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The effect of fly attack (*Bactrocera oleae*) on the quality and phenolic content of *Chemlal* olive oil

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RESUMEN

Efecto del ataque de la mosca (*Bactrocera oleae*) sobre la calidad y contenido en compuestos polifenolicos de aceites procedentes de aceitunas de la variedad *Chemlal.*

Se ha estudiado el ataque de las aceitunas por la plaga *Bactrocera oleae* con el fin de determinar la influencia sobre la calidad del aceite de oliva (acidez libre, índice de peróxidos, extinción UV, calidad sensorial), polifenoles totales e individuales. Este estudio se realizó con el aceite de oliva obtenido a partir de la mayoritaria variedad de Argelia (C*hemlal*).

El análisis cuantitativo y cualitativo de los compuestos fenólicos se realizó por el método colorimétrico y por cromatografía gaseosa acoplada a la espectomertria de masas respectivamente. Los resultados demostraron que el ataque de la mosca, modifica la calidad del aceite de oliva y se confirmó además la existencia de procesos hidroliticos y, en particular oxidativos, que reducen fuertemente la cantidad de compuestos O-difenólicos. El alcance de la modificación fue mucho mayor cuando las aceitunas fueron atacadas en una fase avanzada de maduración. La recolección temprana podría ser una manera eficaz para limitar el daño causado por *B. oleae* y mejorar la calidad del aceite de oliva virgen y preservar los compuestos fenólicos.

PALABRAS CLAVE: Bactrocera oleae – *Calidad del aceite de oliva – Compuestos fenólicos – Maduración.*

SUMMARY

The effect of fly attack (B*actrocera oleae*) on the quality and phenolic content of *Chemlal* olive oil.

The attack on olives by the pest *Bactrocera oleae* has been studied to determine its influence on the olive oil quality (free acidity, peroxide value, UV extinction, sensorial quality), the total polyphenol and the individual phenolic compounds. The Algerian chemlal olive variety was used in this work. Quantitative and qualitative analyses of phenolic compounds were performed using the colorimetric method and the GC coupled with mass spectrometry. The results demonstrated that the attack modified the quality of olive oil and confirmed the existence of hydrolytic and mostly oxidative processess which strongly reduced the amount of O-diphenolic compounds. The extent of modification was much greater when the olives were attacked and harvested at an advanced stage of maturity. Early harvesting could be an effective way to limit the damage caused by *B. oleae* and to improve the quality of virgin olive oil.

KEY-WORDS: Bactrocera oleae – *Olive oil quality – Phenolic compounds – Ripening.*

1. INTRODUCTION

Olive oil is an important source of fat in the Mediterranean diet which has been associated with a lower incidence of coronary heart disease and the prevention of some cancers. The positive effects of olive oil are due not only to its high monounsaturated/saturated fatty acid ratio, but also to its antioxidants like vitamin E and phenolic compounds (Caruso *et al*., 1999). Olive oil phenolics are powerful antioxidants both in vitro and in vivo; and they could contribute to the healthy effect of the Mediterranean diet (Visioli *et al*., 2002). The phenolic compounds also have a positive effect on oxidative stability and sensory properties because they confer a bitter, pungent and astringent taste in the olive oil (Kiritsakis, 1998).

The amount of phenolic compounds in olive oil are variable, depending on several factors such as cultivar, degree of maturation and the infestation by the olive fly *Bactrocera oleae* (Boskou, 2000). A decrease in phenolic content in relation with the attack of the olive fly has been noted by many researchers (Angerosa *et al.,* 1992; Evangelisti *et al*., 1994; Delrio *et al*., 1995; Pereira *et al*., 2004; Tamendjari *et al*., 2004). The oil obtained from olives with nymphal state or with the exit holes of the pest registered a remarkable decrease in phenolic compounds. Total phenolics to O-diphenols ratio varied considerably in olives which exhibited exit holes (Evangelisti *et al*., 1995).

Changes in phenolic compounds in virgin olive oil with the maturation of fruits has also been studied. It usually decreased as the maturation of olives progressed (Uceda and Hermoso, 1997); although there are some exceptions to this rule. Tyrosol and Hydroxytyrosol tended to increase with maturation. On the contrary, both the dialdehydic form and elenolic acid linked to tyrosol or hydroxytyrosol and the oleuropein or ligstroside

aglycons diminished their concentrations in oils with olive maturation (Brenes *et al.,* 1999).

