 ± 0.18	3.15 ± 0.2	1.40 ± 0.13	117.4 ± 12.03 b	1.06 ± 0.1 b	9.7 ± 1	440 ± 3	10490.6 ± 23.84 a

a, b, c, d … homogenous groups.
pH: Hydrogen potential, EC: Electrical Conductivity, S: Salinity, DM: Dry Matter, TSS: Total Suspended Solids MM: Mineral Matter, BOD_5: Biological Oxygen Demand, COD: Chemical Oxygen Demand, BI: Biodegradability Index.

Table 2
ANOVA of the variety effect on the physicochemical parameters of OMWW.

Source	DF	pH	EC (ms/cm)	S (mg/l)	DM (g/l)	TSS (g/l)	MM (g/l)	DBO5	COD	BI
Variety	1	0.16335 ^{ns}	0.15360 ^{ns}	0.00375 ^{ns}	2633.415*	14.16806***	0.135 ^{ns}	150.00^{ns}	1122207.75**	6.10041
Error	4	0.0256	0.02215	0.0109	170.7809	0.00586	0.625	8496.50	37426.868	19.6267

***: very highly significant at 1‰, **: highly significant at 1% *: significant at 5% ns: not significant.

4.2. Effect of variety X fraction on biochemical analysis

The two-factor ANOVA analysis shows significant differences between fractions in terms of yield, OT, ON, and nitrogen content. However, there are no significant differences between fractions in terms of OR, PC, and protein content. Similarly the Variety X Fraction effect outlines significant differences in protein, in yield and ON. Still, there are no significant differences in OR, Nitrogen, and PC.

4.2.1. Study of the fraction effect on biochemical analysis

Comparison of the means by the slight significant difference shows that the AIR fraction gives the best yield and PC, while the SF fraction has the best OT contents, ON, protein, and PC. On the other hand, the best nitrogen content is recorded in the IF fraction (see Table 3).

The results of Table 4 outlines that the SF is the extract with the highest carbohydrate content. The protein level measured in the AIR obtained from the OMWW of this study (2.98 ± 0.25%) is lower than those described by Huisman et al. (1996), Vierhuis et al. (2000), and Nadour et al. (2015) from olive AIR (24.8%, 18%, 3.20%, respectively). This difference is due to the denaturation of proteins during the olive oil extraction process, which uses a three-phase process. It could also t be related the differences between the olive varieties used (Tanilgana et al., 2007). The PC content of the AIR, SF, and IF (between 3.28 and 4.78%) is higher than that of Galanakis et al. (2010a) and Nadour et al. (2015) (between 1.9 and 3.2%). Because of their water solubility which leads to their co-extraction with polysaccharides.

4.2.2. Study of the variety effect on biochemical analysis

The comparison of the means by the small significant difference according to the varieties shows that the variety Chemlal has the best means in terms of yield, OT, Nitrogen, and PC. In contrast, the variety Ferkani is characterized by the best levels of ON, OR, and protein.

The Table 5, results highlights that this data treatment approach is unique in the field, as it compares the two varieties of olives concerning biochemical parameters. Finally, it could be conclude that the Chemlal variety in this study has the best polysaccharide yield compared to Ferkani. Since Chemlal is the most dominant olive variety in Algeria and the yield of this variety differs from one region to another. Still, the Ferkani variety is a specific variety of the region of our study (Khenchela). There are not many studies conducted on this olive variety and it is still unknown to many people, especially in the region and at the national level.

4.3. Study of the interaction between fraction and variety effect on biochemical analyses

Based on the results, six fractions were obtained after extraction of the polysaccharides of the two marginal varieties, three fractions for Chemlal (AIRC, SFC, IFC) and three fractions for Ferkani (AIRF, SFF, IFF).

Table 6 shows the mass yields and composition of total (OT), neutral (ON), reducing (OR), nitrogen, protein, and phenolic compounds. Relative yields are calculated in relation to the mass of dry material used for extraction. Chemlal's marginal AIR yield was (22.8 ± 1.4%) higher than the yield reported by Galanakis et al. (2010a), Nadour et al. (2015) with 64%, and 21.51%, respectively. In contrast Ferkani's AIR yield is (16.55 ± 1.47%). While the SF and IF fractions of (Chemlal and Ferkani) were obtained with respective yields (17.82 ± 2.41%, 5.25 ± 1.12%) and (13.7 ± 1.56%, 6.03 ± 1.02%), which are higher than Nadour et al. (2015). Moreover, the yields in AIR C and SFC are higher than in AIRF and SFF. But the yield of IFF is higher than the IFC. Akrout et al. (2010) explained that yield varies depending on the type of species investigated, regardless of location, geographic separation, and harvest season.

The SFC has the highest OT, at (72.1 ± 17.9%). It is higher than that found by Nadour et al. (2015) 60% and lower than that found by Boual et al. (2014) 82.42%. It was noted that the results of RIAF, SFF, and IFF with OT values between (21 ± 2.77% and 26 ± 5.38) are lower than that of Chemlal (25 ± 6.95% and 72.1 ± 17.9%). ON levels of SFC and SFF are (65.3 ± 6.88%) and (58.4 ± 15.47%) respectively. They are similar to the one noted by Nadour et al. (2015) with (61.73 ± 5.81%) and Addoun et al. (2019) with (63.3 ± 3.17%). The fractions of each variety have the same levels of OR concerning Chemlal or Ferkani.

Chouana et al. (2017) report a protein content of 1.0 g/l, which seems lower than the study's extracts values which ranges between (2.28 ± 0.11 g/l and 4.76 ± 0.57 g/l). Additionally, these values are lower than Zha et al. (2018) value, which equals 5.4 g/l.

Higher levels of nitrogen at IFC and IFF (3.73 ± 0.56 g/l and 3.07 ± 0.48 g/l) are reported. These results are higher than the one recorded in Nadour et al. (2015) or (0.29 ± 0.41 g/l). Contrary to what was found at the SFC and SFF fractions have low nitrogen content (0.84 ± 0.16 g/l; 0.5 ± 0.22 g/l). The values of polyphenols found between (2.24 ± 0.71 μg GAE/mL and 5.01 ± 2.01 μg GAE/mL) are higher than that distinguished by Nadour et al. (2015) (1.90 ± 0.42 μg GAE/mL and 3.23 ± 0.54 μg GAE/mL) and Lukova et al. (2020) or (0.34 ± 0.03 μg GAE/mL). These variances are explained by the temperature influencing of the extraction media. According to Milani et al. (2007), the increase in temperature reduces the protein content in the extract. Due to differences in the chemical components of polysaccharides vary according to various conditions such as the climatic environment (high temperatures, sun exposure, drought, salinity, etc.), geographical location and, origin, harvest period (Saenz et al., 2004), the analytical methods used (Wang & Zhu, 2019), and the polysaccharides primary metabolites. They are used as precursors to other secondary metabolites and as an energy source Zobiri and Hamaiti (2019) or to differences in experimental

Table 3
Mean squares of the variance analysis of the effect of the variety X fraction on the measured parameters.

