Influence of ripening index and water regime on the yield and quality of “Moroccan Picholine” virgin olive oil

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Abstract – The purpose of this work is to evidence the effects of ripening index and water regime on the quantity and quality indices of “Moroccan Picholine” virgin olive oil (VOO) produced in northern Morocco. Olive trees were subjected to full irrigation and rainfed conditions, and olive fruits were collected at different ripening times. Results showed that the extracted volume of VOO increased during ripening, and decreased when full irrigation was applied. In regard to VOO quality, the statistical analysis revealed the predominant effect of ripening index on the majority of the considered parameters, except total phenols content that was strongly influenced by water regime. At more advanced stages of maturity, lower values of peroxide value, K232, carotenoids, chlorophylls and total phenols were registered while more free fatty acids were accumulated. Moreover, full irrigation reduced total phenols and increased free fatty acids, even if a great amount of pigment content was scored. Correlation studies showed significant relationships between pigments content and oxidation indices (peroxide value and K232).

Keywords: irrigation / oil quality indices / oil extraction / olive maturation / VOO

1 Introduction

Olive (Olea europaea L.) is the main crop fruit in the Mediterranean basin, and olive oil is a basic component of the diet in the region (Serra-Majem et al., 2003). Mediterranean countries are the main producers with 97% of the worldwide olive oil production estimated at 3 159 500 tons in the 2016 crop season. Morocco is the sixth world producer of olive oil after Spain, Italy, Greece, Turkey, and Tunisia (IOC, 2016). “Moroccan Picholine” is the most cultivated variety and accounts for more than 96% of cultivated olive trees nationwide (MAPMDREF, 2019).

World consumption of olive oil is still increasing, even in countries that have no history of olive growing, and it is...
The chemical characteristics and quality of virgin olive oil are influenced by several factors, including genotype, tree age, fruit ripening, productions area, pedoclimatic conditions, agronomic and irrigation practices and extraction process (Zamora et al., 2001; Rotondi et al., 2004; Abaza et al., 2005; Ben Temime et al., 2006; Baccouri et al., 2007; Gómez-Rico et al., 2007; Ouni et al., 2011; Jiménez et al., 2013; Yorulmaz et al., 2013; Gouvinhas et al., 2015).

VOO quality is strongly related to fruit maturation. An increase in polyunsaturated fatty acids associated with a loss in total phenols, pigments and oxidative stability, as the olives ripen, was reported in many studies (Gutiérrez et al., 1997; Fitó et al., 2007; Bendini et al., 2007; López-Miranda et al., 2010; Cárdeno et al., 2013).

The present investigation was carried out during the 2017 crop season, on the widely grown olive variety “Moroccan Picholine” in an experimental olive grove located at Taza province (34°12′36″ N, 3°52′0″ W, 530 m asl) in northern Morocco. The soil in the site is a Typic Xerochrept, a weakly developed soil of alluvial contribution with a silty clay loam texture and parental material from middle and old quaternary. The climate is Mediterranean-type with mild and humid winters and dry and hot summers. Total annual rainfall and the average minimum and maximum temperatures during 2017 were 297 mm, 14.0 °C and 27.4 °C, respectively. Annual reference evapotranspiration (ET₀) was 1817 mm. Monthly data (precipitations and air temperatures) are displayed in Figure 1.

Therefore, the purposes of this study were to investigate the effect of olive ripening stage and water regime on the yield and quality of “Moroccan Picholine” VOO (Olea europaea L.) produced in northern Morocco.

2 Materials and methods

2.1 Experimental design and environmental conditions

The chemical and extracted olive oil yield is also under several impacts. Irrigation influence positively the oil content in olive fruits (Inglese et al., 1996; Patumi et al., 1999; Grattan et al., 2006; Tognetti et al., 2006; Gucci et al., 2007; Caruso et al., 2013). In contrast, Iniesta et al. (2009) observed the highest fruit oil content in deficit irrigated orchards. Moreover, moderately irrigated olive trees favored better oil extraction from the olive fresh weight (García et al., 2017). Oil content changes during fruit ripening process were also documented in several studies (Salvador et al., 2001; Zeleke et al., 2012). In fact, oil content accumulates in olive fruits as the ripening progresses (Motilva et al., 2000; Dag et al., 2011; Bakshe et al., 2018; Sönmez et al., 2018). In the same trend, Benito et al. (2013) and Mena et al. (2018) reported an increase in the industrial oil yield during olive maturation.

