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Mild microwaves, ultrasonic and alkaline pretreatments for improving methane production: Impact on biochemical and structural properties of olive pomace

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Abstract

This study aims to investigate the effects of microwaves, ultrasonic and alkaline pretreatments on olive pomace properties and its biomethane potential. Alkaline pretreatment was found to reduce lipid and fiber contents (especially lignin) and to increase soluble matter. The alkali pretreatment at a dose of 8% under 25°C and for 1 day (w/w TS) removed 96% of initial lipids from the solid olive pomace. Unlike NaOH addition, mild microwaves and ultrasonic pretreatments had no impact on lignin. However, in the case of long microwaves pretreatment (450W-10 min), cellulose and lignin contents were reduced by 50% and 26% respectively. Similarly, the combination

of ultrasonic and alkali reagent showed a positive effect on fiber degradation and lipid solubilization as well as a positive impact on methane production. Statistical analysis highlighted the correlation between NaOH dose, solubilization and methane production. The alkaline pretreatment at ambient temperature appeared the most energetically efficient.

Keywords: anaerobic digestion; alkali pretreatment; ultrasounds; microwaves; lignocellulosic biomass;

1. Introduction

The olive oil production in Mediterranean countries represents about 94% of the worldwide production (Albuquerque et al., 2004), which generates annually around 1 Mt of olive pomace (OP) as a by-product (Akay et al., 2015). In some Mediterranean countries the agricultural cooperatives are using traditional process for oil extraction consisting in washing, crushing and blending of olives. The residues (OP) are highly loaded in lipids. Generally, the OP is landfilled or combusted and used to heat traditional ovens. OP is mainly composed of stones, pulp and skin and is rich in lignocellulose, lipids and proteins which can be valorized to bioenergy by anaerobic digestion (AD).

AD is a mature, simple and economically feasible route to valorize organic matter to bioenergy (Vasco-Correa et al., 2018). It consists in the biological transformation of the organic matter to biogas through four-step degradation: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al., 2008). In the case of most solid wastes, hydrolysis is the slowest step in which complex organic matter is degraded into monomers (Hendriks and Zeeman, 2009). However, the olive pomace is one of the most

hardly degraded and recalcitrant **material** due to high lignin content (Pellera et al., 2016) which limits the microbial and enzymatic accessibility (Monlau et al., 2013a; Pellera and Gidakos, 2017). For this reason, the key driver for the successful conversion of OP into biomethane is the selection of an efficient **pretreatment** that maximizes the **sugar, lipid** and protein recovery **while** minimizing the formation of toxic derivatives (Barakat et al., 2012). A pretreatment that is effective for one substrate is not necessarily effective for another **one**, as it depends on biomass composition and reaction mechanisms involved. The choice to use a single or a combination of pretreatments is often due to a compromise between organic matter solubilization efficiency, energy consumption and effluent management. For this, the **selection** of **the** pretreatment should not be based only on its efficiency, but also on **its** environmental impact (Monlau et al., 2015b).

For the last few years, several pretreatments have been developed and applied to OP biomass for enhancing methane production. These pretreatments include mechanical, microwaves, thermal, ultrasonic, dilute acid or alkaline pretreatments (Al-Mallahi et al., 2016; Almansa et al., 2015; Elalami et al., 2018; Leite et al., 2016; Pellera et al., 2016; Rincón et al., 2014; Serrano et al., 2017b; Siciliano et al., 2016). Milling aims to reduce particle size and **to** enhance matter accessibility **which** may have a positive impact on methane production (Elalami et al., 2018). Microwave pretreatment was found efficient to enhance OP solubilization and methane potential. Rincon et al. (2013) reported high methane potential using thermal hydrolysis pretreatment at 180 °C for 180 min, with an increase of 22% compared to untreated OP (Rincón et al., 2013). This was in agreement with de la Lama et al. (2017) who obtained, after treatment at 120 °C, **a** methane potential 45% higher than the untreated OP (de la Lama et al., 2017). However, under a

temperature range of 75–200 °C, microwaves resulted in a negative energy balance (Pellera and Gidakos, 2017). Considering chemical pretreatment applied to OP, Ruggeri et al. (2015) found that CaCO₃ pretreatment was the most energy efficient compared to other salts (FeSO₄, MnSO₄, FeCl₃) (Ruggeri et al., 2015). In the same manner, it has been reported that the addition of NaOH (4%) could significantly modify fiber composition of OP and increased their solubilization and methane production (Pellera et al., 2016). Although the addition of an alkali reagent to fats can lead to their solvation and saponification, the fate of lipids in OP after alkaline pretreatment has never been studied.

The novelty of this study lies in the comparison of mild microwaves (MW), alkaline (NaOH) and ultrasonic (US) pretreatment effects on anaerobic digestion of olive pomace as a lipid rich lignocellulosic waste. In addition, this work aims to identify the relationships between the structural and biochemical composition of OP, the pretreatment conditions and biomethane potential using principal component analysis. Finally, the energy efficiency of different pretreatments was determined, relatively to the control, to identify the most effective pretreatment for olive pomace AD.

2. Materials and methods

2.1. *Substrate*

The olive pomace (OP) used in this study was obtained from a traditional olive oil extraction in the region of Beni Mellal in Morocco. The substrate was dried under the sun, and its particle size was reduced with knife milling using 4 mm-screen (Retsch SM 100, Germany). The substrate was mainly composed of olive stones, skin and pulp. OP contained about 88% of total solids (TS), 97% of TS corresponded to volatile solids

(VS), while an elementary composition of 52% of C, 7.2% of H, 1% of N and 0.1% of S was measured in the dried OP.