Studies concerning the simultaneous action of maturity and the attack by *B. Oleae* on oil quality and phenolic compounds do not exist. The aim of this study was to asses the effect of *Bactrocera oleae* on qualitative parameters and phenolic compounds of the oil obtained from the widspread olive variety in Algeria (*Chemlal*) and to determine the optimal period of harvest in order to preserve the oil quality.

2. MATERIALS AND Methods

2.1. Sampling

Olives from C*hemlal* variety, harvested within the region of Tazmalt (Bejaia) in the center-east of Algeria, were used for the investigation. This variety, characterized by its small fruits and its late maturity, is the most widespread in Algeria (more than 50% of the olive groves).

To evaluate the evolution of the attack by *B. oleae* during olive ripening, fruits with the following color characteristics were chosen at different times: yellow and yellow with reddish spots, purple, purple and black (white pulp), black-violet (purple pulp). Samples were evaluated on 11/11/02, 30/11/02, 20 /12/02 and 16/01/03 and are indicated in the text as 1^{st}, 2^{nd}, 3^{rd} and, 4^{th}.

After the determination of the maturity index according to the Uceda and Frias method (1975) and the attack degree by *B. Oleae* (larval + pupae + the number of exit- holes) for olive samples (30kg) harvested on the 3 first dates of harvesting were divided into 3 samples batches:

1^{st}: non attacked olves by *B .oleae* olives (healthy olives) called S_1, S_2, S_3.

2^{nd}: non selected olives (reflecting real infestation conditions in the field) called N_1, N_2, and N3.

3^{th}: attacked olives (every olive presents at least one hole which is characteristic of the fly exiting the fruit) called A_1, A_2, A_3.

Regarding the 4^{th} sample (black olives with violet pulp) only one sample was prepared. At this time olives were all attacked ($N_{4=}A_4$), so it was impossible to have a sample of healthy fruits as a reference sample.

The extraction of oil was performed by means of a laboratory mill equipped with a blender and a centrifuge separator. A small, established quantity (50ml) of warm water was added to the paste (920g) to make the oil extraction easier.

2.2. Analytical determinations

Free acidity, peroxide value and ultraviolet characteristic determinations

The determination of acidity, peroxide value, specific absorbance at 232 and 270 were carried out according to the EC method (EEC/2568/91).

Sensory analysis

The sensory evaluation of olive oil was performed according to the methodology established by the EC regulation (796/2002) by 8 members of an analytical panel of the Istituto Sperimentale per la Elaiotecnica (Italy).

Total polyphenol

Total phenols were extracted with methanol using an SPE Column of octadecyl C18 (J.T.Backer, Milan, Italy) according to the method of Favati *et a*l.(1994) and evaluated colorimetrically at 765 nm using the Folin-Ciocalteu reagent and the concentrations were expressed as gallic acid ppm.

Extraction and analysis of individual phenolic compounds

The extraction and evaluation of phenolic compounds were performed according the method described by Angerosa *et al*., (1995):

Extraction: Thirty grams of dried virgin olive oil were added to 1 ml of resorcin solution (internal standard 25, 4 mg of resorcin in 50 ml of diethyl-ether solution 0, 5 g/ liters). Extractions of phenolic compounds were performed by means of 3x 30ml of methanol in an Ultra–Turrax apparatus. To remove most of the residual oil, the methanolic solution was kept overnight at -20°C ; after filtration, the solution was concentrated in a vacuum, keeping the bath temperature under 35°C. The syrupy residue was taken up to 10 ml of acetonitrile. To eliminate the residual traces of glycerides, three 20 ml washings with hexane were performed, and the resulting acetonitrile solution was evaporated in a vacuum, keeping the temperature under 35°C, giving a residue that was dissolved in 7 ml of acetone. The resulting acetone solution was submitted to chromatographic or spectroscopic analyses.

HRGC and GC–MS Analyses: These deriving conditions were adopted: 150µl of BSTFA was added to 1 ml of acetone solution; 0.5 ml of this solution was separated and diluted with 0.5 ml of acetone solution.