With respect to water regime, results from previous researches are conflicting. No impact of the tree water status on free acidity, peroxide value, K232 and K270 was documented (Servili et al., 2007; Tognetti et al., 2007; García et al., 2013; Rufat et al., 2018). However, Ismail et al. (1997) and Motilva et al. (1997) noticed higher free fatty acids, peroxide value, total phenols, and oxidative stability in oils extracted from irrigated trees compared to those extracted from water stressed trees. Contrariwise, total phenols, the most affected olive oil components, are significantly higher in VOO from stressed trees than from irrigated ones (Romero et al., 2002; Berenguer et al., 2006; Servili et al., 2007; Fernandes-Silva et al., 2013; García et al., 2017; Gucci et al., 2019).

The chemical and extracted olive oil yield is also under several impacts. Irrigation influence positively the oil content generally in parallel with production rate (Mili, 2006). The significant rise in demand and consumption of olive oil is due to its nutritional value and beneficial health properties including anti-oxidant, anti-atherogenic, anti-inflammatory, anti-tumor, anti-viral, anti-cancer and immune modulator activities (Covas, 2007; Fitó et al., 2007; Bendini et al., 2007; López-Miranda et al., 2010; Cárdeno et al., 2013).

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Thirty adult trees, spaced 6 m apart, were subjected to two water regimes: i) Fully irrigated with trees receiving 100% of water amount needs during irrigation period, and ii) and trees under rainfed condition that did not receive any irrigation.
Each treatment consisted of 15 trees, distributed in three replicates laid out in a randomized block design. The water requirements for the irrigated plot was estimated based on crop evapotranspiration (ETc) calculated using the Penman–Monteith method (Allen et al., 1998), and supplied through a drip irrigation system, with two on-line drippers (4 L/h) per tree, each placed 0.75 cm from the tree-trunk base. The irrigation was applied once a week from June to the beginning of September, which coincides with high atmospheric demands for water vapour, and corresponds to fruit set (BBCH 69) and pit hardening stages (BBCH 71-79) and the period of the fastest oil synthesis (BBCH 80-81) (Gómez del Campo and García, 2013). Trees of the second plot were left unirrigated (rainfed conditions). During the irrigation period, the cumulative rainfall and ET₀ were 13 mm and 883 mm, respectively; while the average minimum and maximum temperatures were 21.3 and 37.0°C, respectively.

Three olive samples (2 kg for each sample) were hand-picked randomly from three olive trees representing each treatment at three ripening stages based on the degree of skin and pulp color. The sampling dates were as follows: 24 September (green skin color), 6 October (green skin color with reddish spots), 20 October (red to purple skin color), 15 November (purple to black skin color with white flesh color), and 27 December (black skin color with violet flesh color).

### 2.2 Ripening index determination

The ripeness index (RI), for olives collected at three maturation stages, was determined according to the method developed at the Agronomic Station of Jaén (Spain) (Uceda et al., 1998). It is based on a scoring system for each stage of colouring of the skin and flesh. The RI was obtained on 100 randomly olive fruits in each sample freshly picked, by applying the following formula:

\[
RI = \frac{A_0 + B_1 + C_2 + D_3 + E_4 + F_5 + G_6 + H_7}{100},
\]

where A, B, C, D, E, F, G, and H are the number of fruits in each of the colour categories 0, 1, 2, 3, 4, 5, 6, and 7, respectively.

### 2.3 Oil extraction

The healthy olive fruits hand-picked at different ripening stages from trees under the two water regimes were immediately transported to the laboratory. Olive oil extraction was performed at the Laboratory of Natural Resources and Environment of the Polydisciplinary Faculty of Taza (Morocco) using a lab-scale instrument reproducing industrial conditions for oil extraction; olives were crushed with a hammer crusher, the resulting paste was slowly mixed at room temperatures for 30 min, and the oil was separated by centrifugation (3000 rpm over 5 min) without addition of warm water. The obtained oil was filtered, transferred into amber glass bottles without headspace, and stored in the dark at 4°C until analyses.