2.2. *Pretreatments*

The olive pomace was subjected to alkaline pretreatment by the addition of different quantities: 0.03, 0.07 and 0.14 g of NaOH pellets (98%) to 2 g of olive pomace to obtain doses of 2%, 4% and 8% (w/w TS) respectively. Afterwards, 100 mL of ultra-purified water was added under a stirring speed of 100 rpm and 25 °C for 4 days, 2 days and 1 day respectively. Alkaline pretreatment at 50°C was carried out in the same pretreatment times, water to solid ratio and stirring conditions. Microwave-assisted pretreatment was carried out to OP containing 88%TS, with OP to water ratio (1:10 w/w), using Minilabotron 2000 (2 kW, 2450 MHz, Sairem, France) at different power conditions 200 W, 450 W, and 700 W. The pretreatment time was 2 min for each power condition. In fact, because of the technical constraints of the microwaves used, it was necessary to work with reduced durations since the device was not designed to support high temperature or pressure. In addition, OP was subjected to microwaves pretreatment for 10 min under 450W. Ultrasonic pretreatment was conducted using a high-intensity sonicator (Fisher bioblock scientific, France, 20 kHz) with a power supply of 450W for 10 min with OP/water ratio of (1:10 w/w). The combined pretreatments were carried out, first, using 4% of NaOH (w/w TS) for 4 hours at room temperature and then the mixture was subjected to microwaves or ultrasonic pretreatment (450W for 10 min). Four flasks were prepared for each pretreatment condition. Three of them were used for the Biochemical Methane Potential (BMP) tests as described in the section (2.4) and the fourth one was used for the analysis. In this flask, solid and liquid phases were separated by centrifugation. The solid phase was then dried and subjected to fiber and

fatty acid analysis while sCOD, sugars and soluble polyphenols were analyzed in the liquid fraction.

2.3. Physicochemical Analysis

The TS and VS contents were determined using the APHA (American Public Health Association) method. After pretreatments, liquid and solid fractions were separated using centrifugation at 7140 rpm for 15 min. Solid samples were then subjected to freeze-drying and stored in a glass desiccator. Hemicelluloses, cellulose and lignin were determined after acid hydrolysis with sulfuric acid at 72%, followed by sugar analysis using High Performance Liquid Chromatography (HPLC) as described in (Sluiter et al., 2008).

The lignin content was then determined by subtracting the ash content from the solid remaining after acid hydrolysis and dried at 105 °C for 24 hours.

The CHNS content was measured by elemental analysis using Thermo Scientific FlashSmart analyzer, via flash combustion at 950 °C.

Two grams of untreated milled OP were macerated in 100 mL of ultrapurified water for 4 hours under stirring at 100 rpm and 25 °C to measure soluble compounds. Soluble chemical oxygen demand (sCOD) and polyphenols were determined from the liquid phase from pretreated and macerated OP. The sCOD was determined by using HACH DR/2000 spectrophotometer at 620 nm (Elalami et al., 2018). Soluble polyphenol content was measured using Folin method. The liquid fraction (100 µL) was added to 500 µL of 10-fold diluted Folin-Ciocalteu solution, and 400 µL of Na₂CO₃ solution at 75 g/L. The mixture was stirred in a vortex mixer, and left 5 min in a bath at 40 °C. It was then analyzed in triplicate using Nanoquant Tecan microplate reader, with a

wavelength of 735 nm using gallic acid as a reference. Thus, soluble polyphenol concentrations were expressed by grams of gallic acid equivalent per gram of volatile matter (Turkmen et al., 2006).

The fatty acids (FA) were quantified in the dried solid fraction of olive pomace before and after pretreatments. Samples were prepared for the transesterification reaction, and then the supernatants were analyzed using gas chromatography as described in (Peydecastaing et al., 2009).

2.4. Biomethane potential tests

The BMP tests were carried out to assess OP anaerobic biodegradability. Batch BMP tests were carried out in triplicate in 500 mL flasks with 400 mL as working volume. Both untreated and pretreated samples (whole slurry) were added to the inoculum at a ratio of 1 gVS OP/g VS inoculum. Trace elements, macro-elements and buffer solutions were added as described in (Monlau et al., 2013b). Flasks were set at mesophilic conditions (35 °C) and were continuously stirred. The calculation of methane volume was based on pressure measurements, on ideal gas law and on the biogas composition obtained using gas chromatography GC CLARUS 480-Perkin Elmer (Monlau et al., 2013b).

The kinetic parameters of the AD process were calculated using the modified Gompertz model (Eq1) (Serrano et al., 2017a).

$$B = B_0 \exp \left(-\exp \left[\frac{R_m(\lambda - t)e}{B_0} + 1 \right] \right) \quad (\text{Eq 1})$$

Where B is the specific methane potential (mL/gVS), B₀ is the ultimate specific methane produced (mL/gVS); R_m is the maximal methane production rate

(mL/gVS. d), λ is the lag-phase time (d) and e is the $\exp(1)$. The coefficient of determination R^2 (Eq2) was calculated to indicate the variation in estimated specific methane potential (Y_i) that is explained by the measured specific methane potential (X_i). Root-mean square error (RMSE) is the standard deviation of the residuals from the N points on regression line (Eq3).

$$R^2 = 1 - \frac{\sum_{i=1}^N (X_i - Y_i)^2}{\sum_{i=1}^N \left[X_i - \frac{\sum_{i=1}^N X_i}{N} \right]^2} \quad (\text{Eq 2})$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (Y_i - X_i)^2}{N}} \quad (\text{Eq 3})$$

The means of BMP results of pretreated OP were compared with that of the untreated OP (control) using a t-test under a Student law at 5% assuming the variance equality, normality and independence of repetitions. The confidence interval was 95%, thus, the null hypothesis was rejected for a probability equal to 5%.

