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Influence of the olive fruit position on the tree in the cv Santulhana fruits and oils composition

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Thank you all!!

Rabeb Medini

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Abstract

The olive tree is a very widespread crop all over the world with a greater presence near the Mediterranean basin. Excellent products are obtained from it, such as olive oil and, to a lesser extent, table olives. The composition of olive oil and its quality are strongly influenced by a combination of several aspects, such as environmental, agronomic, and technological factors. Within the agronomic factors, many conditions can influence the final quality of the oil from the type of cultivar, the number of plants per hectare, the position of the fruit, the sun exposure, the pruning, among others. In this context, this work aimed to study the influence of the olives position, inside and outside of the tree canopy, in the composition of fruits and oils of cv. Santulhana. For this, five trees of the cv. Santulhana were selected, and in three different occasions, olive samples were collected in the interior and exterior of the tree canopy. For each olive samples (outside and inside of olive tree), to sub-samples were constituted. Fruits and stones, one sub-sample, were characterized in respect to some parametric measures like weight and color, and their antioxidant capacity were evaluated from the extracts that were prepared. The other sub-sample, the olive oil was extracted and quality parameters (acidity, peroxide values and extinction coefficients) antioxidant activity, sensory analysis, total and individual phenols content, tocopherols and fatty acid composition were determined.

The comparative analysis revealed that the studied factor significantly affected some parameters. With regard to olives, it was found that color was the only of the parameters affected by the fruit position, with lower luminosities. Concerning to the other evaluated parameters, there were no significant differences between positions. The oil extracted from fruits collected outside of the canopy showed best quality and high amounts of hydroxytyrosol and tyrosol. On the other hand, for the remaining results no significative differences were noted.The results show that picking the olives outside the canopy can be a strategy for the production of oils with a higher content of phenolic compounds and oil quality.

Keywords: Fruit canopy position, olive oil differentiation, quality parameters, chemical composition.

Resumo

A oliveira é uma cultura muito difundida a nível mundial, sobretudo nos países da bacia do Mediterrâneo. Desta planta obtêm-se dois excelentes produtos o azeite e, em menor quantidade, as azeitonas de mesa. A composição do azeite e a sua qualidade são fortemente influenciados pela combinação de vários fatores, ambientais, agronómicos e tecnológicos. Dentro dos fatores agronómicos, são vários os aspetos que podem influenciar a qualidade final do azeite como por exemplo o cultivo, o número de plantas por hectare, a posição do fruto na copa, a exposição solar, a poda. Nesse contexto, este trabalho teve como objetivo estudar a influência da posição do fruto, dentro e fora da copa da oliveira, na composição azeitonas e azeites da cv. Santulhana. Para isso, foram selecionadas cinco árvores da cv. Santulhana, onde foram colhidos frutos no interior e exterior da copa das plantas em três períodos distintos. Para cada amostra de azeitona (exterior e interior da oliveira), foram constituídas duas subamostras. Na primeira subamostra, os frutos e endocarpos foram caracterizados em relação ao tamanho do fruto, diâmetro na posição máxima, peso e cor, e avaliada a capicidade antioxidante Com os restantes frutos, foi extraído o azeite onde foram avaliados parâmetros de qualidade (acidez, índice de peróxidos e coeficientes de extinção), a atividade antioxidante, análise sensorial, o teor de fenóis totais e individuais, tocoferóis e a composição de ácidos gordos.

A análise comparativa revelou que o fator estudado afetou significativamente alguns parâmetros. No que diz respeito às azeitonas, verificou-se que a cor foi um dos parâmetros mais afetados pela posição do fruto com luminosidades mais baixas. No que respeita aos restantes parâmetros avaliados não se verificaram variações significativas entre as duas posições. O azeite extraído das azeitonas localizadas no exterior da copa mostrou ser de qualidade superior, sendo ricos em hidroxitirosol e tirosol. Nos restantes parâmetros analisados não foram observadas diferenças significativas. Os resultados mostraram que a apanha da azeitona no exterior da copa pode ser uma estratégia para a produção de azeites com maior teor de compostos fenólicos e de qualidade do azeite.

Palavras-chave: Posição na árvore, azeites diferenciados, parâmetros de qualidade, composição quimica.

List of abbreviations

ABTS radical: [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]

ANOVA: Analysis of variance

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EC: European Commission

EVOO: Extra virgin olive oil

FID: Flame ionization detector

HPLC: High Performance Liquid Chromatography

MUFA: Monounsaturated fatty acids

IOC: International Olive Council

UPOV: International Union for the Protection of New Varieties of Plants

OOPE: Olive oil polyphenol extract

NS: No statistically significant difference

PAR: Photosynthetically active radiation

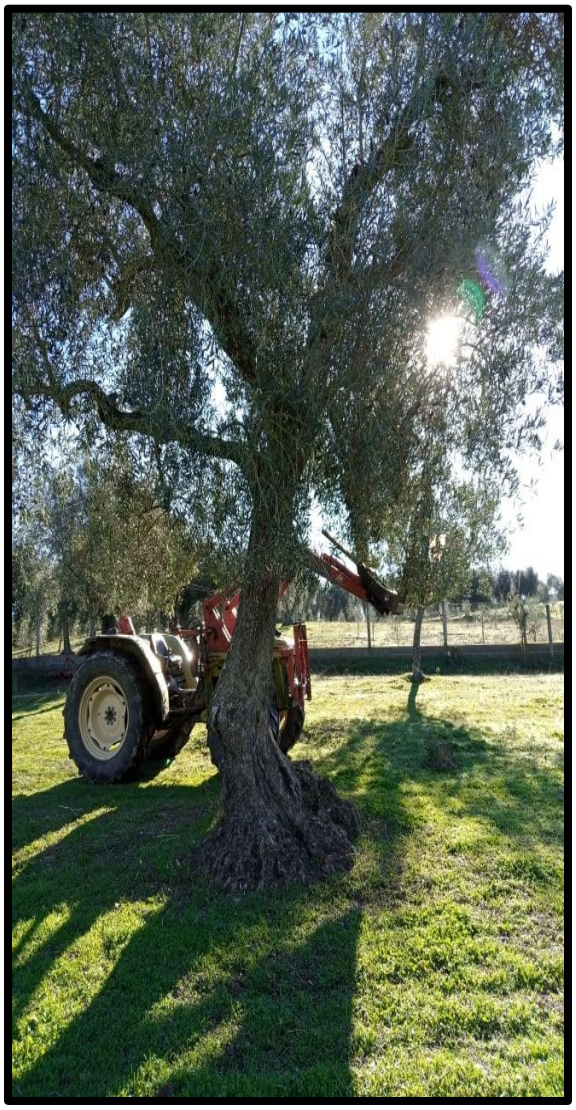
PUFA: Polyunsaturated fatty acids

PVDF: polyvinylidine fluoride

TPC: Total polyphenol content

VOO: Virgin olive oil

Chapter 1. Introduction



1. Introduction

The olive tree, *Olea europaea* L., is part of the *Oleaceae* family (García Martín et al., 2020). Its fruits are very used not only for olive oil extraction but also for table olives preparation. Today, there are nearly one billion olive trees cultivated throughout the world. More than 90 % are grown in the Mediterranean basin, especially in Spain, Italy, Greece (Espadas-Aldana et al., 2019), and Portugal.

Olive oil is the fat part of the olive juice extracted by mechanical or physical means (Visioli et al., 2020), with the operations of extraction, decanting, centrifugation, and filtration (Seçmeler & Galanakis, 2019). And is one essential ingredient of the Mediterranean diet. In fact, some geographical differences exist in the traditional Mediterranean diet, but all share the common feature of using olive oil as the principal culinary fat (Gaforio et al., 2019).

Depending on different physic-chemical parameters and sensory characteristics, according to the European Regulations (Commission regulation (EEC) 2568/91) olive oil can be classified into two categories that could be directly consumed. Firstly, Extra Virgin Olive Oil (EVOO) has a fruity taste and without sensory defects and other physic-chemical parameters according to the European Commission (EC) (Commission regulation (EEC) 2568/91). EVOO is the best kind of olive oil presenting a distinctive organoleptic properties due to is variable composition, with a high oxidativative stability (Iqdiam et al., 2020). Secondly, Virgin Olive Oil (VOO) also has a fruity taste but can have slight sensory faults or some changes in each other physic-chemical parameters according to the EC (Commission regulation (EEC) 2568/91).

Moreover, olive oil is a stable vegetable oil that is difficult to be oxidized, very rich in antioxidants compounds, making it an excellent product for culinary and medicinal consumption. Previous research has indicated that VOO is an efficient alternative for saving pharmaceutical costs. It has been shown that the consumption of this vegetable oil reduces blood pressure considerably (Gaforio et al., 2019).

EVOO turned out to contain anti-carcinogenic agents. Clinical tests have shown that minor components of olive oil, such as hydroxytyrosol, oleuropein, pinoresinol, squalene, and maslinic acid, enhance the anti-tumor response of people with cancer (Mazzocchi et al., 2019).

Other studies have demonstrated that olive oil, and, more particularly polyphenols, it has technological properties such as its antimicrobial activity. Indeed, Guo et al. (2019) have indicated that olive oil polyphenol extract effectively reduces food contamination by *Listeria monocytogenes*.

Concerning the chemical composition, olive oil compounds are classified into two groups: major and minor components. The major component is triacylglycerols, which make up more than 98 % of the total weight of fat. The aliphatic and triterpenic alcohols, sterols, tocopherols, hydrocarbons, and antioxidants, constitute the minor components of olive oil with a proportion ranging from 0.5 to 1.5 % (Oğraş et al., 2016).

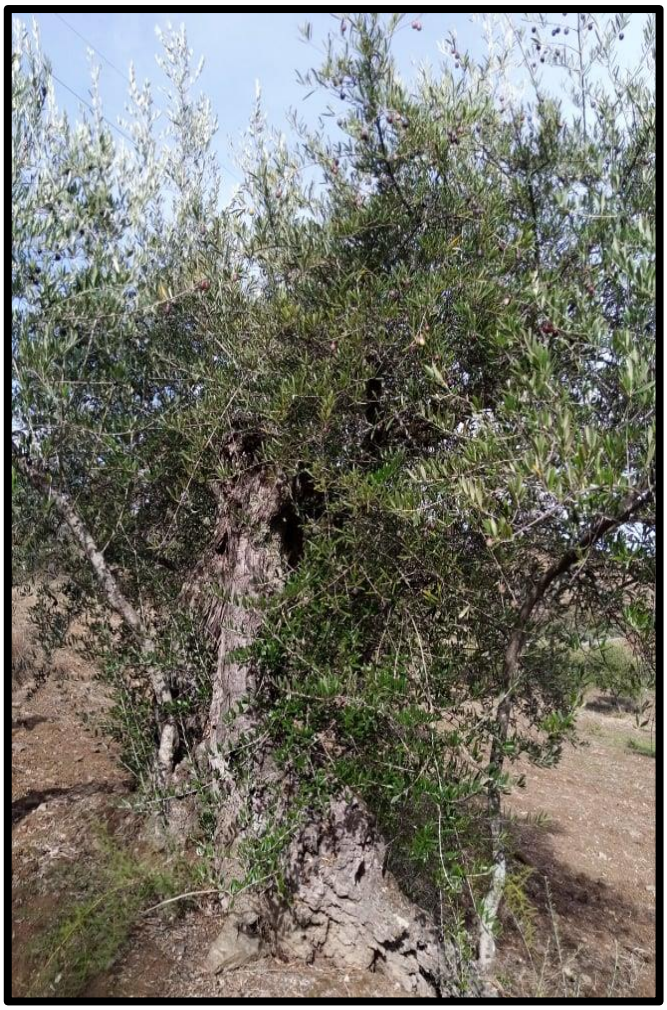
The quality and chemical composition of olive oil are influenced by different factors such as the agronomic practices, cultivar, sun exposition, time of harvesting, harvesting system, the extraction process, mixing of the olive paste, and oil extraction tool between others (Altieri et al., 2020).

For example, the climatic condition of the region, as the desert climate in southern Tunisian, decrease the chlorophyll content and increase the peroxide index on olive oil extracted by *cv. Zelmati* (Rouina et al., 2020). In addition, variety and maturity progress can also affect the quality of the olive oil. According to López-Huertas et al. (2020), some Italian varieties (Frantoio and Cornezuelo) have show a high content of polyphenols in the early ripening stages with a good yield of olive oil.

In most of the previous studies, researchers were mainly focusing on general quality factors (environmental, agronomic, and operational factors) while little attention was granted to how the position of the olives in the tree could affect the quality of its oil as well as its composition.

The present study aimed to evaluate the effect of the fruit position in the tree, inside and outside of the tree canopy, on the biometric parameters, composition, and quality of olives, and on the chemical composition and quality of the extracted olive oil. For this purpose, a physicochemical and sensory characterization was carried out to determine if there is a difference between the characteristics of the olives as well as their oil picked from different parts of the olive trees (from the inside / outside of the tree canopy).

Chapter 2. Theoretical background and literature review



2. Factors that affect the composition and quality of olive oil

The cultivation of the olive tree dates back to the dawn of time. It has never ceased to be an object of attention for researchers from various fields. According to the objectives set in this work, this part of the report is oriented towards the characterization and understanding of the main factors (environmental, agronomic, and technological factors) that affect the quality of olive oil based on previous studies.

2.1.Environmental factors affecting olive oil quality

2.1.1. Climatic conditions

All the climatic conditions affect plant growth and development, and the same occurs for the olive tree. According to the results reported by Nissim et al. (2020), high temperatures during fruit development affect three important characteristics: fruit weight, oil concentration, and oil quality. The decrease in the dried fruit weight at harvest of the "Koroneiki", "Coratina", "Smile", and "Picholine" varieties have been attributed to higher temperatures. The same study has shown that the cultivar "Souri" is more tolerant to a high-temperature environment, allowing it to produce a better olive oil quality than the varieties grown under the same environmental conditions (Nissim et al., 2020).

In the olive tree, high temperatures during fruit growth can increase the percentage of linoleic acid, even exceeding the limits set by regulations. In addition, it deteriorates the quality of olive oil by increasing the free acidity and the peroxide value and reducing the phenolic fraction (Orlandi et al., 2012). On the other hand, water stress and high temperatures reduce the oil yield (Fraga et al., 2020).

Moreover, the hot desert climate leads to increased levels of polyphenols and carotenoids in olive oil from olive trees grown in the region of Kebili-Rjim Maatoug, located in southern Tunisia. Nevertheless, the oleic acid content decreased below 50 %, palmitic acid exceeded 20 %, and linoleic acid exceeded 1 %, which did not conform to the commercial standards of olive oils (Rouina et al., 2020).

