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Running title: Impact of grape harvester on fruit physiology and oil quality from SHD olive hedgerows

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ABSTRACT

BACKGROUND: Super-intensive cultivation facilitates olive mechanized harvesting, determining a substantial savings in the production cost of virgin olive oil (VOO). However, the number of varieties adapted to this type of cultivation is reduced. This paper explores the impact that harvest with grape straddle harvester of 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' olives, grown in a super-intensive cultivation, has on the physiology of the fruit and the quality of the oil, subsequently extracted.

RESULTS: In both cultivars, the fruits mechanically harvested presented higher respiration and ethylene production and a lower firmness than the ones harvested by hand. Their oils exhibited lower phenol contents, oxidative stability and lower presence of positive sensory attributes. However, in these oils the values of parameters used to assess the level of quality of the VOO were kept within the limits required for the best commercial category.

CONCLUSION: Mechanical harvesting of 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' super-high density hedgerows induced physiological alterations in the fruits and a reduction in the contents of natural antioxidants and flavour components in the oils, although it did not determine a loss of the "Extra" level of quality

Keywords: *Olea europaea* L., SHD orchards, grape straddle harvester, ethylene production, oxidative stability, phenols

INTRODUCTION

Super-high density (SHD) hedgerows, with ca. 1500-2000 trees ha^{-1}, are nowadays the most common designs in new olive (*Olea europaea* L.) orchards for oil production, in particular in non-traditional olive producing countries. Main advantages are early yield after planting and minimization of the costs of harvest by using of adapted grape straddle harvesters which allow a dramatic decrease in the both labour costs and time employed[1]. Furthermore, if the harvest is well synchronized with the extraction process, mechanical harvesting allows producers to minimize the time between harvesting and pressing of olives, which has a very positive impact on oil quality.[2] Cultivars must be of early-bearing, consistent annual yields and also of low-vigour to allow the harvesters to pass over the hedgerows and to ensure the illumination of the canopies in the long-term.[3] These are the reasons by which most olive SHD orchards are cultivated with the cultivar 'Arbequina', and in less extent with 'Arbosana' and 'Koroneiki' cv. New cultivars obtained in breeding programs have also recently been introduced to the market for SHD, such as 'Sikitita', 'Askal', 'Fs-17', 'Oliana', and 'Tosca 07'.[3-5] Although 'Arbequina' olive oil is otherwise highly demanded by international market due to its sensorial quality, it must be extracted at an early maturity stage of fruits and during a short period of time because of the loss of intensity in its positive attributes (fruity odour, bitter taste and green-yellow colour) and of the drastic decrease in phenolic compounds and pigment composition (chlrophylls and carotenoids).[6] This decrease, together with that of the reduced monounsaturated/polyunsaturated fatty acids ratio, affects negatively to stability to oxidation of the 'Arbequina' oil, which in fact it is low compared with other cultivars[7-8]

The adapted grape straddle harvesters use rods to shake the canopy. They contact directly with the fruit so they can cause damage which seems to increase as firmness decreases during maturation.[9] However, studies about the impact of these machines on the oil quality from 'Arbequina' hedgerows orchards are really scarce. It's known that, although the harvester may favour the physical oil extraction, it may decrease the oil stability to oxidation in relation to the oil from fruits manually harvested. This decrease is caused by the reduction of both the tocopherol and phenol contents. Moreover, higher values of peroxides and also of K_{232} are also found in the oils from mechanical harvested fruits, which indicates that mechanical harvesting by the grape straddle harvester led to an increase of the primary steps of the oxidation process.[10] These findings are in part similar to those reported in a 'Souri' traditional orchard (with trees at 10 x 10 m) and mechanically harvested by hand-vibrating combs fruits;[11] in particular, a slight increase in peroxide level and a decrease of the total phenol content was also found in the oils from mechanically harvested trees. An increase of the free acidity content was also reported in these oils.

Therefore, regardless the type of harvester employed in an olive oil orchard, internal fruit injures seems to cause the loss of oil quality from mechanical harvested fruits. The selection of the harvesting method must look for the best possible conservation of the fruit integrity, in order to obtain a VOO of high quality. If a particular method causes bruises on the fruit surface as a result of its mechanical impact or compression, olive respiration and the susceptibility to decay at a faster rate, in comparison to undamaged fruit, seems to increase.[12] The oil extracted from these damaged olives can be high in acidity, low in stability and poor in polyphenols and might develop off-flavors due to the enzymatic activities favored by the breakdown of the cells and the contact between enzymes and substrates that were initially compartmented differently.[13] In the light of these considerations, hand picking appears to be the best method for preventing fruit damage,[14] yet, unfortunately, olive manual harvest is quite expensive, so it is fundamental to assess the impact of mechanical harvest on fruit physiology and consequences on oil physicochemical and sensorial quality applied to the different olive varieties.

