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Article

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Figure 1. Total phenols determined by using the Folin−Ciocalteu assay. Results represents means ± standard deviation (n=3); 18, 20, 22, 24 = number of weeks after anthesis; means without a common letter (a-c) indicate significantly different values (P< 0.05).

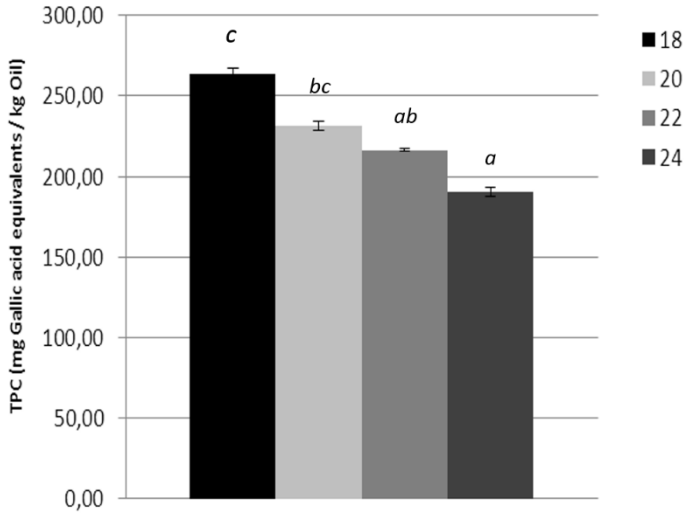


Figure 2. LC-ESI-MS/MS chromatogram of polyphenols found in hybrid palm oil sample at 18 week after anthesis.