2.1.2. Soil type

Soil is an essential matrix for cultivated or maintained to produce food plants. The olive oil quality parameters are impacted by soil type. Olive oils obtained from trees cultivated in clay soils showed lower peroxide values and high phenolic content compared to olive oil from sandy soil (Rached et al., 2017).

2.1.3. Altitude

Altitude has a considerable influence in the chemical composition of olive oil. Fruits grown at low altitudes ripen earlier have the highest average weight. As for the concentration of phenols, the highest concentration was recorded in oils from high altitudes. Concerning the fatty acid composition, significant differences were recorded between the two oils. The oil obtained from olives grown at low altitudes had a higher linolenic and palmitic acid content than oleic and stearic acids (Di Vaio et al., 2013).

2.2. Agronomic factors

2.2.1. Irrigation

Irrigation is one of the agronomic factors needed to maintain food security, public development, and population settlement in rural areas (Elshaikh et al., 2018). It allows to improve the quantity and quality of production, but it has a considerable influence on the chemical composition of the olive oil. The results obtained by García et al. (2020) have revealed that insufficient irrigation reduces the phenol content, oleic acid, and oxidative stability in olive oil from cv. Arbequina. Nevertheless, some vitamins, like vitamins A, E, and F, increase with the irrigation deficit. On the opposite side, Ahumada-Orellana et al. (2018) have observed high total polyphenols in water deficit systems for the same cultivar.

As several quality parameters are affected by the irrigation level, ensuring that the olive trees get an adequate water supply becomes necessary. It was shown that irrigation regulated at 30 % had improved the commercial quality parameters of virgin olive oil (VOO). For instance, pigments, phenolic compounds, and the ratio between oleic acid and linoleic acid are higher than other trees treated with different degrees of irrigation. Nevertheless, tocopherol content and oil yield are not affected (García et al., 2017).

2.2.2. Fertilization

Fertilization is used to enrich the soil with amendments and strengthens the olive trees with main fertilizing elements. A calcium-based fertilizer seemed to have a positive effect on the total polyphenol content. It also significantly increased the percentage of oleic acid and its ratio to linoleic acid. Besides, the monounsaturated fatty acids (MUFA)/polyunsaturated fatty acids (PUFA) ratio was enhanced. Furthermore, nitrogen and boron generated an increase in polyphenols (Zouari et al., 2020).

The results obtained by Dabbaghi et al. (2019) have emphasized the need for Ca^{2+} fertilization. It has been proven that calcium-based fertilizers improve most phenolic and volatile compounds, thus improving the quality and flavor of olive oil.

2.2.3. Olive pests and diseases

The pests and diseases that can affect the olive tree are quite numerous. This section will discuss the principal diseases and pests that could affect olive trees and alter the quality of olive oil. *Bactrocera oleae* is the pest that threatens the quality of virgin olive oil. This fly acts mainly on the chemical composition of olive oil, particularly its free acidity, peroxide value, fatty acid composition, oxidative stability, etc. In addition, *B. oleae* seems to affect the amount of phenols in the oil since it was proven that oils extracted from fruits bitten by this fly have the lowest phenolic fractions (Gómez-Caravaca et al., 2008). This is in accordance with Malheiro et al. (2015) that confirmed the effect of this fly in the chemical composition, quality and functionality of olives and olive oil.

Olive anthracnosis, caused by the fungal plant *Collectotrichum sp*. can also cause high-quality losses. This is because it acts on the quality parameters of olive oil, such as acidity, peroxide value, and extinction coefficient increasing its values (Carvalho et al., 2008; Iliadi et al., 2018). Moreover, oils obtained from olives affected by anthracnose have undergone rapid oxidation (Runcio et al., 2008).

2.2.4. Cultivar

The quality of olive oil is affected by the olive cultivar since its chemical composition changes from a cultivar to another. As mentioned by Essiari et al. (2014), the chemical composition of some cultivars (Picholine, Haouzia, Arbequina, and Menara) cultivated in Morocco was not identical. The comparison between these four varieties revealed that the *cv*. Arbequina was the richest in linoleic acid, palmitic acid, and saturated fatty acids while *cv*. Picholine was the richest in oleic acid and PUFA. Concerning the Haouzia variety, it was less abundant in sterols.

Furthermore, in the case of the Turkish cultivars (Sari Hasebi, Gemlik, and Halhali), it was found that they were not equal in terms of fatty acids, sterols, and total phenolic content. In this regard, the cultivar of Sari Hasebi had the highest oleic acid content. Also, the Halhali cultivar had the greatest antioxidant activity and sterol content Yorulmaz & Konuskan (2017)

2.2.5. Ripeness index

Olive oil quality depends on the maturity stage at the harvest time. Oils obtained from ripe olives at their maximum size and the optimum maturity stage showed a higher percentage of PUFA than those obtained from olives at other stages of maturity. As opposed to green olives (at an early stage of maturity), which had an exceptionally high content of chlorophyllin pigments and a low content of phenolic compounds (well known for their antioxidant properties) (Gharbi et al., 2015).

A high maturity index induced an increase in the extracted oil content. On the other hand, advanced maturity stages led to a decrease in the quality parameters such as peroxide value, specific extinction coefficient (K_{232}), carotenoid content, chlorophyll content, and total phenolics concentration, contrary to the free fatty acid content which accumulated as the olives mature (El Yamani et al., 2020).

2.2.6. Canopy position and light

Since we have chosen, in the present study, the olive position in the tree as an explanatory factor of olive oil quality, this part of the report is devoted to detailing the effect of this factor on a set of parameters defining the quality of olive oil.

2.2.6.1. Maturity index

The position of the fruit is a determining factor for specific physiological processes related to the growth and maturity of the olives. Indeed, the interception of light at the bottom positions of the canopy leads to differences in the extracted fruit and oil. Grilo et al. (2019a) noted a higher maturity index in the top canopy layer, with registered values varying between 0.0 and 2.7, while those values ranged from 0.0 to 1.6 for the bottom layer.

A similar observation was reported by Cherbiy-Hoffmann et al. (2013), who pointed out that different light received levels can decrease the final weight of dried olives and the oil concentration. In addition, the same authors have concluded that fruits with high exposure to the light present high dry weight of the fruit, high oil concentration, and yield. On the contrary, lower light values and a high degree of shadow reduce the amount of oil in the drupes.

The olives located on the top of tree are the most exposed to the sun light, consequently they presented the highest weight, high pulp stone ratio, mesocarp oil content and ripening index compared to those who receiving lower amounts of sun light (Caruso et al., 2017a). In contrast, olives in the lower part had the lowest ripeness index and dry weight (Trentacoste et al., 2018).

2.2.6.2. Biometric parameters and fat contents

As mentioned above, the canopy's interior parts are characterized by their shading, meaning that the photosynthetic radiation reaching them is low. The natural and artificial shadows affect the path of the olive fruit development. These radiations limit fruit growth and slow down the accumulation of fat in the mesocarp cells. Consequently, they were small in size at picking time, and the pulp was weak (Bartolini et al., 2014). In addition, the larger olives had a high proportion of pulp and a small stone, which allowed them to provide a large amount of oil. These olives are located at the top of the canopy (Connor et al., 2012). Bonghi et al. (2019) confirmed these results, finding that fruits exposed to light had a high oil content.

2.2.6.3. Fatty acid composition

Fatty acids are the major components of olive oil. Different researches have indicated that the olive position has a significant influence on the fatty acid composition. In this respect, oleic acid concentration decreases as layer height increases, in contrast to other fatty acid concentrations such as palmitic acid, palmitoleic acid, stearic acid, and linoleic acid, which increase with increasing layer height.

The research carried out by Gómez-Del-Campo & García (2012) on the Arbequina cultivar has reported that the olive oil obtained from the bottom layers, which are the less exposed to light, presented a higher oleic acid concentration. They also reported that the olive oil recovery form the Eastside was richer in oleic acid and that recovery from the Westside the palmitoleic and linoleic acid contents were higher (Gómez-Del-Campo & García, 2012).

Bonghi et al., 2019 also reported that trees without shade provide oils characterized by a low proportion of oleic acid and a high proportion of linoleic and stearic acid compared to shady trees. The increase in photosynthetically active radiation (PAR) values led to an exponential decrease in oleic acid concentration.

Concerning the other fatty acids, it was found that the position in the canopy does not affect their concentration (Rousseaux et al., 2020a).

2.2.6.4. Phenols

Olive oil is considered a functional food due to its richness in beneficial micronutrients, mainly polyphenols (Rodríguez-López et al., 2020). Such compounds play an indispensable role in human health and minimizing olive oil oxidation due to their antioxidant activity.

In this context, VOO extracted from fruits harvested at the top of the canopy were the richest in total phenols. The top layers of the canopy had a greater content of oleuropein and ligstroside compounds than the bottom layers (Gómez-Del-Campo & García., 2012).

Moreover, the north-south direction produced VOO richer in phenol (Gómez-Del-Campo & García, 2012). Romero-Segura et al. (2012) mentioned that the phenolic composition of VOOs was strongly related to the enzyme β-glucosidase in olives. In this respect, it turned out that the enzyme's activity was low in olives exposed to light, which allows us to conclude that this enzyme was sensitive to photosynthetic radiation. It was proved that the enzyme quantity was higher in the fruits located on the outer part of the olive tree. At the same time, at later stages, the activity of β-glucosidase was lower than in the fruits situated on the inner part of the tree (Bartolini et al.,2014). Those findings align with Lémole et al. (2018), who noted that light considerably affected enzymatic activity.

2.2.6.5. Tocopherols

Tocopherols and tocotrienols appear in four different forms (α, β, γ, and δ), which together carry the designation of vitamin E (Albuquerque et al., 2019a). In this regard, Gómez-Del-Campo & García (2012) indicated that only γ -tocopherol is affected by the position in the canopy since it turned out to be higher in the lower parts.

2.2.6.6. Sensory attributes

Chemical analyzes are insufficient to determine the quality of olive oil. Sensory attributes are also important to determine the olive oil quality. Gómez-Del-Campo & García., (2012) concluded that the height difference of the layer or the canopy face does not influence the sensory attributes of the olive oils.

2.2.6.7. Quality parameters

The chemical quality parameters such as free acidity and spectrophotometric indices are not affected by shading. Nevertheless, according to results found by Caruso et al. (2017), the peroxide value of olive oils extracted from the upper area of the tree canopy was higher than those removed from the low-north (L-N) section of the tree canopy. This result was confirmed by Grilo et al. (2019) who also indicated that the quality parameters were not affected by the position within the canopy.

2.3. Harvest time

The olive harvest timing has a significant effect on the quality and yield of the oil (López-Bernal et al., 2021).

The early winter harvest produced olive oil with better chemical and sensory attributes. While a delayed harvest deteriorated the sensory qualities of the oil. Concerning the chemical composition, it was found that the concentrations of palmitic acid, stearic acid, linoleic acid, and PUFA in the oil increased at late harvest times. Conversely, oleic acid and MUFA were reduced (Alowaiesh et al., 2018).

Another study has indicated that a late harvest caused a decline in oil quality. Indeed, an increase in free fatty acids combined with a rapid decrease in polyphenol content and the ratio of MUFAs and PUFAs in oils from late-harvested olives was recorded (A. Dag et al., 2011).

3. Santulhana cultivar

Olives trees have been around for along time with a long history dating back to ancient civilizations. Portugal is a country known for its strategic geographical location and it has had a considerable olive-growing heritage resulting in a landscape marked by olive trees without forgetting its oil quality. Trás-os-Montes region is known for its high-quality oil and its authenticity. Furthermore, this oil is acknowledged by the European Union and has been awarded the Protected Designation of Origin “Azeite de Trás-os-Montes” (Peres et al., 2011a).

The santulhana cultivar (*cv. santulhana*) is one of cultivars cultivated in this region, precisely, in Bragança, Vimoso and Macedo de Cavaleiros (Figure 1). It is characterized by its considerable level of natural antioxidants such as tocopherols and phenols with concentrations respectively varying between 150-300mg and 200-300 mg (Azeites de Portugal., 2018)

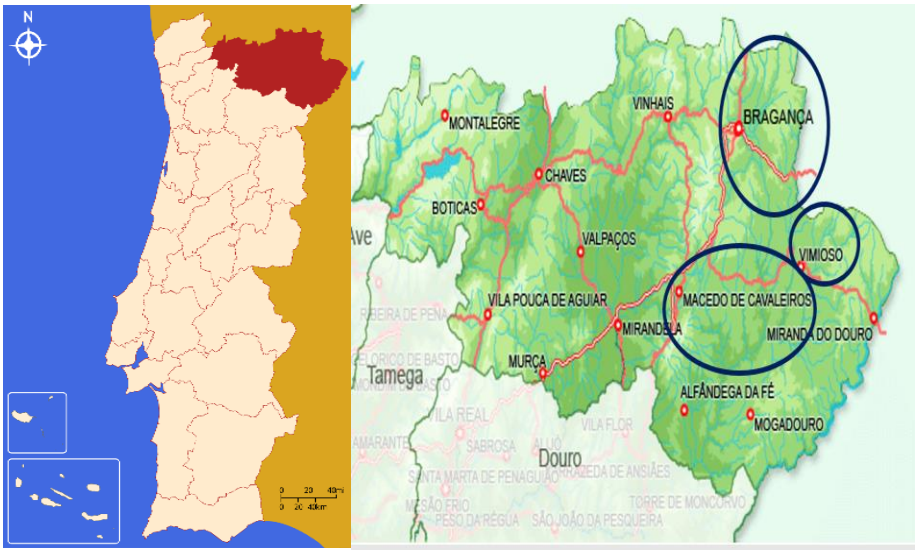


Figure 1. On the left, Portugal map, with the Trás-os-Montes region signalized. On the right, are marked the three municipalities with cv. Santulhana.

Chapter 3. Material and methods

3. Material and methods

3.1. Experimental design

3.1.1. Sampling

To carry out this work, a lot with five olive trees of cv. Santulhana located in the Santulhão region in the Bragança district in northern Portugal, where the collection of internal and external samples was carried out on three different dates (October 26, November 22, and December 14, 2020). At each harvest, approximately three Kg of olives were harvested per plant from inside and outside the canopy, where a part was used to make the morphological parameters of the fruits and the physicochemical determination. The remaining fruits were used to extract the oil. In the extracted oils, quality parameters (free acidity, peroxide value, specific extinction coefficients), chemical composition (fatty acids, tocopherol, and volatile compounds), total phenolic compounds and antioxidant activity by determination of free radical blocking effect 2,2-diphenyl-1-picrylhydrazyl (DPPH), resistance to oxidation (Rancimat method) and the sensory profile of the obtained oils were determined.