The scarcity of suitable olive cultivars is, therefore, an important disadvantage of SHD orchards. Olive breeding is necessary to produce new cultivars adapted to these orchards with hedgerow management. Furthermore, it is necessary the study of the suitability of traditional olive cultivars not only to be grown in SHD orchards but also to produce oil with better quality than the current ones. In this sense, Morales-Sillero *et al*.[15] have recently showed that 'Manzanilla de Sevilla and 'Manzanilla Cacereña' trees are high-yield and may be mechanically harvester by a grape straddle harvester when grown under SHD hedgerows. The work also highlights the efficiency of mechanical harvesting to remove green fruits from both cultivars (98%) and the short time required for harvesting (< 1.7 h). These cultivars are usually cultivated in intensive orchards not only in Spain but also in USA, Israel, Argentina ('Manzanilla de Sevilla') and Portugal ('Manzanilla Cacereña'), and the productions are mainly destined for table olive. The oil contents are not usually high, but they are quite appreciated by their stability against oxidation, their green colour and their elevated content on the more characteristic sensory attributes of the VOO, such as fruity, bitter or pungent.[16-17] In fact, a substantial part of the production (10 – 15 %), mainly corresponding to the fruit with inadequate appearance for table market, routinely goes to olive oil extraction industries, where the oil is habitually used for blending with other VOOs to improve its sensory quality. In a continuation of this work, the main aim of this study is to evaluate how the grape straddle harvester affect the physiology of fruits of both cultivars ('Manzanilla de

Sevilla' and 'Manzanilla Cacereña') and the impact on the oil physicochemical characteristics and sensory attributes. Results will also contribute to determine to what extent the impact depends on the cultivar.

EXPERIMENTAL

Orchard description

The field experiment was made in 2012, in a commercial orchard of Elvas (Portugal) (latitude 38,56'N; longitude 7º02'O; altitude 201 m) with five-year-old 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' (*Olea europaea* L.) hedgerows at a spacing of 3.75 m x 1.35 m (1,975 trees ha^{-1}) in a north-south orientation. The area is characterized by a Mediterranean climate, with an average annual rainfall of 516.4 mm and ET_o of 1296.5 mm. The soil is a loam with effective depth of 0.6 m. Four random rows of trees (ca. 90) per cultivar ('Manzanilla de Sevilla' and 'Manzanilla Cacereña') and per harvesting treatments (Hand, in which fruit were picked up by hand; and Mechanical, in which trees were harvested with a grape straddle harvester), were selected for the trial.

Trees were irrigated from March to October with 280 mm ha^{-1} with a lateral per tree row with two 2.43 L h^{-1} compensating drippers per tree, 0.60 m apart. Fertilization was made according to foliar analyses to non-limiting nutrient conditions. Weeds were controlled by non-residual herbicides in the tree rows and natural ground cover between them. The branches growing towards the centre of the rows were removed in winter. Three months before the experiment, 'Manzanilla de Sevilla' hedgerows were 2.8 m tall and 2.0 m wide and those of 'Manzanilla Cacereña' were 2.4 m and 1.5 m, respectively. Fruit yields were ca. 10,000 kg ha^{-1} for 'Manzanilla de Sevilla' cv. and ca. 18,000 kg ha^{-1} for 'Manzanilla Cacereña' cv.[15]

Fruit harvesting and oil extraction

Harvesting was made in December 10th when fruits had a ripening index between 3 and 4.[18] Fruit samples of 5 kg by experimental unit, each one of the four random rows, were handpicked around the canopy of 25 trees randomly selected in the manual treatment. Later, the rest of the fruits of the same replicate were mechanical harvested with a model VX 7090 (Olive, CNH Global, Belgium) and samples of 5 kg by experimental unit were also randomly taken from the total amount. The fruits were transported the same day to the Instituto de la Grasa in Seville, stored at 5 ºC during the night and analysed early in the morning.

Fruit characteristics: Colour, firmness, respiration rate and ethylene production

Fruit skin colour was determined on the equatorial zone of 50 healthy fruits per experimental unit using a Minolta CR200 (Minolta Camera Co., Osaka, Japan) chromameter with a measuring area of 8 mm in diameter, diffuse illumination and a viewing angle of 0º. The International Commission on Illumination colour notation system (ICI L*a*b*) was applied to determine the parameters L*, a*, and b*; with L* as the lightness, a* the colour axis from green to red, and b* the colour axis from blue to yellow. The following equation (colour index, CI), previously used to monitor colour changes during olive cold storage,[19] was applied:

$$CI = L^* \times (b^* - a^*) \times 10^{-2}$$

Fruit firmness was also evaluated on the equatorial zone of the same fruits, using a Zwick 3300 hand densimeter (Zwick GmbH & Co., Ulm, Germany). The consistency of each fruit was measured without rupture by the pressure in a 5 mm diameter disk. Results were expressed in N.

Fruit samples of 500 g by replicate were placed into 2.0 L glass jar and stored at 2 ºC for 24 hours. These jars were them hermetically sealed during 3 h. Later, a G100 portable gas analyser (Geotechnical Instrument Ltd. Leamington Spa, UK) was used to measure the CO_2 content of the head space, to estimate the respiration rate of the fruit. The ethylene content was simultaneously evaluated, with an ICA portable ethylene analyser (International Controlled Atmosphere Ltd., Paddock Wood, UK). Respiration rate values were expressed as mL CO_2 L^{-1} kg^{-1} h^{-1} and ethylene production mean values were expressed as µL Ethylene L^{-1} kg^{-1} h^{-1}.

