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A newly developed method based on surface physicochemical proprieties, for measuring the maturation level of olive fruit

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Abstract: The maturation process is an important determining factor to initiate the fermentation process in olive fruit. Level of maturation classically determined by the color of the olive fruit. In this study, we aim to develop a measurable criterion based on physicochemical properties and surface roughness of two important olive varieties grown in Morocco. The hydrophobic/hydrophilic properties and the electron donor-acceptor character of the olives were calculated. The obtained results showed a very clear change in the electron donor character and the surface roughness of the two studied varieties. This change depended on the degree of maturity and the nature of the cultivar. The electron donor character decreased (two times) with an increasing degree of maturity for the Arbequina, contrary to the Picholine variety, which showed a significant increase (4 times). Surface roughness increased 10 times for the Arbequina and decreased 5 times for the Picholine as maturity progressed from the green to the black stage. These findings may be useful in the management/prediction of the process of table olive fermentation.

Keywords: contact angle; geometry; hydrophobicity; maturity index; olive surface

The olive tree *(Olea europaea* Linnaeus*)* belongs to the family of Oleaceae, one of the earliest-known agricultural corps in history (Gabriel de Oliveira et al. 2015). The olive tree adapts to any pedoclimatic conditions and an integral part of the Mediterranean culture (Mouhtadi et al. 2014). Morocco alone produces approximately 90 000 tons of table olives. The determining factor for initiating the olive fermentation process is fruit maturity. This is the most important parameter of the determining olive fruit quality (Beltrán et al. 2004; Cunha et al. 2006) and is quantified in the maturation index (MI).

The MI is the expression of a common marker of fruit maturation level. The important soluble components present in the olive tissues are sugars and phenolic compounds (Lanza et al. 2013)· They cause the olive surface to have certain physicochemical properties and a rough surface texture. Multiple studies have examined the relationships between olive maturity, the quality of olive oil produced (Ben Youssef et al. 2010; Zipori et al. 2010), and the cultivar (Damak et al. 2008).

The fermentation process is a phenomenon governed by the dhesion of lactic acid bacteria to the olive surface. This adhesion is the result of interfacial interactions between two surfaces that depend on the physicochemical properties and roughness of the substratum.

The maturation process is measured using two methods: the MI, which relies on fruit color and is therefore an imprecise method, the dosage of phenolic component (Morelló et al. 2004), fatty acids,

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and the antioxidant activity (Damak et al. 2008). These methods require expensive and heavy equipment. In this study, we attempted to identify that is measurable and easy-to-use, utilizing the measurement of contact angle (Hamadi and Latrache 2008), and the surface roughness (Bengourram et al. 2009) of the olive fruit. In general, these two methods were used to study the phenomenon of bio interface interactions, which controls many fields including bio adhesion and fermentation processes.

To our knowledge, no studies have examined the physicochemical proprieties of olive surface and their relation to the degree of maturation. The aim of this study was to determine the surface physicochemical proprieties of two olive varieties grown in Morocco and their relation to the degree of maturity in order to develop a new method to estimate the maturation level.

MATERIAL AND METHODS

Sampling. The materials investigated were the Moroccan Arbequina and Picholine cultivars. Olive fruit was collected from the region of Beni Mellal in Morocco. The samples of the two varieties were divided into 4 groups: green, rotating green, rotating, and black (Table1).

Preparation of olive surfaces. The surfaces of the two varieties studied were removed (1 × 1 cm) and characterized using the contact angle, which was measured using a goniometer (GBX, France) by the sessile drop method. The surfaces were also characterized using a roughometer to determine the surface roughness. The sample used to measure the contact angle and the surface roughness was taken at random.

Table 1. Dates of fruit harvests and dominant fruit color at the respective sampling date

Harvest	Sampling date	Fruit color
1 (green)	Sept 9	green
2 (green)	Oct 11	green
3 (green)	Oct 26	green
4 (green rotating)	Nov 8	green
5 (green rotating)	Nov 23	right green
6 (rotating)	Dec 7	small reddish spots
7 (rotating)	Dec 23	turn color
8 (black)	Jan 26	purple
9 (black)	Feb 20	black

Contact angle measurements and surface tension components. The olive's surface characteristics were identified from measured contact angles between the olive surface and the probe liquids (Figure 1) (Szyszka and Szczepanski 2015) using a goniometer: Two polar liquids (water and formamide) and one non-polar liquid (diiodomethane) were used as illustrated in Table 2.