Source	DF	Yeild	OT	ON	OR	Protein	Nitrogen	Phenolic compound
Fraction	2	314.72***	1181.35***	1751.95***	4.94 ^{ns}	0.68 ^{ns}	11.80***	3.93^{ns}
Variety	1	45.984**	1409.805***	1393.92**	106.58*	2.97**	1.67 ^{ns}	0.99 ^{ns}
Variety *Fraction	2	19.49*	925.75**	724.87*	0.26 ^{ns}	3.13***	0.092 ^{ns}	6.30 **
Error	12	2.4429	71.188	87.579	8.5771	0.17081	0.28236	1.1489

***: very highly significant at 1‰, **: highly significant at 1% *: significant at 5% ns: not significant.
OT: Total Oses, ON: Neutral Oses, OR: Reducing Oses, PC: Phenolic Compound, AIR: Alcool Insoluble Fraction, SF: Soluble Fraction, IF: Insoluble Fraction.

Table 4
The means of the measured parameters according to the fractions.

Parameters Fraction	Yeild (LSD = 1.96)	OT (LSD = 10.61)	ON (LSD = 11.77)	OR (LSD = 3.68)	Protein (LSD = 0.519)	Nitrogen (LSD = 0.66)	PC (LSD = 1.34)
AIR	19.67 ± 3.65 a	25 ± 4.94 b	29.80 ± 7.54 b	16.70 ± 2.55	2.98 ± 0.25 b	2.59 ± 0.79 b	4.78 ± 1.37
SF	15.76 ± 2.89 b	49.05 ± 7.88 a	61.85 ± 11.35 a	14.90 ± 2.95	3.52 ± 1.40 a	0.67 ± 0.25 c	3.50 ± 1.36
IF	5.64 ± 1.04 c	24.5 ± 4.62 b	35.55 ± 9.3 b	16 ± 5.17	2.89 ± 0.46 b	3.40 ± 0.59 a	3.28 ± 0.95

a, b, c … homogenous groups.

Table 5
The means of the parameters measured according to the varieties.

Parameters varieties	Yeild (LSD = 1.60)	OT (LSD = 8.66)	ON (LSD = 9.61)	OR (LSD = 3.008)	Proteine (LSD = 0.42)	Nitrogen (LSD = 0.54)	PC LSD = 1.10)
Chemlal	15.29 ± 7.97 a	41.70 ± 24.81 a	33.6 ± 24.75 b	13.43 ± 1.24 b	2.72 ± 0.44 b	2.52 ± 1.42	4.09 ± 1.70
Ferkani	12.09 ± 4.85 b	24 ± 419 b	51.2 ± 11.73 a	18.30 ± 3.55 a	3.54 ± 1.006 a	1.91 ± 1.17	3.62 ± 1.17

a, b, c, d … homogenous groups.

Table 6
The biochemical composition of the polysaccharide extracts of OMWW.

Parameters fractions	Yields	OT (%DM)	ON (%DM)	OR (%DM)	Protein (g/l)	Nitrogen (g/l)	PC (μg GAE/mL)
AIRC	22.8 ± 1.4 a	25 ± 6.95 b	12 ± 1.52 c	14.5 ± 0.79 a	3.04 ± 0.35 b	3.01 ± 0.99 a	5.01 ± 2.01 a
SFC	17.82 ± 2.41 b	72.1 ± 17.9 a	65.3 ± 6.88 a	12.3 ± 0.96 a	2.28 ± 0.11 c	0.84 ± 0.16 c	4.77 ± 1.19 a
IFC	5.25 ± 1.12 d	28 ± 3 b	23.5 ± 6.46 c	13.5 ± 1.03 b	2.86 ± 0.43 b	3.73 ± 0.56 a	2,5 ± 0.63 b
AIRF	16.55 ± 1.47 b	25 ± 3.57 b	47.6 ± 9.22 b	18.9 ± 1.1 a	2.93 ± 0.18 b	2.18 ± 0.31 b	4.56 ± 0.71 a
SFF	13.7 ± 1.56 c	26 ± 5.38 b	58.4 ± 15.47 a	17.5 ± 0.77 a	4.76 ± 0.57 a	0.5 ± 0.22 c	2.24 ± 0.71 b
IFF	6.03 ± 1,02 d	21 ± 2.77 c	47.6 ± 10.48 b	18.5 ± 6.86 a	2.93 ± 0.59 b	3.07 ± 0.48 a	4.07 ± 0.18 a
ppds	2.78	15.01	16.65	5.21	0.73	0.94	1.9

a, b, c, d … homogenous groups.

conditions in the laboratory (extraction time, extraction number, and extraction temperature) (Kir et al., 2O20; Beretema et al., 2021).

4.4. Qualitative and quantitative analysis of the monosaccharide composition of the fractions of the margins by Anion Exchange Chromatography coupled to pulsed amperometric detection (HPAEC-PAD)

The identification of the monosaccharide composition constituting all polysaccharide fractions extracted from Chemlal and Ferkani OMWW was studied by HPAEC-PAD (Table 7) (see Table 8).

Table 7 describes the heterogeneity of monosaccharides in all samples. Glucose (Glc) is the major monosaccharide and fucose (Fuc) appears as the minor monosaccharide. Other monosaccharides have been identified, namely Rhamnose (Rha), Galactose (Gal), Arabinose (Ara), Mannose(Man), Xylose(Xyl), Galacturonic Acid (AGal), and Glucuronic Acid (AGlc).