Physical and chemical extraction of olive oil: VOO yield, humidity, total oil yield and physical extractability

One kilogram of olives was randomly taken from each experimental unit for oil extraction and analysis. The oil was extracted with the Abencor analyzer (Comercial Abengoa S.A., Seville, Spain), which simulates the industrial process of VOO yield at laboratory scale[20]. Each sample was milled separately. From the resulting paste in each sample, 800 g were collected in a metallic pitcher, homogenized with a spatula and the oil was physically extracted. After centrifugation, the oil was decanted into a graduated tube to measure the volume, and the VOO yield so determined was expressed as percentage of fresh weight, considering oil density at ambient temperature of 0.916 kg L^{-1}. The extracted oil was then paper filtered and stored at -20 °C under N_2 atmosphere until analysis. Samples of 50 g, taken from the leftover paste obtained for the physical extraction of the oil, were dried at 105 ºC up to constant weight to evaluate the humidity and the dry weight of the olive fruits. The oil of the dried pastes were chemically extracted with hexane, using the Soxhlet method,[21] to evaluate the total oil yield of the paste as the percentage referred to the fresh weight. The physical extractability was calculated as the percentage of total oil yield that represents the physically extracted VOO yield.

Oil analysis

In each oil sample, free acidity, peroxide index value, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) and the overall grading of the sensory quality of the oils were evaluated according to the European Union Standard Methods.[22] The sensory intensities of the negative attributes (rancid, fusty, winey, musty, etc.), of the bitterness and of the pungency were graded by a panel of eight trained tasters using a line scale, and the results converted into a numerical score by measuring the position of the placed mark along a 10 cm line. This allowed calculating averages among taster sensory scores. The overall sensory quality of each oil sample was graded by the same panel according to a structured scale of nine points, "1" being the value for the poorest quality possible and "9" for the best, being "6.5" the lowest limit value for the best level of sensory quality (named "Extra") for VOOs.

Oxidative stability was measured by using the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g oil samples exposed to streams of dry air (20 L h^{-1}) heated to 100 °C. [23]

The contents of chlorophyll and carotenoid pigments in the oils were evaluated by their absorbances at 470 and 670 nm, respectively, and the results were expressed as mg kg^{-1}. [24]

Tocopherol composition was determined by HPLC using the IUPAC method (1992).[25]

The phenolic fraction was isolated by solid-phase extraction and analyzed by reversed-phase high performance liquid chromatography (HPLC) using a diode-array UV detector.[26] The quantification of phenolic compounds (except flavones and ferulic acid) was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, while flavones (luteoline and apigenine) and ferulic acid were quantified at 335 nm using *o*-coumaric acid as an internal standard. The results were expressed in mg kg^{-1}. Fatty acid composition was determined by gas chromatographic analysis of methyl esters for each oil sample.[27] This was performed on an Agilent 6890 equipped with a flame ionization detector, fitted with a fused-silica capillary column (SP-2380, 60 m/0.25 mm I.D.) coated with cyanopropylsilicone (0.20 mm film thickness). Hydrogen was employed as carrier gas at flow of 1mL min^{-1}. The oven temperature was maintained at 185 ºC and the injector (split 1:20) and detector at 225 ºC. The results were expressed in g kg^{-1}.

Statistical analyses

Analyses were performed using StatGraphics Plus 5.1 (StatGraphics Software, Inc., La Jolla, California, USA). Data were subjected to statistical analysis using variance analysis to determine the effect of treatments and cultivars. When necessary, to achieve normality and homogenize the variance, data were previously transformed using the arcsine of the square root or Box-Cox power transformations.[28]

RESULTS AND DISCUSSION

Fruit characteristics: Colour, firmness, respiration rate and ethylene production

Chromatic parameters L*, a* and b* of the skin of olive fruits were not significantly affected by the method of harvest (Table 1). Regardless the cultivar tested, no significant effects on CI values were detected in the fruits as a consequence of the different system of harvesting, which agrees with results of Yousfi *et al.*[10] who found no effect due to this factor on CI values of 'Arbequina' olives skin from a hedgerow orchard.

Fruit firmness was clearly affected by mechanical harvesting (Table 1). Manually harvested fruits exhibited significantly higher values of this parameter whatever the cultivar, as it was previously found by Morales-Sillero *et al*.[15] in the same experimental orchard with green fruits. In contrast, Yousfi *et al*.[10] did not find this effect on 'Arbequina' olives. It seems that the cellular tissues of both 'Manzanilla' olives are more sensitive than those of 'Arbequina' to the harvester's mechanical action, which would induce a deterioration of their cell walls.