2.5. Principal component analysis

Principal component analysis (PCA) and linear regression of experimental data were carried out using SIMCA from UMETRICS (13.0). The methane potential, composition of liquid (sCOD) and solid (lignin, cellulose and hemicellulose and FA) fractions were included in the PCA loadings plot as dependent variables. In addition, pretreatment parameters: NaOH dose, microwaves and ultrasonic energies, temperature and alkali pretreatment time were considered in the PCA as independent variables. Correlation coefficient R^2 was calculated to assess the statistical relationship between two variables, while p-value was estimated to describe the probability that the null hypothesis was true.

2.6. Relative efficiency calculation

The specific energy consumed during knife milling (E_M) was measured with a wattmeter and calculated as follows:

$$E_M (J/gTS) = \frac{\int_0^t (P_M - P_{M0}) dt}{MOP} = \frac{\int_0^t \Delta P_M dt}{MOP} = 140 \text{ (Eq 4)}$$

Where P_M (watts) is power consumed during knife milling at a time t (s), P_0 (watts) is the power consumption during knife milling under idle conditions (without OP), and MOP (gTS) is the mass of olive pomace.

Energy consumed (E_c) for NaOH production ($E_c(\text{NaOH})$) was calculated using the Ecoinvent (2010) database where the global energy requirement (GER) is estimated to be around 5980 kJeq/kg_{NaOH} (Ruggeri et al., 2015). When OP was pretreated with NaOH at 50 °C, the energy consumption of the heating system was added to the NaOH production energy. This energy was theoretically calculated using (Eq 6). The calorific capacity of the liquid phase was considered equivalent to that of water, as the solid to the liquid ratio was 1:50.

$$Q (J/gTS) = \frac{M_{\text{water}} \cdot C_{p\text{water}} \cdot (T - 298)}{MOP} = 5937 \text{ (Eq 6)}$$

Where Q is the energy consumed during the alkaline pretreatment at 323°K, M_{water} is the water quantity (g), $C_{p\text{water}}$ is the calorific capacity of water (4.18 J/g.°K) and T is equal to 323°K and M_{OP} (gTS) is the quantity of olive pomace to be treated.

Then the total energies consumed by the alkaline pretreatments at 25°C and 50°C were calculated taking into account the knife milling energy. The total energies consumed (tEc) are given in Eq 7 and Eq 8 .

$$tEc (25^\circ C) (J/gTS) = E_M + E_c (\text{NaOH}) = E_M + GER \times \text{NaOH}(\%) \text{ (Eq 7)}$$

$$tEc (50^{\circ}C)(J/gTS) = EM + Q + Ec (NaOH) = EM + Q + GER \times NaOH(\%) \quad (Eq 8)$$

Energy consumed by microwave oven ($E_c(MW)$) was determined by subtracting the specific energy reflected (data not shown) from the energy supplied (Table 1). The energy supplied was calculated as follow (Eq 5):

$$E_{supplied}(J/gTS) = \frac{P_{MW} \cdot t_{MW}}{M_{OP}} \quad (Eq 5)$$

Where P_{MW} (watts) is the power consumed by the magnetron, t_{MW} (s) is the microwaves pretreatment duration and M_{OP} (gTS) is the quantity of olive pomace to be treated.

The total energy consumed for MW pretreatment is given by (Eq 9). It is important to mention that both ultrasonicator and the microwave reaction vessel setups were not designed to minimize the energy reflected. The energy consumed by ultrasound pretreatment was given by the sonicator system at the end of the pretreatment ($E_c(US)$). The total energy consumed for US pretreatment is given by (Eq 10).

$$tEc (MW)(kJ/kgTS) = EM + Ec(MW) \quad (Eq 9)$$

$$tEc (US)(kJ/kgTS) = EM + Ec(US) \quad (Eq 10)$$

The relative efficiency was calculated using the equation (Eq 11). It enables measuring the difference between the energy gained during the pretreatment and that of the knife milling (control). It is important to note that this term remains related to the control since it does not include energies consumed during AD or the production of nutrient solutions added during the BMP test.

$$Reff(\%) = \frac{(E_{out} - tEc) - (E_{out0} - EM)}{(E_{out0} - EM)} \times 100 \quad (Eq 11)$$

Where, E_{out} (J CH₄/gTS) and E_{out0} (J CH₄/gTS) are the methane energies produced after the AD of pretreated and untreated OP respectively. The methane energy was calculated using the higher heat value of methane which is estimated to 55.6 kJ/g.

3. Results and discussion

3.1. Effects of pretreatments on olive pomace characteristics

3.1.1. Soluble chemical oxygen demand, sugars and polyphenols

Table 2 shows the effect of the different pretreatments studied on sCOD. It was found that all pretreatments enhanced the sCOD and especially **the alkaline one** at a dosage of 8% under 50 °C which attained 6-fold the sCOD of the untreated OP. **Regarding the microwaves-assisted pretreatment**, the **sCOD** increased with **the** energy supplied.

However, it remained lower than the sCOD after NaOH pretreatments. Contrarily to Doğan and Sanin (2009), the solubilization of OP after the combined microwaves and alkaline method was lower than the sum of individual pretreatments (Doğan and Sanin, 2009). This finding may show the effect of **pretreatment time** on the effectiveness of alkaline pretreatment, as the combined pretreatment was performed after only 4 hours of contact between NaOH and the biomass **while single alkaline pretreatment was carried out for 1, 2 or 4 days depending on alkali doses**. Thus, a higher alkaline pretreatment time can significantly improve **the** methane production (Thomas et al., 2018). Indeed, all pretreatments used in this study increased the solubilization of OP. **Ultrasonic and alkaline pretreatment combination resulted in 67% higher COD release compared to alkaline pretreatment at 4% and 25 °C for 2 days**. Higher sCOD content was obtained compared to individual pretreatments. Thus, ultrasonic **pretreatment** can be efficient when a preliminary degradation has already been done. In fact, the order of alkali and ultrasonic pretreatments can have an impact on the pretreatment results. Jin et al. (2009)

showed that more sCOD was obtained when alkali reagent was followed by US than the opposite case (Jin et al., 2009).