The industrial oil yield (extracted oil), given in percentage of fresh olive paste weight (W) and considering the olive oil density (D) at ambient temperature of 0.915 g/mL⁻¹, was determined using the formula (Mena et al., 2018):

\[
\text{Extracted oil (\%)} = \frac{V \times D}{W} \times 100,
\]

Where V is the volume of olive oil obtained (mL).

### 2.4 Olive oil analysis

#### 2.4.1 Quality indices

Determination of free fatty acids, peroxide value, and specific wavelength absorbance at 232 nm and 270 nm (K232 and K270) were carried out, following the analytical methods described in Regulations EEC/2568/91 and later modifications of the Commission of the European Union (EEC, 1991, 2003).

Free fatty acids, expressed as % of oleic acid, was determined by titration of a mixture of oil sample (20 g) dissolved in ethanol (50 mL) with ethanolic solution of potassium hydroxide (0.1 N). Phenolphthalein was used as indicator.

Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq O₂/kg), was measured in the following procedure: olive oil sample (5 g) was dissolved in a solution of chloroform-acetic acid (30 mL) then the mixture was left to react with a solution of potassium iodide in darkness. The liberated iodine by the peroxides was titrated with standardized sodium thiosulphate solution using starch as indicator.

K232 and K270 were calculated from absorption at 232 and 270 nm, respectively, with a UV spectrophotometer (SPECUVIS1; UV-Visible), using a 1% solution of olive oil in cyclohexane (1 g/100 mL) and a path length of 1 cm.

#### 2.4.2 Pigments

The pigment contents (mg/kg of oil) were determined colorimetrically using SPECUVIS1 spectrophotometer; UV-Visible, following the method described by Minguez-Mosquera et al. (1991). A sample of 7.5 g oil was dissolved in 25 mL of cyclohexane. The absorbance of this solution was read at 670 and 470 nm for chlorophylls and for carotenoids, respectively. The values of the specific extinction coefficients used were 613 for pheophytin as major component in the chlorophyll fraction, and 2000 for lutein as major component in the carotenoid fraction. Thusly, pigment contents were calculated using the following equations:

\[
\text{Chlorophylls (mg/kg)} = \frac{A_{670}}{613} \times 10^6 \times \frac{L}{L},
\]

\[
\text{Carotenoids (mg/kg)} = \frac{A_{470}}{2000} \times 10^6 \times \frac{L}{L},
\]

Where A is the absorbance and L is the spectrophotometer cell thickness (1 cm).

#### 2.4.3 Total phenols

Total phenols were isolated according to the method described by Zunin et al. (1995). Olive oil samples (10 g)
dissolved in n-hexane (10 mL) were extracted three times with aqueous methanol (60/40, v/v, 10 mL). The concentration of total phenols was determined spectrophotometrically (SPEC-UVIS1; UV-Visible) following the method of Folin and Ciocalteu (1927). Folin–Ciocalteau reagent was added to a suitable dilution of the extract, and the absorbance was measured at 750 nm using as standard the caffeic acid (Sigma-Aldrich, St. Louis, MO, USA). Values for total phenols content are given as mg caffeic acid/kg oil.

2.5 Statistical analyses

All determinations were performed in three replicates. The two-way analysis of variance (ANOVA) was carried out over ripening index and water regimes. Least significant difference (LSD) values were calculated at the 5% probability level. The relationships between the studied parameters were established on the mean data of all the replicates. The STATGRAPHICS Centurion XVII package (Stat point Technologies, Inc., Virginia, USA) was used for all the calculations.

3 Results

3.1 Extracted oil

Mean values of the extracted olive oil from the olive fresh weight (%) at each ripening index and water regime are shown in Figure 2. The VOO extracted showed a significant variation among the considered maturation stages of olive fruits; it increased consistently with RI and ranged from 7.43% at the early stage to 16.10% at the latest one, with an average value of 10.89%. In relation to water regime, higher extracted oil was scored in olive fruits from trees under rainfed conditions (11.63%), in comparison to those from fully irrigated regime (10.15%). In addition, evolution of extracted oil amount with ripening index was slightly greater in rainfed regime than in the fully irrigated one.