Mechanical harvesting also induced in the fruit of both cultivars a significant increase of the respiration rate and, specially, of the ethylene production (Table 1). Moreover, this increase of the ethylene was greater in 'Manzanilla de Sevilla' fruits than in those of 'Manzanilla Cacereña'. Segovia-Bravo *et al*.[12] had already found that simulating the mechanical harvesting of 'Manzanilla de Sevilla' fruits, those which are bruised show higher respiratory activity than the unbruised ones. This suggests that the fruit has undergone further physical damage with mechanical harvesting than with manual harvesting. Olive is usually classified as a non-climacteric fruit, therefore it shows no drastic increases in their CO_2 and ethylene production rates during ripening. In contrast, climacteric fruits, since a determined moment of the ripening process (climacteric point) produce increased levels of ethylene, which is accompanied by a respiration peak.[29] At this time, all the physiological mechanisms associated to fruit maturation and senescence such as: colour changes, softening, chlorophyll degradation, phenol oxidation and aroma development are accelerated in the fruits.[30] However, ethylene production may also be a response of a plant to injury.[31-32] Thus, the stimulation of ethylene production found in the mechanical harvested fruits of the studied cultivars seems to be a response to the internal injury caused to the fruits by the grape straddle harvester. Moreover, this increase of ethylene production could have favoured that of the respiration rate. A similar emission of ethylene was reported by Yousfi *et al.*[13] in 'Arbequina' fruits harvested with a similar machine. Results here shown therefore support that mechanical harvesting causes internal damage in olive fruits, as Dag *et al.*[11] and Yousfi *et al.*[10, 13] previously suggested.

VOO yield, total oil yield, humidity and physical extractability

VOO yield was significantly higher in 'Manzanila de Sevilla' than in 'Manzanilla Cacereña', but regardless of the cultivar, no differences in the physical oil extraction were found in the mechanically harvested fruits when compared to the manually harvested (Table 1). These results do not agree with those of Yousfi *et al.*[10] which found a higher virgin oil yield in 'Arbequina' mechanically harvested fruits by a grape straddle harvester when compared to the handpicked ones.

Since no difference between both harvesting systems was found in the total oil yield, which is chemically extracted, the physical extractability was also similar. In the same way, no effect due to the different system of harvesting was detected in the fruit humidity. Nevertheless, 'Manzanilla Cacereña' olives showed higher humidity values, than 'Manzanilla Sevillana' fruits, although only those mechanically harvested reached a statistical significant difference. This higher humidity could explain the significant lower VOO yield and physical extractability values that presented this variety ('Manzanilla Cacereña'), because the presence of water hinders the VOO physical extraction.[32] The VOO yields did not reach high values, in agreement with previous results related to both cultivars, although contents up to 20% have been also indicated, in particular for the 'Manzanilla de Sevilla' cv.[34-36]. The low extractability of

'Manzanilla Cacereña', either by an 'Abencor' system or on an industrial scale, has also been recently reported, and it may be even lower as fruit ripening increase.[35] However, the high fruit yields of the 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' trees cultivated in the SHD hedgerows compensated at least in part the low VOO yield.

Oil quality

All the extracted oils were classified as "Extra", the best commercial category of olive oil, given the mean values of free acidity, peroxide index, $K_{232,}$ K_{270} and panel test (Table 2). However, the mechanical treatment increased significantly the free acidity in the 'Manzanilla de Sevilla' oils, from 0.3 to 0.7%, thus close to the limit (0.8%) established by the EU-legislated standards for this category. Previously, Dag *et al.*[11] found that free acidity increased when 'Souri' fruits from trees under different irrigation conditions (from non-irrigated to irrigated with 125% ET_0) were harvested by hand-vibrating combs, compared to those manually harvested. Although this effect was observed regardless the irrigation regime of the trees, those fruits from highly irrigated trees reached the highest values of free acidity (0.8). In contrast, Yousfi *et al.*[10] did not find differences on the free acidity of the oils extracted from 'Arbequina' fruit manually and mechanically harvested by a grape straddle harvester. Probably, this induction of the free acidity increase is related with the activation of the internal olive lipase, which would act during the process of virgin olive oil extraction. A similar increase of free acidity values of the virgin oil is observed coinciding with the progress of fruit ripening.[37] Taking in account that ethylene is the most efficient inductor of the maturity-related enzymatic activities, it seems reasonable to think that this phenomenon, the increase of free acidity in the virgin oil, is linked with the increase of ethylene biosynthesis.

The parameters used to assess the level of oxidative deterioration in the VOO (peroxide value, K_{232} and K_{270}) were not affected by the harvesting system (Table 2). These results do not agree with the ones obtained by Dag *et al.*[11], using 'Souri' olives and Yousfi *et al.*[10] with 'Arbequina' olives, who observed that oil extracted from mechanically harvested olives presented significantly higher peroxide values. Moreover, Yousfi *et al.*[13] also found significantly higher K_{232} values in these oils. Probably, these differences were due to that the internal damage suffered by these cultivars determined a more closed contact of the oil contained in the fruit with the atmospheric oxygen.