Alkali reagent presence induces solvation and saponification (Hendriks and Zeeman, 2009) which explains the improved solubilization. Also, it causes the disruption of lignocellulosic matrix and the release of phenolic compounds and sugars (such as mannose, xylose and glucose). The soluble sugar and polyphenol concentrations in the supernatant of pretreated OP are presented in **Table 2**. The sugar analysis from untreated OP revealed that it contained about 10.7 mg of soluble sugars per gVS. The release of sugars was enhanced after all alkaline pretreatments. The addition of 2% sodium hydroxide resulted in 88% more soluble sugars compared to untreated OP at 25°C. Besides, at 50 °C, a dosage of 8% NaOH increased soluble sugar concentration by 114%.

Soluble sugar concentration after microwave pretreatment depended highly on the energy consumed, which agrees with Binod's study (Binod et al., 2012). In fact, after only 2 min of a 200 W microwave pretreatment, sugars released concentration was quite similar to the untreated OP, while sCOD increased by 48% compared to untreated OP. However, when increasing the microwave pretreatment time at 450W from 2 to 10 min, 77% more sugars were released. An increase of 116% of soluble sugar concentration was obtained after the combination of ultrasonic and alkaline pretreatment. This confirms the high impact of the combined US and NaOH on OP solubilization.

Also, the pH of liquid samples was understandably affected by alkaline pretreatment, while microwaves did not seem to have an impact on the final pH of the liquid sample. Soluble polyphenol release was enhanced after all the studied pretreatments. However,

the highest soluble polyphenol content in the liquid phase was obtained after combining NaOH and US which led to a 233% increase compared to the soluble polyphenol concentration in untreated OP (after soaking in water). It should be mentioned that polyphenols are AD inhibitors, but in the case of this study the polyphenol content remained lower than the inhibition threshold (1 g/L) (Monlau et al., 2014).

3.1.2. Fatty acids concentrations

Table 2 presents the fatty acids (FA) concentrations in solid fractions of pretreated OP. After alkaline pretreatment, the concentration of fatty acids decreased in solid fraction dependently on NaOH dose, while all lipids remained in the solid fraction after microwave pretreatment. However, the combined alkali and microwave pretreatment had the highest impact on FA concentrations. The high impact of ultrasonic pretreatment was obvious. A significant decrease in lipids was noticed even without NaOH addition. Around 75% of initial lipids were solubilized after US. This reduction was mainly due to lipid particles disintegration occurring throughout US pretreatment. At a supplied energy of 26 MJ/kgTS of microalgae, Passos et al. (2015) found that ultrasonication increased the soluble FA content achieving 3-fold the soluble FA content in the untreated microalgae (Passos et al., 2015). Similarly, lipids in food waste were highly reduced after ultrasonic pretreatment (Jiang et al., 2014). Only 7% of initial lipids remained in the solid residue at a specific energy of 4320 kJ/kgTS (Jiang et al., 2014).

Fig 1 presents the FA content in untreated and pretreated samples. The dried olive pomace was composed of 16% of lipids that mainly contained linoleic acid (C18:2n-6) which accounted for 68% of total fatty acids, followed by linolenic acid (C18:3n-3)

(14%). Overall, after all pretreatments, FA distribution remained stable, and linoleic acid was the most abundant. In fact, olive pomace contains polyunsaturated fats which undergo saponification in presence of alkali reagent and water (Al Hatrooshi et al., 2020). Thus, alkali pretreated solids were poor in fats. After the addition of 2% of NaOH at 25 °C, FA content in the pretreated solid was around 4% (w/w TS).

Microwave pretreatment had no effect on fatty acids accessibility and solubilization as most of them remained in the solid fraction. The combination of alkaline and microwave pretreatments decreased the lipid content, as 11% of lipids were found in the solid fraction. Similarly, the combination of NaOH and US led to the solubilization and saponification of 95% of initial lipids.

Moreover, it was assumed that saponification was more related to NaOH dose than temperature, especially when a high NaOH dose was added (above 4%). Theoretically, 3 moles of NaOH are needed to produce soap from 1 mole of triglycerides which corresponds to 11 mmol of NaOH/g of lipids. The addition of 8 g of NaOH per 100 g of dried olive pomace leads to a dose of 12 mmol/g of FA initially present in OP. This was also confirmed by the pH values of pretreated OP. In fact, at 8% NaOH dose, the final pH was high (around 10–11) compared to 2% and 4% doses which was clearly due to an excess of alkali reagent.

3.1.3. Fiber composition

Table 2 presents the fiber content in untreated and pretreated OP. Compared to untreated OP, lignin content decreased significantly after alkaline pretreatments. This finding was in agreement with literature where alkali reagents were reported to be effective in delignification of grass and woody species (Kim et al., 2016). Alkali

pretreatment (8% at 25°C for 1 day) resulted in hemicellulose and cellulose concentration decrease by 44% and 53%, respectively. However, the highest impact was found to be on lignin degradation (reduced by 67% at 8% at 50 °C for 1 day). In the present work, the observed actions of NaOH were: i) solubilization of organic matter, ii) saponification of lipids contained in olive pomace, and iii) degradation of lignin and polyphenol solubilization. These effects have already been reported (Zhen et al., 2017). In fact, NaOH promotes the decomposition of lignin into low molecular weight compounds. This occurs through the breakdown of the alky-aryl ether bond (β -O-4) under the action of sodium ion which polarizes the bond, making it easier to break (Poletto, 2018).