4 Olive oil quality

4.1 Data variability

The combined analyses of variance (ANOVA) of “Moroccan Picholine” VOO produced from olive fruits harvested at different ripening index in trees grown under fully irrigated and rainfed regimes are summarized in Table 1. The ANOVA test showed that peroxide value, pigment (carotenoids and chlorophylls) contents and K232 were predominantly influenced by the ripening index that assigned more than 78% of the observed variance. Total phenols were mainly under the impact water regime (93%). Free fatty acids were highly affected by ripening index (64%) and in lesser extent by water regime (29%). The interaction “ripening index × water regime” had minor influence on all analyzed parameters.

4.2 Effect of ripening index

The mean values of the parameters considered in this work with regard to ripening index are shown in Table 2. Free fatty acids increased significantly from 0.28% at early ripening stage (RI = 0.89) to 0.35% at later stage (RI = 5.05). In contrast, a significant decrease was observed for peroxide value and K232 when RI increased. Similarly, pigment contents showed a reduction tendency towards the progressing of olive maturation. The minimum levels are reached at black pigmentation (RI = 5.06), and were of 0.92 and 1.45 mg/kg for carotenoids and chlorophylls, respectively. Total phenols content also varied markedly with RI; the lowest value (442.85 mg/kg caffeic) was scored in oils produced from fruit olives with the RI equal to 5.06.
Effect of water regime

Concerning water regimes, statistical analyses evidenced some significant differences in tested oils (Tab. 2). The highest values for free fatty acids, carotenoids and chlorophylls (0.32%, 1.84 mg/kg and 3.26 mg/kg, respectively) were measured in olive oils obtained from fully irrigated trees. In contrast, the rainfed conditions favored the production of total phenols (469.63 mg/kg caffeic) compared to the full irrigation (441.36 mg/kg caffeic). No significant differences were revealed for peroxide value, K232 and K270 between the two water regimes.

4.4 Relationships among parameters and factors

Changes of the analytical parameters regarding both ripening index and water regime were plotted in Figure 3. An increase in free fatty acids was observed during maturation, which was more promoted by irrigation. In contrast, a significant decrease was detected during olive fruit ripening in peroxide value, K232 and K270 that appeared unaffected by the water regime. A similar behavior was shown for pigments with a slight effect of water regime. For total phenols, the same response to ripening index was observed under the two regimes; they decreased progressively as maturation progressed, however, oils produced from fully irrigated trees had lower contents than rainfed ones.

Results of the correlation study between the considered parameters of olive oils obtained from fruit olives at different ripening index from fully irrigated trees and those under rainfed conditions are presented in Table 3. The most significant relationships were highlighted between pigment content and oxidation indices. Chlorophylls were strongly and positively associated with carotenoids ($r = 0.971^{***}$), K232 ($r = 0.936^{***}$) and peroxide value ($r = 0.927^{***}$). Carotenoids were highly correlated to peroxide value ($r = 0.965^{***}$) and K232 ($r = 0.891^{**}$). A significant association was also shown between Peroxide value and K232 ($r = 0.822^{**}$). The other correlations were not significant and of minor importance.

5 Discussion

The analytical parameters examined in this study confirmed the effects of olive fruits ripeness and water regime on the industrial yield of extracted VOO and its quality. In fact, significant increase in extracted oil yield was observed during...
Fig. 3. Evolution of free fatty acids (A), peroxide value (B), K232 (C), K270 (D), carotenoids (E), chlorophylls (F) and total phenols (G) in “Moroccan Picholine” virgin olive oil produced from olive fruits harvested at three successive ripening index (RI = 0.89; 3.05 and 5.06) from trees grown in Taza province under two water regimes (Fully irrigated and rainfed) during the 2017 crop season.
ripening progress of olives fruits. Our results are consistent with previous studies. Mena et al. (2018) reported an increase from 12.14% to 15.48% of industrial oil yield produced from “Castellana” variety grown in Spain, during olive ripening, and explained this change by the loss of moisture of the olives as they ripened. The oil accumulation in olive fruits as the maturity progressed was also observed for several olive varieties such as “Leccino”, “Messinese”, “Picholina”, “Etnea”, “Itrana”, “Coratina” and “Zaituna” (Zeleke et al., 2012, Zeleke and Aytom, 2014). Bakshi et al. (2018) attributed the rise in oil content during olive fruits growth to the biosynthesis of triglycerides until the fruit reached full maturity. In addition, Sanchez (1994) reported that oil accumulation in olives takes place before the start of the ripening process, when the fruit is green and photosynthetically competent, while the triacylglycerols formation reached a plateau when the color of the fruit turn from green to purple and then black.