The oil extracted from olives mechanically harvested of both cultivars showed systematically lower intensity of positive sensory attributes such as olive fruity or bitter, exhibiting the oils from 'Manzanilla Cacereña' of this treatment, also significantly lower intensity of pungent (Table 2). In consequence, the overall grading of the sensory quality evaluated by panel test found that oil extracted from manually harvested olives presented a significant higher level of sensory quality. In contrast, Yousfi *et al.*[10] found no significant differences between the panel test values of the oils extracted from 'Arbequina' olives manually or mechanically harvested. This suggests that the sensory quality of the oils contained in the cells of 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' olives is more sensitive to the internal damage caused by the mechanical harvesting than 'Arbequina' oils. Enzymatic activities such as lipoxygenase, hydroperoxide lyase, alcohol dehydrogenase or alcohol acyltransferase, related with the release of the positive sensory attributes of the VOO, and/or other enzymes responsible for the phenolic content of the VOO, such as glucosidases and phenoloxidases should be implied in this fact. Probably, the higher production of ethylene in the mechanically harvested fruits favored the inhibition of these activities. The progress of fruit maturation coincides with the reduction of these positive attributes.[37]

Mean values of oxidative stability were ≥ 85 h. (Table 3). They are high if compared to 'Arbequina' oils, the most grown cultivar under SHD conditions worldwide, for which it is usually lower than 40 h.[5, 10, 38] The stability to oxidation, i.e the resistance to rancidity, decreased significantly in the oils from mechanically harvested fruits both in 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' oils. Yousfi *et al*.[10] found that the oxidative stability decreased significantly by 9 h in the 'Arbequina' oils from mechanically harvested fruits as compared to the manually harvested ones. In our study it decreased by 13 h in the 'Manzanilla Cacereña oils', and up to 25 h in 'Manzanilla de Sevilla' ones. These decreases were probably related to the changes in the phenol content in both cultivars (Table 4). Moreover, the tocopherols contents in 'Manzanilla de Sevilla' (Table 3) could have probably affected the oxidative stability, given the antioxidant capacity of these compounds.[39] In fact, the tocopherols α, β and total decreased by more than 50% in the 'Manzanilla de Sevilla' oils from the mechanically harvested fruits, while no differences between treatments were found for this parameter in the 'Manzanilla Cacereña' oils, which otherwise had only about one third of the total tocopherols as compared to 'Manzanilla de Sevilla'. No differences in tocopherols contents were found by Yousfi *et al*.[10] in oils from 'Arbequina' olives manually or mechanically harvested, which exhibited a similar amount of total tocopherol content than the oils of 'Manzanilla de Sevilla' from manually harvested olives in our experiment.

Chlorophylls and carotenoids are the main responsible for the colour of VOO,[40] and carotenoids display an antioxidant action.[38] The mechanical harvest did not affect the chlorophylls content in any of both cultivars (Table 3). In contrast, although no differences were found on the carotenoids content in the 'Manzanilla de Sevilla' oils, the 'Manzanilla Cacereña' oils extracted from mechanically harvested fruits showed higher values of them than the ones from manually harvested olives. Yousfi *et al*.[10] previously found no effect due to the different harvesting system on these pigment contents of ‘Arbequina’ oils. The pressure and the beating suffered by the fruit during mechanized harvesting can facilitate an easier breaking of chloroplasts during the subsequent fruit grinding and olive paste kneading of the virgin olive oil extraction, favoring the release of a greater amount of chlorophylls and carotenoids. However, the greater presence of ethylene, which was synthesized as response to this mechanical damage, could stimulate enzymatic activities such as chlorophyllase or lipoxygenase that lead to the oxidation of these pigments.[41]

The total phenol content differed clearly between cultivars (Table 4). Thus, the ‘Manzanilla de Sevilla’ oils from the manual treatment had more than double than those of ‘Manzanilla Cacereña’(>539 and 259 mg kg^{-1}, respectively). The high antioxidant capacity of the ‘Manzanilla de Sevilla’ in relation to the phenol content oils has been previously indicated. Several works show even more than 1000 mg kg^{-1}of total phenols.[36, 42] The mechanical harvest decreased the mean values of total phenol content, total orthodiphenols and, in particular, those of the total secoiridoids, the most abundant phenolic compounds in VOO, which are related with the bitter and pungent attributes, as well as the stability of VOO against oxidation.[43-44] In both cultivars, differences were due to the lower contents of the dialdehydic form of the decarboxymethyl oleuropeine aglycone (3,4-DHPEA-EDA), and of the hydroxytyrosyl elenolate (3,4-DHPEA-EA) in the oils from the mechanically harvested fruits. Both compounds simultaneously belong to the phenolic groups of orthodiphenols and of secoiridoid derivatives. The lignans (pinoresinol and acetoxypinoresinol) were also significantly lower in the oils of ‘Manzanilla de Sevilla’ olives mechacnically harvested, while in the similar ‘Manzanilla Cacereña’ oils the pinoresinol, the *p*-HPEA-EDA, and the vanillic acid, achieved a significantly lower content. Nevertheless, the contents of hydroxytyrosol and tyrosol in 'Manzanilla de Sevilla', and those of hydroxytyrosolacetate in 'Manzanilla Cacereña', were higher in the oils from mechanically harvested fruits. Our results confirm those of Dag *et al.*[11] and Yousfy *et al.*[10], therefore the idea that mechanically harvesting affects negatively the phenol content regardless the cultivar and type of harvester. However, when fruits are harvested by grape straddle harvesters, the response of phenol composition differs between cultivars. Segovia-Bravo *et al.*[12, 45] found local tissue degradation together with an output of intracellular water and the oxidation of phenolic compounds in 'Manzanilla de Sevilla' green fruits, when simulated the mechanical harvesting.