The majority of monosaccharides identified in the OMWW have also been identified by other studies (Vierhuis, Korver, Schols, & Voragen, 2003; Galanakis et al., 2010a), except for AGlc and Fuc. These authors found that the AIR of OMWW was mainly composed of AGal, Ara, Glc, or Gal. The predominance of Glc in AIR and other extracts obtained in this study could be explained by the presence of glucans or xyloglucans, similar to those detected in other olive pulp studies (Vierhuis et al. (2000); 2001); and by the extraction procedure, cultivar specificity, harvest season and ripening stage of the olive used as a raw material (Huisman et al., 1996; Vierhuis et al. (2000); Cardoso et al., 2010). For instance, the impact of the ripening stage of olives on the monosaccharide composition of polysaccharides in their cell walls has been well documented. During maturation, the Ara and Xyl content decrease while the Glc content increases due to the accumulation of cellulose (Huisman et al., 1996; Vierhuis et al. (2000)). However, this result was not observed in all cases. Indeed, Cardoso et al. (2010) reported that black olives of the variety Taggiasca were rich in pectic sugars (AGal and Ara) and poor in Glc and Xyl while the variety Conservolea had a higher content of Glc and Xyl.

Table 7
Monosaccharide composition of polysaccharide Fraction extracts of margins after analysis by HPAEC-PAD (%DM).

Sample Monosaccharide (% DM)	AIRC	SFC IFC AIRF	SFF IFF
Fuc	0.04	0.03 0.04 0.05	0.10 0.02
Rha + Ara	1.47	1.68 1.91 1.15	2.01 0.97
Gal	1.23	2.18 1.00 1.33	2.91 0.49
Glc	7.61	9.12 7.66 4.43	3.86 7.01
Xyl	0.40	0.30 0.65 0.26	0.39 0.31
Man	0.30	0.31 0.41 0.36	0.62 0.29
AGlc	3.24	3.03 3.92 2.20	4.71 1.35
AGal	0.16	0.27 0.09 0.21	0.46 0.08
Total (% DM)	14.44	16.93 15.68 10	15.05 10.52

Alcohol Insoluble Residue Chemlal (AIRC), Alcohol insoluble residue Ferkani (AIRF),Water Soluble Fraction Chemlal (SFC), Water Soluble Fraction Ferkani (SFF), Water Insoluble Fraction Chemlal (IFC), Water Insoluble Fraction Ferkani (IFF).

The results concerning the composition of the SF and IF fractions of our varieties (Chemlal and Ferkani) are not consistent with the results of Galanakis et al. (2010a) who found that the soluble fraction is composed only of AGal and Ara. Nevertheless, these finding are similar to that of Nadour et al. (2015) with a difference in the percentages of monosaccharides, which can be explained by the conditions and analytical column used in HPAEC-PAD (CarboPac PA-20 instead of CarboPac PA-1). This difference in the monosaccharide composition of SF and IF for (Chemlal, Ferkani) could be related, to the difference in the extraction temperature used (50 °C instead of 10 °C), in addition to the factors mentioned above. This monosaccharide composition of the AIR of margins, SF, and IF may suggest the presence of partially soluble cellodextrins, pectins, and hemicelluloses (Nadour et al., 2015).

4.5. Evaluation of the antioxidant activity of the soluble fractions (SF) of the two varieties

4.5.1. Analysis of the variance of the evaluation of the antioxidant activity of soluble fractions

Analysis of the variance of antioxidant activity shows that significant

Table 8
ANOVA of the antioxidant activity of soluble fractions (SF) of the OMWW of two varieties.

Source	DF	CUPRAC A 0,5 µg/ml	Phenanthroline A 0,5 µg/ml	DPPH IC 50 µg/ml	ABTS IC 50 µg/ml	PER HYD IC 50 µg/ml
Model	5	100001.4117***	28784.5272***	116678.797***	19822.258***	64789.36***
Error	12	88.8475	0.9065	26.5316	4.27063	1089.663

***: very highly significant at 1‰.

differences exist between the solid fractions of the OMWW of the two Chemlal and Ferkani varieties and the used standards ($p \leq 0.0001$).

Regarding the evaluation of antioxidant activity by DPPH, ABTS, CUPRAC, and phenanthroline, the results of IC 50(Table 9) show that SFC and SFF have a low activity compared to the standards used. Xu et al. (2019), suggest that the outcome of in vitro antioxidant activity depends largely on the chosen trapping test because a different in vitro test at different mechanisms and antioxidant activity has been attributed to different mechanisms. The IC 50 levels of SFC and SFF explain by the presence of many free hydroxyl groups in the polysaccharide structure and most likely by the presence of residual PC. In addition, Wu et al. (2014) reported that the presence of Ara in the polysaccharide structure could reduce the production of hydroxyl radicals by chelation of Fe^{+2} (a pro-oxidant). This hypothesis was reinforced by Chevalier et al. (2001) who reported that ribose and Ara glycosylated proteins had significant antioxidant properties. This role of Ara in antioxidant activities could be related to its association with PC, such as ferulic acid, and p-coumaric.

The hydrogen peroxide antioxidant test shows that the SFF fraction (285.46 ± 7.4 µg/ml) belongs to the same BHT standard group (288.47 ± 15.87 µg/ml) indicating that they have the same effect, and the SFC fraction (94.86 ± 1.37 µg/ml) belongs to the same group of standard BHA (144.38 ± 22.78 µg/ml) which indicates that they have the same effect. So BHA and BHT standards can be replaced by SFC and SFF fractions. Several studies have postulated that the protein or peptide fragment in the polysaccharide is responsible for part of the radical sweep effect. Indeed, Residues of polysaccharides-polyphenols have been shown to have significant antioxidant functions in many reports (Wang et al., 2016b). The resulting SFC and SFF extracts contain an important amount of protein (4.76 ± 0.57 µg GAE/mL and 2.28 ± 0.11 µg GAE/mL respectively) which explains the strong antioxidant activity. In addition, the presence of mannose (Man) has a positive influence on antioxidant activity (Zhang & Li, 2015). High content of Gal and uronic acid has also been reported as beneficial for the antioxidant activity of polysaccharides, to their ability to bind with hydroxyl radicals resulting from hydrogen peroxide (Wang et al., 2017).

Several factors influence the antioxidant activity of polysaccharides, including monosaccharide composition, molecular weight, and chain conformation. Consequently, due to various structural characteristics, polysaccharides of various origins exhibit a different antioxidant activity (Li, Sun, et al., 2017). Polysaccharide monosaccharide composition and monosaccharide ratios may have an apparent effect on antioxidant capacity (Jiang et al., 2015; Liu et al., 2015). Moreover, the extraction method may affect antioxidant activity (Chen et al., 2018b). Hot water-soluble extraction is the most effective method for preserving the antioxidant activity of polysaccharides (Liu et al., 2015).

The CUPRAC, Phenanthroline, and Hydrogen Peroxide antioxidant tests are used for the first time in the evaluation of the antioxidant activity of OMWW water-soluble polysaccharides. From this study it can be said that our fractions (SFC and SFF) in the hydrogen peroxide have the best antioxidant activity.