The contact angle measurements for each surface were repeated at least 3 times. These measurements were performed using the sessile drop method. In this method, the image of a droplet of liquid placed on the flat solid surface is taken to measure the tangent of the liquid-vapor interface between the angle and the solid-liquid interface. The hydrophobicity of the olive surface was evaluated through water contact angle measurements (Figure 2). The contact angle measurements and the approach of Van Oss et al. (1988), were used to estimate the Lifhitz–van der Waals [$\gamma^{(LW)}$], electron acceptor [$\gamma^{(+)}$], and electron donor [$\gamma^{(-)}$] components of the surface tension. In this approach, the contact angle is expressed as (Equation 1):

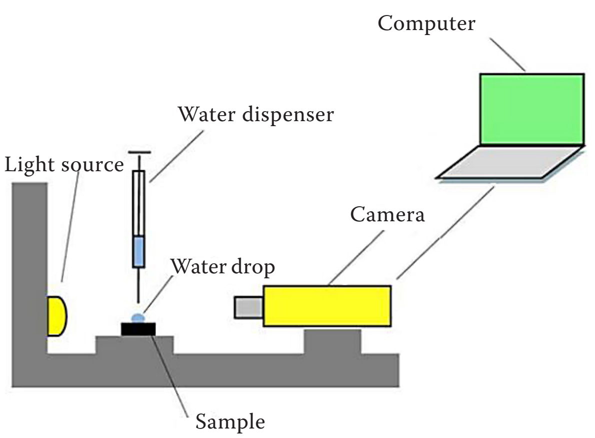

Figure 1. Schematic representation of the system to measure the contact angle

Table 2. Energy characteristics of pure liquid used to measure the contact angle (mJ·m^{-2})

Liquid	$\gamma^{(LW)}$	$\gamma^{(+)}$	$\gamma^{(-)}$
Water	21.6	25.4	25.4
Formamide	38.7	2.3	39.4
Diiodomethane	50.5	0.7	0.0

The total surface tension components were calculated from the contact angle; $\gamma^{(LW)}$ – Lifshitz-van der Waals; $\gamma^{(+)}$ – electron acceptor; $\gamma^{(-)}$ – electron donor

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$$\cos\theta = -1 + 2 \times \frac{\sqrt{\gamma_s^{LW} - \gamma_s^{LW}}}{\gamma_L} + 2 \times \frac{\sqrt{\gamma_s^{+} - \gamma_L^{-}}}{\gamma_L} + 2 \times \frac{\sqrt{\gamma_s^{-} - \gamma_L^{+}}}{\gamma_L} \quad (1)$$

where: L – the pure liquid; s – the solid; $\gamma^{(LW)}$ – Lifshitz–van der Waals; $\gamma^{(-)}$ – electron donor; $\gamma^{(+)}$ – electron acceptor.

The Lewis acid-base (γ_S^{AB}) components component (Equation 2):

$$\gamma_S^{AB} = 2 \times \sqrt{\gamma_s^{-} \times \gamma_s^{+}} \quad (2)$$

The free energy is given by the (Equation 3):

$$\gamma_S = \gamma_S^{LW} \times \gamma_S^{AB} \quad (3)$$

Surface roughness measurements. Olive surface roughness was measured using a surface roughometer (Federal Surf®, country?). For each surface tested, one parameter, mean surface roughness (R_a), was chosen for analysis as it was identified as being representative of the surfaces. Three samples were produced for each olive variety for the four degrees of maturity: green, rotating green, rotating, and black.

Maturation index. The MI was determined according to the method proposed by the National Institute of Agronomical Research of Spain, San Jae'n Station. Briefly, the empirical procedure consists in distributing a randomly taken sample of 100 olives in 8 groups according to the skin colors: bright green (group N = 0), green yellowish (group N = 1), green with reddish spots (group N = 2), reddish-brown (group N = 3), black with white flesh (group N = 4), black with < 50% purple flesh (group N = 5), black with g 50% purple flesh (group N = 6), black with 100% purple flesh (group N = 7). The index is given by Equation (5):

$$\sum \frac{(N_i \times n_i)}{100} \quad (5)$$

where: N – the group number; n – the number of olives number in that group.

MI values range from 0 (N = 0 bright green olives, n = 100) to 7 (N = 100 purple flesh olives, n = 100).