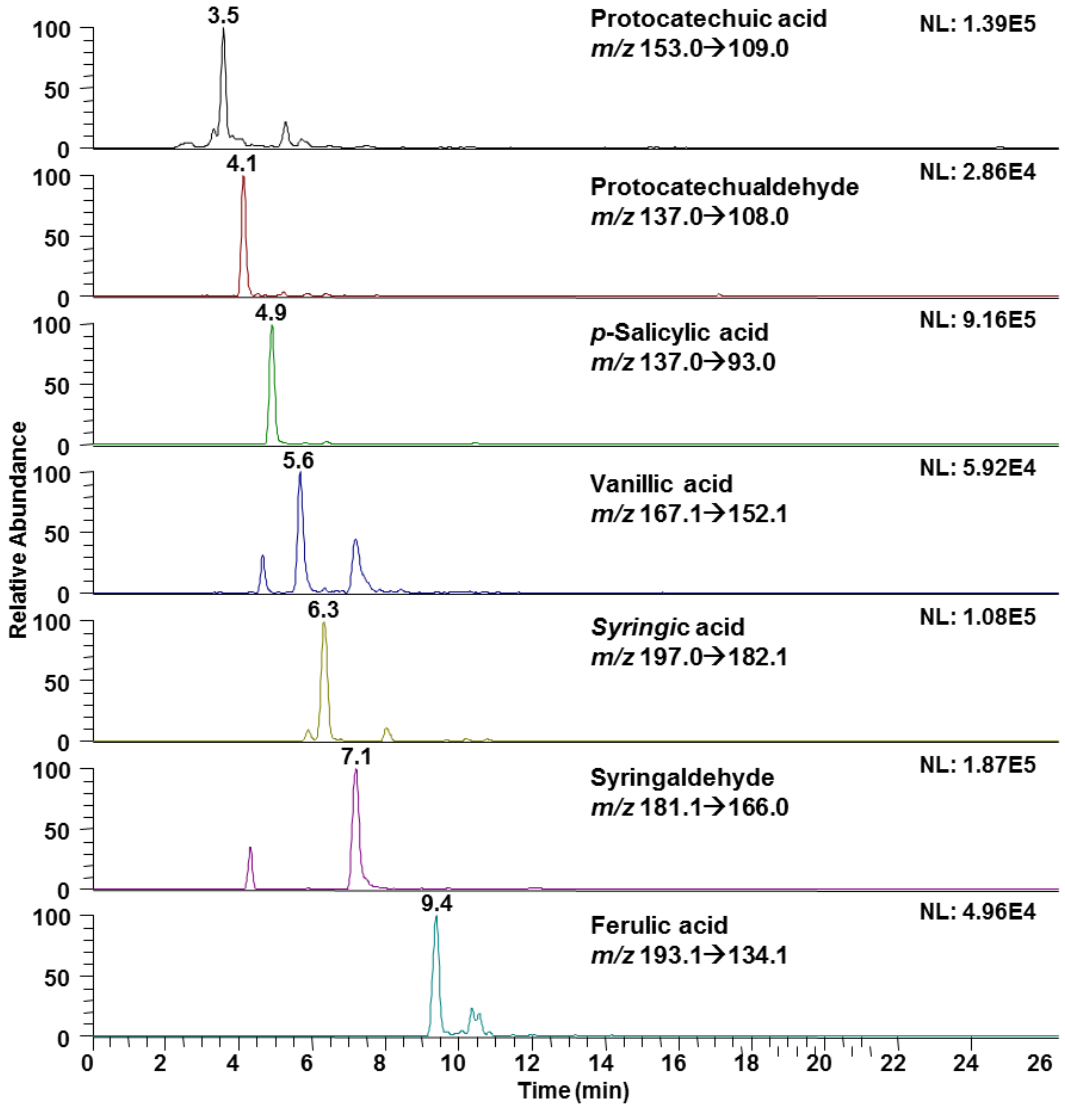
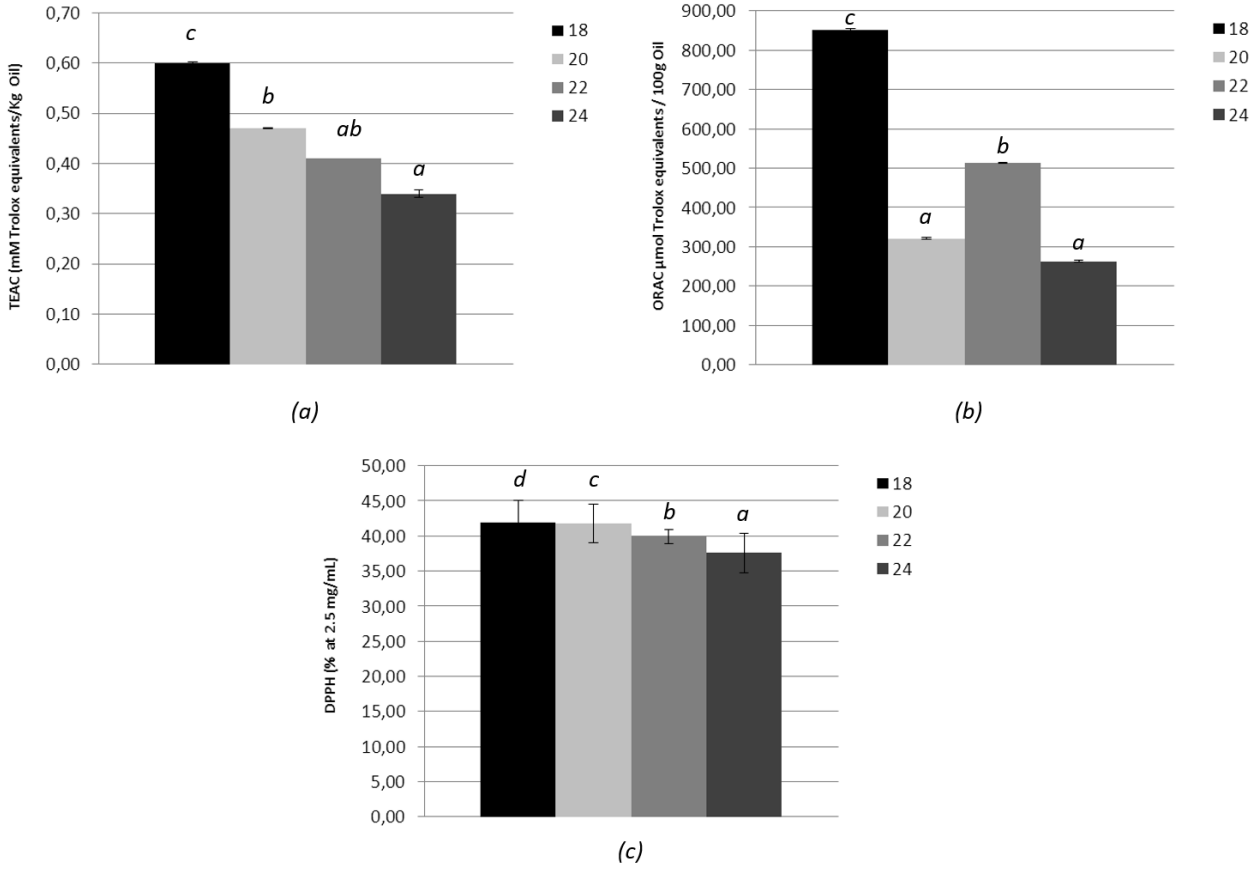


Figure 3. *In vitro* antioxidant activity [(*a*): TEAC, (*b*): ORAC; (*c*): DPPH assay]. Results represents means ± standard deviation (n=3); 18, 20, 22, 24 = number of weeks after anthesis; means without a common letter (*a*-*d*) indicate significantly different values (P< 0.05).