As for the olives, the fruits were submitted to biometric measurements, color (CIELAB method), and antioxidant (total phenolic compounds, DPPH assay, radical scavenging activity of [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical (ABTS), and reducing power).

3.1.2. Olive oil extraction protocol

Olive oil extraction was performed using a laboratory oil mill working with Abencor system (School of Agriculture of Castelo Branco, Portugal). The fruits were extracted shortly after harvest, i.e., less than 24 h after harvest, in a pilot extraction plant with an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain), with three main units: a mill, a thermobeater where malaxation takes place at controlled temperature, and a centrifuge. Olives were milled, and the obtained paste was homogenized, and about 700 g were transferred to the thermobeater unit (25 ºC, 20 min) for malaxation using a thermostatic water bath at 25 ºC. In the final 5 min of each malaxation, 100 mL of water at 27 ºC was added to aid the olive oil separation. The mixture was centrifuged, decanted, and the olive oil collected. After that, the oils were filtered (Whatman paper nº 4) using anhydrous sulfate to remove the solid particles and residual water. Finally, the olive oils were put in 125 mL dark bottles and stored in the dark at room temperature. All the assays were carried out between one and two months after extraction and were made in triplicate.

3.2. Olive fruit characterization

3.2.1. Olive fruit and stones characterization

All fruit samples were morphologically characterized by its length (mm) and maximum diameter (mm), using for both the fruit and the endocarp of the same fruit according to International Union for the Protection of New Varieties of Plants (UPOV) guidelines for *Olea europaea* (UPOV, 2011). The weight (g) of each fruit was also evaluated.

At each harvest time, about 40 olives (20 olives fruit collected from the outer part of the olive trees while the others collected from the internal part) per tree were selected to make biometric measurements.

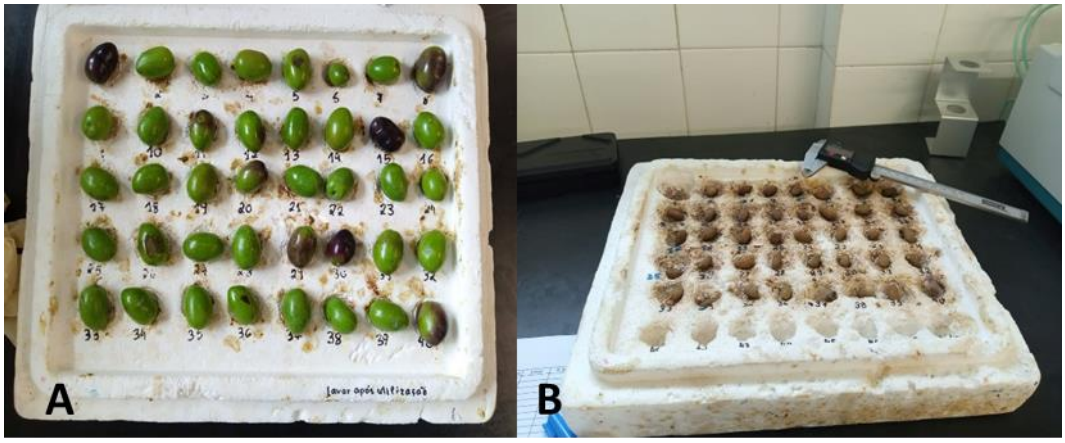


Figure 2. Olive fruit and stones biometric analysis from cv. Santulhana: A- the olive fruits in the support to undergo; B- the biometric analysis of the dried stones after having eliminated the flesh to undergo the biometric measurements.

3.2.2. Color measurement

Olives fruit color was assessed with a Konica Minolta model CR-400 colorimeter equipped with computer software. The chromatic ordinates evaluated were L*, a*, and b*. The parameter L* is a measure of luminance or lightness component, and it varies from 0 to 100 (black and white); The coordinate a* varies from negative to positive (green to red respectively) and b* which it varies also from negative to positive (blue to yellow respectively).

In order to do so, 40 olives (20 olives fruit harvested from the external part of the olive trees while the others harvested from the internal part) were picked from each olive tree (total of 200) at the three sampling times.

3.2.3. Antioxidant activity

3.2.3.1. Preparation of olive extract

After removing the endocarp, the olives were frozen and lyophilized. After complete dehydration, they were crushed with a Moulinex until obtaining a more or less homogeneous powder. Thereafter, about 1.5 g was weighed into a beaker and 50 mL of methanol was added (Figure 3). The mixture was left under stirring for one hour. Subsequently, the extract was filtered and washed with methanol. Stirring for one hour and filtration was then repeated three times.

Figure 3. Olive extract preparation from cv. Santulhana fruits.

The extract obtained was filtered into flasks, and the methane was recovered on a rotary evaporator to remove solvent residues. After 30 min, in the dry flasks, an appropriate volume of methanol for High Performance Liquid Chromatography (HPLC) was added. To complete the extraction of bioactive compounds, the biofilm sealed balloons were placed in the ultrasound extraction equipment. Finally, the filtrate was collected and stored in falcon tubes.

3.2.3.2. Determination of free radical blocking effect 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The evaluation method used to detect the ability to block DPPH free radicals from olive fruit extracts was described by Hatano et al. (1988). From the obtained extract, different concentrations were prepared in triplicate test tubes. Then, 0.3 mL of the different extract concentrations from each sample were mixed with 2.7 mL of DPPH solution. Thereafter, the mixture was stirred vigorously and allowed to stand in a dark place for 60 min. The reduction of the DPPH radical was quantified by continuous monitoring of the decrease in absorbance in the spectrophotometer at λ= 517 nm. The values obtained are expressed as a percentage of reduction of DPPH at 60 min of reaction, and the calculation is made by the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{AB - AS}{AB} \times 100$$

where AB and AS represent, respectively, the absorbance at 517 nm of the blank solution and of the sample extract.

3.2.3.3. Radical scavenging activity of the ABTS

The formation of the ABTS radical [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] is the basis of one of the spectrophotometric methods that has been applied to measure the antioxidant activity of products. The scavenging activity of olive fruits from cv. Santulhana was performed following the method of Sánchez et al. (2007). The method is based on the ability of a sample to inhibit the ABTS radical compared to an antioxidant reference standard (Trolox). Before starting the analysis, 25 mL of ABTS was enriched with 0.440 mL of potassium persulfate ($K_2S_2O_8$), and allowed to stand in the dark at room temperature for 12 to 16 h. Afterward, the samples were prepared by adding 2 mL of ABTS to 100 μL of plant extract sample. Absorbance readings were taken by an ultraviolet and visible spectrophotometer (UV-VIS/UV-1280 Shimadzu) at 734 nm.

3.2.3.4. Reducing Power

To assess the reducing power of the extracts, the method described by Berker et al. (2007) were performed. Thus, 1 mL of the extract solution was used, 2.5 mL of a 0.2 M sodium phosphate buffer solution with a pH of 6.6, and 2.5 mL of potassium ferrocyanide ($C_6FeK_4N_6$) was added at 1 %. The mixture formed was shaken and incubated at 50 °C for 20 min in a water bath. After cooling the samples, 2.5 mL of trichloroacetic acid 10 % (w/v) were added, stirring vigorously. 2.5 mL of the supernatant was removed from the mixture, and 2.5 ml of distilled water and 0.5 mL of 0.1 % iron (III) chloride ($FeCl_3$) were added. After the mixture was ready with all the necessary reagents, it waited 2 min, and the reading to evaluate the reducing capacity was made at an absorbance of 700 nm, in an ultraviolet and visible spectrophotometer (UV-VIS/UV-1280 Shimadzu).

3.2.3.5. Total phenols content

To evaluate total phenols content the method of Singleton and Rossi (1965) was used, with some modifications. Thus, in 1 mL of samples, 1 mL of Folin-Ciocalteu reagent (Adrich Chemistry) was added and vortexed, allowing the reaction to stand for 3 min. After the

estimated time had elapsed, 1 mL of saturated sodium carbonate solution (Na_2CO_3) and 7 mL of distilled water were added, stirring the mixture again.

The final solution was left in the dark for an hour and a half, then absorbance was measured in an ultraviolet and visible spectrophotometer (UV-VIS/UV-1280 Shimadzu) at 725 nm. Gallic acid was used to calculate the calibration curve to obtain the final data, and the results were expressed in milligrams of gallic acid per gram of extract (mg GAE /g extract).

3.3. Olive oil characterization

3.3.1. Olive oil quality parameters

3.3.1.1. Free acidity

The free acidity (FA) analyzes were carried out in accordance with the methodology present in Regulation (EEC) No 2568/91 of the European Commission of 11 July 1991, in which the following procedure was defined: 5.00 g of olive oil was weighed in a 50 mL Erlenmeyer flask and then 50 mL of a 1:1 solution of ethanol and ethyl ether and 3 drops of a solution of 2 % phenolphthalein. After homogenization of the sample, it is titrated with 0.1 N sodium hydroxide (NaOH), and the solution changes from a yellow color to a rust color at its turning point. All samples were determined in duplicate, and the result was expressed as a percentage of free oleic acid in the sample, using the following equation:

$$Free\ acidity\ (\%) = \frac{V * c * M}{10 * m}$$

That is:

V-Volume of sodium hydroxide consumed in the titration (mL);

c - Exact concentration of the sodium hydroxide solution in moles per liter;

M - Molar mass of oleic acid in g/mol;

m – Weight of the olive oil sample studied, in grams;

3.2.1.2. Peroxide value

The peroxide value (PV) analyzes were carried out according to the methodology present in Regulation (EEC) No 2568/91 of the European Commission of 11 July 1991, in which the following procedure was defined: 1.20 g of olive oil in a 50 mL Erlenmeyer flask, 15 mL of glacial acetic acid, 10 mL of chloroform and 1 mL of a saturated solution of potassium

iodide were added, then the tubes were capped with parafilm and shaken for approximately 1 min. After stirring, the sample was stored for 5 min in the dark, and then 75 mL of distilled water and three drops of starch solution at 1.0 g/100 mL were added (Figure 4).

Figure 4. Determination of peroxide value using titration.

For the titration, a standard solution of 0.01 N sodium thiosulfate was used, and the solution changes from a dark color to a completely transparent color when reaching the turning point. All tests were performed in duplicate, and to calculate the peroxide value the following formula was used:

$$IP\left(mEq.\frac{O2}{Kg}\right) = \frac{V * N * 1000}{m}$$

That is:

V - Volume of sodium thiosulfate consumed in the titration, taking into account the blank test;

N - Exact normality of the sodium thiosulfate solution (0.1N);

m – Weight of the olive oil sample studied, in grams.

3.2.1.3. Specific extinction coefficients

The absorbance analyzes were performed according to the methodology present in Regulation (EEC) No 2568/91 of the European Commission of 11 July 1991, with some

changes described below: 0.60 g of oil was weighed in falcon tubes 15 mL and then made up to volume with isooctane (2,2,4-trimethylpentane) until 10 mL of solution was reached. After the first dilution, a second dilution with isooctane must be carried out for reading at the appropriate wavelengths, which is normally used 1:25 for reading at 232 nm and 1:5 for lengths 264, 268, 272 nm. The results must show values between 0.100 and 0.800. If they are not within this range, the second dilution must be adjusted for this purpose. The reading at the respective wavelengths was done on a Shimadzu UV-VIS/UV-1280 spectrophotometer.

The formulas used to calculate the extinction coefficients at 232 nm, 268 nm, and ΔK were:

$$K232 = \frac{A232 * D.F}{m * 10}$$

$$K268 = \frac{A268 * D.F}{m * 10}$$

$$\Delta K = K268 - \frac{(\mathrm{A264} + \mathrm{A272})}{2}$$

That is:

A232, A264, A268, and A272: Absorbances;

D.F: The dilution factor;

m: The weight of the olive oil sample.

3.3.1.4. Sensorial analysis

The sensory evaluation of the different oils was carried out by a trained panel from the Escola Superior Agrária de Bragança, based on the methodology described by Rodrigues et al. (2020). The model of the oil profile sensor sheet is attached. In each sample, in its respective time, the olfactory (fruity, other fruits, herbal sensations, and harmony), gustatory (fruity, sweet, bitter, spicy, fruity, herbaceous, and harmony), and olfactory-taste (complexity and persistence). To establish the sensory profile and not influence the tasters, blank spaces were left to identify possible sensations without the expected attribute. The profile sheet used

included the sensory attributes described for each profile, the intensity being assessed using a 10 cm unstructured scale with an intensity ranging between 0 and 10.

The analysis was performed in appropriate blue-colored glasses to eliminate the interference of color in the taster's assessment. The ideal oil temperature to perform this analysis is around 28 °C, a temperature at which there is a greater release of aroma and flavor compounds, which leads to a more reliable result. The analysis began with assessing the olfactory profile, followed by the gustatory profile, and finally, the olfactory-gustatory profile.

3.3.2. Oxidative stability

The olive oil stability was conducted as suggested by Rodrigues et al. (2019) using the Rancimat 743 conductivity method, Methom Ltd., Switzerland, was used, a device that performs the analysis automatically and continuously. This methodology consists of bubbling air streams, filtered, cleaned, and dried at a speed of 20 L/h through the sample heated to 120 ± 1.6 °C. 3.0 g were weighed per samples, this analysis being done in duplicate. Oxidation compounds that are formed during storage are more polar than triglycerides, so they are carried away by the air flow that deposits them in the aqueous solution. The analysis is completed when each sample reaches a conductivity of 300 μS/cm. The stability time is calculated by the computer program associated with the equipment, from the calculations of the tangents to the curve obtained by the analysis.

3.3.3. Olive oil antioxidant activity

3.3.3.1. Preparation of olive oil micro extraction

The micro methanol-water extraction, was done according to Pizarro et al. 2013, where in a 2 cm^3 Eppendorf tube, 1 cm^3 of MeOH-H_2O 80 % (v / v) was added to 0.5 g of olive oil, stirred for 1 min in vortex, the maximum speed was subsequently centrifuged (Minispin 5452 Eppendorf), for 5 min at 13.200 rpm. The supernatant was removed into a 5 cm^3 volumetric flask. This procedure was repeated two more times, replacing 1 cm^3 of MeOH-H_2O 80 % (v / v) in Eppendorf. The 3 extracts were collected in the same flask, which in the end was made up of ultrapure water. Each extraction was performed in triplicate.