Table 9
IC50 averages of antioxidant activities with standards.

	DPPH (IC50 µg/ml)	ABTS (IC50 µg/ml)	CUPRAC (A 0.5 µg/ml)	hydrogen peroxide ++ (IC50 µg/ml)	Phenanthroline (A 0.5 µg/ml)
SFC	478.14 ± 10.08 a	199.52 ± 5.05 a	350.33 ± 18.50 b	94.86 ± 1.37 d	63.56 ± 0.96 b
SFF	244 ± 7.47 b	89.9 ± 0.31 b	373.67 ± 13.8 a	285.46 ± 7.4 c	249 ± 6.93 a
BHA	6.14 ± 0.41c	1.29 ± 0.30 c	8.97 ± 3.94 c	144.38 ± 22.78 d	4.29 ± 0.01 cd
BHT	12.99 ± 0.41c	1.81 ± 0.10 c	8.97 ± 3.94 c	288.47 ± 15.87 c	4.81 ± 0.01 c
Trolox	3.21 ± 0.06c	3.21 ± 0.06 c	8.69 ± 0.14 c	364.31 ± 43.18 b	5.21 ± 0.27 c
ascorbic acid	3.04 ± 0.05c	3.04 ± 0.05 c	8.31 ± 0.15 c	282.11 ± 4.96 a	3.08 ± 0.02 d
LSD	9.1634	3.6764	16.769	58.725	1.6938

a, b, c, d … homogenous groups.

4.6. Analysis of main components

The two axes (1 and 2) of the analysis in the main components describe 47.94 and 7.57% respectively, or almost 90.09% of the information (see Fig. 1).

The group formed by the variables PC and Glc activities is positively correlated with ACP axis 1 and 2. It opposes the group made up of the following compounds: OR, AGlc, Fuc, Man, Loten and Rha + Ara which are negatively correlated with it (Fig. 2) (see Fig. 3).

From the results of the distribution of the variables, it is found that the yield is due to the high content of Phenolic compounds (PC), Ot, On, Gal, Agal and Glc.

The projection of the individuals on the plane (1 and 2) indicates that the group composed of the soluble fraction of the Chemlal variety and the AIR fraction were positively correlated with axes 1 and 2of the ACP. Conversely, the soluble fraction of the Ferkani variety is negatively correlated with axis 1 and axis 2 of the ACP (Fig. 2).

The superposition of the two parameters and individuals projections indicates that the soluble fraction of the Chemlal variety is characterized by the best yield that is very rich in PC, OT, and Glc. Unlike the soluble fraction of the Ferkani variety which is characterized by a high AGlc, Fuc, Man, Loten and Rha + Ara contents. The IF fraction of chemlal is characterized by a high nitrogen content.

Based on the obtained results of evaluation of antioxidant activity, the soluble fraction of chemlal is characterized by the highest activity with phenanthroline method and this is due to the high PC and OT contents; and the soluble fraction of Fercani variety is characterized by the high antioxidant activity with ABTS method due to the presence of AGlc, Fuc, Man, Loten and Rha + Ara.

The polysaccharides antioxidant capacity is closely related to their structure and composition, including molecular weight, monosaccharide composition, uronic acid content, molecular conformation, types of glycosidic bonds, etc. (Li et al., 2015). Although the antioxidant mechanism of polysaccharides is still uncertain, it is believed that hydrogen atoms on aldose rings as well as the C–H bonds of uronic acids in polysaccharides can stabilize free radicals. The radical scanning activity of polysaccharides is positively correlated to the hydrogen donation capacity of the hydroxyl groups (Xiao et al., 2019). Low molecular weight polysaccharides are can expose an abundant amount of terminal saccharide residues to participate in reduction reactions compared to other polysaccharides of the same concentration (Li, Wang, et al., 2017; Qiu et al., 2019).

Polyphenols bind quickly and spontaneously to the polysaccharides of the cell walls of foods rich in dietary fiber when the break of fruits and vegetables releases them during consumption (grinding, chewing) or