Effects of the fruit ripening stage on antioxidant capacity, total phenolics, and polyphenolic composition of crude palm oil from interspecific hybrid *Elaeis oleifera*×*Elaeis guineensis*

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ABSTRACT

In the present study, we assessed for the first time the changes in the antioxidant capacity, total phenolic content, and polyphenolic composition of interspecific hybrid palm oil extracted from *Elaeis oleifera* x *Elaeis guineensis* (O×G, Coari × La Mé cultivar) during the fruit ripening process 18, 20, 22, and 24 weeks after anthesis. A progressive decrease ($p<0.05$) of phenolic content occurred during fruit development together with marked changes in polyphenol profiles. Significant negative correlations were established between antioxidant activity measured by TEAC ($R = -0.954$, $p<0.05$) and ORAC ($R = -0.745$, $p<0.05$) and fruits ripening stage while positive correlation between total phenolic content was found using either the TEAC assay or the ORAC assay. Highest DPPH radicals scavenging activity was also obtained with oils extracted at 18 WAA. These results highlight that O×G fruits of early ripeness represent a better source of phenolic compounds and may provide extracts with higher antioxidant activities when hybrid palm oil is aimed to be used as functional ingredient for the development of food or food products with antioxidant properties.

Keywords: ripening, interspecific hybrid palm, phenols, dietary antioxidants, HPLC-ESI-MS/MS

INTRODUCTION

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage.[1,2] Endogenous free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions.[3] However, unhealthy lifestyle, such as smoking, stress, physical inactivity, and an inadequate diet may promote radicals formation.[4] When the production of these molecules exceed the endogenous antioxidant mechanisms, oxidative stress appears in the body, which has been related to the occurrence of different pathologies such as neurodegenerative diseases, cardiovascular diseases, cancer, liver cirrhosis, atherosclerosis, cataracts, diabetes, and inflammation.[5,6]

Within this context, an important field of research today is the control of 'redox' status by consuming foods with high antioxidant properties.[7-9] Natural antioxidants present in certain foods (i.e., fruits, vegetables, nuts, wines, and oils) increase the resistance to oxidative stress and they may have impact on human health by preventing oxidative stress-related diseases.[4-6,10] For this reason, investigation in antioxidants has dramatically increased in the past years, and a huge number of studies dealing with the identification and characterization of antioxidant-rich foods for the development of natural products and functional foods or supplements have now been published.[5,11,12]

Examples of common food antioxidant compounds include tocols, ascorbic acid, carotenes and phenols, among others.[10] Various factors such as genetic, environmental, and technological aspects may affect the chemical composition of plant foods and may have a significant role in determining the content, composition, and activity of these bioactive compounds.[13] Maturity stage is another extremely important factor that may influence the compositional quality of fruit and vegetables. During fruit ripening, several biochemical, physiological, and structural modifications happen, thus affecting the content of health-related phytochemicals.[13-14]

Palm oil, which is mainly extracted from the fruit of the African oil palm (*Elaeis guineensis* Jacq.), is currently the most consumed edible oil in the world. However, because of its partial resistance to

the bud rot disease, several producers are now increasingly planting the O×G interspecific hybrid, a cross between the American palm [*Elaeis oleifera* (Kunth) Cortes)] and African palm (*E. guineensis*).[15-17] In addition to agronomic advantages of *Elaeis oleifera* x *Elaeis guineensis* oil palm, recent studies have also proved that hybrid palm oil (HPO) has interesting chemical and nutritional characteristics. For instance, in our latest investigation[18] we found that the consumption of 25 mL/day of HPO for a period of 3 months had a favorable effect on plasma lipids pattern related to cardiovascular risk factors, such as total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) and that this effect was not statistically different from that of extra-virgin olive oil. In fact, beside its high percentage of oleic acid (54.6 ± 1.0 %) and low saturated fatty acid content (33.5 ± 0.5 %), HPO also represents an extremely valuable source of antioxidant compounds, such as carotenes, tocopherols and tocotrienols that might contribute to lower the risk of certain diseases.[19-22] Nevertheless, it is important to recognize that the content of antioxidants in HPO, and thus its antioxidant capacity, may significantly vary during the ripening process because of the different metabolic changes that occur in the fruit.[23-26] In a recent study we reported the chemical characterization of O×G interspecific hybrid palm oil (fatty acid composition, triglycerides composition, unsaponifiable matter composition) during fruit maturation.[19] However, to date, no information is available about the evolution of phenolic compounds and antioxidant activity of HPO during ripening. Therefore, because of the increasing importance of O×G hybrid for palm oil production and because of the need to improve knowledge of HPO antioxidant properties, the aim of this work has been to study, for the first time, the effect of fruit maturation process on the antioxidant capacity, total phenolics, and polyphenolic composition of oil from interspecific hybrid *E. oleifera* × *E. guineensis* (Coari × La Mé cultivar). The study has been conducted during the last six weeks of fruit ripening before the optimal harvest time (at 24 weeks after anthesis). The total phenolic content was assessed with the Folin–Ciocalteu method while the total flavonoid content and phenolic profile were determined using aluminum chloride colorimetric method and LC-ESI-MS/MS, respectively. Finally, four different assays [trolox

equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing activity power (FRAP)] were used to determine the antioxidant activity of HPO samples.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were purchased from purchased from Sigma-Aldrich (Steinhein, Germany) and VWR International (Milan, Italy) and, unless specified otherwise, were analytical grade or higher.