3.3.3.2. Determination of free radical blocking effect 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant capacity of olive oil samples was investigated, and the radical scavenging activity (DPPH) was assessed by antioxidants present in the extracts of olive oil

was used, thus generating a decrease in absorbance at 517 nm. The color of DPPH is then changed from purple to yellow. A DPPH control solution was prepared with 0.1 mL of methanol and 3.9 mL of DPPH (0.06 mM), homogenized in the vortex for 15 s and read at 517 nm on the UV-VIS/UV-1280 Shimadzu spectrophotometer.

The extracts were prepared in triplicate, left in the dark for 30 min and then read the absorbances. The values obtained are expressed as a percentage of reduction of DPPH at 30 min of reaction, and the calculation is made by the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{AB} - \text{AS}}{\text{AB}} \times 100$$

where AB and AS represent, respectively, the absorbance at 517 nm of the blank solution and of the oil sample extract.

3.3.3.3. Total phenols content

To determine the total phenols contents, a solution was prepared with 1500 mm^3 of water, 100 mm^3 of phenolic extract, and 100 mm^3 of reagent Folin-Ciocalteu, vortexed for 3 s and allowed to react for 3 min. Subsequently, 300 mm^3 of 20 % Na_2CO_3 (w/v) was added, vortexed for 3 s, and allowed to react for 60 min in the dark and at room temperature (20-22 ºC). This procedure was carried out in triplicate, in the extracts obtained in the MeOH-H_2O 80 % extraction. All were evaluated by VIS spectroscopy, on a UV-VIS/UV-1280 Shimadzu spectrophotometer and detected at 765 nm. The results were expressed in gallic acid equivalent (mg EAG Kg^{-1}).

3.3.4. Olive oil chemical characterization

3.3.4.1. Vitamin E content and profile

Vitamin E contents were analyzed by HPLC with fluorescence detection, according to ISO 9936, with some modifications as detailed by Rodrigues et al. (2020). Tocopherol's standards (α, β and γ) were purchased from Sigma (Spain), while the internal standard 2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol (tocol) was from Matreya Inc. (Pleasant Gap, PA). Individual standards purity was monitored by spectrophotometry (UV-1800, Shimadzu, Japan) based on their molar attenuation coefficients. N-hexane was HPLC grade from Sigma-Aldrich (Germany), 1,4-dioxane was from Sigma-Aldrich (p.a., USA). Filtered olive oil (50 mg) was mixed with internal standard solution (tocol), diluted in n-hexane, and homogenized. The

mixture was centrifuged for 5 min at 13.000 rpm at room temperature, and the supernatant obtained analysed by HPLC, using a normal phase silica column (SupelcosilTM LC-SI; 7.5 cm×3 mm; 3 mm) (Supelco, USA), conditioned at 25 °C and eluted with a mobile phase of 1, 4-dioxane in n-hexane (2.5 %, v/v), at a flow rate of 0.75 mL/min. Analyses were carried out using an integrated system with a data transmitter (Jasco LC – NetII/ADC, Japan), pumps (Jasco PU – 4180, Japan), an auto-sampler (Jasco AS – 4050, Japan), oven (ECOM Eco2000, Czech Republic), a DAD (Jasco MD – 4010, Japan), and fluorescence detector (FLD, Jasco FP – 4025, Japan) programmed for excitation at 290 nm and emission at 330 nm. Data were analyzed with the ChromNAV Control Center - JASCO Chromatography Data Station (Japan). The different compounds of vitamin E were identified by comparing the retention times with authentic standards, confirmed by their UV spectra and spectral purity, and quantified by individual calibration curves, being expressed in mg/Kg of olive oil.

3.3.4.2. Indiviual phenolic compounds identification

The phenolic compounds were firstly extracted following the method described by IOC (International Olive Council, 2017), with some modifications as reported by Rodrigues et al. (2019). About 25 μL of a MeOH-H_2O (80/20 v/v) solution containing syringic acid (0.15 mg/mL; Sigma-Aldrich) were added to 0.4 g of each olive oil sample, the whole is subsequently vortexed for 30 s. Afterwards, 5 mL of the same MeOH-H_2O solution were added and then the solution was agitated. To ensure the proper elimination of fat, about 5 mL of hexane was added and the mixture was then homogenized using the horizontal mechanical shaker (Edmund Bühler GmbH). The obtained solution was later centrifuged at 5000 rpm for 5 min. The hydrophilic phase (bottom phase) was recovered and filtered using a 0.22 μm polyvinylidine fluoride (PVDF) microfilter. The solution was then dried under a gentle stream of nitrogen (40 °C) and instantly re-diluted with 100 μL of methanol. The olive oil phenolic methanolic extract profiles were analysed in the HPLC coupled to a diode array detector (DAD) along with a data transmitter (LC–NetII/ADC). The HPLC system consisted of two integrated pumps (PU–4180), an auto-sampler (AS–4050), a column oven (ECOM Eco2000, Czech Republic), and the DAD (MD–4010). The different phenolic compounds were separated, at 35 °C, by a C18 reversed-phase column (Kinetex C18; particle size: 2.6 μm; pore size: 100 Å; LC length: 100 mm; internal diameter: 3.00 mm, Phenomenex), with an eluent made up of water and acetonitrile, both with 0.1 % of formic acid, at a flow rate of 0.8 mL/min. Peak identification was carried out by matching the retention times (RT) and UV/Vis spectra (200–600 nm) with those of the corresponding standards (apigenin, apigenin 7–glucoside).

3.3.4.3. Fatty acid composition

Fatty acids were evaluated as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution (Commission Regulation (EEC 2568/91 of 11th July) and extraction with n-heptane. The fatty acid profile was determined with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a flame ionization detector, an autosampler Chrompack CP-9050 and a 50 m x 0.25 mm i.d. fused silica Select FAME capillary column (Varian). Helium was used as carrier gas at an internal pressure of 110 kPa. The temperatures of the detector and injector were 270 ºC and 250 ºC, respectively. The split ratio was 1:50, and the injected volume was of 1 μL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area eluting between myristic and lignoceric methyl esters. A control sample (olive oil 47118, Supelco) and a fatty acids methyl esters standard mixture (Supelco 37 FAME Mix) was used for identification and calibration purposes (Sigma, Spain).

3.4. Statistical analysis

All data were reported as the mean ± standard deviation (SD), with at least three replications for each sample (olive fruit and olive oil). All statistical analyses were done with SPSS version 19 software. First, the homogeneity of the variance and the normal distribution of the residuals were verified. The statistical variance analysis, ANOVA one-way test, was used to compare different means. Variance analysis tests were carried out at a 5 % significance level.

Chapter 4. Results and discussion

4. Results and discussion

4.1. Olive fruits characterization

4.1.1. Fruits and stones characterization

The characterization of the Santulhana fruits and endocarps, performed according to UPOV parameters, are summarized in Table 1. The cv. Santulhana produced drupes with an average fruit length and horizontal diameter of 24.23 and 17.46 mm, respectively. These values are nearly comparable to those reported in the literature. Giuffrè (2017), in its study conducted in Italy to evaluate the biometric parameters of twelve olive cultivars under rainfed conditions, recorded a maximum value for the longitudinal diameter of 15 mm and 25 mm for horizontal diameter for the cv. Nocellara Messinese variety. Also, we noticed fruit weight values in 5.01-6.12 g rang. The International olive council (IOC) categorizes olives into three main groups: small size (less than 3 g), medium size (weight varying between 3 and 5 g), and large size (> 5 g). By referring to the IOC classification, olives of the cv. Santulhana cultivar are considered as being large in size since we recorded a minimum weight of 5.01 g in the first collection and are collected from the outer part. No significant differences were recorded in these parameters when fruits from outside and inside of the three canopies were compared (Table 1).

Furthermore, a low stone weight is an attractive feature in the olive table industry or for high yields in the oil extraction (Giuffrè, 2017b). In that respect, a large stone complicates oil extraction considering the rupture force, rupture energy, and specific deformation increased according to the increase of the pit size for all compression axes (Kiliçkan & Güner, 2008). For the olives stone biometrical parameters, we recorded an average of 18.73 mm for stone lenght, 8.72 mm for stone diameter, and 0.90 g for the stone weight. These results are consistent with those recorded for Greek olive cultivar cv. Kalamon has a diameter equal to 8.85 mm (Blazakis et al., 2017). In addition, any significant differences were observed in the fruit stones from the outside and inside of the three canopy (Table 1).

These biometric parameters findings also corroborate with Peres et al. (2011) when classifying the olives cultivars from the Trás-os-Montes region in Portugal's northeast. They reported for the cv. Santulhana cultivar an average weight of 4.31 g for the fruit and 0.84 g for the endocarp. In addition, these same authors recorded values for the fruit length ranging from 18.70 to 30.90 mm and for the fruit diameter (D_{max}) from 6.35 to 11.60 mm. For the endocarp weight, the authors cited above mentioned an average value of about 0.84 g.

Table 1. Length (mm), maximum diameter (mm)s and weight (g) of fruits and stones of fruit and endocarp (mean ± standard deviation), collected from the inside and outside part of olive tree at three different harvest times.

Harvest time / Parameters in fruit and stone		Olive fruit			Stone		
		Length (mm)	**Max. diameter (mm)**	**Weight (g)**	**Length (mm)**	**Max. diameter (mm)**	**Weight (g)**
October,26,2020	**Outside**	24.83 ± 0.22	17.73 ± 0.15	5.01 ± 0.12	18.65 ± 0.18	8.70 ± 0.09	0.89 ± 0.02
	Inside	25.12 ± 0.17	17.60 ± 0.12	5.08 ± 0.09	18.99 ± 0.16	8.76 ± 0.07	0.94 ± 0.02
	α	0.31	0.49	0.65	0.16	0.61	0.12
November, 22,2020	**Outside**	25.13 ± 0.24	18.25 ± 0.28	5.53 ± 0.11	18.64 ± 0.17	8.86 ± 0.14	0.88 ± 0.02
	Inside	24.96 ± 0.23	17.72 ± 0.16	5.56 ± 0.12	18.92 ± 0.19	8.65 ± 0.09	0.86 ± 0.02
	α	0.61	0.11	0.84	0.25	0.19	0.67
December 14,2020	**Outside**	24.23 ± 0.17	16.71 ± 0.13	6.12 ± 0.61	18.76 ± 0.17	8.69 ± 0.08	0.92 ± 0.20
	Inside	24.14 ± 0.22	16.75 ± 0.14	5.67 ± 0.13	18.41 ± 0.19	8.65 ± 0.09	0.90 ± 0.02
	α	0.75	0.82	0.47	0.17	0.69	0.50

For the factor under study, the ANOVA results demonstrated that there is no statistical difference between the two parts of the tree, which means that the olive fruit length and diameter, and weight were unaffected by the olives position in the tree. These results arein accordance with Grilo et al. (2021) who observed that fruit weight was not impacted by planting density or canopy position. In contrast, this finding does not align with the outcomes reported by Trentacoste et al. (2016). The authors found that the olive fruits from *cv.* Arbequina, which are most disposed to the light, have the largest size and mesocarp cells. Nevertheless, the endocarp fresh weight was not affected by the position of the fruit in the canopy.

4.1.2. Olive fruit color measurement

The skin and pulp color of the olives are important factors for deciding the harvest time (Krapac et al., 2018). The descriptive CIELAB color coordinates of olive fruits analyzed as a function of the canopy position are illustrated in table 2.

Brightness values ranged from roughly 27.74 to 48.42. The chromatic coordinate a* values fluctuated from -15.06 to 3.61. The blue–yellow coordinate b* had values averaged between 8.47 and 24.11. The Chroma (C*) value is a measure of the color intensity or saturation. Moreover, the hue angle (h*) is a good factor for assessing changes in olive fruits color. This measure correlates with the a* and b* values. The h* values obtained were between 42.29 and 212.74. As indicated, the a* parameter did not behave in the same way during the three collections. Being negative in the olive samples collected in the first and second collection, for the third collection it became positive to reach a value of 3.16. All b* values are positive. Nevertheless, as the ripening process progresses, this color parameter declines significantly from 24.11 to 7.96 and tends towards the blue region. Concerning the light parameter L*, initially, the values were equal to 47.56 and 48.42 for the external and internal parts of the olive tree, respectively. As the fruit matures, the brightness parameter decline. These observations can be explained by the fact that during olive fruit ripening, the photosynthetic activity, the chlorophylls concentrations, and carotenoids progressively decline, while other compounds having red pigments, especially anthocyanins, are produced (Borges et al., 2019). Thus, at advanced stages of maturity, the fruits become darker and lose their yellow colour which explains the low values of L* and b*.

Table 2. Olive fruit from cv. Santulhana colour parameters (mean ± standard deviation, with ANOVA results) collected from the outside and inside part of olive tree at three different harvest times.

Evaluated parameters	October, 26, 2020			November,22,2020			December,14,2020		
	Outside	Inside	α	Outside	Inside	α	Outside	Inside	α
L*	47.56 ± 0.60	48.42 ± 0.31	0.21	40.89 ± 0.97	44.64 ± 0.9	**0.01**	27.74 ± 0.86	27.90 ± 0,90	0.90
a*	-12.67 ± 0.73	-15.05 ± 0.24	**0.00**	-2.07 ± 0.99	-4.92 ± 0.95	**0.04**	2.17 ± 0.80	3.61 ± 0,55	0.14
b*	21.62 ± 0.94	24.11 ± 0.37	**0.02**	8.47 ± 1.35	12.80 ± 1.22	**0.02**	8.70 ± 1.23	7.96 ± 1.08	0.65
C*	26.48 ± 0.82	28.46 ± 0.42	**0.03**	15.63 ± 1.04	18.29 ± 0.96	0.06	12.423 ± 1.18	11.12 ± 0.90	0.40
h*	141.00 ± 6.52	122.85 ± 0.93	**0.01**	212.74 ± 11.42	177.45 ± 10.72	**0.03**	42.29 ± 5.07	42.38 ± 5.70	0.99

The olive fruit position significantly affected the green-red coordinate (a*), the blue-yellow coordinate (b*), chromaticity coordinates (C*), and hue (h*), during the first and second collection. L* did not show significant differences between the two positions during the first and the third harvest. However, for the second harvest, a statistically significant difference was observed. The lowest L* values were found in olive fruits obtained from the outside part, indicating that these fruits are the darkest. These findings suggest that the olives grown in the inside part are green at the first and second collections. Then, during the third collection, they tended to change color to become reddish. As mentioned above, all values of b* are positive and decrease as maturation progresses. Even so, statistically significant differences between samples were noted. Regarding C*, the most significant values were observed in the samples taken from the canopy inside, which proves a brighter green color for the samples collected from the inside part of the olive tree during the first and second collections. As for the third collection, no significant differences were detected. Finally, the h*that correlates with the a* and b* values. It is a good indicator of changes in color characteristics in olive fruit. In this study, the h*values ranged from 94.42 to 96.14, thus placing the samples very close to the first quadrant in CIELAB space, so these olive fruits present a greenish-yellow hue. As stated in the work of Wrolstad et al (2005), the hue angle is given on a 360° chart where 0°=blue-red, 90°=yellow, 180°=green and 270°=blue. As previously mentioned (see Table 2), the olives collected in the first collection belong to the 90° angle. The second collection samples belong to the 180° angle. Finally, the third collection gave olives belonging to the 0° angle. Therefore, the first harvest samples had a yellow hue, the second collection olive samples showed a greenish hue, and the last harvest olive fruit sample presented a reddish hue.