The microwave-assisted pretreatment at low energy supplied had no significant impact on fiber content. However, the effect of microwave pretreatment for 10 min on fiber degradation was observed. In fact, cellulose and lignin contents were reduced by 50% and 26% respectively, which is due to the increase of energy and exposure time. In fact, microwave is used for its heating effect, which occurs due to the molecular motion and rotation of dipolar species. Then, the friction between the molecules generates heat (Aguilar-Reynosa et al., 2017). Depolymerization of cellulose can occur at temperatures above 150 °C due to the hot spots formation caused by the electromagnetic field (Aguilar-Reynosa et al., 2017). It was assumed that below these temperatures, microwaves can partially solubilize the matter (de la Hoz et al., 2005). However, combining NaOH and microwaves allowed better degradation of lignin (+28% compared to microwaves only). In fact, the combined NaOH and MW can lead to the dissolution of lignocellulosic components compared to MW alone, which is due to the higher polarity of the aqueous NaOH compared to water (Tumuluru, 2018). Similarly,

fiber content after the combined alkali and MW pretreatment was lower than that obtained after NaOH alone. In fact, microwaves promote and/or accelerate chemical reactions caused by NaOH, which is in agreement with (Zhu et al., 2005).

Cellulose content in ultrasonicated OP was reduced by 19%, while hemicellulose and lignin contents remained constant. In fact, ultrasounds allow the depolymerization of matter by acoustic cavitation following the formation, growth and implosion of bubbles that promote the generation of free radicals which initiate the matter degradation (Gogate and Prajapat, 2015). The addition of alkali reagent before US enhanced significantly the fiber degradation. In fact, 18% more lignin was degraded after combining US and NaOH pretreatment compared to the sum of individual pretreatments. As NaOH weakens the lignocellulosic matrix, the bubbles generated by ultrasound penetrate more easily into olive pomace. Then, it allows a better delignification compared to NaOH pretreatment alone, which is in agreement with the results of (Subhedar and Gogate, 2014). Consequently, the ultrasonication can reduce both the needed alkali reagent dose and the pretreatment time.

3.2. Effects of pretreatments on biomethane potential and kinetics

Table 3 presents the methane production after each pretreatment. At a dosage of 8% of NaOH for 1 day, an improvement of 30% of methane was obtained; however mild temperatures (25–50°C) did not seem to have a significant effect on methane potential. Microwaves (MW) and ultrasonic (US) pretreatments had no impact on methane production, which can be explained by their low effect on fiber degradation. Radiation is often inadequate to bring a complete degradation to an easily digestible and fermentable product. The cellular structure of lignocellulosic biomass is inherently

complex and difficult to penetrate so that the breakdown of the matter requires predominantly chemical reactions in addition to physical de-structuring. In fact, the combination of MW and US with alkali reagent addition increased methane production by 13% and 16% respectively, which was identical to the methane potential found after the alkaline pretreatment at 4% at 25 °C for 2 days. Therefore, MW and US reduced the time required for alkaline pretreatment.

Pellera et al. (2016) obtained 23% higher methane potential from OP after its thermoalkaline pretreatment at 4% for 4 hours and 90°C compared to untreated OP. Similar effect of alkaline pretreatment at the same dose was found in this study, but for 2 days at 50°C. Serrano et al. (2019) reported that steam explosion of olive pomace at 170°C for 60 min increased by 44% the methane produced which was higher than the results found in this study. However, the main disadvantage of thermal pretreatment is the high energy consumption. Microwaves are often used to substitute the thermal pretreatment; but Pellera et al. (2017) did not find any effect of MW (75°C-200°C) on methane potential of olive pomace.

Table 3 also shows the kinetics parameters of the methane production from the pretreated samples. Pretreatments have a different impact on the kinetics of methane production which depends on the mechanisms of each method and its effects on the samples structure and composition. In the case of OP, the modified Gompertz model was suitable in describing the kinetics of AD (Serrano et al., 2017a). Indeed, it can be observed that the alkaline pretreatment increased the values of R_m (rate of methane production) and methane potential (B_0). The lag phase time (λ) was reduced after the pretreatments at doses of 2% and 4 % while it increased at 8%. In fact, the sodium inhibition hypothesis can be rejected because the Na^+ concentration in the BMP flask

was lower (0.08 mol/L as the maximum achieved) than the inhibition threshold reported in the literature (0.35 mol/L) (Ahring et al., 1991). In the case of this study, a slow hydrolysis may be caused by high pH conditions, resulting in a lag phase.

Pretreatments with MW reduced the rate of methane production and lag phase time, while slightly improving the B_0 . In contrast, methane production rate (R_m) was increased after US pretreatment (3-fold the R_m of the control) but the lag time was increased by 53% which may be attributed to the solubilization of complex organic matter. The combination of pretreatments highly improved the methane production rate mainly due to the enhanced solubilization of OP (Table 2).

Bolado-Rodríguez et al. (2016) reported that methane production rates increased when lignin degradation was caused by the pretreatment (Bolado-Rodríguez et al., 2016). In this study, combined pretreatments and alkaline pretreatment at 8% and 50°C for 1 day showed the highest lignin reductions as well as higher R_m values. Similarly, Pellerá et al. (2016) reported that at 4% and 90°C for 4 hours, the R_m was increased compared to 25°C at the same dose and for 16 hours (Pellerá et al., 2016), while Pellerá and Gidarakos (2018) reported that pretreatment with citric acid decreased the R_m and lag phase time (Pellerá and Gidarakos, 2018). Similarly, ultrasonic pretreatment at different conditions was found to increase the methane production rate from olive pomace (Rincón et al., 2014). Those findings are in agreement with the present study.