The main phenolic compounds found in the olive fruit are oleuropein, ligstroside and demethyloleuropein. They are glycosides that do not dissolve in the oil. The presence of phenolic derivatives in VOO depends mainly on the action of enzyme activities, such as glycosidases and estearases that transform these compounds in other oil soluble molecules. Thus, the secoiridoid derivatives resulting from the enzymatic hydrolysis of these glycosides identified as 3,4-DHPEA-EDA, the dialdehydic form of decarboxymethyl ligstroside aglycone (*p*-HPEA-EDA), the 3,4-DHPEA-EA and the aldehydic form ligstroside aglycone (*p*-HPEA-EA), are usually the most abundant phenolic components found in most VOO.[46-47] The differences found in this study on phenolic content due to the different system of harvesting only can be explained by a partial inhibition of these activities induced by the mechanical harvesting. As the phenolic content of the VOO decreases during fruit maturation,[37] probably the increase of ethylene biosynthesis induced by the grape straddle harvester in the olive cells is also related with this fact.

Fatty acid composition was not significantly altered by the mechanization of the harvest with a grape straddle harvester. In consequence, no differences in the monounsaturated/polyunsaturated (MUFA/PUFA) and saturated/unsaturated (SFA/UFA) ratios were found (data not shown). These results confirmed the ones previously obtained by Yousfi *et al.*[10] testing 'Arbequina' olives, who found no effect on fatty acid composition due to the harvesting system. Greater contents of different fatty acids were found in 'Manzanilla de Sevilla' than in 'Manzanilla Cacereña'. In any case, it is noticeable the high contents in oleic acid in the oils from both cultivars when they are cultivated under superintensive conditions. These contents are considerably higher than those normally found in the oils extracted from 'Arbequina' olives (600-700 g kg^{-1}).[48] The elevated presence of oleic acid contributes to the high stability of the oils and is also a dietary, nutritional and therapeutic improvement.[49]

The current work demonstrated that the use of a grape straddle harvester in 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' SHD hedgerow orchards induced relevant changes in physiological parameters of fruits, such as decreases of firmness and clear increases of the respiration rate and ethylene emission. Probably as response to these physiological alterations of the fruit, the VOO extracted suffered a clear deterioration of its quality characteristics. Thus, the stability against oxidation and the overall rating of the sensory quality significantly decreased, likely due to a reduction of the phenol contents. Furthermore, the oils extracted from mechanically harvested 'Manzanilla de Sevilla' olives showed a significantly higher titratable acidity and a lower tocopherol content. Despite these disadvantages, the parameters of quality of the oils from mechanical harvesting were kept within the established limits for the best commercial category of quality. In consequence, under a commercial point of view, exclusively considering the quality level of the oil extracted, the mechanical harvesting of these olives grown in hedgerow would be feasible. Furthermore, taking into account the sensorial quality and the oxidative stability of these oils, particularly interesting from the commercial point of view would be the mixture of these 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' VOOs with those of the 'Arbequina', their combination would result in a "coupage" with better balanced flavour and stability. Bearing in mind that these results are preliminary, the complementarity of the VOOs of these cultivars seems to suggest the advisability of including some hedgerows of these cultivars in the SHD 'Arbequina' orchards, to improve the sensory quality, nutritional value and the stability of these oils through the processing of all these olives together.

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Table 1. Physiological and physical variables in fruits from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' cultivars grown in SHD hedgerow orchards, and harvested by hand or mechanically. Each value is the mean ± SD of four replicates.

Variables/Harvesting system	'Manzanilla de Sevilla' Hand	'Manzanilla de Sevilla' Mechanical	'Manzanilla Cacereña' Hand	'Manzanilla Cacereña' Mechanical	Interaction[a]
L*	45.9 ± 14.0	30.9 ± 9.8	40.0 ± 15.8	37.0 ± 11.2	Ns
a*	3.0 ± 14.4	8.0 ± 8.0	13.2 ± 12.7	11.6 ± 8.8	Ns
b*	23.4 ± 16.0	9. 3± 11.6	14.1 ± 14.6	15.4 ± 11.0	Ns
Color index [L*(b*-a*)/100]	13.3 ± 17.7	1.9 ± 9.3	3.8 ± 14.8	3.2 ± 9.1	Ns
Firmness (N)	32 ± 4b	24 ± 8a	28 ± 4b	22 ± 5a	Ns
Respiration rate (mL CO_2 kg^{-1} h^{-1})	3.9 ± 0.0aA[b]	4.9 ± 0.0b	6.4 ± 0.1aB	7.9 ± 0.5b	Ns
Ethylene production (µL $kg^{-1}L^{-1}h^{-1}$)	2.1 ± 0.2aA	12.0 ± 1.5bB	5.4 ± 0.1aB	13.3 ± 0.2bB	Ns
Virgin oil yield (g kg^{-1})	88.2 ± 4.1B	79.3 ± 11.4B	45.2 ± 8.1A	54.0 ± 8.2A	*
Humidity (g kg^{-1})	645.4 ± 21.2	660.8 ± 12.1A	676.2 ± 34.1	695.2 ±13.9B	Ns
Total oil yield (g kg^{-1})	119.2 ± 17.7	97.8 ± 6.3A	114.5 ± 3.3	126.0 ± 8.5B	**
Extractability (g kg^{-1})	754.1 ± 98.1B	803.4 ± 81.7B	393.8 ± 67.0A	428.7 ± 70.8A	Ns

[a] Cultivar × harvesting system

[b] Mean values in the same row followed by different lowercase letters indicate significant differences in harvesting system for each cultivar at $P \leq 0.05$. Mean values in the same row followed by different capital letters indicate significant differences in cultivars for each harvesting system at $P \leq 0.05$. No letter means non significant effect.