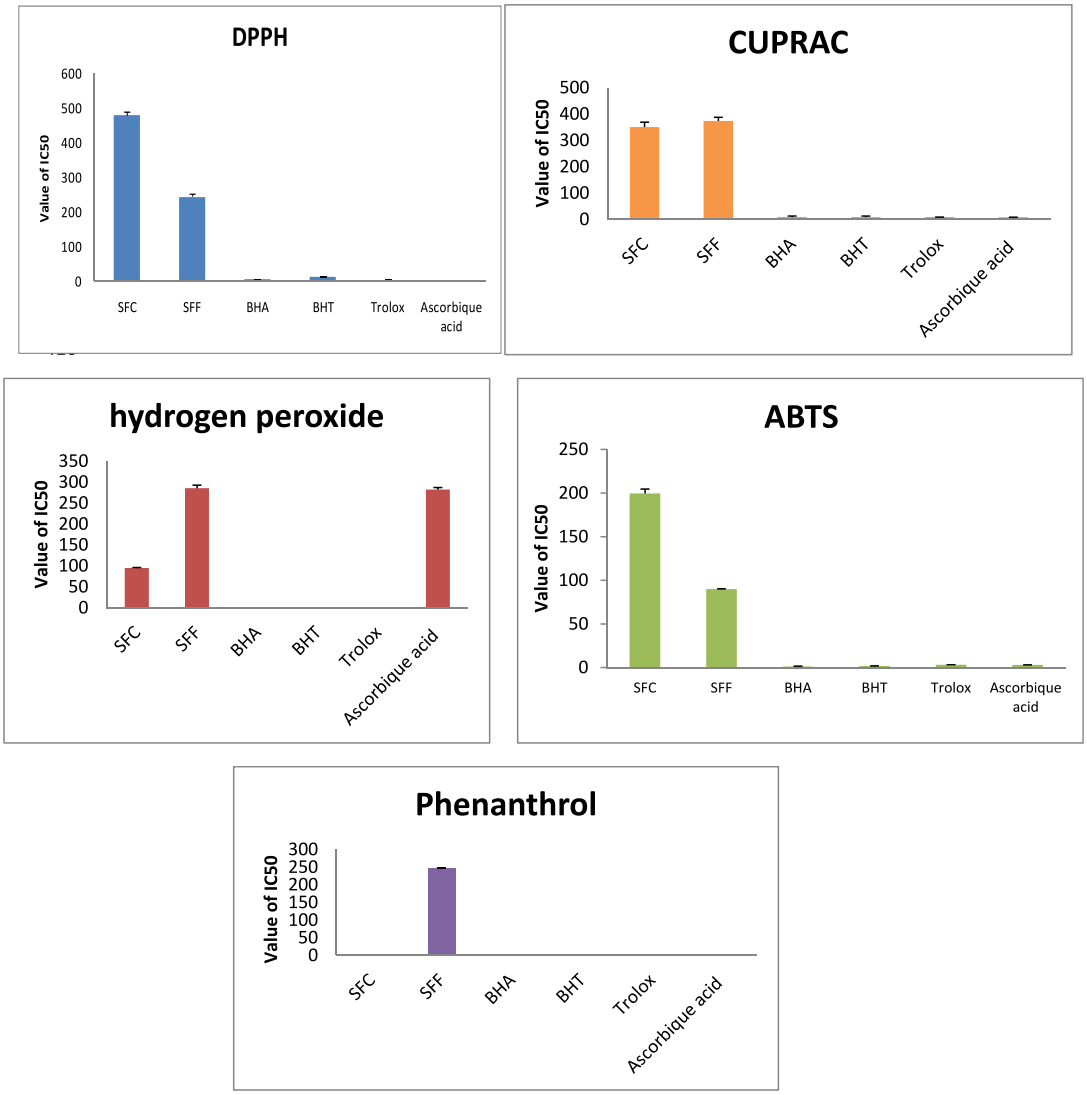

Fig. 1. Graphic representation of antioxidant activities.

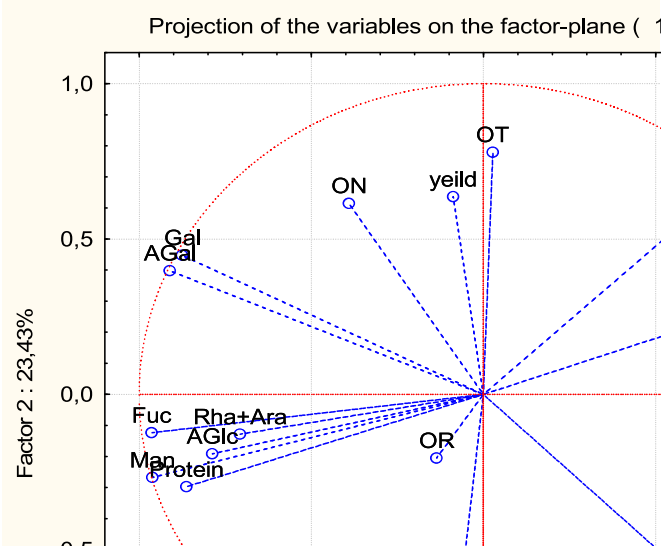

Fig. 2. Projection of the measured parameters (variables) in the (1, 2) main component analysis.

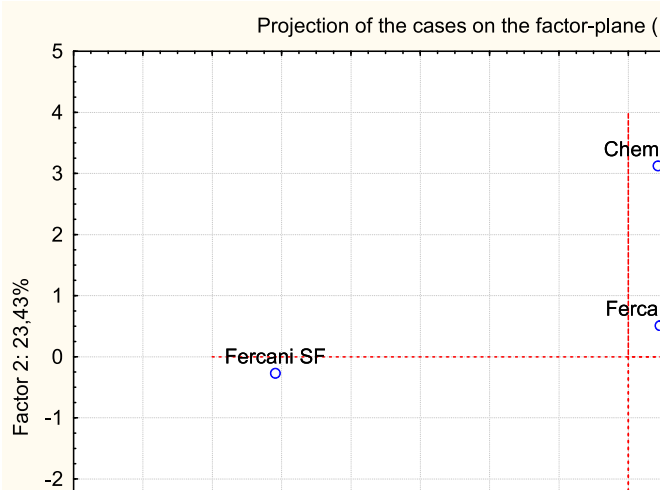

Fig. 3. Projection of individuals (fractions and varieties) at the plane (1, 2) of the main component analysis.

processing (boiling, autoclaving or freeze-drying) (Liu et al., 2017). Dietary fiber components (polysaccharides) have the ability to bind phenolic compounds (Liu et al., 2017; Saura-Calixto, 2011) and, in some cases, these complexes have a significant antioxidant capacity (Pérez-Jiménez et Saura-Calixto, 2015; Wu et al., 2011).

Polysaccharides low in Mw and uronic acid are generally considered to have a higher antioxidant activity (Mateos-Aparicio et al., 2010), the antioxidant capacity of polysaccharides could be either the existence of hydroxides in the polysaccharide molecule or high galacturonic acid content (Hamed et al., 2020). The combination the two components properties would provide a unique material able of recovering free radicals and countering the effect of dietary pro-oxidants (Bermúdez-Oria et al., 2019).

5. Conclusion

This study investigates the polysaccharides extracted from Olive Mill Wastewater (OMWW) of two endemic varieties from the Khenchela region (Chemlal and Ferkani), OMWW includes considerable quantities of simple sugars. Furthermore their preventive effects on oxidative degeneration and pathological processes due to oxidizing stress. In addition to their use in the agrifood industry, cosmetics, and pharmacies, plants polysaccharides represent a highly rich source of natural antioxidants. Therefore, we suggest recovering food waste as it includes a huge quantity of antioxidant-wealthy components.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Abichou, M., Gargouri, K., Rhouma, A., & Khatteli, H. (2014). Long term effect of olive mill waste water spreading on microbial population and the natural floristic composition of a sandy soilAgric. *Science Engineering Technology Research, 1*, 16–25.

Achak, M., Ouazzani, N., Yaacoubi, A., & Mandi, L. (2008). Caractérisation des margines issues d'une huilerie moderne et essais de leur traitement par coagulation-floculation par la chaux et le sulfate d'aluminium. *Rev. Des Sci. L'eau/J. Water Sci., 21*, 53–67.

Addoun, N., Boual, Z., Delattre, C., Ursu, A. V., Desbrières, J., Le Cerf, D., Christine Gardarin, C., Hentati, F., Ould El-Hadj, M. D., Michaud, M., & Pierre, G. (2019). Structural features and rheological behavior of a water-soluble polysaccharide extracted from the seeds of Plantago ciliata Desf. *International Journal of Biological Macromolecules, 155*, 1333–1341.

AFNOR T 90-103. (1975). *Essais des eaux-détermination de la demande biochimique en oxygène(DBO)*.

AFNOR T 90-105. (1978). *Essais des eaux. Détermination des matières en suspension*.