Plant Materials

The research was carried out using 9-years-old O×G (Coari × La Mé) interspecific hybrid palms planted in the municipality of Cumaral (Department of Meta, Llanos Orientales, Colombia) on the geographic grid reference longitude 73° 16’ W and latitude 4° 16’ N, at 305 mamsl. In order to assess the variation of polyphenols content and composition, flavonoids content, and *in vitro* antioxidant properties during fruit ripening in the bunch, 12 palms of the same palm oil plantation were selected at random and female inflorescences were tagged at the anthesis stage at the beginning of the experiment. The inflorescences were hand-pollinated and in each stage at 18, 20, 22, and 24 WAA, which indicatively corresponded respectively to 803, 805, 806, and 807 BBCH phenological scale for the OxG hybrid,[27] 3 whole bunches were randomly selected and harvested from three distinct palms (one bunch per individual). Bunches were then processed by cold pressing and subsequent clarification by sedimentation, as reported in previous papers.[15,19,20] Briefly, the collected fresh fruit bunches were heated with steam at a pressure ranging from 1.4 to 3 atm for about 45 min. In the next stage, the fruits were separated from the bunches by a threshing machine and mashed by rotating stirrer arms before being fed into screw presses to extract the crude palm oil. Then, the crude oil/water mixture was passed to a vibrating screen, heated to 90 °C and pumped

to vertical tanks where a gravity separation of oil from water took place. We decided to study the chemical composition of oil samples obtained from the mesocarp of fruits in the range from 18 to 24 WAA since it has previously been demonstrated that at 18 WAA the mesocarp from OxG (Coari x La Mé) interspecific hybrid contained less than 8% of total lipids (based on fresh weight of bunch) whereas the oil content increased rapidly in the following weeks reaching the maximum oil content of 21.6% at 24 WAA.

Extraction of Polyphenols

Polyphenols extraction was performed according to the method of Minioti and Georgiou[28] with slight modification. Briefly, HPO sample (0.5g) was diluted 1:1 (v:v) in n-hexane. Samples were then extracted by two 0.50 mL portions of methanol:water 80:20 (v:v) solvent, each time by vortex-mixing vigorously for 2 min. After separation from the lipidic fraction by 10 min of centrifugation at 3500 rpm, the polar extracts were combined and stored at -20 ºC until for further analysis.

Determination of Total Phenols

The concentration of total phenols was determined by the Folin–Ciocalteau colorimetric method of Singleton *et al.*[29] with some modifications. Sample extracts (50 µL) were placed into test tubes, and 250 µL of Folin–Ciocalteu reagent (1N) were added and vortexed for 5min at room temperature. After 1 min, 750 µl of 20% (w/v) aqueous Na_2CO_3 were added, and the volume was made up to 2.0 ml with H_2O. The solutions were kept in the dark at 25 ºC for 2h and the absorbance was measured at 680 nm. The results were expressed as gallic acid equivalents (mg GAE/kg HPO) based on the calibration curve (R^2=0.995) generated using standard solutions of gallic acid within the range of 0–400 mg/L.

Determination of Total Flavonoids

The flavonoids content was determined as previously described.[30] Extract (1 mL) was added to 4

mL of distilled water. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) $AlCl_3$ was added, and then at 6 min 2 mL of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm. The levels of total flavonoid content were expressed as mg of quercetin equivalents *per* g of dry extract.

LC-ESI-MS/MS Analysis of Phenolic Compounds

The determination of the phenolic profile of HPO sample extracts was performed by means of liquid chromatography-electrospray tandem mass spectrometry, as already described elsewhere.[31] Briefly, chromatographic separation was performed using a Phenomenex Kinetex C18 reversed-phase column (100 x 4.6 mm, 2.6 μm particles) on an Accela liquid chromatography system (Thermo Fisher Scientific, San José, CA, USA), equipped with a quaternary pump, an autosampler and a column oven. Gradient separation was created from solvent A (0.1 % formic acid aqueous solution) and solvent B (methanol) as follows: 0-3 min, linear gradient from 5 to 25 % B; 3-6 min, isocratic step at 25 % B; 6-9 min, linear gradient from 25 to 37 % B; 9-13 min, isocratic step at 37 % B; 13-18 min, linear gradient from 37 to 54 % B; 18-22 min, isocratic step at 54 % B; 22-26 min, linear gradient from 54 to 95 % B; 26-29 min, isocratic step at 95 % B; 29-29.15 min, back to initial conditions at 5 % B; and from 29.15 to 36 min, isocratic step at 5 % B. The column temperature was kept at 25 °C. The mobile phase flow rate was 1 mL/min.