Ns and *, ** indicate non-significant and significant at $P \leq 0.05$ and 0.01, respectively

Table 2. Legally established parameters to evaluate the level of quality of virgin olive oils extracted from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' cultivars grown in SHD hedgerow orchards, and harvested by hand or mechanically. Each value is the mean ± SD of four replicates.

Variables/Harvesting system	'Manzanilla de Sevilla' Hand	'Manzanilla de Sevilla' Mechanical	'Manzanilla Cacereña' Hand	'Manzanilla Cacereña' Mechanical	Interaction[a]
Acidity (% Oleic acid)	0.3 ± 0.1a[b]	0.7 ± 0.1bB	0.3 ± 0.0	0.3 ± 0.0A	***
Peroxide value (meq O_2 kg^{-1})	3.6 ± 0.6	3.6 ± 0.4	4.4 ± 0.6	4.8 ± 0.9	Ns
K_{232}	1.63 ± 0.24	1.47 ± 0.18	1.48 ± 0.02	1.45 ± 0.10	Ns
K_{270}	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10± 0.01	Ns
Sensory attributes					
Fruity	4.6 ± 0.7bB	3.6 ± 0.8a	3.7 ± 0.6bA	2.6 ± 0.8a	Ns
Bitter	3.1 ± 0.6b	2.3 ± 0.8a	2.6 ± 0.9b	1.6 ± 0.4a	Ns
Pungent	3.2 ± 0.7	2.9 ± 0.7B	2.7 ± 0.2b	1.6 ± 0.5aA	*
Overall grading (1, worst- 9, best)	7.8 ± 0.4b	7.4 ± 0.2a	7.4 ± 0.4b	6.9 ± 0.3a	Ns

[a] Cultivar × harvesting system

[b]Mean values in the same row followed by different lowercase letters indicate significant differences in treatments for each cultivar at $P \leq 0.05$. Mean values in the same row followed by different capital letters indicate significant differences in cultivars for each treatment at $P \leq 0.05$. No letter means non significant effect.

Ns and * and *** indicate non-significant and significant at $P \leq 0.05$ and 0.001, respectively

Table 3. Oxidative stability, tocopherol composition and photosynthetic pigments content of virgin olive oils extracted from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' cultivars grown in SHD hedgerow orchards, and harvested by hand or mechanically. Each value is the mean ± SD of four replicates.

	'Manzanilla de Sevilla'		'Manzanilla Cacereña'		Interaction[a]
	Hand	Mechanical	Hand	Mechanical	
Oxidative stability (h)	113.5 ± 7.2bB[b]	89.0 ± 10.5a	98.6 ± 4.5bA	85.2 ± 4.7a	Ns
α-Tocopherol (mg kg^{-1})	405.1±98.5bB	170.0 ± 59.3a	144.2 ± 47.3A	171.4 ± 22.4	**
β-Tocopherol (mg kg^{-1})	41.8 ± 9.6bB	8.8 ± 9.9a	ND	ND	**
γ-Tocopherol (mg kg^{-1})	ND	ND	ND	ND	Ns
Total tocoferols (mg kg^{-1})	446.9 ± 107.0bB	178.8 ± 68.9a	144.2 ± 47.3A	171.4 ± 22.4	**
Chlorophylls (mg kg^{-1})	7.8±1.7	7.6±1.5	6.9±1.8	7.5±1.9	Ns
Carotenoids (mg kg^{-1})	6.0±1.1	5.4±0.6A	5.7±0.4a	7.3±1.2bB	*

[a] Cultivar × harvesting system

[b]Mean values in the same row followed by different lowercase letters indicate significant differences in treatments for each cultivar at $P \leq 0.05$. Mean values in the same row followed by different capital letters indicate significant differences in cultivars for each treatment at $P \leq 0.05$. No letter means non significant effect.

ND, Ns and *, ** indicate non detected, non-significant and significant at $P \leq 0.05$ and 0.01, respectively

Table 4. Phenolic composition ($mg\ kg^{-1}$) of virgin olive oils extracted from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' cultivars grown in SHD hedgerow orchards, and harvested by hand or mechanically. Each value is the mean ± SD of four replicates.

