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Evaluation of processing factors for selected organic contaminants during virgin olive oil production: distribution of BTEXS during olives processing

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Abstract

The presence of BTEXS (benzene, toluene, ethylbenzene, xylenes and styrene) in virgin olive oils can be attributed to environmental contamination, but also to biological processes during oil lipogenesis (styrene). In this work, the processing factor of BTEXS from olives to olive oil during its production was evaluated at lab-scale with an Abencor system. Benzene showed the lowest processing factor (15%), whereas toluene and xylenes showed an intermediate behavior (with 40-60% efficiency), and ethylbenzene and styrene were completely transferred (100%). In addition, an attempt to examine the contribution of potential sources to olives contamination with BTEXS was carried out for the first time. Two types of olives samples were classified according to their proximity to the contamination source (road). Although higher levels of BTEXS were found in samples close to roads, the concentrations were relatively low and do not constitute a major contribution to BTEXS usually detected in olive oil.

Keywords: BTEXS, headspace, GC-MS, olives, olive oil, processing factor

Chemical compounds studied in this article: benzene (PubChem CID: 241); toluene (PubChem CID: 1140); ethylbenzene (PubChem CID: 7500); o-xylene (PubChem CID: 7237); m-xylene (PubChem CID: 7929); p-xylene (PubChem CID: 7809); styrene (PubChem CID: 7501).

1. Introduction

Olive oil industry is a billion-dollar business in Spain that stands out as the main economic sector in selected areas such as the province of Jaén. Virgin olive oil is already an outstanding commodity due to its health promoting benefits. Considering the olive oil production figures and the increasing demand of consumers for high quality products (Preedy & Watson, 2010; Owena, Giacosac, Hullb, Haubnera, Würtelea, Spiegelhaldera & Bartscha, 2000), the assessment of high quality and food safety standards is of paramount interest, particularly regarding the absence of chemical contaminants.

Benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) are monocyclic aromatic hydrocarbons whose presence in the environment is usually attributed to incomplete fuel combustion (Meniconi, Gabardo, Carneiro, Barbanti, Cruz da Silva & Massone, 2002; Alberici, Zampronio, Poppi & Eberlin, 2002). Some of them have been used as industrial solvents and products for painting (Wang, Li, Fingas, Sigouin & Ménard, 2002; Lopes & Bender, 1998). BTEXS are a subclass of volatile organic compounds, with boiling points between 80 and 150 °C, and they are considered hazardous carcinogenic and neurotoxic compounds, being issued as priority pollutants by Environment Canada (Fingas, Laroche, Sergy, Mansfield, Cloutier & Mazerolle, 1991) and the U.S. Environment Protection Agency (EPA). These organic compounds are very volatile and, consequently widely distributed in the environment and, even in foods (Peña, Cárdenas, Gallego & Valcárcel, 2004). Due to their high volatility and relatively high solubility in oils and fats, BTEXS can enter and contaminate olives, particularly if they are stored in enclosed spaces nearby vehicles or other fuel-based engines (Biedermann, Grob & Morchio, 1995; Biedermann, Grob & Morchio, 1996). The presence of BTEXS was first reported in virgin olive oil by Olías-Jiménez (Olías-Jiménez, Gutiérrez-Rosales, Dobarganes-García, Gutiérrez González-Quijano, 1980). In addition, styrene can be present in virgin olive oil samples by natural causes (Biedermann et al., 1995) or by transference from the plastic package (Carrillo-Carrión, Lucena, Cárdenas & Valcárcel, 2007). Peña et al. (Peña, Cardenas, Gallego & Valcárcel, 2004) proposed a cut-off threshold value of 200 $\mu g \cdot L^{-1}$ for the sum of BTEXS results in virgin olive oils, to consider it as positive finding.

Several studies have been carried out on the determination of BTEXS in virgin olive oils using different methodologies such as head-space solid-phase microextraction (HS-SPME) (Cavalli, Fernández, Lizzani-Cuvelier & Loiseau, 2004; Vichi, Pizzale, Conte, Buxaderas & López-Tamares, 2005), purge-and-trap with GC-MS (Ollivier & Guérère, 2001), thermal desorption (Zunin, Boggia, Lanteri, Leardi, De Andreis & Evangelist, 2004; Cavalli, Fernández, Lizzani-Cuvelier & Loiseau, 2004) and headspace combined with mass spectrometry (Peña et al., 2004; Carrillo-Carrión et al., 2007; Gilbert-López, Robles-Molina, García-Reyes & Molina-Díaz, 2010; Toledo, Enríquez, Garrido, Fernández-Band & Richter, 2010). While factual information is available on the concentration levels of BTEXS in virgin olive oil, scarcely any literature is available for olives. Gilbert-López et al. (Gilbert-López, Robles-Molina, García-Reyes & Molina-Díaz, 2010), found up to 332 $\mu g \cdot kg^{-1}$ of BTEXS in commercial extra virgin olive oils. Vichi et al., (2005), reported up to 372 $\mu g\ kg^{-1}$ of toluene in one sample. As regards to data on olives, Biedermann et al. (Biedermann, Grob & Morchio, 1996) proposed up to 250 $\mu g \cdot kg^{-1}$ for the sum of benzene, toluene, and the C_2-benzenes as possible background concentrations in olives.