4.1.3. Olive fruits antioxidant activity

4.1.3.1. DPPH radical scavenging capacity

DPPH assay is commonly used to assess the free radical scavenging activity of antioxidants. Indeed, antioxidants react with the stable free radical DPPH, initially with a dark purple color, and convert it to 2,2-diphenyl-1-picryl hydrazine. The antioxidant activity of olive fruit extracts on DPPH radical scavenging is due to their ability to provide hydrogen, which reduces the stable purple radical from DPPH to the yellow-colored DPPH-H. Therefore, the role of antioxidants is to capture the oxidation chain of free radicals and hence form stable free radicals, preventing the spread of oxidation. As shown in Figure 5, the scavenging capacities of the tested samples ranged between 38.83 % and 49.09 %. It can be observed from the same illustration that the scavenging capacity of olive fruit extracts increased depending on the

harvest time progress. Samples from the third harvest showed a powerful DPPH radical-scavenging ability (47.52 % and 49.09 % for samples collected from the outside and inside part of the olive tree, respectively) compared to other samples collected during subsequent harvest times. The samples from the first collection presented a weaker inhibition percentage than samples collected in the third collection but exceeded olive fruit extract gathered in the second collection. Our results were in agreement with the findings reported by Malheiro et al. (2011). In their study of the cultivar effect on the phenolic composition and antioxidant activity of stoned table olives, they have mentioned that cv. Cobrançosa and Santulhana cultivars presented higher antioxidant potential with a percentage of inhibition equal to 50 %. Within this same context, Xie et al. (2015) indicated similar values for olive extracts of Chinese cultivars.

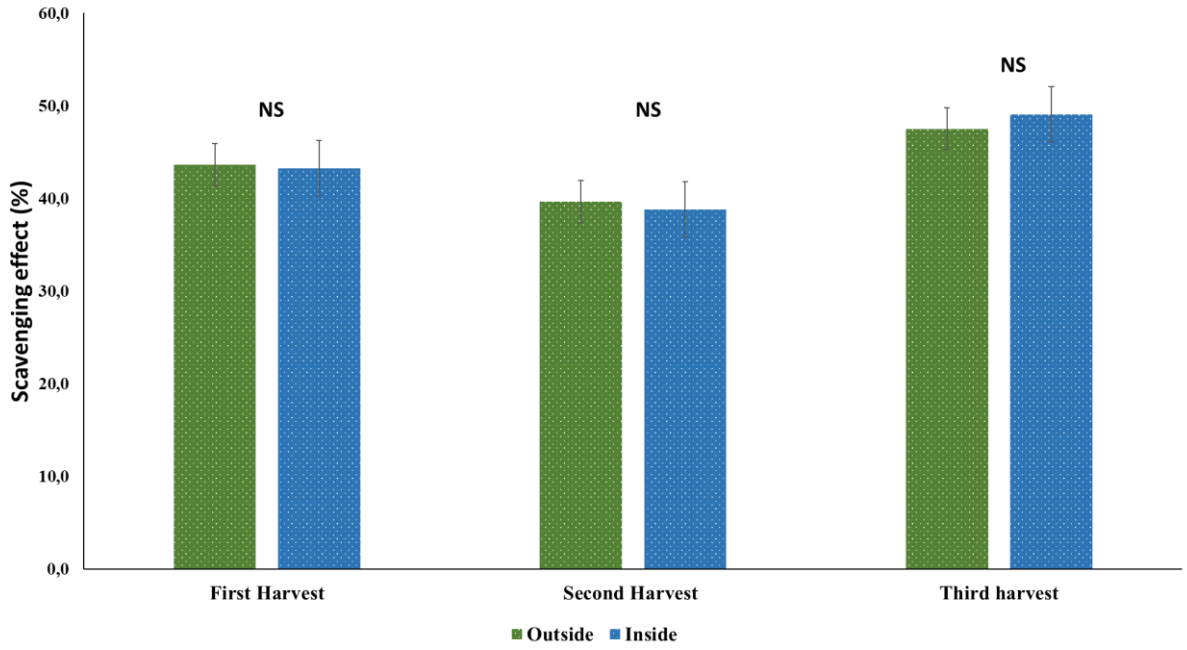


Figure 5. Average values of the DPPH radical scavenging activity (%) of olive fruit extracts from cv. Santulhana variety collected from the inside and outside part of olive tree at three different harvest times. N.S. no significant differences, * P≤0.05.

Also, from the results shown in the same figure it can be inferred that DPPH assay did not differ among position.

4.1.3.2. The ABTS scavenging ability assay

The ABTS test aims to measure the olive fruit extracts antioxidant capacity, able to neutralize the 2,2 azinobis (3- ethylbenzthiazolin-6-sulfonic acid) ($ABTS^{+}$). The intensity of the blue-green coloration of this stable radical cation decreases in the presence of antioxidants.

The ABTS scavenging ability is represented in Figure 6. As shown, all olive fruit extracts presented an inhibitory potential in opposition to ABTS free radical. However, this capacity has declined along with the progress of the harvest time. From October to December 2020, the scavenging percentages decreased from 45.51 % (first harvest, inside part) to 26.06 % (third harvest, inside part). Thus, the antioxidant activity of cv. Santulhana olive extracts can be attributed to the presence of phenolic compounds. As previously reported by Benlarbi et al. (2018b) the green types of all cultivars have a higher antioxidant activity than the black types. These statements account for the antioxidant power of olives of the santulhana cultivar, which are characterized by their green color during the early stages of ripening. Some previously published bibliographic data have confirmed the outcomes of this study. The ABTS inhibitory rates of Chinese olive fruit methanol extract fluctuated approximately between 25 and 58 % (Kuo et al., 2015).

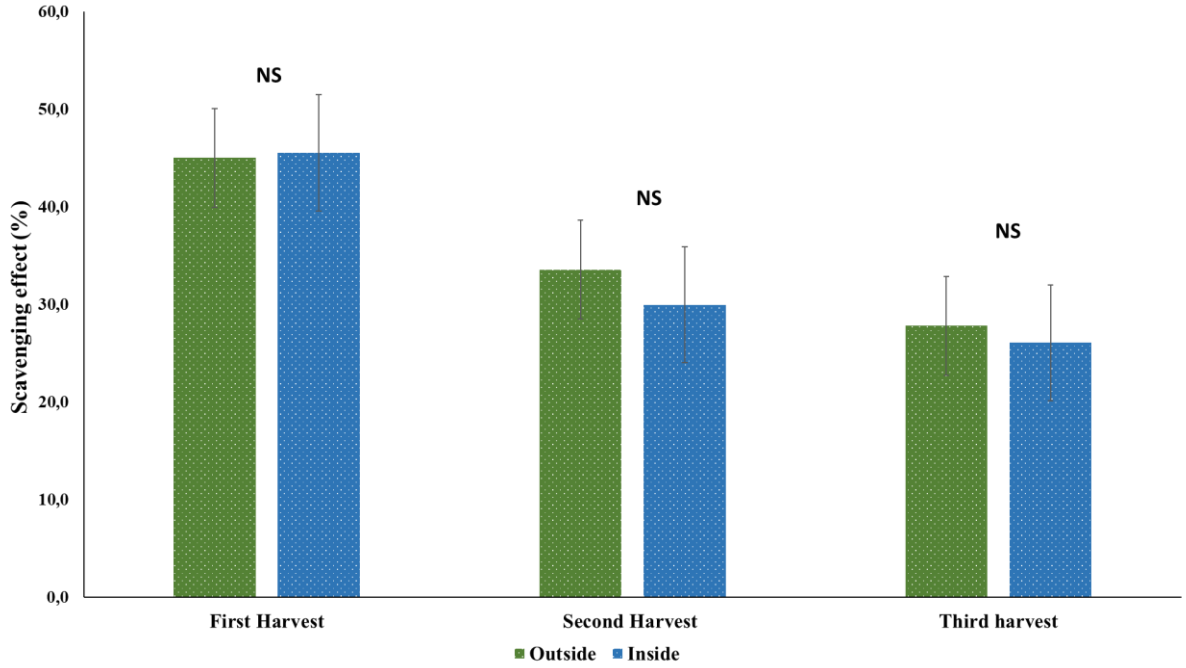


Figure 6. Average values of the ABTS radical scavenging activity (%) of olive fruit extracts from cv. Santulhana collected from the inside and outside part of olive tree at three different harvest times. N.S. no significant differences, * P≤0.05.

As it happens for DPPH assay, the antioxidant activity evaluated by the ABTS method response to different positions has been inconsistent.

4.1.3.3. Olive fruit Reducing Power

The reducing power test is a method to assess the total antioxidant capacity using the ferric ion. The presence of reducing agents, namely antioxidants, leads to the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form by donating an electron (Sousa et al., 2014). The results appearing in Figure 7 indicate that the ability of cv. Santulhana olive fruit extract samples to reduce Fe^{3+} to Fe^{2+} varied from 43.18 to 56.90 mg Trolox/g of olive fruit extract. As it can be seen, the highest reducing power was detected in samples of the second harvest, which are located in the outside part of the olive tree. However, the lowest values were recorded for the olives from the third collection, equal to 44.51 and 43.18 mg Trolox/g of olive fruit extract respectively for the samples from the outer and inner part. These results suggest that cv. Santulhana olive extracts contain antioxidant agents, such as total phenolic compounds, hydroxytyrosols and tocopherols.

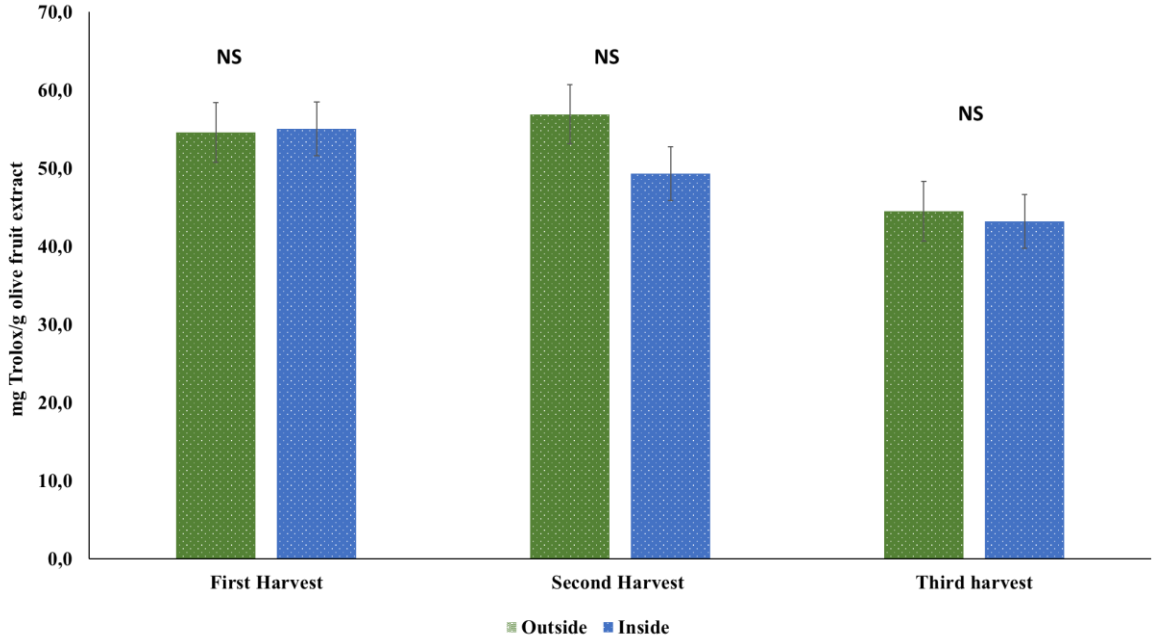


Figure 7. Average values of the reducing power (mg Trolox /g olive fruit extract) of olive fruit extracts from cv Santulhana collected from the inside and outside part of olive tree at three different harvest time. N.S. no significant differences, * $P \leq 0.05$.

These compounds are characterized by their redox properties, they constitute hydrogen donors, which allow them to act as reducing agents. The decreasing observed in reducing power for the cv. Cobrançosa olive aqueous extracts variety, whose reducing power decreased as the maturity process advanced (Sousa et al., 2014b). Regarding the position effect, the statistical study showed no difference between the two positions.

4.1.4. Olive fruit total phenolic contents

The phenolic composition of olives represents a rich and unique source of health-promoting molecules due to the presence of specific phenolic compounds (Ferro et al., 2020). The results obtained in the measurement of the total phenolic contents of cv. Santulhana olive extracts picked from the inside and outside part of olive tree at three different harvest time are illustrated in Figure 8. As expected, the results revealed that the total phenols content diminished depending on the advancement of the ripening process, a fact confirmed by Benlarbi et al., (2018a) for Dahbia cultivar. This decrease is perhaps due to the increase of the hydrolytic enzyme's activity during the maturity of olive fruits. The highest content was detected in olive fruit extract during the first harvest (October 26, 2020) and collected from the outside of the tree (742.01 mg gallic acid/ g olive fruit extract).