HRGC was carried out with a Carlo Erba Mega Series 5160, equipped with an on column injection system and a FID detector, an Altech silica capillary AT-5 column (30 m length; 0, 32 i.d.; 0, 25 µm film thickness). The oven temperature program was a follows: from 80 to 135°C at 4,5°C /min, 5min at 135°C , from 135 to 174 at 3°C/min , 1 min at 174°C, from 174 to 315°C at 2,5°C /min , 15 min at 315°C . The temperature of the detector was held at 315°C. The carrier gas was H_2 and the carrier pressure on the head of the column was 35 KPa. Quantification was done by peak area integration with Carlo Erba Mega Series integration.

GC-MS was performed with an HP model 5890A, equipped with an on column injection

system and mass selective detector model HP 5970B, a Nordian silica capillary SE - carrier pressure on the column was 10 Kpa. The oven temperature program was the same used for the HRGC determination; the transfer line temperature was held at 300°C.

3. RESULTS AND DISCUSSION

Table 1 reports the ripening degree of fruits and the real *Bactrocera* infestation from the first ten days of November until the middle of January.

Results showed that the infestation increased continuously from the first to the 4th stage. It is important to consider the increase in exit-holes of olives which indicates that the parasite completed its larval development and metamorphosis. These olives whose integrity have been compromised are more exposed to atmospheric conditions and thus to alteration. This trend can be explained by the favorable climatic conditions that prevailed during the year, particularly the temperature which is considered as an important factor of development of the olive fly and its biology. The low production of olive fruits during the campaign 2002/2003 was another factor that promoted the extension of infestation. Gaouar and Debouzie (1991) pointed out the presence of four generations of *B. oleae* per year in Algeria.

3.1. Free acidity, peroxides value, U.V

Data of acidity, peroxide value and U.V specific absorbance of the oil samples are shown in Table 2. An increase in all parameters is observed in oils obtained from infested olives, especially in samples obtained at more advanced stages of ripeness (3rd and 4th stage). Besides the 4 stage of maturation, the values for the parameters don't exceed the limit established for the best commercial quality olive oil designated extra virgin (regulation EC 2568/1991). This evolution is a result of the hydrolytic and oxidative processes that are favored by the exit holes of the olive fly, which caused an extensive exposure of olive pulp to the environmental influence. Also, the olives are more sensitive to various microorganisms (bacteria, yeasts and Smaller moulds) (Torres-Villa *et al*., 2003) and mechanical damage.

3.2. Sensory analysis

The results (Table 2) of the sensory analysis of oils show that the ripening stage and the infestation affected the sensorial quality.

As far as ripeness is concerned, the oils of all samples belong to the extra virgin category, but there is a weakening of the intensities of fruity, bitter and pungent attributes when olives get riper. The oil marked S_2 obtained at the end of November presented the best equilibrium in the positive attributes. Garcia *et al*. (1996) found that the sensory quality of olive oil of some Spanish varieties decreased during fruit ripening.

The worsening of organoleptic quality is more pronounced when the oils are obtained from attacked olives harvested in an advanced stage of ripeness. Indeed, except for the samples N1, N_2 A_1, extracted from the early stage (olives yellow or yellow with reddish spots and purple), which were all classified as extra-virgin oils, the other samples were classified as virgin because tasters perceived, in addition to the weakening of all positive attributes in all oil samples, the fusty defect with slight intensity in A_2, and the fusty defect accompanied by the winy and grubby sensation in A_3 and N_3. The last A_4 sample, when the olives were black, gave oil with the worse quality; all tasters perceived fusty, musty, winy and grubby defects with quite high intensity. The fruity and pungent attributes had practically disappeared. The high intensity of the fustiness defect has been noted by Angerosa *et al.,* (1992) in the oil obtained from olives with 50% infested olives.

3.3. Total phenolic compounds

Phenolic compounds, being responsible for the oil bitterness and the resistance to oxidation, contribute considerably the quality of virgin olive oils. The total phenolic concentration (Table 3), determined by means of the colorimetric method, decreases with ripeness and attack of the olives by *B. oleae*. By considering only the ripeness, a maximum value of their concentration is observed in oils from the first stage; it decreased gradually in the oil extracted from healthy olives as ripening degree advanced. This loss, in relation to samples S_1, in agreement with results of Brenes *et al.* (1999); Gutierrez *et al.,* (1999) and Caponio *et al*., (2001), was of about 30% after the third stage of ripeness.