Effect of water regime on extracted oil content was also revealed in the present work, which was in harmony with previous studies indicating that irrigation caused a decrease of mechanically extractable oil (Grattan et al., 2006; Morales-Siller et al., 2011). The highest amount of oil physically extracted in relation to the fruit fresh weight was scored in the less-irrigated treatments (Iniesta et al., 2009; Ramos and Santos, 2010; Garcia et al., 2017). Zeleke and Aytom (2014) noticed that the irrigation treatment significantly influenced the extractable olive oil that was higher in non-irrigated treatment compared to the fully and partially irrigated ones. The same authors announced that the mechanical extraction efficiency decreased with the fruit water content, since the irrigation treatment was not contrasting enough to affect the oil accumulation in olives. The greater oil yield (expressed as fresh weight of fruit) in stressed olives could be the result of higher water content in olives from the irrigated trees, which may affect negatively oil extraction (Motilva et al., 2000).

Concerning the VOO quality indices, the free fatty acids were influenced mainly by ripening index but also water regime. It increased significantly as fruit ripening progressed, which was in agreement with the results published previously (Gutiérrez et al., 1999, Salvador et al., 2001; Rotondi et al., 2004; Yousfi et al., 2006; Dag et al., 2011; Bakshi et al., 2018). The high level of free fatty acids in VOO from olives at advanced maturation stage was attributed to an increase in enzymatic activity, especially by lipolitic enzymes, and to higher sensitivity to pathogenic infections (Martínez-Suárez, 1973; Salvador et al., 2001). Between water regimes, the highest score of free fatty acids was observed in VOO from fully irrigated trees. Chehab et al. (2013) have also observed a tendency of increasing free fatty acids of virgin olive oil with increased irrigation levels. In addition, minor effects of water irrigation on free fatty acids were indicated in several studies (Servili et al., 2007; Allalout et al., 2009; Dabbou et al., 2011; 2015). However, no statistical differences between water regimes were reported in other investigations (Tovar et al., 2001; Caruso et al., 2014; Rufat et al., 2018). The high sensitivity to fly attack of olive fruits with higher water content from fully irrigated compared to rainfed trees could explain the differences detected in our study, as reported by Gómez-Rico et al. (2009) and Chehab et al. (2013).

The peroxide value was totally under the impact of ripening index. The lower values were obtained at the latest stage of olives maturity. The reduction in peroxide value during maturation process is well documented (Salvador et al., 2001; Baccouri et al., 2008; Ben Youssef et al., 2010; Dag et al., 2011). The behavior observed for the peroxide value was a consequence of a decrease in the lipoxygenase activity (Gutiérrez et al., 1999). The results obtained for K232 that is related to the primary oxidation of oil, were relatively similar to those for the peroxide value and are in conformity with other studies (Gómez-Rico et al., 2007; Baccouri et al., 2008). K270 is an indicator of the secondary oxidation products (aldehyds and ketones) in olives and was slightly affected by both the ripening index and water regime, but no statistical differences were detected. The effect of water regime on oil oxidation indices (peroxide value, K232 and K270) was of negligible magnitude, in contrast to the results of other authors who mentioned a significant effect of irrigation level on these indices (Gómez-Rico et al., 2007; Dabbou et al., 2015; García et al., 2017).

The changes in pigments of extracted VOO were controlled by ripening index. In fact, losses of both chlorophylls and carotenoids were observed as the olive fruits

| FFA | PV | K232 | K270 | Car | Chl | TP |
|-----|----|------|------|-----|-----|----|
| −0.786* | −0.803* | −0.125 | −0.833** | −0.764* | −0.699* |
| PV | 0.822** | 0.540 | 0.965*** | 0.927*** | 0.250 |
| K232 | 0.377 | 0.891** | 0.936*** | 0.366 |
| K270 | 0.468 | 0.565 | −0.447 |
| Car | 0.971*** | 0.267 |
| Chl | 0.170 |
| TP | | | | | | |

FIA: free fatty acids, PV: peroxide value, K232 and K270: extinction coefficients at 232 nm and 270 nm, Chl: chlorophylls, Car: carotenoids, TP: total phenols.