3.3. Principal component analysis

The PCA analysis was conducted in order to link the methane production to the characterization results (for both solid and liquid fractions) and operating conditions of the pretreatments. Fiber and FA contents were used as properties of solid samples. It

was assumed that the sCOD refers to the overall measure of solubilization. **The two principal components accounted for 44.3% and 24.8% of the total dataset variability.**

The PCA shows that alkali dose was negatively correlated to hemicelluloses and lignin content in the remaining solid and much less to cellulose. In fact, as previously mentioned, alkaline pretreatment at low temperatures partially degraded hemicelluloses and lignin while cellulose remained intact. In fact, NaOH dose was highly correlated with methane production ($R^2= 0.90$, $p < 0.001$) and sCOD ($R^2= 0.89$, $p < 0.001$) (**Table 4**). Negative correlation was also observed between NaOH dose and FA in solid fractions. Alkali reagent addition reduced FA contained in the solid due to **the** solubilization and saponification of lipids. In addition, FA and lignin were indirectly correlated because of the impact of NaOH on both of them. Temperature was only related to MW energy supplied and no significant relation was found between methane and MW energy, cellulose and FA. Contrarily to NaOH dose, the influence of alkali pretreatment time, temperature and MW and US **consumed energies** on methane production were not statistically significant.

Indeed, NaOH dose was the most influencing pretreatment parameter on methane produced volume, whereas lignin, sCOD and hemicelluloses also **affected** the methane potential of OP. It was clear that BMP was positively correlated only with NaOH dose and sCOD. In fact, all organic materials present in the solid fraction (lignin, hemicellulose and FA) were found negatively correlated with methane, this simply means that organic matter that remained in the solid was **less accessible and available for AD compared to the already solubilized matter.** MW energy and temperature had the lowest impact on methane production, which was already reported in the previous sections (**Tables 2 and 3**). It must be mentioned that those findings cannot be projected

to higher values of MW energies as the conclusions reached were related to mild conditions exclusively. Higher MW energy, temperature and pressure can significantly impact methane production and matter solubilization (Alqaralleh et al., 2019; Kainthola et al., 2019).

3.4. Relative energy efficiency

Table 5 presents the results obtained. **Microwaves pretreatment** at low energy supplied improved slightly **the** methane production, and the relative energy efficiency **R_{eff}** was negative even for the softest conditions, but remained better than alkaline pretreatment at 50 °C. Sonication pretreatment resulted in **a** negative **R_{eff}**. **However, the combination of alkaline and US pretreatments resulted in an increase** in **R_{eff}** **value**. Unlike the other pretreatments, alkaline pretreatment was more energetically efficient at 25°C compared to the control (knife milling). At a dose of 8%, the highest **R_{eff}** was achieved (25%). The efficiency of alkaline pretreatment at 50 °C can be enhanced when considering heat recovery and higher solid to liquid ratio (Monlau et al., 2015a). If the agronomic valorization of digestates is targeted, it is necessary to carefully select the alkaline reagent to be used. Thus, it is important to consider NaOH substitution by another alkali reagent that is more beneficial for soil such as KOH or cheaper like CaO.

4. Conclusion

Alkali pretreatment influenced biochemical properties of the substrate. Single ultrasonic and mild microwave pretreatment had no significant impact on OP composition and methane production, contrarily to their combination with alkaline pretreatment which **led** to lipid saponification and lignin degradation. However, in the case of **olive pomace**, the main profit of the alkali reagent remains the lignin degradation.

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Fig.1 Fatty acids profile in pretreated samples

Fig.2 Loading plot of principal components analysis

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Tables

Table 1 Pretreatment conditions and energy consumption

Pretreatments	Conditions	Max temperature achieved (°C)	E _{supplied} (kJ/kgTS)	E _c (kJ/kgTS)	tE _c (kJ/kgTS)
	Untreated	-	0	140 ^a	140
NaOH	2% -25 °C-4 days	25 ^b	0	120 ^c	260
	4% -25 °C-2 days		0	240 ^c	380
	8% -25 °C-1 day		0	478 ^c	618
	2% -50 °C-4 days	50 ^e	0	6057 ^d	6197
	4% -50 °C-2 days		0	6177 ^d	6317
	8% -50 °C-1 day		0	6415 ^d	6555
MW	200W - 2 min	37	2727	1415	1555
	450W - 2 min	61	6136	2297	2437
	700W - 2 min	95	9545	3675	3815
	450W - 10 min	98	30682	11484	11624
NaOH+MW	4% -25 °C-4 hours	98	30682	11854	11994
	450W-10 min				
US	450W - 10 min	48	30682	2128	2268
NaOH+US	4% -25 °C-4 hours	65	30682	2204	2464
	450W-10 min				

^a E_M

^b mean laboratory temperature

^c GER*NaOH(%)

^d Q+GER*NaOH(%)

^e maintained temperature

Table 2 composition of liquid and solid phases of the pretreated olive pomace

Pretreatment	Liquid phase					Solid phase				
	pH _f	Soluble sugars (mg/gVS raw)	Soluble polyphenols (mg/gVS raw)	sCOD (mg/gVS raw)	Cellulose (%TS raw)	Hemicellulose (%TS raw)	Lignin (%TS raw)	FA (mg/gTS raw)	Pretreated solid (%TS raw)	
Untreated	6.3	10.7±0.6	5.4±0.3	69±1	12.3±0.1	8.9±0.1	34±3	161.9±8.1	100	
NaOH	2% -25 °C-4 days	7.1	20.1±1	9.6±0.3	290±3	6±0.3	7.6±0.2	23±2	41.3±2.1	75.0
	4% -25 °C-2 days	7.7	20.5±1	9.6±0.1	364±2	7.9±0.2	7.8±0.2	21±0.1	25.3±1.3	73.1
	8% -25 °C-1 day	11.1	20.2±1	12.7±0.3	537±3	5.8±0.2	5±0.2	16±1	3.7±0.3	55.8
	2% -50 °C-4 days	7.0	20.2±1	13.9±0.3	287±2	10.2±0.2	8.4±0.1	25±1	64.1±3.2	75.0
	4% -50 °C-2 days	7.1	21.2±1.1	11.4±0.2	337±6	10.5±0.4	9.4±0.3	22.0±1.6	30.3±1.5	75.8
	8% -50 °C-1 day	10.4	22.9±1.1	12.7±0.2	625±2	6.7±0.3	5.7±0.2	11±0.9	3.6±0.2	47.0
MW	200W - 2 min	6.2	10.1±0.5	3.0±0.2	102±1	10.9±0.6	10.5±0.6	32±3	147±3	98.3
	450W - 2 min	6.3	13.9±0.6	6.0±0.1	132±2	9.7±0.3	10.8±0.0	32±4	138±6	95.2
	700W - 2 min	6.2	14.5±0.7	8.6±2.3	160±1	7.4±0.5	11.5±0.5	33±2	140±4	93.5
	450W - 10 min	6.2	24.8±1.2	15.0±0.2	259±1	6.1±0.2	8.8±0.4	25±2	132±7	83.6
NaOH+MW	4% -25 °C-4 hours	8.4	22.2±1.1	14.0±0.6	445±1	6.6±0.1	7.5±0.1	18±1	15.9±0.8	65.0
US	450W - 10 min	6.1	15.9±0.8	9.5±0.1	222±1	10.3±0.7	9.9±0.5	30.6±2.5	27.6±1.4	87.3
NaOH+US	4% -25 °C-4 hours	7.1	23.1±1.1	18.0±0.1	610±1	7.5±0.7	6.1±0.5	14.7±0.9	7.9±0.4	48.5
	450W-10 min									