Table 1
Evolution of the color, ripening index and degree of attack on the olive samples from the trees (real infestation=infestation field)

Date of sampling	Color	Ripening Index	Degree of attack (%)	
			Larval, pupae and exit-holes (%)	Exit-holes (%)
11/11/2002	Yellow and yellow with the reddish spot	1.5	64.0	44
30/11/2002	Purple	2.6	68,0	56
20/12/2002	Purple and black(white pulp)	3.5	84,0	63
16/01/2003	Black (violet pulp)	5.0	100,0	92

Table 2
Analytical characteristics of the oils produced at different olive sampling times (mean + standard deviation)

Samples	11/11/02 S_1	N_1	A_1	30/11/02 S_2	N_2	A_2	20/12/02 S_3	N_3	16/01/03 A_3	N_4 (A_4)
Acidity (oleic acid)	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4± 0.0	0.5± 0.0	1.1 ± 0.1
Peroxide number (meq d'O_2/Kg)	6 ± 1.0	11 ± 0.7	15 ± 0.6	11 ± 0.7	16 ± 1.0	19 ± 0.7	10 ± 0.6	18 ± 0.7	20 ± 1.0	22 ± 1.0
K_{232}	2.09 ± 0.05	2.17 ± 0.02	2.23 ± 0.03	2.18 ± 0.03	2.35 ± 0.05	2.43 ± 0.05	2.36 ± 0.04	2.43 ± 0.03	2.50 ± 0.05	2.85 ± 0.08
K_{270}	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.18 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.28 ± 0.02
Sensory evaluation	extra-virgin	extra-virgin	extra-virgin	extra-virgin	extra-virgin	extra-courante	extra-virgin	extra-courante	extra-courante	extra-courante
Positives attributes (median)										
– Fruity	3.6 ± 0.3	4.0 ± 0.4	3.1± 0.6	4.4 ± 0.5	2.9 ± 0.4	2.6 ± 0.5	2.8 ± 0.4	1.6 ± 0.4	1.6 ± 0.5	0.9 ± 0.3
– Bitter	4.0 ± 0.5	4.2 ± 0.4	3.0 ± 0.5	3.7 ± 0.6	2.1 ± 0.5	2.1 ± 0.6	2.8 ± 0.5	2.9 ± 0.6	1.0 ± 0.3	0.4 ± 0.2
– Pungent	3.9 ± 0.5	3.6 ± 0.5	3.3 ± 0.6	4.0 ± 0.4	2.9 ± 0.6	2.3 ± 0.5	3.6 ± 0.6	3.2 ± 0.5	1.7 ± 0.5	0.4 ± 0.2
Defects:										
– Fusty						3.2 ± 0.5		0.7 ± 0.3	3.4 ± 0.6	3.9 ± 0.6
– Grubby						0.4 ± 0.3		1.4 ± 0.4	1.8 ± 0.4	2.6 ± 0.5
– Winey						0.4 ± 0.2		1.5 ± 0.4	0.7 ± 0.3	3.1 ± 0.5
– Rancid										2.2 ± 0.5
– Musty										2.5 ± 0.6

Table 3
Evolution of phenolic composition of olive oil with ripening and attack on olives by *Bactrocera oleae