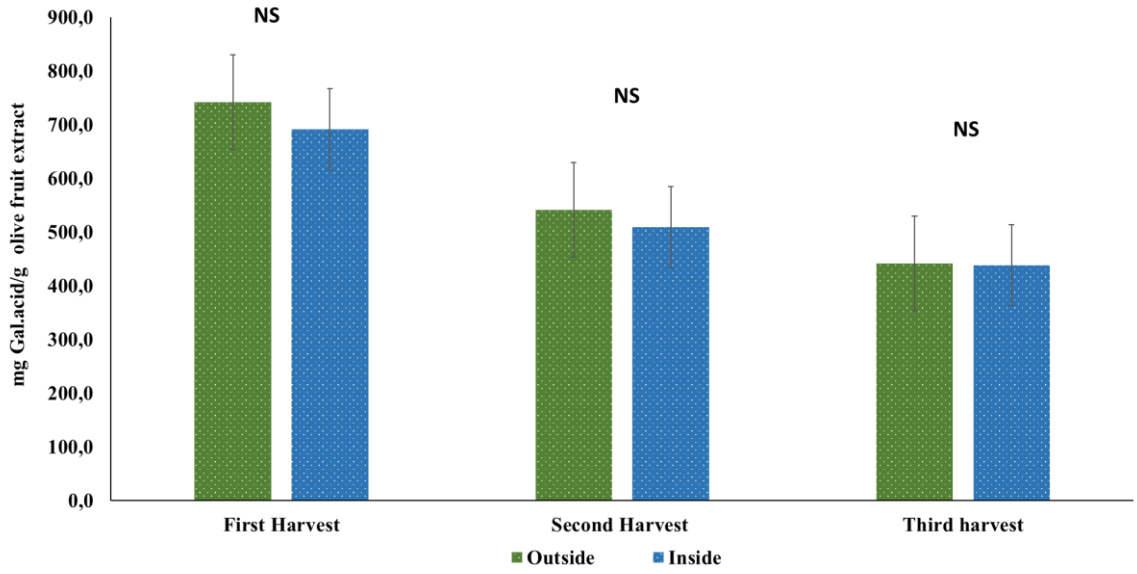


Figure 8. Average values of the total phenols content (mg Gallic acid/g olive fruit extract) of olive fruit extracts from cv Santulhana variety collected from the inside and outside part of olive tree at three different harvest time. N.S. no significant differences, * P≤0.05.

These values were close to those found by Salazar-García et al. (2019), which recorded a total phenolic content average of 570 mg/ kg for the Spanish cultivars "Mas Blanc". Moreover, the results point out that there is no statistically relevant difference between the samples from the outside and inside parts.

4.2. Olive oil

4.2.1. Olive oil quality parameters

This part of the report will be devoted to evaluating the commercial quality level of the different extracted oil samples concerning the Commission Delegated Regulation (EU) 2015/1830, Commission of July 8, 2015. The analyzed param&eters include free acidity, peroxide value, specific extinction values, and the sensory profile. The results are summarized in the table 3.

Free acidity is the result of the hydrolysis of triglycerides in the presence of water and by the catalytic action of the enzyme lipase. During this process, triglycerides are broken down into glycerol, mono- and di-glycerides, and fatty acids. This process begins in the fruit, especially when the skin has been damaged by insects such as *Bactrocera oleae*, or if it is injured by hail or when the olive fruits are harvested. Free acidity values are presented in table 3. As stated, the free fatty acid content of all samples did not go beyond the standard limit of 0.8 %, and values ranged from 0.24 to 0.31 %. Therefore, with reference to the Regulation of the Commission [EU] 2015/1830, 2015, the samples were classified as "Extra Virgin Olive Oil". Our results showed that the lowest free acidity value was found in the oil extracted during the first collection from the outside part of the tree (0.237 %), while the highest value (0.311 %) was found in the oil extracted during the second harvest from the outside part. These results coincide with Ahumada-Orellana et al. (2018b), which registered a free acidity value range, for the Arbequina cultivar, between 0.21 and 0.32 %. Regarding the position effect, we did not record any significant difference between the olive oils obtained from the two parts of the olive tree. These findings are not consistent with earlier investigations. Consider that the olives located in the inside part of the olive trees are less exposed to the solar rays and therefore are located in the shaded parts. In this context, Lémole et al. (2018) reported that olive oil from the unshaded parts and those from the parts with total shading had similar free acidity.

Table 3. Olive oil quality parameters (mean ± standard deviation) with ANOVA results of olive oils extracted from cv. Santulhana collected from the inside and outside part of olive tree at three different harvest times.

Quality parameters	First Harvest			Second Harvest			Third Harvest		
	Outside	**Inside**	***p-value***	**Outside**	**Inside**	***p-value***	**Outside**	**Inside**	***p-value***
FA (%)	0.24 ± 0.01	0.24 ± 0.01	0.10	0.31 ± 0.02	0.29 ± 0.01	0.35	0.25 ± 0.03	0.27 ± 0.02	0.62
PV (mEq.O_2/Kg)	4.24 ± 0.40	5.81 ± 0.60	**0.04**	3.90 ± 0.25	3.74 ± 0.18	0.61	3.49 ± 0.21	3.73 ± 0.19	0.39
K_{232}	1.74 ± 0.04	1.77 ± 0.07	0.89	1.93 ± 0.01	2.01 ± 0.06	0.26	1.83 ± 0.05	1.90 ± 0.04	0.22
K_{268}	0.14 ± 0.00	0.14 ± 0.00	0.16	0.13 ± 0.01	0.14 ± 0.05	**0.05**	0.14 ± 0.00	0.15 ± 0.00	**0.02**
ΔK	0.000	0.000	-	0.000	0.000	-	0.000	0.000	-
Sensorial analysis	**EVOO**								

The peroxide value (Table 3) is related to the oxidation of unsaturated fatty acids contained in an oil and the formation of hydroperoxides. In contact with oxygen from the air, olive oil oxidizes due to free radicals of unsaturated acids. The oil oxidation reaction takes place in several stages and includes initiation, propagation, and termination reactions. Oxidized olive oils develop an unpleasant odor and taste that make them unpalatable and ultimately inedible. From the results shown in Table 3, it is obvious that all olive oil samples are within the standards set by Commission Regulation [EU] 2015/1830, 2015 since we have recorded peroxide values ≤ 20 mEq O_2/Kg, the values vary between 3.49 ± 0.21 mEq.O_2/Kg and 5.81±0.60 mEq.O_2/Kg. As noted, the peroxide value decreased over the ripeness from 5.81 mEq.O_2/Kg in the first harvesting time to 3.49 mEq.O_2/Kg in the last date. This tendency was not observed for the oils obtained from cv. Memecik cv. and cv. Ayvalık at later advanced stages of maturity exhibited higher peroxide value, especially in November (Sönmez et al., 2018). Furthermore, the ANOVA analysis of variance has revealed that olive oil extracted from the inside part during the first collection gave an oil with a significantly higher ($P \leq 0.05$) peroxide value (5.81±0.60 mEq.O_2/Kg) than the rest of the samples. However, in the second and third harvest, the position effect did not occur. By contrast, Caruso et al. (2017b) have demonstrated that olive oil extracted from the top part of the canopy provided the most important peroxide values.

Absorbance measurement at 232 and 268 nm is an essential factor for assessing the oxidation degree of olive oil and, therefore, its quality. The absorbance at 232 nm provides information on diene conjugates, which are formed during the olive oil oxidation process. Regarding K_{268} reveals the presence of conjugated trienes, which are the secondary oxidation products in particular aldehydes and ketones (Hashm Rasul et al., 2020). As shown in Table 3, the K_{268} content of all olive oil samples did not exceed the limits set by the Commission Delegated Regulation (EU) 2015/1830 of July 2015. In fact, all samples showed levels ranging from 0.13 ± 0.01 to 0.15 ± 0.00. Results of the present work are consistent with the findings of some previous workers. Rodrigues et al. (2019) in their study of the characterization of oils from varieties cultivated in the north of Portugal (cvs. Lentisca, Rebolã, Madural, Redondal, Verdeal and Verdeal Transmontana), have stated K_{232} values lower than 2.03 for all the investigated varieties during the two years of study. In the case of K_{268}, the values reported have varied from 0.11 to 0.23. In addition, the existence of the position effect was confirmed for only K_{268} values in the second and third collections. These results partially agree with those found by Caruso et al. (2017), since they stated that there is no significant difference in olive position for all specific extinction parameters.

Overall, the olive oils from the outer part are apparently more stable since we have noted minimal peroxide (at the first harvest) and K_{268} values (at the second and third harvest), as also observed by Gómez-del-Campo & García. (2011). These authors reported that fruits in the upper part of the canopies produced more stable oil than those obtained from less illuminated fruits.

4.2.2. Sensorial analysis

Sensory analysis is a complementary analysis to physicochemical assays. It allows us to evaluate both the state of the olives before crushing and the progress of the ageing of the olive oil. The sensations that can be encountered with olive oil are: olfactory or gustatory, tactile sensations. Therefore, each sensation will be presented separately.

Referring to the results obtained and which are illustrated in Table 3, we can see that no defects were recorded, so all the samples of olive oil analyzed can be classified as extra virgin olive oil (EVOO). The olfactory profile (Figure 9) reveals that the perception intensity varies between 0.0 and 8.0. All olive oil samples have a fruity olfactory sensation intensity higher than 0.0, but in the samples from the first collection and the second collection, this sensation was "green" and its intensity varied between 2.3 and 7.3. In olive oil from the third collection, the olfactory sensation was "mature" with an intensity ranging from 2.8 to 5.5. These findings do not correlate with Caporaso et al. (2021) statements, which mentioned that the "fruity" peak appeared only during the third collection. Moreover, all olive oil samples provided a high intensity for "apple" olfactory attribute, with a mean value of 4.8. These findings are in accordance with the results reported by Rodrigues et al. (2019) which have recorded values ranging from 0.0 to 5.9 for this same attribute. Similarly, "tomato" and "dry fruit" sensations were present in tested samples, with a broad range of perceived intensities that varied from 0.0 to 5.7 and from 0.0 to 4.7, respectively. Low intensities were observed for "banana" olfactive perception at the first collection, with mean values ranging from 2.0 to 3.8. This attribute was not felt in the samples from the second collection, although higher intensities, graded from 2.6 to 4.9, were recorded during the third harvest. The "cherry" and "fresh grass" notes were only perceptible at first and the second harvest, with averages around 0.1 and 2.3, respectively. These results are in line with previous studies. Rodrigues et al. (2019), when studying the olive oil differentiation from a sensory point of view, recorded similar fruity sensations, including tomato, apple, banana, fresh herbs, cabbage, and tomato leaves. Regarding herbaceous olfactory sensation, fresh grass, tomato leaves, cabbage and olive leaves were found with different scores ranging from 0.0 to 2.3. Interestingly, grass sensation was the dominant notes.

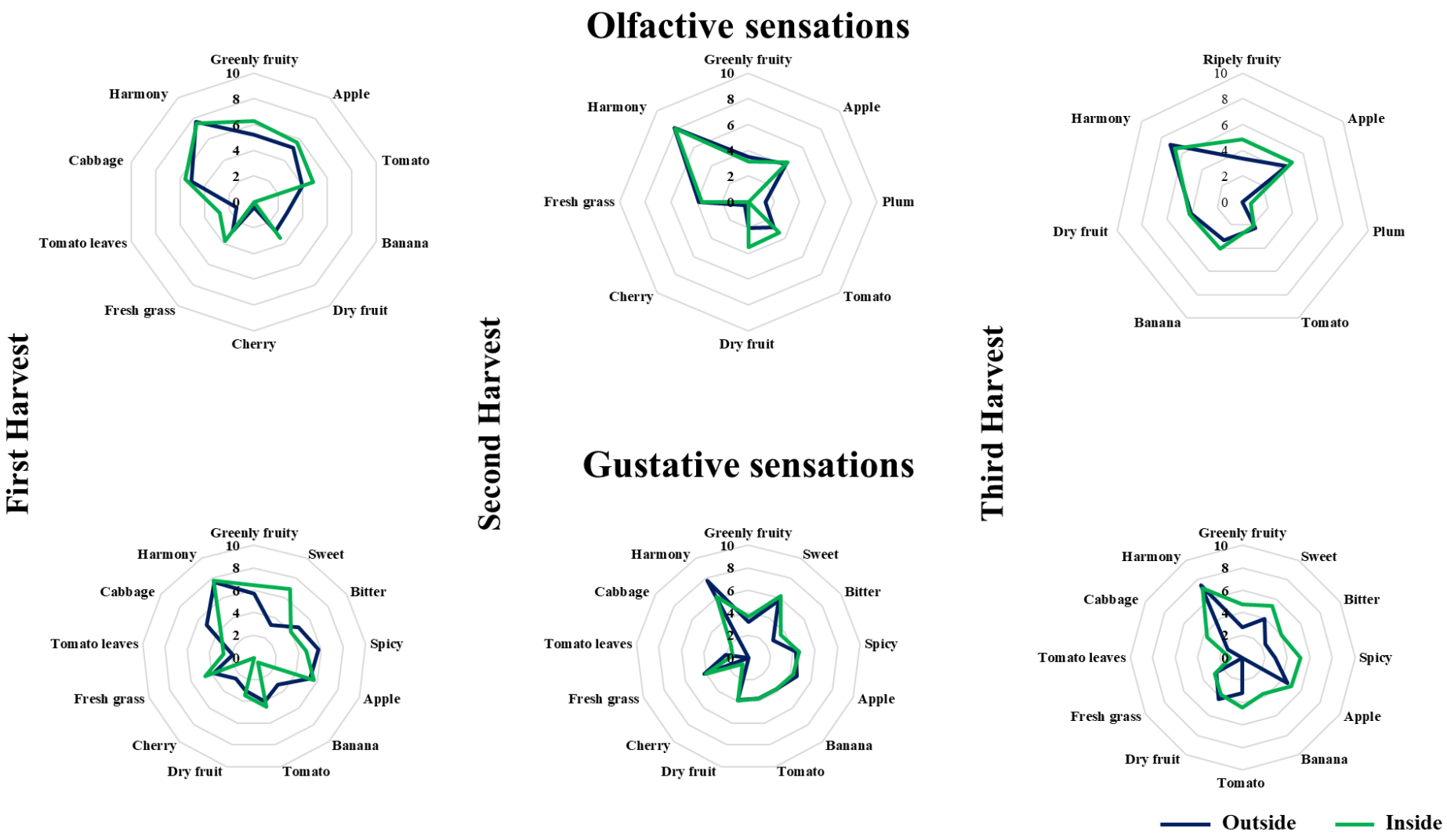


Figure 9. Sensory profile of olive oils obtained from cv. Santulhana and collected from the inside and outside part of olive tree at three different harvest time.

Concerning gustatory sensations (Figure 9), “apple”, “banana”, “tomato”, “dry fruit” were outstanding in almost tested olive oil samples during all the collections periods, while cherry gustatory sensation appeared only in the first collection and in some samples resulting from the second collection (0.1).

The results of the one-way ANOVA showed some differences between the olfactory sensory attributes, namely “apple”, “dry fruit" and "cherry" depending on the position of the olive fruits in the olive tree ($P \leq 0.05$). The same outcomes proved that samples from the inside part scored the highest values for apple (5.0) and dry fruit (3.6) notes (See Figure 9).