Aggoun, M., Arhab, R., Cornu, A., Portelli, J., Barkat, M., & Graulet, B. (2016). Olive mill wastewater microconstituents composition according to olive variety and extraction process. *Food Chemistry, 209*, 72–80.

Ak, T., & Gülçin, İ. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions, 174*(1), 27–37.

Akrout, A., EljaniL, H., Amouri, S., & Neffati, M. (2010). Screening of antiradical and antibacterial activities of essential oils of Artemisia campestris L., Artemisia herba-alba Asso. and Thymus capitatus Hoff et Link. growing wild in the southern of Tunisia. *Recent Research in Science and Technology, 2*, 29–39.

Apak, R., Guclü, K., Ozyurek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence ofneocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry, 52*, 7970–7981.

Apha. (1992). *American Public Health Association 1992. Standard methods for analysis of waste and waste water*. Washington, DC: APHA .Pub, 18thEd.

Arabi, M., Elias, A., Ziane, K., Ait younes, Y., Mansouri, B., & Toumert, I. (2018). Characterization of olive mill wastewater and gamma irradiation effects on some parameters of its composition. *Journal of Radioanalytical and Nuclear Chemistry, 317*, 1095–1106.

Benyahia, N., & Zein, K. (2003). *Analyse des problemes de l'industrie de l'huile d'olive et solutions récemment développées*. Lausanne, Suisse: 2ème Conférence Internationale Swiss Environmental Solution.

Bermúdez-Oria, A., Rodríguez-Gutiérrez, G., Fernández-Prior, Á., Vioque, B., & Fernández-Bolaños, J. (2019). Strawberry dietary fiber functionalized with phenolic antioxidants from olives. Interactions between polysaccharides and phenolic compounds. *Food Chemistry, 280*, 310–320.

Berritima, B. D., & Medjour, H. (2021). *Extraction et Caractérisation physico-chimique des polysaccharides des noyaux des dattes cultivars Deglet-Nour de BISKRA, 48*. Université of Eloued.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable Free Radical. *Nature, 4617*(181), 1119–1200.

Boual, Z., Kemassi, A., Hamid Oudjana, A., Michaud, Ph., & Ould el hadj, M. D. (2014). Caractérisation partielle des polysaccharides hydrosolubles des feuilles de *MALVA PARVIFLORA* L. (MALVACEAE): Activité prébiotique. *Lebanese Science Journal, 14*, 41–51.

Boughrara, S., Boucatta, N., Lecheb, F., Medjboub, K., & Belkhir, M. (2021). Characterization and valorisation of olive by-products from a traditional oil mill in Béjaia. *Algerian Journal of Environmental Science and Technology .March edition, 7*, 1819–1824.

Bozin, B., Mimica-Dukic, N., Samojlik, I., Goran, A., & Igic, R. (2008). Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). *Food Chemistry, 111*, 925–929.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. *Analytical Biochemestry, 72*, 248–254.

Caputo, A. C., Scacchia, F., & Pelagagge, P. M. (2003). Disposal of by-products in olive oil industry: Waste-to-energy solutions. *Applied Thermal Engineering, 23*, 197–214.

Cardoso, S. M., Mafra, I., Reis, A., Nunes, C., Saraiva, J. A., & Coimbra, M. A. (2010). Naturally fermented black olives: Effect on cell wall polysaccharides and on enzyme activities of Taggiasca and Conservolea varieties. *LWT–Food Science and Technology, 43*, 153–160.

Chen, F., & Huang, G. (2018b). Antioxidant activity of polysaccharides from different sources of ginseng. *International Journal of Biological Macromolecules, 13*.

Chevalier, F., Chobert, J. M., Genot, C., & Haertle, T. (2001). Scavenging of free radicals, antimicrobial, and cytotoxic activities of the Maillard reaction products of α-lactoglobulin glycated with several sugars. *Journal of Agricultural and Food Chemistry, 49*, 5031–5038.

Chouana, T. (2017). *Caractérisation structurale et activités biologiques des polysaccharides d'Astragalus gombo bunge. Thèse de doctorat*. Universite Kasdi Merbah d'Ouargla.

Chouana, T., Pierre, G., Vial, C., Gardarin, C., Wadouachi, A., Cailleu, D., Le Cerf, D., Boual, Z., Ould El Hadj, M. D., Michaud, P., & Delattre, C. (2017). Structural characterization and rheological properties of a galactomannan from Astragalus gombo Bunge seeds harvested in Algerian Sahara. *Carbohydrate Polymers, 175*, 387–394.

Cui, M., Wu, J., Wang, S., Shu, H., Zhang, M., Liu, K., & Liu, K. (2018). Characterization and anti-inflammatory effects of sulfated polysaccharide from the red seaweed Gelidium pacificum Okamura. *International Journal of Biological Macromolecules, 129*, 377–385.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry, 48*, 1532–1552.

Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Galanakis, C. M., Tornerberg, E., & Gekas, V. (2010a). A study of the recovery of the dietary fibres from olive mill wastewater and the gelling ability of the soluble fibre fraction. *LWT-Food Science and Technology, 43*, 1009–1017.

Gueboudji, Z., Addad, D., Kadi, K., Nagaz, K., Secraf, M., BenYahya, L., Lachehib, B., & Abdelmalek, A. (2022a). Biological activities and phenolic compounds of olive oil mill wastewater from Abani, endemic Algerian variety. *Scientific Reports, 12*, 6042.

Gueboudji, Z., Kadi, K., Mahmoudi, M., Hannachi, H., Nagaz, K., Addad, D., Ben Yahya, L., Lachehib, B., & Hessini, K. (2022b). Maceration and liquid–liquid extractions of phenolic compounds and antioxidants from Algerian olive oil mill wastewater. *Environmental Science and Pollution Research*. https://doi.org/10.21203/rs.3.rs-1423045/v1

Hamed, M., Bougatef, H., Karoud, W., Krichen, F., Haddar, A., Bougatef, A., & Sila, A. (2020). Polysaccharides extracted from pistachio external hull: Characterization, antioxidant activity and potential application on meat as preservative. *Industrial Crops and Products, 148*, Article 112315.

Huisman, M. M. H., Schols, H. A., & Voragen, A. G. J. (1996). Changes in cell wall polysaccharides from ripening olive fruits. *Carbohydrate Polymers, 31*, 123–133.

Jiang, P., Yuan, L., Cai, D., Jiao, L., & Zhang, L. (2015). Characterization and Antioxidant activities of the polysaccharides from mycelium of *Phellinus pini* and *culturemedium*. *Carbohydrate Polymers, 117*, 600–604.