The mass spectrometer was a TSQ Quantum Ultra AM (Thermo Fisher Scientific) triple quadrupole equipped with heated-electrospray (H-ESI). Selected reaction monitoring (SRM) acquisition mode (mass resolution of 0.7 *m/z* FWHM on both Q1 and Q3), with a scan width of 0.5 m/z and a scan time of 0.01 s, was used for quantification purposes by monitoring two SRM transitions for each compound. Twenty-six selected analytes belonging to different phenolic classes [gallic acid, (+)-catechin hydrate, *p*-coumaric acid, *p*-salicylic acid, caffeic acid, chlorogenic acid, (−)-epicatechin, (−)-epigallocatechin, ethyl gallate, ferulic acid, fisetin, gentisic acid, homogentisic acid, polydatin,

protocatechuic acid, protocatechualdehyde, quercetin dehydrate, quercitrin hydrate, resveratrol, syringic acid, syringaldehyde, taxifolin, umbelliferon, sinapic acid, kaempferol, and vanillic acid] were monitored.

Determination of the antioxidant activity

Trolox equivalent antioxidant capacity (TEAC) assay

This assay was based on the method previously described elsewhere with slight modifications.[32,33] The ABTS radical cation ($ABTS^+$) was prepared by reacting a 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was stored in the dark at room temperature for 16 h before use. The $ABTS^+$ solution was diluted in ethanol to an absorbance of 0.70 ± 0.05 at λ = 734 nm. After addition of 2.0 mL of this diluted solution to aliquots (25 µL) of sample or Trolox standard, absorbance at λ = 734 nm was measured and the total antioxidant activities of HPO samples were then expressed in mM Trolox equivalents *per* kg of HPO sample (mM eq Trolox/kg HPO).

Oxygen radical absorbance capacity (ORAC) assay

The Oxygen Radical Antioxidant Capacity (ORAC) used fluorescein as fluorescent probe and was an adaptation of the protocols proposed by Prior *et al.*[34] and López-Alarcón *et al.*[5]. The analysis was performed using a microplate spectrophotometer FLUOstar Optima (BMG Labtech). Aliquots (20 µL) of diluted sample or Trolox standard were mixed with 120 µl of flourescein (80 nM) and incubated at the 37ºC for 15min in the microplate. The radical AAPH (25µl) was then added manually using a multichannel pipette and the microplate was shaken. All the procedure (< 2 min) was realized in an area protected against light. The fluorescence (λ excitation = 485 nm, λ emission = 520 nm) was registered each 90 s over 1.5 h in order to obtain the Area Under the Curve (AUC). The results were analyzed as proposed in Stockham *et al.*[35] and were expressed in Trolox equivalents (µM eq Trolox/g HPO).

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

DPPH radical scavenging activity was determined according to the technique previously described.[36] A mixture of DPPH methanol solution (1.0×10^{-4} M) and extracts was prepared and kept in the dark for 30 min. The bleaching of DPPH was determined by measuring the absorbance at 517 nm (UV-Vis Jenway 6003 spectrophotometer). The DPPH radicals scavenging activity was calculated as follows: $[(A_0 - A_1/A_0) \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample. Ascorbic acid was used as positive control.

Ferric Reducing Activity Power (FRAP) Assay

The FRAP test is based on the redox reaction that involves TPTZ (2,4,6-tripyridyl-*s*-triazine)-Fe^{3+} complex.[37] FRAP reagent was prepared by mixing 10 mM TPTZ solution with 40 mM HCl, 20 mM $FeCl_3$ and 0.3 M acetate buffer. The absorption was measured at 595 nm. The FRAP value represents the *ratio* between the slope of the linear plot for reducing Fe^{3+}-TPTZ reagent by extracts compared to the slope of the plot for $FeSO_4$. Extracts were dissolved in methanol and tested at 2.5 mg/mL. BHT was used as positive control.

Statistical analysis

The results reported in this study are the averages of at least three repetitions (n = 3). Chemical data were analyzed by the IBM SPSS (19 Version) statistical software (SPSS Inc., Chicago, IL, USA). The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey's test. Correlations were estimated using Pearson's correlation coefficient (R).

RESULTS

Total Phenolic and Flavonoids Content

The changes in total phenolic content (TPC) during ripening are presented in **Fig. 1**. There was a significant negative correlation ($R = -0.903$, $p<0.05$) and a linear dependence between phenolic content and maturation stages. TPC values varied between 190.4 ± 11.8 and 263.8 ± 4.7 mg GAE/kg HPO, with samples at 18 WAA being the richest source of phenolic compounds. After this stage, total phenolic content decreased significantly ($p<0.05$) as the maturation state increase reaching the lowest TPC value at 24 WAA, which correspond to the consolidated harvest time for HPO.

In HPO samples flavonoids were not detected, regardless of the fruit maturation stage.

Phenolic Compound Composition

The phenolic profile of the investigated HPO samples at different stages is shown in **Table 1**. Only 7 polyphenols of the 26 compounds monitored by the LC-ESI-MS/MS method have been detected in the analyzed oil samples (**Fig. 2**).