Table 3 Effects of pretreatments on methane production and kinetic parameters of AD

Pretreatment	Methane production (mL/gVS)	BMP Improvement (% untreated)	Kinetic parameters					
			<i>Modified Gompertz model</i>					
			$B=B_0 \exp(-\exp((R_m - (\lambda - t) e/B_0)+1))$					
			B_0 (mL/gVS)	R_m (mL/gVS.d)	λ (d)	RMSE	R^2	
Untreated	215±10	-	227	21.8	3.8	8.30	0.995	
NaOH	2% -25 °C-4 days	252±16	+8	247	24.8	0.8	5.73	0.997
	4% -25 °C-2 days	255±5	+17	243	25.9	0	9.11	0.998
	8% -25 °C-1 day	280±3	+30**	277	46.0	4.9	4.81	0.999
	2% -50 °C-4 days	240±15	+9	226	22.4	0.1	7.83	0.998
	4% -50 °C-2 days	264±11	+23*	252	26.9	0.1	9.49	0.998
	8% -50 °C-1 day	280±13	+30**	276	50.9	5.6	4.08	0.999
MW	200W - 2 min	229±2	+6	236	18.8	3.0	4.20	0.998
	450W - 2 min	244±10	+13	239	16.4	0	10.57	0.995
	700W - 2 min	217±5	-1	218	17.3	2.7	3.25	0.998
	450W - 10 min	226±2	+5	224	23.7	1.9	2.03	0.999
NaOH+MW	4% -25 °C-4 hours 450W-10 min	244±5	+13*	250	76.2	5.4	7.04	0.999
US	450W - 10 min	207±15	-4	202	65.9	5.8	3.97	0.999
NaOH+US	4% -25 °C-4 hours 450W-10 min	250±13	+16*	254	74.8	5.1	7.02	0.999

* ($\alpha < 0.1$); ** ($\alpha < 0.05$)

Table 4 Correlation matrix of all variables

	Methane	%NaOH	Temperature	Time	MW energy	US energy	sCOD	Cellulose	Hemicelluloses	Lignin	FA
Methane	1										
%NaOH	0.90***	1									
Temperature	-0.29	-0.23	1								
Time	0.38	0.23	-0.43	1							
MW energy	-0.24	-0.2	0.80***	-0.37	1						
US energy	-0.26	-0.08	0.04	-0.27	-0.22	1					
sCOD	0.76***	0.89***	0.03	0.15	-0.05	0.24	1				
Cellulose	-0.48	-0.51	-0.32	-0.11	-0.39	0.09	-0.63*	1			
Hemicelluloses	-0.73**	-0.86***	0.27	-0.25	0.13	-0.11	-0.9***	0.56	1		
Lignin	-0.82***	-0.91***	0.02	-0.25	0	-0.09	-0.9***	0.64*	0.89***	1	
FA	-0.65**	-0.80***	0.21	-0.35	0.22	-0.34	-0.8***	0.43	0.76**	0.84***	1

p-value for correlations significance: (***) <0.001; (**) <0.01; (*) <0.05; (.) <0.1; () <1

Table 5 Relative energy efficiency of the studied pretreatments

Pretreatment	Conditions	tEc (kJ/kgTS)	E _{Out} (kJ/kgTS)	R _{eff} (%)
	Control	E _M =140	E _{Out0} =7842	0
NaOH	2% -25 °C-4 days	260	9190	+16
	4% -25 °C-2 days	380	9300	+16
	8% -25 °C-1 day	618	10213	+25
	2% -50 °C-4 days	6197	8535	-70
	4% -50 °C-2 days	6317	9636	-57
	8% -50 °C-1 day	6555	10220	-52
MW	200W- 2 min	1555	7918	-19
	450W - 2 min	2437	8436	-22
	700W - 2 min	3815	7330	-54
	450W - 10 min	11624	7814	-
NaOH+MW	4% -25 °C-4 hours	11994	8436	-
	450W-10 min			
US	450W - 10 min	2268	7550	-31
NaOH+US	4% -25 °C-4 hours	2464	9118	-14
	450W-10 min			