	S1	S2	S3	N1	N2	N3	A1	A2	A3	SNA
Total phenolic compounds (folin-ciocalteu) method (mg/kg)	396.3 ± 11.5	317.3 ± 8.8	259.9 ± 6.9	367.2 ± 7.9	272.5 ± 4.7	198.5 ± 8.0	290.3 ± 9.0	222.3 ± 10.0	148.8 ± 6.3	17.0 ± 1.0
Total phenolic compounds (GC determination)	247.94 ± 13.39	234.97± 14.36	199,67± 11.61	210,61± 10.41	158,20± 8.27	118,62± 6.36	132,76± 7.04	115,62± 7.03	92,86± 4.29	12,92± 0.51
Dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol	4,12 ± 0.16	6,78 ± 0.60	2,00± 0.02	9.59± 0.50	10.73± 0.07	1.97± 0.20	7.63± 0.60	11.33± 0.38	2.55± 0.15	00
Free tyrosol	13,41± 0.30	18,00± 1.22	26,71± 1.08	15,07± 0.60	20,99± 1.05	22,48± 1.40	25,31± 0.70	21,86± 2.10	19,17± 0.50	8,42 ± 0.20
Free hydroxytyrosol	2,48± 0.10	2,35± 0.30	5,41± 0.50	2,11± 0.20	1,10± 0.10	1,62± 0.16	1,38± 0.20	1,21± 0.12	0,98± 0.09	1,00± 0.10
Linked phenol containing Tyrosol (mg/kg)	159,74± 9.97	147,96± 8.73	113,27± 6.15	131,08± 6.78	84,58± 4.29	54,19± 2.53	62,90± 3.58	48,11± 2.58	39,50± 1.77	2,65± 0.12
Linked phenols containing Hydroxytyrosol mg/kg)	68,19 ± 2.86	59,88± 3.51	52,28± 3.86	52,76± 2.33	40,80± 2.76	38,36± 2.07	35,54± 1.96	33,11± 1.95	30,66± 1.78	0,85± 0.09
Linked form of Tyrosol and hydroxytyrosol (mg/kg)	227,93± 12.83	207,84± 12.24	165,55± 10.01	183,84± 9.11	125,38± 7.05	92,55± 4.60	98,44± 5.54	81,22± 4.53	70,16± 3.55	3,50± 0.21

*(mean ± standard deviation)

The attack on olives by *B. oleae* caused important losses in the total amount of phenolic compounds, particularly in oils from olives in advanced stages of ripeness. The reduction of the phenolic concentration in oils from olives totally attacked at stages 1, 2 and 3, in relation to corresponding samples from healthy fruits, was about 34, 46 and 49 % respectively. Sample A_4 showed the lowest level of phenolic compounds.

The losses in phenolic compounds under the effects of attack and the ripening process induced a considerable reduction in the positive bitterness and pungent attributes. Bitterness in virgin olive oil has been linked generally to the total phenol content (Angerosa *et al.,* 2000; Beltran *et al*., 2005). According to Morello *et al,* (2006), the total phenol cotent better described the bitterness index than 3,4 DHPEA-EDA and HPEA-EDA, probably due to the high diversity of phenolic compounds implied in the biterness of virgin olive oil.

3.4. Evolution of different phenolic compounds

Figure 1 represents the chromatogram of olive oil phenolic compounds and the concentrations of different considered compounds are presented in Table 3. The exam of these results shows the predominance of oleuropein and derivatives containing hydroxytyrosol and tyrosol respectively. The free forms of tyrosol and hydrotyrosol are minor. If we consider the result of the healthy samples, maturation occurs with a decrease in the total polyphenols, oleuropein, and the complexes that contain tyrosol and hydroxytyrosol, with an increase in the free form of tyrosol and hydroxytyrosol. This evolution, which is similar to that noticed by Brenes *et al*., (1999), suggests an enzymatic hydrolysis such as glucosidases or esterases.

The attack on olives by *B. oleae* cause further, more important reduction of linked forms of tyrosol and hydroxytyrosol. The 100% affected sample and harvested at the last stage (olive black) contains only 0.25 ppm of phenolic complex.

The evolution of the free form of tyrosol and hydroxytyrosol are disparate, the hydroxytyrosol tends to decrease with the attack in various maturation stages, and the tyrosol undergoes a decrease only at the third stage while increases were recorded for the first and the second stage of maturation. The increased content of free tyrosol in samples at the first and second stage shows a strong hydrolytic process which involved hydrolysable phenolic compounds containing its structure. In fact, Perrin (1992) reported that in damaged olives, esterase activity and pH contibute to a remarkable development of hydrolysis. The reduction of both compounds (tyrosol and hydroxytyrosol) in the third stage coincides with a rise in oxidation parameters. The losses of the amount of phenolic compounds were probably a consequence of the presence of pupae and/or exit holes, which caused an extensive exposure of olive pulp to environmental influences. Furthermore, the tissue breaking could have promoted the activity of polyphenol-oxidase which is present in olive pulp (Perrin, 1992).