Statistical analysis. Data obtained from these experiments were subjected to statistical analysis using IBM SPSS Statistics software (version 23). Differences in the variation of the MI, the hydrophobicity, and the donor/acceptor electron of the olive surfaces were examined by an ANOVA techniques. Significance was determined at the P < 0.05 level. All experiments were replicated 3 times on separate days.

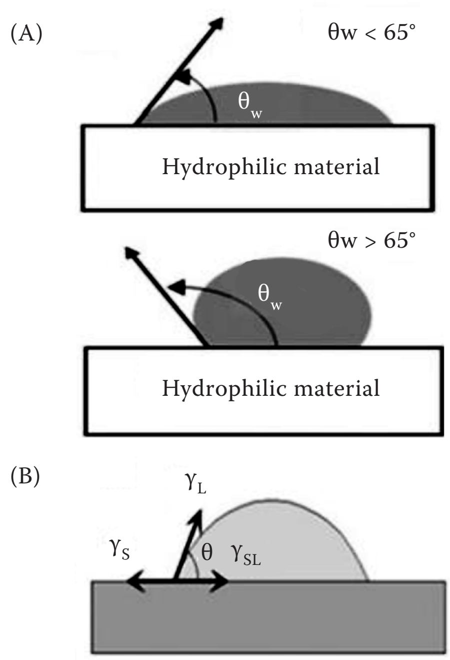

Figure 2. Schematic representation of contact angle measurement according to (A) Vogler's approach and (B) Van Oss's approach (1997)

γ_L – total surface tension of a liquid; γ_S – total surface tension of a solide; γ_{SL} – measurement of a surface-liquid interactions; θ_w – water contact angle

RESULTS

Physicochemical characterization of Moroccan olive surfaces. The determination of such physicochemical characteristics as Lifshitz van der Waals, electron donor, and acceptor proprieties of the olive surface can be very important in different areas, particularly in bacterial adhesion phenomena. The results of physicochemical characterization using contact angles, roughness measurements, and the MI are listed in Table 3.

The results of the water contact angle measurements of the Moroccan Arbequina in different degrees of maturity- green olives (immature), rotating green, rotating and black (mature) – showed that hydrophobicity depended on the degree of maturity, which was particularly increased for the black group. The surface tension of the

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Table 3. Contact angles of parameters obtained with Arbequina and Moroccan Picholine

Cultivar	Surface tension: Components and parameters ($mJ \cdot m^{-2}$)						R_a (μm)	MI
	θ diiométhane	θ formamide	θ water	$\gamma^{(LW)}$	$\gamma^{(+)}$	$\gamma^{(-)}$		
Arbequina								
Green	12.25	46.32	52.3 ± 3.39***	49.51	0.41 ± 0.54	32.7 ± 10*	0.132 ± 0.04***	0.15 ± 0.01***
Rotating green	34.2	53.07	61.3 ± 1.41***	42.29	0.11 ± 0.02	28.9 ± 5.47*	0.57 ± 0.06***	2.68 ± 0.28***
Rotating	45.8	47	62.9 ± 1.91***	36.5	061 ± 0.091	17.3 ± 3.78*	0.76 ± 0.12***	4.7 ± 0.12***
Black	50	59	70.9 ± 1.05***	34.2	0.06 ± 0.05	15.54 ± 1.5*	0.93 ± 0.11***	5.6 ± 0.44***
Moroccan Picholine								
Green	50	70	70.9 ± 0***	34.2	0.62 ± 0***	24.9 ± 0***	0.97 ± 0.12*	0 ± 0***
Rotating green	46.6	54.2	58.5 ± 0.28***	36.05	0 ± 0***	28.9 ± 3.81***	0.95 ± 0.17*	2.89 ± 0.1***
Rotating	55.5	64.85	54.15 ± 0.21***	31.1	0.5 ± 0.56***	47.55 ± 0.35***	0.86 ± 0.03*	4.44 ± 0.06***
Black	57.9	74.7	44.15 ± 0.3***	29.72	3.03 ± 0.3***	83.28 ± 0.02***	0.63 ± 0.02*	5 ± 0.23***

***, *$P < 0.001$ and 0.05, respectively; values are the mean of 3 replicates ± a SD; $\gamma^{(LW)}$ – Lifshitz–van der Waals; $\gamma^{(-)}$ – electron donor; $\gamma^{(+)}$ – electron acceptor; R_a – mean surface roughness; MI – maturity index

Moroccan Arbequina was calculated from contact angle values using a dispersive polar approach. Our results showed that Arbequina surfaces had an important donor electron character since the $\gamma^{(-)}$ basic character was higher than the $\gamma^{(+)}$ acid character (Table 3). A large variation was obtained when increasing the degree of maturity, with a twofold decrease from $\gamma^{(-)} = 32.7\ mJ \cdot m^{-2}$ (green) to $\gamma^{(-)} = 15.54\ mJ \cdot m^{-2}$ (black).