For the “cherry” aroma, higher intensities were attributed to the samples coming from the outside part (0.3). Despite the variety of values observed for the other fruity sensations, no significant differences were detected. A similar situation was found for all gustatory attributes, that is to say, no significant differences were observed between the two positions of the olive tree. The two other variables assessed were “complexity” and “persistence”. The complexity values that were evaluated by the combination of the different positive sensations perceived for each sample ranged from 5.2 to 7.2. Persistence is meant to describe the durability that is the length of time during which the sensations perceived in a retro nasal way persist in the senses such as the mouth. The obtained values for this parameter were in the order of 7.5 to 5.3. And, the highest values were recorded for the olive oils obtained from the outside part of the olive tree and the first collection. In contrast, the lowest values were registered for the olive oil samples issued from the second collection and the inside part of the tree. But no significant differences were observed between positions for both of these evaluated parameters.

4.2.1. Oxidative stability

The Rancimat method is used to assess the oxidative stability of edible oils and fats. Using this method, it is possible to determine the induction time required for maximum oxidative damage to an oil or fat (Sanaeifar & Jafari, 2019). Induction time under Rancimat conditions 120 ºC and 20 L/h air flow of all olive oil samples is depicted in figure 10. The results, as illustrated, varied between 14.73 and 16.30 h. Similar results were outlined in earlier studies. El-Gharbi et al. (2018) investigated the impact of geographical location on the chemical properties of Zarazi VOO produced in the south of Tunisia. The samples were analyzed by Rancimat test and it was observed that Sfax oil samples was able to withstand up to 16.21 h before deteriorating. Thus, the ripeness of the olives can affect the oil's oxidative stability since it can lead to changes in some compounds having antioxidant activity. This connection turned

out that the oxidative stability was mainly influenced by the fatty acid profile, polyphenols and carotenes (el Yamani et al., 2020).

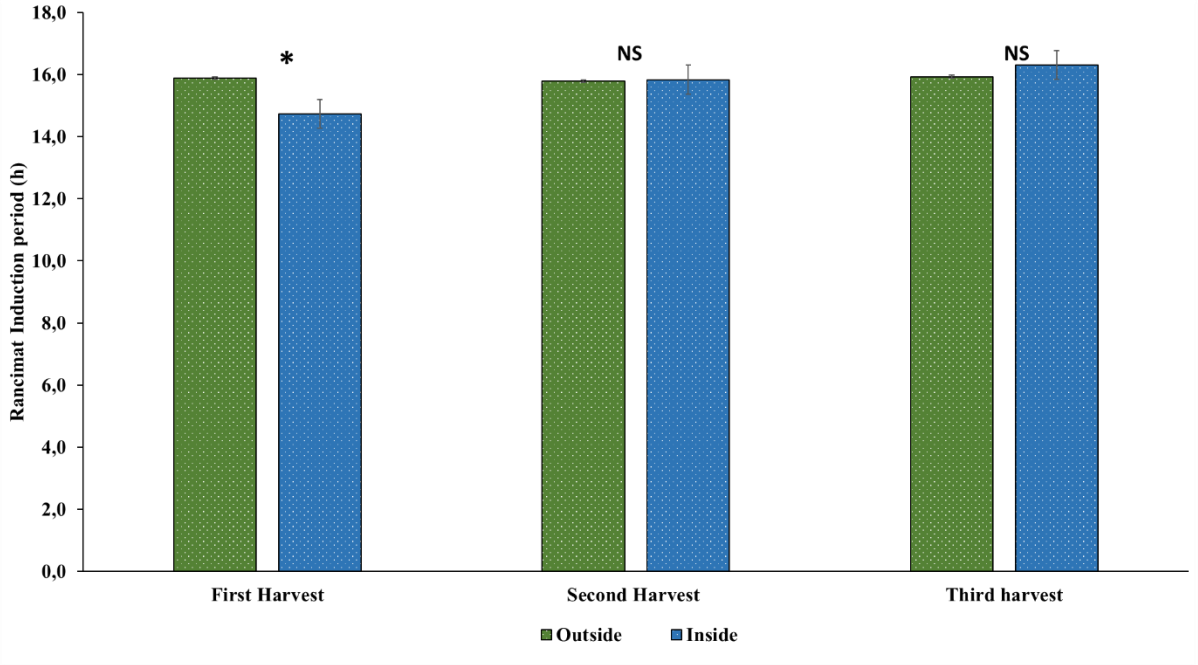


Figure 10. Average values of olive oil oxidative stability extracted from cv. Santulhana and collected from the inside and outside part of olive tree at three different harvest times. N.S. no significant differences, * P≤0.05.

Moreover, the dependent variable, the fatty acid content, contributed very strongly to the oxidative stability of the virgin olive oil from Moroccan Picholine cultivar since it occupied the most significant weight in the stepwise regression to explain the relationship between the composition of the oils and their oxidative stability (Ceci & Carelli, 2010).

Furthermore, ANOVA test showed that the position effect was significant only for samples resulting from the first collection ($P \leq 0.05$). It turned out that the olive oil of the outside part is more stable. These findings have been supported by some authors. In a study conducted on the effect of the location of fruit in canopies of hedgerow olive trees, Gómez-Del-Campo & García (2012) reported that olive oil resulting from higher layers was significantly more resistant to oxidation.

4.2.2. Olive oil antioxidant activity-DPPH scavenging assay

The antioxidant properties of olive oil samples were evaluated by DPPH scavenging activity assay. This test aims to measure the reducing ability of olive oil antioxidants toward the DPPH radical. As illustrated in Figure 11, the scavenging activity of all tested samples was

within the range of 28.5 (olive oil from the inside, third harvest) to 51.8 % (olive oil from the inside, first harvest). In contrast, Quintero Flórez et al. (2018) have reported lower values for Blanqueta and Sevillana cultivars, the reported values varied between 15 and 19 %. Also, it can be seen that free radical scavenging activity of cv. Santulhana oils decreased according to the advancement of the harvest time. Samples from the first harvest and from the outside of the olive tree part had the highest scavenging activity (51.8 %). However, the lower inhibition activity within harvest dates occurred in the samples collected from the third collection (21.5 %).

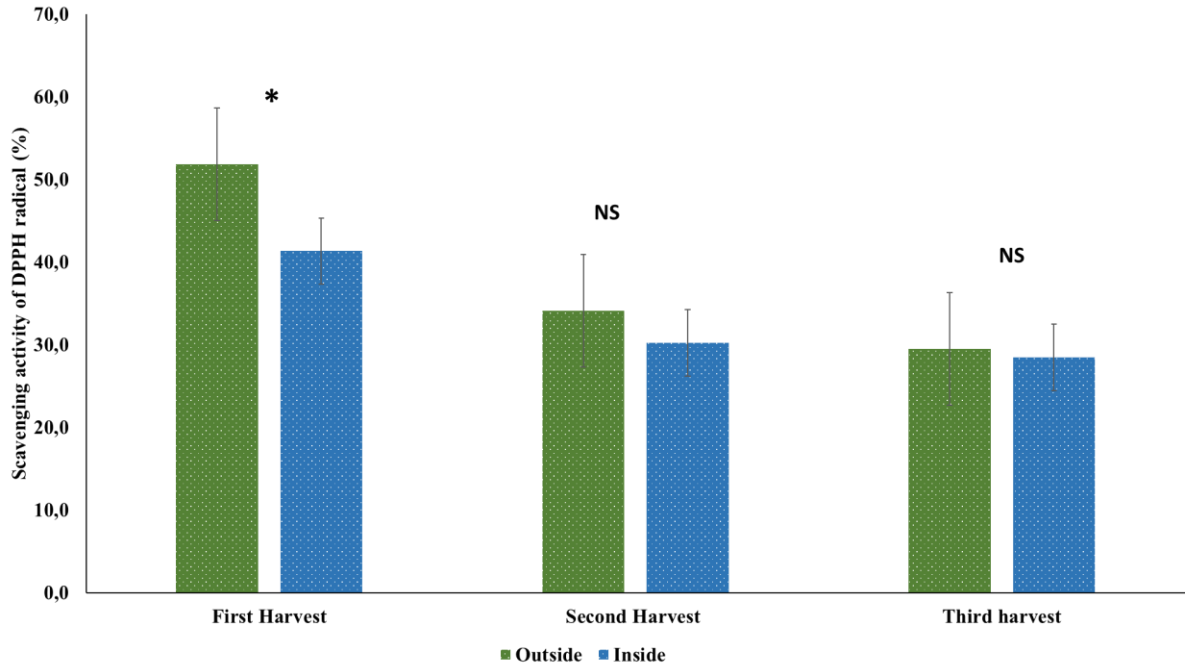


Figure 11. Scavenging activity of olive oil extracts from cv Santulhana collected from the inside and outside part of olive tree at three different harvest times. N.S. no significant differences, * $P \leq 0.05$.

The higher inhibition activity of cv. Santulhana olive oils can be explained by the higher contents of bio compounds in early harvest time. Similar pattern was observed for olive oil obtained from Manzanilla, Barnea and Kadesh cultivar. These cultivars showed lower levels of total phenols and a weaker antioxidant activity in the later ripening periods (el Qarnifa et al., 2019). From the Figure 11, it can be also stated that olive oil antioxidant activity was unaffected by the position.

4.2.3. Olive oil total phenols contents

Total phenolic contents are the main antioxidants in olive oil, which help improve the product's nutritional properties and shelf life. The results of the tested samples are shown in Figure 12, the total phenolic contents of olive oils extracted at different harvest times and from both positions ranged between 136.3 and 308.43 mg gallic acid /Kg olive oil. The amounts of total phenols content were highest at the first harvest. Our results were in line with those reported by Abdallah et al. (2018). Their findings showed that initially, the Tunisian cultivars Chemchali and Fouji displayed total phenols content equal to 461.9 and 432.6 mg gallic acid /Kg olive oil, respectively.

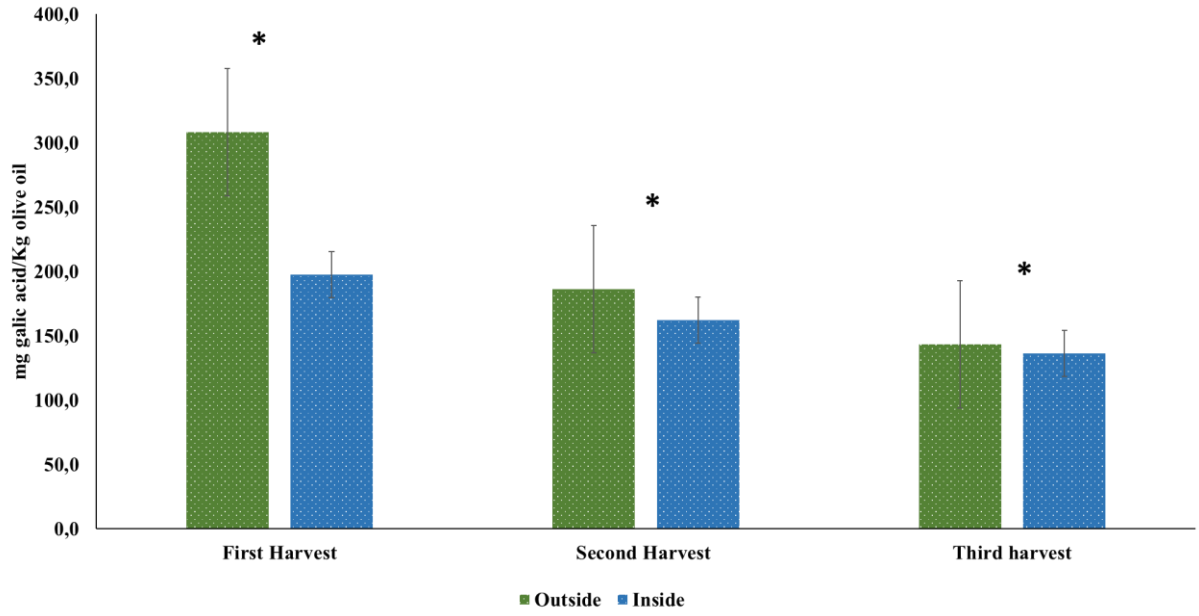


Figure 12. Total phenols content in olive oils extracted from *cv*. Santulhana collected from the inside and outside part of olive tree at three different harvest time. N.S. no significant differences, * P≤0.05.

Upon further ripening, a decrease in polyphenols content was recorded to attain values equal to 234.9 and 219.4 mg gallic acid /Kg olive oil, respectively. According to a previous study, oleuropein is the main polyphenol content in olive fruit. Its concentration is highest in the early stages of development then declines with the progression of ripening, coinciding with the increase in the hydrolytic enzyme's activity (Ferro et al., 2020). In respect to the position, olives located in the outside part produced oil richer in polyphenols in all collection periods. These results were confirmed by Grilo et al. (2021), Grilo et al. (2019), and Gómez-del-Campo

& García (2012), who reported significantly lower values in olive oil from lower layers in Arbequina cultivar.

4.2.4. Tocopherol content

Tocopherols are methylated phenols, the majority of which are characterized by vitamin E activity. Vitamin E exists as at least eight naturally occurring compounds, including α-, β-, δ- and γ-tocopherol and α-, β-, δ- and γ-tocotrienol. They are present in varying amounts in the minor fraction of olive oil (Cayuela & García, 2017). Three forms of tocopherols have been identified using HPLC analysis and designed as α-, β- and γ tocopherols.

As shown in Table 4, the α-tocopherol level decreased throughout ripening progress. Indeed, olive oil samples presented an α-tocopherol content ranging from 269.42 mg/Kg to 303.78 mg/Kg, in olive oils from the third harvest and olive oil samples obtained from the first harvest, , respectively. Besides, the tocopherol content was not influenced by the position factor

Table 4. Tocopherol content quantified (mean ± standard deviation, with ANOVA results) in olive oils extracted from cv. Santulhana variety collected from the interior and exterior part of olive tree at three different harvest times.