Figures

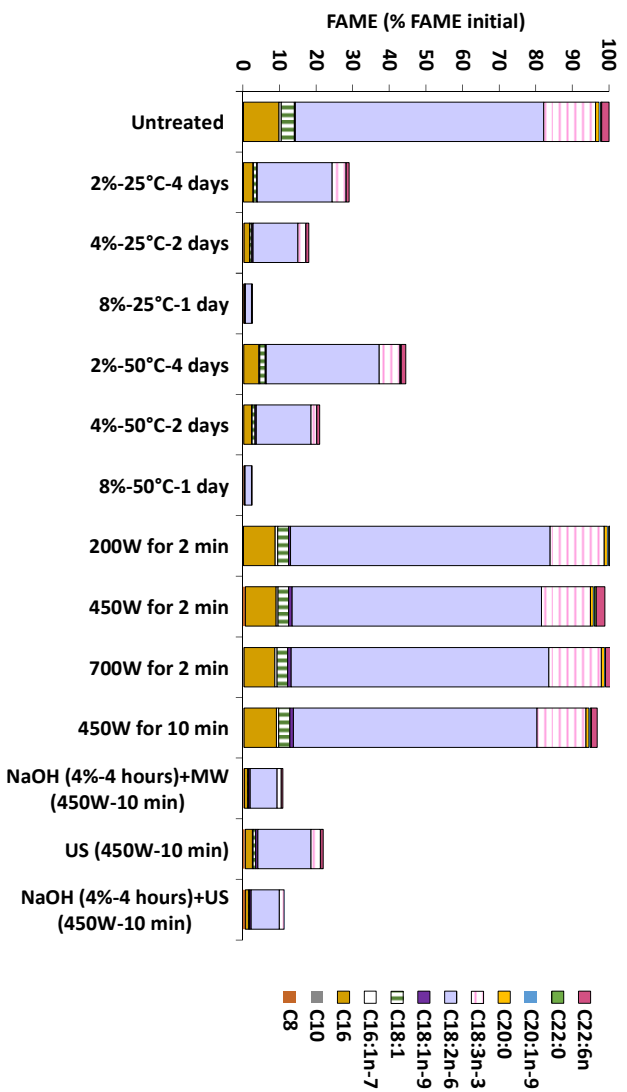


Fig.1 Fatty acids profile in pretreated samples

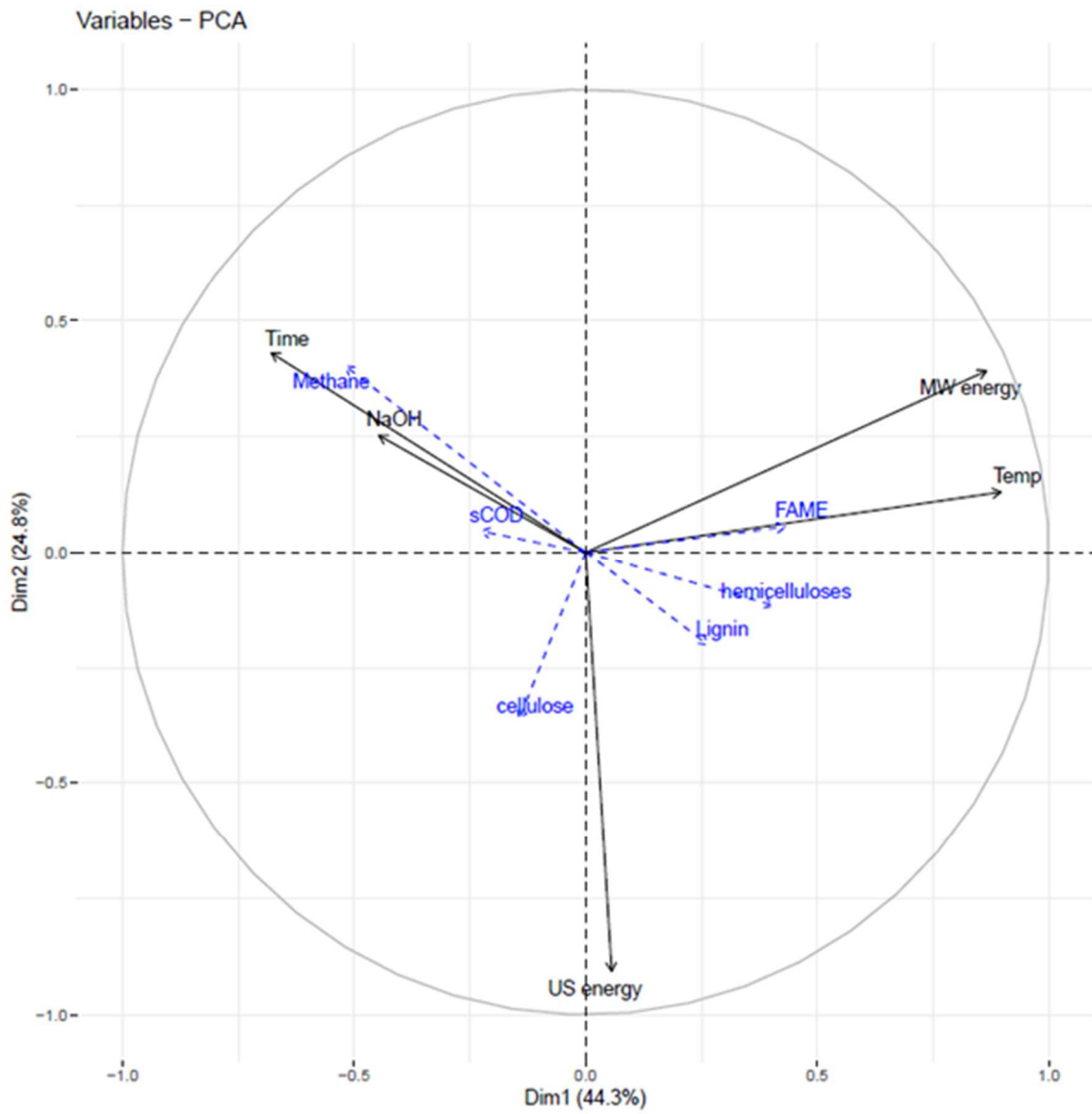


Fig.2 Loading plot of principal components analysis