As for the Arbequina surface, the results of the physicochemical characterization of Picholine also showed that surface hydrophobicity was dependt on the degree of maturity. Contrary to the surface of Arbequina, however, the Picholine become less hydrophobic with increasing degrees of maturity [black Picholine (θ_w = 44.15°)]. The Moroccan Picholine surfaces also had an electron donor character (i.e. a Lewis base), ranging from $\gamma^{(-)} = 24.9\ mJ \cdot m^{-2}$ to $\gamma^{(-)} = 83.29\ mJ \cdot m^{-2}$; this character increased with an increase in the degree of maturation (Table 3.)

Measurement of Moroccan olive surface roughness. Examination of the Moroccan Picholine and Arbequina surface roughness demonstrated that surface roughness was influenced by the degree of maturity and cultivar nature. The Moroccan Picholine surface roughness decreased with a decreasing degree of maturity, which ranged from R_a = 0.976 μm to R_a = 0.639 μm (Table 3). For the Moroccan Arbequina, on the other hand, surface roughness increased with an increase in the degree of maturity, with a tenfold increase from 0.1326 μm to 0.963 μm.

DISCUSSION

Maturation is one of the most important parameters related to the fruit evolution and vegetable quality. It is regular to take into account that there are three phases for the lifetime of the olive fruit (Shulman and Lavee 1976). In this study, we have compared the MI, to physicochemical properties of the surface of olives in order to determine measurable parameters to quantify the level of maturity. Which is related to the phenolic profile (Amiot et al. 1989).

The phenolic profile is influenced by agronomic conditions including different methods of irrigation of olive trees (Tovar et al. 2001; Romero et al. 2002). Oleuropein (heterosidic ester of elenolic acid and 3,4-dihydroxyphenylethanol) is the main phenolic compounds in olive fruit (Gariboldi et al. 1986;

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Amiot et al. 1989). It has considerable technical importance due to its intensely bitter taste and browning capacity (Sciancalepore and Longone 1984; Sciancalepore 1985).

Moreover, oleuropein represents more than 14% of the dry weight of the olive fruit (Bianco et al. 2006). Oleuropein levels increase during the development phase of the olive, then decrease during maturation, following the activation of esterases, until it disappears in the black olive maturation process (Amiot et al. 1989; Visioli and Galli 1998). This may explain the obtained results for the Moroccan Picholine, which became more hydrophilic with increasing maturity (θ_w = 44.15°). Oleuropein and ligstroside constitute the main polar (hydrophilic) phenolic fraction of the olive fruit (Tsimidou 1998). One of the essential changes that occurs during olive ripening is the development of color; it is thought to be associated with a decrease in chlorophyll levels and the appearance of anthocyanins responsible of olives' black color (Saija and Uccella 2000; Wichers et al. 2000). The hydrophobic character of anthocyanins is confirmed by their polyphenolic structure (Khoo et al. 2017). Furthermore, the obtained results for the Arbequina, when oleuropein (the polar fraction) fell during the maturation process (from the green to the black stage), the hydrophilicity decreased. The high hydrophobic character at the black maturation stage (θ_w = 70.9°) of olives could be explained by the appearance of the anthocyanins, which are characterized by their hydrophobicity (Khoo et al. 2017).

A varietal effect exists for the surface hydrophobicity as showen in Table 3. The surface of Arbequina was more hydrophobic at the black maturation stage, possibly due to the increase in oil content at this stage (Zamora et al. 2001). In contrast to the Arbequina, the surface of the Picholine olive became more hydrophilic as it progressed to the black maturation stage. This could be due to the non-exhaustive fall of the polar fraction mostly due to a fall in oleuropein (Tsimidou 1998) to 25 $mg \cdot g^{-1}$ dry weight during the black maturation stage (Amiot et al. 1989). Moreover, the black maturation stage of Picholine is characterized by the accumulation of elenolic acid glucoside (Amiot et al. 1989). Visioli et al. (2002) reported that this molecule presents a polar and hydrophilic form, which could explain the hydrophilic character of the black maturation process of Picholine olive surfaces (Visioli et al. 2002).