A general trend towards a significant decrease of the levels of phenolic compounds with maturation time was confirmed. Particularly, a rapid and pronounced decrease ($p<0.05$) of each phenol concentration was observed from 18 to 20 WAA samples. Afterwards, an increase of the levels of all phenol compounds occurred between 20-22 WAA. However, such increase resulted significant ($P<0.05$) only for protocatechuic and *p*-salicylic acids. Finally, the 24 WAA samples showed a significant lower level of each phenolic compound than all the other samples, including 22 WAA samples. As result, the phenolic profile of 18 WAA samples was clearly different from those of the last stage of ripening samples (24 WAA). In fact, *p*-salicylic acid was the most abundant phenol at 18 WAA (8.691 ± 0.04 mg/kg HPO) followed by vanillic acid (5.145 ± 0.06 mg/kg HPO), syringaldehyde (4.982 ± 0.15 mg/kg HPO), and syringic acid (2.410 ± 0.11 mg/kg HPO). At 24 WAA, the preponderant phenolic compound was syringaldehyde (1.135 ± 0.07 mg/kg HPO), while lower amounts of *p*-salicylic (0.390 ± 0.07 mg/Kg HPO), vanillic acid (0.412 ± 0.06 mg/Kg HPO), and syringic acid (0.257 ± 0.01 mg/Kg HPO) were recorded. Finally, at the last stage of ripening,

significantly lower concentrations within the range of 0.006 - 0.047 mg/kg oil have been observed for protocatechuic acid, ferulic acid, and protocatechualdehyde.

***In vitro* antioxidant properties**

The relative antioxidant activity as measured by the TEAC assay is presented in **Fig. 3a**. As already observed for TPC, TEAC also decreased as the ripening stages increase with values ranging from 0.34 ± 0.01 (24 WAA) to 0.59 ± 0.02 mM eq Trolox/kg HPO (18 WAA). The antioxidant capacity of oil samples obtained at 18 WAA was significantly higher than that of all other samples, whereas a significant difference ($p<0.05$) between oils extracted at 20 WAA (0.47 ± 0.04) and 24 WAA has been observed. A significant correlation between TEAC values and maturation stages ($R= -0.954$, $p<0.05$) or phenolic content ($R = 0.887$, $p<0.05$) was recorded.

Antioxidant capacity measured by ORAC significantly decreased as the palm fruit became ripe, and varied from 8.5 ± 0.8 to 2.6 ± 0.2 µM eq Trolox/g HPO (**Fig. 3b**). Indeed, the highest ORAC value was obtained with HPO extracted at 18 WAA, as already observed for TEAC analysis. However, beside the fact that oil from fruits of more advanced ripeness (24 WAA) were lower in their ORAC values than less ripe fruit (18 WAA), a significant temporary increase ($p<0.05$) of antioxidant capacity was found at 22 WAA. On the contrary, no significant differences were recorded between 20 and 24 WAA samples. The antioxidant capacity measured by ORAC was correlated with maturation state ($R = -0.745$, $p<0.05$). Analysis of relationship between phenolic content and ORAC data showed a positive and significant correlation between these parameters ($R = 0.750$, $p<0.05$). The radical scavenging activity as measured by the DPPH assay is presented in **Fig. 3c** and varied from 41.94 to 37.56% at the maximum concentration tested of 1 mg/mL, for sample 18 and 24, respectively. Indeed, the highest DPPH radicals scavenging activity was obtained with HPO extracted at 18 WAA. Analysis of relationship between phenolic content and DPPH data showed a positive and significant correlation between these parameters ($R = 0.83$ $p<0.001$). The effect of

HPO samples on the iron, strongly involved in oxidative processes, was analyzed by FRAP assay. All tested samples were not active at the concentration of 2.5 mg/mL.

DISCUSSIONS

Phenolic compounds play an important role in the quality of edible oils, given that they are responsible for the oxidative stability of oil and, therefore, for its shelf-life.[38] Furthermore, many data have suggested the potential human health benefits of polyphenol-rich foods, with green tea, red wine and olive oil being probably the main dietary sources of such beneficial antioxidants.[10,39] Recently, oil palm (*Elaeis guineensis*) fruit extract has been proven to contain significant amounts of different types of phenols such as gallic acid, caffeic acid and vanillic acid,[40] which indicates that hybrid O×G may also be an important source of these compounds as well. However, and to the best of our knowledge, there is no information available about phenolic fraction and antioxidant capacity of HPO neither about the evolution of these parameters during the ripening stages of hybrid O×G fruit.