The evolution of simple and hydrolysable phenolic compounds confirms the existence of

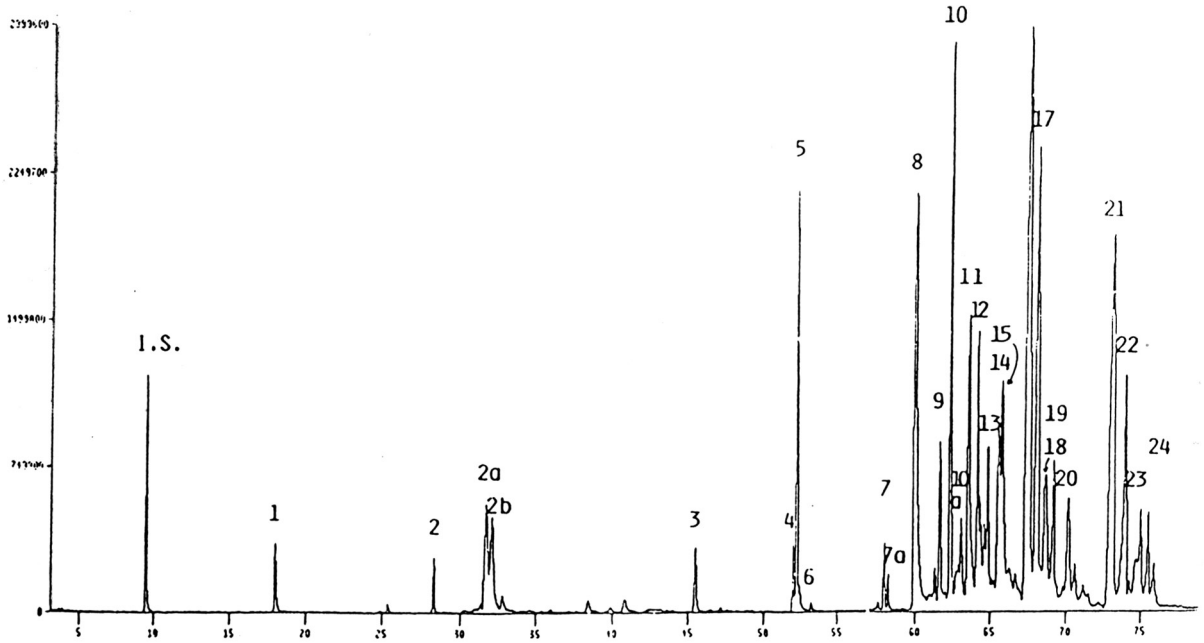

Figura 1
Chromatographic profile of derived olive oil methanolic extract. Peaks:1, tyrosol; 2, hydroxytyrosol; 2a and 2b, unknown; 3, palmitic acid; 4, linoleic acid; 5, oleic acid; 6, stearic acid; 7 and 7a, unknown; 8, dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol; 9, 10, 10a, 14, 16, 17, 18, and 19, linked phenols containing tyrosol; 11, 13, 15, 20 ,21, 22, 23 and 24, linked phenols containing hydroxytyrosol; 12, monoglyceride.

hydrolytic and mostly oxidative processes which reduces the amount of phenolic compounds. Gomez–Caravaca *et al*. (2008) observed that the samples strongly attacked by the olive fly contain the lowest phenolic content, O-diphenols and particularly some secoiridoid derivatives.

4. CONCLUSIONS

Results show that both ripeness and the *B. oleae* attack have repercussions on oil quality and phenolic compounds. Concerning the ripeness factor, it affects the sensory characteristics with a flattening of most attributes. Throughout ripening, several metabolic processes take place in olives with subsequent variations on profiles of some compounds. The changes are reflected in the oil quality grade, sensorial characteristics and phenolic compounds. The damage of olive drupes by *B. Oleae* involves a decrease in organoleptic characteristics and significant losses in phenolic compounds. The detriment is more evident in oils obtained from fruits showing important levels of infestation and an advanced stage of ripeness. The results of this investigation allow for advice to harvest when olives are turning purple or have purple skin in order to minimize the damages caused by *B. oleae*. This occurs from the end of November and the first ten days of December. The anticipation of the harvesting could represent an effective mean of prevention from the damages of *B. oleae* attack and consequently an improvement in virgin olive oil quality, and in many cases the only possible remedy since no treatments against the olive fly are performed in Algeria.

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