The surface of the two varieties express an important electron donor character [high value of $\gamma^{(-)}$] Table 3. This may be due to the difference in the chemical composition of the two varieties studied, but no relationship has been etablished between the elemental composition, functional composition (expressed as a percentage), and the electron-donor character (Hamadi et al. 2012).

The variation of the surface roughness has been reported to be related to changing physicochemical proprieties or changing hydrophobicity (Bengourram et al. 2009). which confirms and explains the correlations of hydrophobicity and surface roughness seen in this study (surface roughness increased with an increase in surface hydrophobicity), these results are in accordance with Hamadi et al. (2008).

In order to complete our analysis, multiple correlations were established between the maturity index and the various physicochemical parameters. For the Arbequina, a positive correlations was obtained between θ_w, surface roughness, and maturity index. However, donor electron character and the MI were negatively correlated (Table 4). For the Picholine, a negative correlation between surface hydrophobicity, surface roughness, and maturity index was found. A positive correlation was found between electron donor character and MI (Table 4). The results in the Table 4 show that the MI was related to the olive's physicochemical properties, including electron donor, surface hydrophobicity, and surface roughness.

These parameters were studied in order to establish guidance for identifying the optimal stage of ripening for harvesting and processing in the Arbequina and Picholine cultivars We found precise and measurable parameters to determine the maturity of these olives, for example, if a Picholine with green maturity is needed, a hydrophobicity of 70.9° can be used to identify the correct maturation, as our results showed a significant correlation between hydrophobicity and degree of maturity. Additionally, by using this information along with the XDLVO approach, we can predict the adhesion of LAB on the olive surface for improving and optimizing the fermentation process, as the fermentation process is the result of the metabolism of LAB attached to the surface olives. This attachment is the result of physicochemical interactions between the bacterial surface and the olive surface. All of these considerations can help improve the olive industry.

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Table 4. Correlation between physicochemical properties, sutface roughness, and MI found in Arbequina and Picholine

	Correlations				
	θ_w	$\gamma^{(+)}$	$\gamma^{(-)}$	R_a	MI
Arbequina					
θ_w	1.000	–	–	–	–
$\gamma^{(+)}$	–0.395	1.000	–	–	–
$\gamma^{(-)}$	–0.682*	0.238	1.000	–	–
R_a	0.933**	-0.157	–0.702*	1.000	–
MI	0.918**	-0.134	–0.806**	0.955**	1.000
Picholine					
θ_w	1.000	–	–	–	–
$\gamma^{(+)}$	–0.678*	1.000	–	–	–
$\gamma^{(-)}$	–0.899**	0.894**	1.000	–	–
R_a	0.744**	–0.754**	–0.799**	1.000	–
MI	–0.959**	0.499	0.782**	–0.638*	1.000

**,* $P < 0.01$ and 0.05, respectively; θ_w – contact angles of water; $\gamma^{(-)}$ – electron donor; $\gamma^{(+)}$ – electron acceptor; R_a – mean surface roughness; MI – maturity index

CONCLUSION

This study indicates a new method for measuring the maturation level of olive fruit based on measurement of physicochemical properties. The results of our study showed that the MI influenced by hydrophobicity level and the surface roughness.

For the Picholine; the surface hydrophobicity decreased with the increasing of the MI (from hydrophobe for the green fruit [θ_w = 70.9°] to hydrophile for the black fruit [θ_w = 44.15°). Also, the surface roughness decreased with the increase of the maturation index (from R_a = 0.976 μm for the green fruit to R_a = 0.639 μm for the black fruit),

For the Arbequina: the surface hydrophobicity increased with the increase of the maturation index (from hydrophile for the green fruit [θ_w = 46.32°] to hydrophobic for the black fruit [θ_w = 70.9°]). Though the surface roughness decreased with the increase of the maturation index (from R_a = 0.93 μm for the green fruit to R_a = 0.123 μm for the black fruit).

These differences depend on the maturation process and the nature of the cultivar. As well as the phenolic compounds, which influence the fermentation process. These findings may be useful in the management/prediction of table olive fermentation.

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