The results of this study reveal for the first time that, from 18 WAA to 24 WAA [which is considered the optimal harvest time because of the oil content of O×G fruit],[41] a significant decrease of phenols content occurred, thus confirming that total phenolic content in hybrid O×G oil is dependent on the fruit maturity stage. Anyway, at the latest ripening stage (24 WAA) the phenolic content of HPO samples (190.8 ± 11.8 mg GAE/kg oil) was comparable to that of other oils, including extra-virgin olive oil where phenols are the bioactive compounds with the highest antioxidant capacity.[42,43] In this respect it should be stressed that phenolic total amount and composition of olive oil varies from 50 to 1000 mg/kg [44], depending on cultivars, place of origin, olive ripening and technological process for oil production.

Concerning the degree of fruit ripeness, as we revealed for HPO samples, a decreasing trend was observed in the phenolic content of olive oils during the olive ripening process.[45,46] Other reports

have been written on the decline in phenolic content of the fruit (mesocarp), during the ripening, or when the fruit tissue is injured by pathogens, or mechanical damages [47-49]. Such decline was linked to the oxidation of phenolic content by polyphenol oxidase that characterizes the final stage of the fruit ripeness process. As an alternative, Amira et al. [50] suggested that the decrease of phenolic acids content in the date palm (*Phoneix dactylifera* L.) revealed during fruit ripening could be a result of a progressive incorporation of the phenolic acids into the cell walls. In fact, the accumulation of phenolics esters into cell walls is considered an important mechanism by which plants defend themselves against pathogens and strengthen their cell walls. Additionally, the accumulation of these esters protects the cells against membrane damage caused by reactive oxygen species.

With regard to the composition of polyphenols in relation to fruit ripening, the used LC-ESI-MS/MS methodology was able to provide a comprehensive evaluation of twenty-six selected phenols belonging to different phenolic classes, such as benzoic and cinnamic acids, flavanols and flavones. A total of 7 compounds were identified with *p*-salicylic acid and syringaldehyde being the most abundant compounds in HPO at 18 and 24 WAA, respectively. As has been already reported, *p*-salicylic acid is one of the major cell wall-bound phenolics in the genera of Palmae as well as a possible taxonomic marker because of its preferential accumulation in mesocarp of coconut husk and other species of palm.[40,51,52] Overall, our results draw quite different phenolic profiles from those achieved by Neo *et al.* (2010)[40] in palm fruit extracts: HPO samples revealed the presence of protocatechualdehyde and syringaldehyde while the absence of some other phenols such as gallic acid, caffeic acid, and *p*-coumaric acid was recorded. However, it is somewhat difficult to compare our findings with other results published by other researchers because no studies have been specifically conducted on palm oil with the exception of one study, reporting the presence of 3, 4 hydroxybenzyaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid in Nigerian red palm oil (*Elaeis Guineensis*) without, however, providing any quantitative information.[53] When analyzing changes occurring during the harvesting stages, a significant final decline of all the identified phenolic compounds during the fruit ripening was confirmed. This

finding is in general agreement with the results of total phenolic content that highlighted a significant negative correlation between TPC and maturation stages. Furthermore, in addition to what it was observed with TPC, the antioxidant capacity measured by TEAC assay showed a significant variation as well. In fact, a decrease of TEAC values has been observed as the ripeness increased, being the stage with highest antioxidant capacity the 18 WAA. At the same time, we register also the highest radical scavenging activity by DPPH method. This behavior was observed in other studies conducted in olive oil, where the antioxidant capacity and phenol content significantly drop as the maturity of fruits increased.[26,54]

Results obtained by the ORAC method also showed a general tendency of the antioxidant capacity to decrease during the different maturity stages; however, contrarily to TEAC results, a temporary increase was observed at the 22 WAA stage. It is important to take into account that the contribution of particular phenolic compound to the total antioxidant activity may vary. Therefore, in some cases a predominant specific type of phenol compound can lead to an elevated or diminished expression of the ORAC values,[40] which can be the factor influencing the results of this study. For instance, in HPO samples, while TPC value decrease from 231.4 ± 3.8 mg GAE/kg oil at 20 WAA to 216.3 ± 25.9 mg GAE/kg oil at 22 WAA, phenolic composition analysis showed a simultaneous significant increase of protocatechuic acid and *p*-salicylic acid content. In fact, while all other compounds mainly remained unchanged during this period, protocatechuic acid increase from 0.256 ± 0.02 (20 WAA) to 0.435 ± 0.02 mg/Kg (22 WAA) while *p*-salicylic acid almost doubled over the two weeks reaching a value at 22 WAA of 1.156 ± 0.12 mg/kg oil.