	'Manzanilla de Sevilla'		'Manzanilla Cacereña'		Interaction[a]
	Hand	Mechanical	Hand	Mechanical	
Hydroxytirosol	5.7 ± 2.2aB[x]	11.2 ± 3.7b	1.5 ± 0.2A	4.7 ± 5.3	Ns
Tyrosol	9.0 ± 1.6a	13.7 ± 2.9bB	8.1 ± 0.4	8.3 ± 0.9A	*
Vanillic acid	0.6 ± 0.0B	0.6 ± 0.1B	0.5 ± 0.0bA	0.3 ± 0.0aA	*
Vanilline	ND	ND	0.1 ± 0.0	0.1 ± 0.1	Ns
p-Coumaric acid	1.7 ± 0.3B	1.8 ± 0.5B	0.3 ± 0.0A	0.3 ± 0.0A	Ns
hydroxytirosol acetate	5.0 ± 2.1	7.3 ± 0.6B	2.8 ± 0.4a	3.5 ± 0.4bA	Ns
3, 4-DHPEA-EDA[c]	107.7 ± 25.2bB	48.5 ± 27.1a	45.1 ± 3.7bA	31.6 ± 5.9a	Ns
Tirosol acetate	ND	ND	ND	ND	Ns
p-HPEA-EDA[d]	65.9 ± 28.6	47.6 ± 8.1B	41.5 ± 4.1b	27.4 ± 3.3aA	Ns
Pinoresinol	2.7 ± 0.6bA	0.8 ± 0.9aA	3.7 ± 0.1bB	3.0 ± 0.5aB	Ns
Cinamic acid	ND	ND	ND	ND	Ns
Acetoxypinoresinol	25.5 ± 2.9b	12.5 ± 7.4a	9.6 ± 1.7	5.6 ± 3.0	Ns
3, 4-DHPEA-EA[e]	219.0 ± 57.3bB	130.9 ± 42.2a	107.1 ± 9.8bA	75.7 ± 13.7a	Ns
p-HPEA-EA[f]	21.2 ± 8.6	17.2 ± 2.4	18.4 ± 0.9	19.4 ± 0.5	Ns
Ferulic acid	70.1 ± 15.0B	86.7 ± 9.2B	10.6 ± 1.4A	10.9 ± 2.6A	Ns
Luteoline	3.2 ± 0.5A	3.5 ± 0.4A	6.3 ± 0.4B	6.3 ± 1.0B	Ns
Apigenine	1.8 ± 0.2A	1.6 ± 0.3A	3.0 ± 0.1B	3.3 ± 0.4B	Ns
Σ Phenols	539.1 ± 86.7bB	383.9 ± 79.5aB	258.6 ± 16.2bA	200.4 ± 36.0aA	Ns
Σ*O*-diphenols	340.6 ± 66.1bB	201.4 ± 66.7aB	162.8 ± 13.1bA	121.8 ± 13.8aA	*
ΣSecoiridoid derivatives	413.8 ± 93.5bB	244.2 ± 75.6aB	212.1 ± 14.5bA	154.1 ± 30.1aA	Ns

[a] Cultivar × harvesting system
[b]Mean values in the same row followed by different lowercase letters indicate significant differences in treatments for each cultivar at $P \leq 0.05$. Mean values in the same row followed by different capital letters indicate significant differences in cultivars for each treatment at $P \leq 0.05$. No letter means non significant effect.
ND, Ns and * indicate non detected, non-significant and significant at $P \leq 0.05$, respectively
[c]Dialdehydic form of the decarboxymethyl oleuropeinaglycone.
[d]Dialdehydic form of the decarboxymethyl ligstrosideaglycone.
[e]Hydroxytyrosylelenolate.
[f]Tyrosylelenolate.

Table 5. Fatty acid composition (g kg^{-1}) of virgin olive oils extracted from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' cultivars grown in SHD hedgerow orchards, and harvested by hand or mechanically. Each value is the mean ± SD of four replicates.

Fatty acid[a]	'Manzanilla de Sevilla'		'Manzanilla Cacereña'		Interaction[a]
	Hand	Mechanical	Hand	Mechanical	
Palmític (16:0)[c]	140.1 ± 2.2B[b]	139.0 ± 1.1B	129.3 ± 2.4A	127.3 ± 0.1A	Ns
Palmitoleic (16:1)	13.2 ± 0.1	14.4 ± 0.2B	12.0 ± 0.0	11.2 ± 0.0A	Ns
Estearic (18:0)	28.1 ± 0.2B	30.2 ± 1.1B	15.4 ± 0.3A	15.5 ± 1.1A	Ns
Oleic (18:1)	749.3 ± 6.3A	746.2 ± 3.3A	784.2 ± 1.1B	786.2 ± 3.3B	Ns
Linoleic (18:2)	55.2 ± 4.2B	56.0 ± 3.3B	44.0 ± 1.1A	43.4 ± 2.2A	Ns
Araquídic (20:0)	4.0 ± 0.1	4.1 ± 0.2	3.8 ± 0.4	4.2 ± 0.4	Ns
Linolenic (18:3)	2.0 ± 0.2A	2.0 ± 0.5A	4.4 ± 0.4B	4.3 ± 0.4B	Ns
Gondoic (20:1)	7.0 ± 0.3	7.2 ± 0.4	8.0 ± 0.3	8.2 ± 0.3	Ns
Behenic (22:0)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1± 0.1	Ns

[a] Cultivar × harvesting system
[b]Mean values in the same row followed by different lowercase letters indicate significant differences in treatments for each cultivar at $P\leq 0.05$. Mean values in the same row followed by different capital letters indicate significant differences in cultivars for each treatment at $P\leq 0.05$. No letter means non significant effect.
Ns indicate non-significant
[c](Carbon number : unsaturation number)