*Significant at 0.05 probability level; **Significant at 0.01 probability level; ***Significant at 0.001 probability level.
ripened. These findings match those already found by other authors (Gutiérrez et al., 1999; Beltrán et al., 2005; Ben Youssef et al., 2010; Bakshi et al., 2018; Piscopo et al., 2018). The progressive reduction of pigments with olive maturation is due firstly to decrease in the photosynthetic activity (Salvador et al., 2001) and also to the formation of other colored compounds, such as anthocyanins (Roca and Mínguez-Mosquera, 2001). Significant differences were found for both chlorophylls and carotenoids between rainfed and fully irrigated trees. The highest contents were associated with the greatest level of irrigation, which was in agreement with the finding by Motilva et al. (2000) and Baccouri et al. (2008).

Total phenols were influenced by both the water regime and the ripening index, decreasing in fully irrigated treatment and as olives ripened. The changes in these substances regarding the mentioned factors confirmed the results obtained by Motilva et al. (2000) and Rufat et al. (2018). In the same way, it was indicated that higher levels of irrigation reduced the total phenols content in olive oils (Salas et al., 1997; Tovar et al., 2001; Romero et al., 2004; Gómez-Rico et al., 2007; Servilli et al., 2007; Dag et al., 2008); while the highest concentrations were recorded at deficit-irrigated, and severely stressed trees or those grown under rainfed conditions (Motilva et al., 2000; Caruso et al., 2014; Gucci et al., 2019). Moreover, differences in total phenols amounts were related to many environmental conditions, especially the amount of water applied (Berenguer et al., 2006; Chehab et al., 2013; Marra et al., 2016; Sastre et al., 2016). In fact, it is known that the plant water status implied changes in the activity of L-phenylalanine ammonia lyase (PAL); the main enzyme responsible for phenolic compounds synthesis in drupes; its activity is reduced as the amount of water applied increased (Patumi et al., 1999; Tovar et al., 2002; Morelló et al., 2005).

In relation to olive maturation, a reducing tendency of total phenols was observed during ripening, declining to the lower levels at RI of 5.06, in accordance with the findings reported by Vázquez-Roncero et al. (1971), Serman et al. (2011) and Piscopo et al. (2018). Similarly, a clear negative correlation was obtained between total phenols and RI (Rotondi et al., 2004; Dag et al., 2011). The total phenols content increased gradually until reaching a maximum at RI between 2 and 4, after which it decreased (Salvador et al., 2001, Baccouri et al., 2008, Ben Youssef et al., 2010).

The correlation matrix from mean data presented in our work revealed a close positive relationship between pigments content (chlorophylls and carotenoids) and indicators of oil oxidation (peroxide value, K232 and K270). However, other authors reported a negative correlation and confirmed the antioxidant potential of carotenoids and chlorophylls (Velasco and Dobarganes, 2002; Szydlowska-Czerniak et al., 2011; El Yamani et al., 2019). The chlorophylls and their derivatives in presence of light promote the first phases of the autodestruction process (Cichelli and Pertesana, 2004; Ben Tekaya and Hassouna, 2007). The positive correlation between pigments content (especially carotenoids) and oxidation indicators could be due to their similar response to ripening index and water regime. A negative but not significant correlation was observed between total phenols and oxidation indicators, in agreement with numerous authors (Sif et al., 2001; Torres and Maestri, 2006; El Yamani et al., 2019) who indicated the role of phenolic compounds in resisting to oxidation.

6 Conclusions

This work reports the changes in the extraction yield and different quality parameters of VOO from “Moroccan Picholine” in relation to olive fruit ripening and water regime. Our findings could be useful and of great importance to the olive oil industry for providing the optimal VOO quality with the high industrial oil yield. The optimum harvest period was clearly inconsistent with either early or advanced ripening stages. In fact, it is recommended that the harvesting should be carried before reaching advanced maturity of olive fruits, in order to get maximum oil yield while keeping its good quality. With regard to water regime, the best scores either for extraction yield or quality of olive oil were found for rainfed conditions. However, the influence of deficit irrigation levels needs further investigations.

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Conflicts of interest. The authors declare no conflict of interest.

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