Finally, many authors have studied correlations between bioactive compounds and antioxidant activities in numerous fruits and vegetables.[55] However, as commented before, there is no information concerning these types of correlations in O×G palm. In the present study, the assessment of the antioxidant capacity of HPO extracted from O×G palm fruit during four stages of maturation and ripening using TEAC, ORAC and DPPH assays revealed that antioxidant activity was strongly related to the total phenolic content. This is probably because the antioxidant capacity

368 measured on the hydrophilic phase of the oil extracts increases or decreases depending on the
369 phenols content of the extract. And these correlations confirm that the phenolic compounds are
370 probably the main phytochemicals contributing to the antioxidant activities of HPO. Several studies
371 conducted on fruits, vegetables, wine, oils and other plants have already shown a high correlation
372 between the antioxidant activities and the TPC.[48,56,57] Previous investigations in olive oil and palm
373 fruit extracts indicate a higher correlation between total phenolic content and TEAC (ABTS) assay
374 than between TPC and ORAC method.[57,58] This is in accordance with our finding on HPO where a
375 stronger correlation between TPC and both TEAC (R=0.887) and DPPH (R=0.83) compared to
376 TPC and ORAC (R=0.750) was observed. These results may be explained by the fact that
377 Folin−Ciacolteu and the radical scavenging methods ABTS and DPPH share the same reaction
378 mechanism (electron transfer) whereas ORAC method is based on hydrogen atoms transference
379 reactions. The absence of flavonoids in HPO is also supported by the literature, in fact Das &
380 Pereira (1990)[59] reported the addition of different flavonoids to palm oil to stabilize it and prevent
381 the thermal autoxidation. In another study, Van Dyck *et al.* (2004)[60] reported that the good oxidative
382 stability of palm oil mainly contains mono-unsaturated fatty acids. However, it was no stable under
383 certain stress conditions, such as storage of the oil at elevated temperature and the effect of pro-
384 oxidants such as carotenoids and metal ion contamination. The oxidative stability of crude palm oil
385 is mainly attributed to its content in tocopherols. The removal of these phytochemical determines
386 the halve of the oxidative stability. Moreover, carotenoids contained in palm oil could act as a pro-
387 oxidant, determine the acceleration of the oxidation process. So, in order to protect palm oil from
388 oxidation during its shelf-life a stabilization of the oil with antioxidants is necessary.
389 In conclusion, this study reveals that HPO represents a valuable source of antioxidant compounds.
390 However, the antioxidant characteristics of HPO strongly depend on the fruit maturation process,
391 with progressive reduction in the phenolic content and its antioxidant capacity with increasing
392 degree of ripeness. It has previously been demonstrated that at 18 WAA the mesocarp from O×G
393 (Coari × La Mé) interspecific hybrid contained less than 8% of total lipids whereas the oil content

increased rapidly in the following weeks reaching the maximum oil content of 21.6% at 24 WAA.[36] Therefore, 24 WAA obviously represents the optimal harvest time for quantitative (i.e., extraction rate, industrial applications, etc.) characteristics of the O×G interspecific hybrid oil. At this stage HPO oil has also been shown to present maximum levels of tocols and oleic acid.[18] On the other hand, our study revealed that earlier ripening stages could also be taken into account when HPO is intended to be used as ingredient for preparation of polyphenols-rich food and/or nutraceuticals with functional antioxidant properties.

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Figure Captions

Figure 1. Total phenols determined by using the Folin−Ciocalteu assay. Results represents means ± standard deviation (n=3); 18, 20, 22, 24 = number of weeks after anthesis; means without a common letter (*a*-*c*) indicate significantly different values (P< 0.05).

Figure 2. LC-ESI-MS/MS chromatogram of polyphenols found in hybrid palm oil sample at 18 week after anthesis.

Figure 3. *In vitro* antioxidant activity [(*a*): TEAC, (*b)*: ORAC; (*c)*: DPPH assay]. Results represents means ± standard deviation (n=3); 18, 20, 22, 24 = number of weeks after anthesis; means without a common letter (*a*-*d*) indicate significantly different values (P< 0.05).

Table 1. Phenolic compounds (mg/kg oil) found in hybrid palm oil samples during ripening of fruits.

	WAA			
Compound	**18**	**20**	**22**	**24**
Protocatechuic acid	0.839±0.04 [d]	0.256±0.02 [b]	0.435±0.02 [c]	0.047±0.01 [a]
Protocatechualdehyde	0.184±0.01 [c]	0.038±0.01 [b]	0.044±0.00 [b]	0.006±0.00 [a]
p-Salicylic acid	8.691±0.04 [d]	0.614±0.07 [b]	1.156±0.12 [c]	0.390±0.07 [a]
Vanillic acid	5.145±0.06 [c]	0.805±0.05 [b]	0.939±0.05 [b]	0.412±0.06 [a]
Syringic acid	2.410±0.11 [c]	0.396±0.02 [a,b]	0.457±0.02 [b]	0.257±0.01 [a]
Syringaldehyde	4.982±0.15 [b]	1.340±0.14 [a]	1.289±0.05 [a]	1.135±0.07 [a]
Ferulic acid	0.477±0.02 [c]	0.043±0.00 [a,b]	0.069±0.00 [b]	0.031±0.00 [a]

Results represents means ± standard deviation (n=3); WAA, week after anthesis; means within the same row without a common letter (a-d) indicate significantly different values ($P < 0.05$).

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