Kadi, K., Mrah, R., Hamli, S., Lekmine, S., Dib, D., Addad, D., Boukeria, S., Gueboudji, Z., & Hafsaoui, I. (2020). Evaluation of the anticoagulant activityof margins from olives extraction in the KHENCHELA region. *Journal of Fundamental and Applied Sciences, 12*, 634–649.

Kir, C., & Reguioua, A. (2020). *Contribution à l'étude des polysaccharides hydrosolubles de Plantago albicans L. issue de la région d'El Oued, 43*. Université of Eloued.

Li, C., Huang, Q., Fu, X., Yue, X. J., Liu, R. H., & You, L. J. (2015). Characterization, antioxidant and immunomodulatory activities of polysaccharides from Prunella vulgaris Linn. *International Journal of Biological Macromolecules, 75*, 298–305.

Li, J., Sun, Q., Meng, Q., Wang, L., Xiong, W., & Zhang, L. (2017). Anti-fatigue activity of polysaccharide fractions from Lepidium meyenii Walp. (maca). *International Journal of Biological Macromolecules, 95*, 1305–1311.

Liu, D., Martinez-Sanz, M., Lopez-Sanchez, P., Gilbert, E. P., & Gidley, M. J. (2017). Adsorption behaviour of polyphenols on cellulose is affected by processing history. *Food Hydrocolloids, 63*, 496–507.

Liu, J., Wen, X., Zhang, X., Pu, H., Kan, J., & Jin, C. (2015). Extraction, characterization and in vitro antioxidant activity of polysaccharides from black soybean. *International Journal of Biological Macromolecules, 72*, 1182–1190.

Li, J. E., Wang, W. J., Zheng, G. D., & Li, L. Y. (2017). Physicochemical properties and antioxidant activities of polysaccharides from Gynura procumbens leaves by fractional precipitation. *International Journal of Biological Macromolecules, 95*, 719–724.

Lukova, P., Karcheva-Bahchevanska, D., Nikolova, M., Ilia, N., Iliev, I., & Mladenov, R. (2017). Comparison of structure and antioxidant activity of polysaccharides extracted from the leaves of *Plantago major* L., P. media L. and P. lanceolata L. *Bulgarian Chemical Communications, 49*, 282–288.

Lukova, P., Nikolova, M., Petit, E., Elboutachfaiti, R., Vasileva, T., Katsarov, P., Manev, H., Gardarin, C., Guillaume, P., Michaud, P., Iliev, I., & Delattre, C. (2020). Prebiotic activity of poly- and oligosaccharides obtained from plantago major L. Leaves. *Applied Sciences, 10*, 2648.

Magdich, S., Ben ahmed, C., Boukhris, M., Ben rouina, B., & Ammar, E. (2015). Olive mill wastewater spreading effects on productivity and oil quality of adult chemlali olive (Olea europaea L.) in the South of Tunisia. *International Journal of Applied Agricultural Research, 6*, 56–67.

Mateos-Aparicio, I., Mateos-Peinado, C., Jiménez-Escrig, A., & Rupérez, P. (2010). Multifunctional antioxidant activity of polysaccharide fractions from the soybean by product okara. *Carbohydrate Polymers, 82*, 245–250.

Mekersi, N., Kadi, K., Silvia Casini, S., Addad, D., Bazri, K. E., Marref, S. E., Lekmine, S., & Amari, A. (2021). Effects of single and combined olive mill wastewater and olive mill pomace on the growth, reproduction, and survival of two earthworm species (*Aporrectodea trapezoides, Eisenia fetida*). *Applied Soil Ecology, 168*, Article 104123.

Milani, M., Emam-djomeh, Z., Rezaee, K., Safari, M., Ganbarzadeh, B., & Gunasekaran, S. (2007). Extraction and physicochemical properties of Barijeh (Ferula galbaniflua) gum. *International Journal of Agriculture and Biology, 9*, 80–83.

Miller, G. L. (1956). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426–428.

Mirzadeh, M., Arianejad, M. R., & Khedmat, L. (2019). Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: A review. *Carbohydrate Polymers, 229*, Article 115421.

Monsigny, M., Petit, C., & Roche, A. C. (1988). Colorimetric determination of neutral sugars by a resorcinol sulfuric acid micromethod. *Elsevier. Analytical Biochemistry, 175*, 525–530.

Munir, J., Rusan, M., Ammar, R., Albalasmeh, A., & Malkawi, H. I. (2016). Treated olive mill wastewater effects on soil properties and plant growth. *Water, Air, and Soil Pollution, 227*, 135–144.

Nadour, M., Laroche, C., Guillaume, P., Delattre, C., Moulti-mati, F., & Michaud, Ph. (2015). Structural characterization and biological activities of polysaccharides from olive mill wastewater. Springer. *Applied Biochemistry and Biotechnology, 177*, 431–445.

Ouabou, E., Anouar, A., & Hilali, S. (2014). Traitement de la margine brute d'huile d'olive par distillation suivi de neutralisation par la chaux. *J. Appl.Biosci., 79*, 6867–6872.

Pérez-Jiménez, J., & Saura-Calixto, F. (2015). Macromolecular antioxidants or nonextractable polyphenols in fruit and vegetables: Intake in four European countries. *Food Research International, 74*, 315–323.

Qiu, J., Zhang, H., & Wang, Z. (2019). Ultrasonic degradation of polysaccharides from Auricularia auricula and the antioxidant activity of their degradation products. *LWT - Food Science and Technology, 113*, Article 108266.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cationdecolorizationassay. *Free Radical Biology & Medicine, 26*, 1231–1237.

Rodier, J. (1984). *L'analyse de l'eau: Eaux résiduaires, eau de mer, 7ème édition* (p. 1365). Paris, France: DUNOD, BORDAS.

Rodier, J. (1996). *L'analyse de l'eau : Eaux naturelles, eaux résiduaires, eau de mer, 8ème édition* (p. 1384). Paris, France: DUNOD, BORDAS.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Caranogen, 10*, 1003–10008.

Salama, A. (2020). Cellulose/calcium phosphate hybrids: New materials for biomedical and environmental applications. *International Journal of Biological Macromolecules, 127*, 606–617.

Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants: An essential physiological function. *Journal of Agricultural and Food Chemistry, 59*, 43.

Simangunsong, E., Ziegler-Devin, I., Chrusciel, L., Girods, P., Wistara, N. J., & Brosse, N. (2018). Steam explosion of beech wood: Effect of the particle size on the Xylans recovery. *Waste and Biomass Valorization, 11*, 625–633.