Harvest time		α-Tocopherol	β-Tocopherol	γ-Tocopherol	∑ -Tocopherol
		(mg/Kg of olive oil)			
October, 26, 2020	**Exterior**	303.78 ± 16.54	2.24 ± 0.29	14.65 ± 2.67	320.67 ± 17.30
	Interior	298.12 ± 10.40	2.22 ± 0.15	13.85 ± 1.52	314.19 ± 10.90
	p-Value	0.372	0.847	0.421	0.324
November, 22, 2021	**Exterior**	280.61 ± 15.54	1.98 ± 0.18	14.67 ± 2.62	297.26 ± 15.50
	Interior	290.79 ± 726	2.14 ± 0.84	14.72 ± 3.05	307.65 ± 9.80
	p-Value	0.077	0.210	0.969	0.90
December, 14, 2020	**Exterior**	269.42 ± 11.73	1.76 ± 0.13	15.03 ± 1.41	286.21 ± 11.50
	Interior	281.09 ± 16.13	1.86 ± 0.17	15.47 ± 2.53	298.42 ± 13.60
	p-Value	0.081	0.155	0.637	0.070

The registered values can be considered low compared to those found in some previous studies, namely in Tunisia. Faghim et al. (2021) recorded values varied between 335.51 mg/Kg and 415.24 mg/Kg. Nevertheless, olive oil from cv. Santulhana displayed a slightly higher α-

tocopherol concentration than olive oil from cv. Arbequina in Spain, the reported values varied between 92 and 208 mg/Kg (Borges et al., 2017). In what concerns β-tocopherol, values ranged from 1.76 to 2.24 mg/Kg. The highest amount was observed in samples resulting from the first harvest and outside part the canopy; however, the lowest content was recorded in the olive oil samples from the last collection and from which the olive fruits were gathered from the outside part. These findings were consistent with the literature. In an overview of research concerning the composition of Portuguese olive oils and table olives, a range between 1.16 and 3.37 mg/Kg for β-tocopherol was reported for olive oil from Trás-os-Montes region (Albuquerque et al., 2019). In contrast to Dag et al. (2015), who mentioned lower values varying from 1.26 to 1.97 mg/Kg. In this study, γ-tocopherol values were within the range 13.58 and 15.47 mg/Kg. These results are in accordance with Beltrán et al. (2010), who reported an intermediate value of 14 mg/Kg for Lentisca cultivar. Considering the totality of tocopherols, the concentration decreased from 320.67 mg/Kg (first collection) to 286.21 mg/Kg (last collection), expect for the second harvest, a slight increase in the samples from the inside part was noted compared to the third collection 307.65 mg/Kg. These tocopherol concentration decrease can be attributed to many factors, such as cultivar, cultural practices and environmental factors. Besides, tocopherols are considered an essential element of non-enzymatic stress protection (Kafkaletou et al., 2021).

4.2.5. Hydroxytyrosol and tyrosol content

Phenols are characterized by their antioxidant and anti-inflammatory activity. The European Union Regulation (432/2012) permits a health claim on the olive oil label regarding the protective role of polyphenols against oxidative stress only if the oil contains at least 5 mg of hydroxytyrosol and its derivatives (including tyrosol) per 20 g of olive oil. In this context, our experimental results showed that hydroxytyrosol was the principal group among the phenolic compounds recognized with a mean content fluctuating from 236.68 to 298.9 mg/Kg and hence consistent with the standards set (Figure 13). However, tyrosol levels were lower than those fixed by the same standard (250 mg/Kg) since the values varied between 135.31 (second harvest, inside part) and 222.71 mg/Kg (first harvest, outside part). Moreover, as can be inferred from the Figure 13, during the first and second harvest, the olive oil samples from the exterior part are more abundant in hydroxytyrosol, whereas, during the third collection, the samples did not show the same pattern as we noticed a higher concentration of hydroxytyrosol in the samples from the interior part (296.28 mg/Kg of olive oil). Furthermore, the analysis of

variance test used revealed that the statistically significant difference was only detected in the first collection ($P \leq 0.05$).

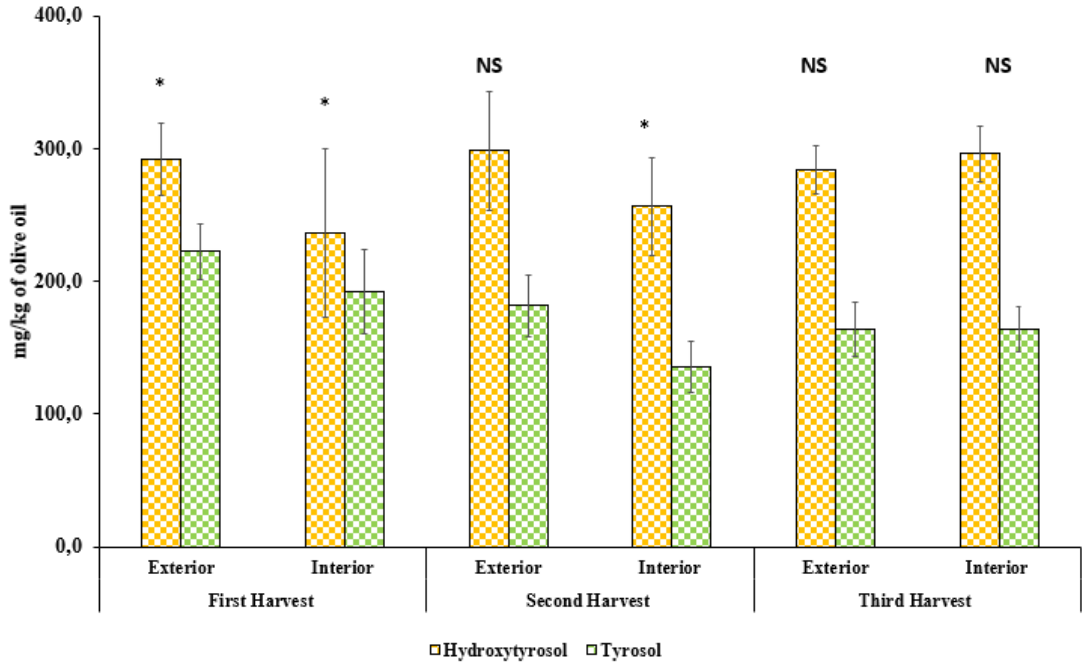


Figure 13. Phenolic compound quantified (mean ± standard deviation) in olive oils extracted from cv. Santulhana collected from the inside and outside part of olive tree at three different harvest time.

Regarding tyrosol, a higher value above 222 mg/Kg was observed in olive oil samples collected during the initial harvest from the olive tree exterior part. Still, no significant statistical difference were detected for the third and second harvest. Our findings were in line with previously published works. Tyrosol and hydroxytyrosol assessment in Portuguese monovarietal olive oils revealed an hydroxytyrosol mean value of about 262.95 mg/Kg for Blanqueta cultivar, similarly, a tyrosol average amount of 229.05 mg/Kg for the Cobrançosa cultivar (Pereira et al., 2020).

4.2.6. Fatty acid composition

Fatty acid profiling of cv. Santulhana olive oil is essential to identify its nutritional value. Indeed, it has been proven that a high percentage of MUFA, especially oleic acid, helps to reduce the risk of atherosclerosis and protects against various types of cancer. Further, the fatty acid composition influences the stability of the oil through the influence of PUFA on the olive oil rancidity (Riachy et al., 2019). Fatty acid profiles of cv. Santulhana olive oil samples are summarized in Table 5. The major fatty acids was oleic acid ($C_{18:1}$, 66.77 - 67.5 %),

succeeded by palmitic acid ($C_{16:0}$, 13.28 - 14.75 %), linoleic acid ($C_{18:2}$, 12.55 - 13.32 %), stearic acid ($C_{18:0}$, 2.94 - 3.19 %). The foregoing works apparently confirm these results. Instead, Rodrigues et al. (2021) studied the effect of olive cultivar and crop year on fatty acid composition from olive oils of Portuguese centenarian trees. It turned out that olive oil from cv. Madural presented oleic acid ($C_{18:1}$) proportions ranged from 68.3 to 72.9 %. Besides, Palmitic acid ($C_{16:0}$), which was similarly the second major fatty acid, its quantities varied between 13.28 and 14.75 % during the study years. About linoleic acid, our findings were very close to those obtained in Abou Kanani cultivar, the variability range was between and 3.34 and 27.12 % (Hernández et al., 2021). In addition, it can be observed from data in table 5 that during the olive fruit development, the oleic acid ($C_{18:1}$) content was raised whereas the palmitic acid ($C_{16:0}$) showed an opposite trend. This concentration change can be explained by the active triacylglycerol biosynthesis that occurs throughout the fruit ripening leading to a decrease in the relative percentage of palmitic acid content of the oil. As expected, the MUFA/PUFA ratio decreased as ripening progressed. Green olives give, indeed, oil higher content in MUFAs than late-harvested ones. As mentioned, the results verified that the fatty acid profile of cv. Santulhana was affected by the canopy olive position since some variations were noticed in a fatty acid set between the two positions. Stearic acid (2.94 %, first harvest) and arachid acid (0.46; first harvest) amounts were higher in olive oil that arose from the interior part. During the third collection, olive fruit position did not induce any significant modification in fatty acid composition. The obtained results partially agree with the outcomes of the fruit canopy position effect and artificial shading on the fatty acid composition of olive oils. As a matter of fact, oleic acid content in Arbequina cultivar exhibited a significant decrease depending on the increase in the photo-synthetically active radiation (PAR) quantities received daily from the different canopy positions. Likewise, the same study demonstrated that olive trees under artificial shade increased stearic and linolenic acid concentrations. Contrary to palmitic, palmitoleic, and linoleic acid proportions, were not influenced by shading (Rousseaux et al., 2020b).

Table 5. Fatty acid composition (%) (mean ± standard deviation, with ANOVA results) of olive oils extracted from cv Santulhana collected from the outside and inside part of olive tree at three harvest times.

Fatty acids	First Harvest			Second Harvest			Third Harvest		
	Exterior	**Interior**	**p-Value**	**Exterior**	**Interior**	**p-Value**	**Exterior**	**Interior**	**p-Value**
$C_{14:0}$	0.02 ± 0.01	0.01 ± 0.00	0.07	0.02 ± 0.00	0.01 ± 0.00	**0.02**	0.01 ± 0.00	0.01 ± 0.01	0.14
$C_{16:0}$	14.75 ± 0.14	14.58 ± 0.18	**0.03**	13.79 ± 0.35	13.77 ± 0.20	0.88	13.28 ± 0.23	13.31 ± 0.29	0.80
$C_{16:1}$	0.89 ± 0.08	0.85 ± 0.04	0.16	0.93 ± 0.12	0.87 ± 0.10	0.26	0.87 ± 0.06	0.87 ± 0.10	0.92
$C_{17:0}$	0.10 ± 0.00	0.10 ± 0.00	1.00	0.10 ± 0.00	0.10 ± 0.00	1.00	0.10 ± 0.00	0.10 ± 0.00	1.00
$C_{17:1}$	0.10 ± 0.00	0.10 ± 0.00	1.00	0.10 ± 0.00	0.10 ± 0.00	1.00	0.10 ± 0.00	0.10 ± 0.00	1.00
$C_{18:0}$	2.94 ± 0.08	3.03 ± 0.05	**0.01**	3.19 ± 0.1	3.18 ± 0.13	0.85	3.04 ± 0.10	3.13 ± 0.17	0.18
$C_{18:1}$	66.77 ± 0.06	66.86 ± 0.46	0.73	67.45 ± 1.33	67.25 ± 0.96	0.70	67.5 ± 0.80	67.4 ± 0.96	0.84
$C_{18:2}$	12.57 ± 0.68	12.55 ± 0.32	0.93	12.60 ± 0.84	12.85 ± 0.05	0.45	13.32 ± 0.48	13.23 ± 0.77	0.76
$C_{18:3}$	0.86 ± 0.05	0.89 ± 0.06	0.23	0.85 ± 0.05	0.90 ± 0.07	0.08	0.87 ± 0.04	0.90 ± 0.00	0.07
$C_{20:0}$	0.45 ± 0.01	0.46 ± 0.01	**0.01**	0.45 ± 0.01	0.46 ± 0.01	0.27	0.43 ± 0.01	0.43 ± 0.01	0.40
$C_{20:1}$	0.28 ± 0.01	0.29 ± 0.01	0.15	0.28 ± 0.01	0.29 ± 0.01	**0.04**	0.28 ± 0.02	0.28 ± 0.01	0.63
$C_{22:0}$	0.15 ± 0.01	0.16 ± 0.01	0.06	0.15 ± 0.01	0.15 ± 0.01	0.71	0.15 ± 0.01	0.15 ± 0.00	0.36
$C_{24:0}$	0.06 ± 0.00	0.06 ± 0.00	0.29	0.05 ± 0.00	0.05 ± 0.00	0.33	0.05 ± 0.01	0.05 ± 0.00	0.58
MUFA	68.06 ± 0.61	68.12 ± 0.43	0.68	68.78 ± 1.23	68.51 ± 0.88	0.58	68.75 ± 0.75	68.67 ± 1.17	0.85
PUFA	13.47 ± 0.68	13.48 ± 035	0.63	13.49 ± 0.84	13.78 ± 0.60	0.38	14.20 ± 0.46	14.16 ± 0.78	0.90
SUFA	18.44 ± 0.15	18.37 ± 0.13	0.95	17.7 ± 0.41	17.69 ± 0.29	0.91	17.02 ± 0.42	17.14 ± 0.37	0.48
MUFA/PUFA ratio	5.07 ± 0.3	5.06 ± 0.16	0.94	5.12 ± 0.39	4.98 ± 0.28	0.38	4.85 ± 0.21	4.87 ± 0.36	0.89

Chapter 5. Conclusion and perspectives

5. Conclusion

Olive oil quality as well as product that are derived from it depends on a combination of factors including the ripening, extraction method, soil type, climatic conditions, harvesting time and varieties. The scope of this work was the evaluation of the effect of the olive position in the olive tree on their quality and the oil extracted from them. The comparative analysis revealed that the factor studied has shown an effect on some olive fruit parameters. It turned out that the color parameters were the only parameters affected by the position factor. However, for the remaining parameters no difference was detected. This work has also examined the position effect on the olive oil quality, its sensorial profile as well as its chemical composition. The findings demonstrated there were significant changes in the samples tested on both sides of the olive tree. Overall, it seems that the outside samples are of superior quality. Indeed, its main characteristics were a low peroxide value, a lower absorbency at 268 nm, a high concentration of total polyphenols on all collection dates, high oxidative deterioration resistance and an important scavenging activity against the DPPH radical. In conclusion, collecting the olives from the outside of the olive tree seems more suitable to produce a high quality EVOO from cv. Santulhana. These results can be attributed to the quantity of light intercepted. Obviously, the olive fruits located outside are the most exposed to the light as opposed to those located inside which are covered by branches and leaves preventing them from receiving sufficient light. The present study could be useful for the horticulturists in helping to organize the olive orchards of point of view light interception and radiation distribution. As a perspective, it would be interesting to complete the investigation by including other parameters namely the photosynthetically active radiation percentage.

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