Song, Q., Jiang, L., Yang, X., Huang, L., Yu, Y., Yu, Q., & Xie, J. (2019). Physicochemical and functional properties of a water-soluble polysaccharide extracted from Mung bean (*Vigna radiate* L.) and its antioxidant activity. *International Journal of Biological, 138*, 874–880.

Hillis, W. E., & Swain, T (1959). The phenolic constituents of*Prunus domestica* . II.†—the analysis of tissues of the Victoria plum tree. *Journal of the Science of Food and Agriculture, 10*, 63.

Szydlowska-Czerniaka, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szlyk, E. (2008). Determination of antioxidant capacities of vegetable oils byferric-ion spectrophotometric methods. *Talanta, 76*, 899–905.

Tanilgana, K., Özcanb, M. M., & Ünverb, A. (2007). Physical and chemical characteristics of five Turkish olive (Olea europea L.) varieties and their oils. *Grasas y Aceites, 58*(2), 142–147.

Tel, G., Apaydın, M., Mehmet, E., & Duruet, M. E. (2012). Antioxidant and cholinesterase inhibition activities of three tricholoma species with total phenolic and flavonoid contents: The edible mushrooms from anatolia. *Food Anal, 5*, 495–504.

Thiex, N. J., Anderson, S., & Persson, J.Å. (2002). Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: Collaborative study. *Journal of AOAC International, 85*, 309–317.

Tosti, S., Accetta, C., Fabbricino, M., Sansovini, M., & Pontoni, L. (2013). Reforming of olive mill wastewater through a Pd-membrane reactor. *International Journal of Hydrogen Energy, 38*, 10252–10259.

Tosti, S., Fabbricino, M., Pontoni, L., Palma, V., & Ruocco, C. (2016). Catalytic reforming of olive mill wastewater and methane in a Pd-membrane reactor. *International Journal of Hydrogen Energy, 41*, 5465–5474.

Verhuis, E., Schols, H. A., Beldman, G., & Voragen, A. G. J. (2001). Structural characterization of xyloglucan and xylans present in olive fruit. *Olea europaea cv koroneiki).Carbohydr. Polym., 44*, 51–62.

Wang, Y., Lai, L., Teng, L., Li, Y., Cheng, J., Chen, J., & Deng, C. (2019). Mechanism of the anti-inflammatory activity by a polysaccharide from *Dictyophora indusiata in* lipopolysaccharide-stimulated macrophages. *International Journal of Biological Macromolecules, 621*, 1158–1166.

Wang, W., Wang, X., Ye, H., Hu, B., Zhou, L., Jabbar, S., & Shen, W. (2016a). Optimization of extraction, characterization and antioxidant activity of polysaccharides from Brassica rapa L. *International Journal of Biological Macromolecules, 82*, 979–988.

Vierhuis, E., Korver, M., Schols, H. A., & Voragen, A. G. J. (2003). Structural characteristics of pectic polysaccharides from olive fruit (*Olea europaea cv moraiolo*) in relation to processing for oil extraction. *Carbohydrate Polymers, 51*, 135–148.

Vierhuis, E., Schols, H. A., Beldman, G., & Voragen, A. G. J. (2000). Isolation and characterisation of cell wall material from olive fruit (*Olea europaea* cv koroneiki) at different ripening stages. *Carbohydrate Polymers, 43*, 11–21.

Wang, H., Xu, Z., Li, X., Sun, J., Yao, D., Jiang, H., & Yue, R. (2017). Extraction, preliminary characterization and antioxidant properties of polysaccharides from the testa of Salicornia herbacea. *Carbohydrate Polymers, 176*, 99–106.

Wang, S., & Zhu, F. (2019). Chemical composition and health effects of maca (Lepidium meyenii). *Food Chemistry, 288*, 422–443.

Wu, G. H., Hu, T., Li, Z. Y., Huang, Z. L., & Jiang, J. G. (2014). In vitro antioxidant activities of the polysaccharides from Pleurotus tuber-regium (Fr.) Sing. *Food Chemistry, 148*, 351–356.

Wu, Z., Ming, J., Gao, R., Wang, Y., Liang, Q., Yu, H., & Zhao, G. (2011). Characterization and antioxidant activity of the complex of tea polyphenols andOat β-glucan. *Journal of Agricultural and Food Chemistry, 59*(19), 10737–10746.

Xiao, H., Fu, X., Cao, C., Li, C., Chen, C., & Huang, Q. (2019). Sulfated modification, characterization, antioxidant and hypoglycemic activities of polysaccharides from Sargassum pallidum. *International Journal of Biological Macromolecules, 121*, 407–414.

Xu, Y., Wu, Y., Sun, P., Zhang, F., Linhardt, R. J., & Zhang, A. (2019). Chemically modified polysaccharides: Synthesis, characterization, structure activity relationships of action. *International Journal of Biological Macromolecules, 132*, 970–977.

Yaakoubi, A., Aganchich, B., Jaouab, Y., Meddich, A., & Wahbi, S. (2021). Réponses morpho-physiologiques et biochimiques de la plante du féverole (Vicia faba. L) dans le sol amendé par les margines de centrifugation, essai en pots. *Afrique SCIENCE, 18*, 38–51.

Yu, X. H., Liu, Y., Wu, X. L., Liu, L. Z., Fu, W., & Song, D. D. (2017). Isolation, purification, characterization and immunostimulatory activity of polysaccharides derived from American ginseng. *Carbohydrate Polymers, 156*, 9–18.

Zaier, H., Chemingui, W., Rajhi, H., Bouzidi, D., Roussos, S., & Rhouma, A. (2017). Physico-chemical and microbiological characterization of olive mill wastewater (OMW) of different regions of Tunisia (North, Sahel, South). *Journal of new science. Agriculture and Biotechnology, 48*(2), 2897–2906.

Zhang, S., & Li, X. Z. (2015). Inhibition of α-D-glucosidase by polysaccharides from the fruit hull of Camellia oleifera Abel. *Carbohydrate Polymers, 115*, 38–43.

Zha, Z., Wang, S. Y., Chu, W., Lv, Y., Kan, H., Chen, Q., & Yin, H. (2018). Isolation, purification, structural characterization and immunostimulatory activity of water-soluble polysaccharides from Lepidium meyenii. *Phytochemistry, 147*, 184–193.

Zobiri, O., & Hamaiti, M. (2019). Contribution à l'étude de l'activité anti - inflammatoire des polysaccharides hydrosolubles d'Astragalus gombo chez les rats Wistar albinos. *Université of Eloued., 37*.