The preliminary determination of BTEXS in olives can anticipate the possible contamination subsequently obtained in the final product. The study of the processing factor and behavior of BTEXS during virgin olive oil production is of paramount importance. In this sense, previous studies on the processing factors of selected pesticides during olive oil production stage are available (Cabras, Angioni, Garau, Melis, Pirisi & Minelli, 1997; Amvrazi & Albanis, 2008). Pesticide processing factors have been calculated in soybean-processed products (Zhao, Ge, Liu & Jiang, 2014), apple juices and raisin products (Shabeer, Banerjee, Jadhav, Girame, Utture, Highmire & Oulkar, 2015) amongst other derivate commodities. Consequently, polar pesticides presumably do not tend to preconcentrate in the olive-oil phase during its production, while fat-soluble pesticides tend to concentrate in the extent of the olive oil production yield from olives (García-Reyes, Ferrer, Gómez-Martos, Fernández-Alba & Molina-Díaz, 2007). Thus, the main aim of this work is to study processing factors of BTEXS from olives to virgin olive oil during the production stage at lab-scale. In addition, an attempt to examine the contribution of potential sources to olives contamination with BTEXS was carried out. Two types of olives samples were classified according to their proximity to a possible contamination source (such as gas stations or roads).

2. Experimental

2.1 Chemicals and materials

Analytical-grade standards of benzene, toluene, ethylbenzene, the three isomers of xylenes (ortho, meta and para) and styrene, were purchased from Riedel de-Haën (OEKANAL® quality) (Seelze, Germany). Stock solutions of each analyte were prepared in methanol at a concentration of 1.0 mg·mL^{-1} and stored in amber glass capped vials at -20 °C. A standard solution containing the mixture of BTEXS at individual concentrations of 100 µg·mL^{-1} was prepared in methanol by appropriate dilution of the stock solutions. Working standard solutions were prepared by spiking the standard solution to olives and olive oil samples (previously analyzed to check the initial content of BTEXS). HPLC-grade methanol was obtained from Merck (Darmstadt, Germany). 20 mL glass flat-bottomed vials (22.7 mm OD x 75 mm) as well as magnetic PTFE-silicone seals (3.0 mm i.d.) were purchased from Supelco (Madrid, Spain). PTFE-encapsulated magnetic stirring bars (6 mm x 12 mm) were purchased from Varian Inc. (Walnut Creek, CA, USA). Potassium chloride (reagent grade) was obtained from Panreac (Barcelona, Spain). A Mili-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses.

The olives samples were picked up from several plots of olive grove from Andalucía region, while two different virgin olive oil samples were treated. One by extraction from olives by Abencor method and a second group were purchased at local market.

2.2. Head Space Gas chromatography/mass spectrometry

2.2.1 *Gas chromatography/mass spectrometry*. The separation of the species targeted was carried out using a CP-3800 gas chromatograph (Varian Inc. Walnut Creek, CA, USA) equipped with electronic flow control (EFC) and a 1079 universal capillary injector that allows programmed temperature injection (a PTV injection port). The gas chromatograph was also equipped with an autosampler (CombiPAL autosampler, CTC Analytics) with capacity for 32 headspace vials composed of an oven for sample heating/headspace generation and a robotic arm where the headspace syringe was place.

Helium was employed as carrier gas. The operating conditions of the headspace were: incubation time: 30 min; incubation temperature: 90 °C; magnetic stirring speed: 700 rpm. Then 1 mL of the vial headspace was injected into the GC inlet heated at 200 °C, operated in Splitless mode and equipped with a split open deactivated insert liner of 5 mm OD x 54 mm x 3.4 mm ID (Varian Inc., Walnut Creek, CA, USA). The column oven temperature was set at 30 °C and remained constant during 10 min. After this time, the temperature was raised up to 60 °C at 5 °C/min, and after 1 min it raised up again to 200 °C at 20 °C/min. Then, it was kept at 200 °C for 2 min. A constant column flow of 1.5 mL/min of helium was used. A Varian FactorFour VF-5ms capillary column of 30.0 m x 0.25 mm i.d. and 0.25 µm of film size (Varian Inc., Walnut Creek, CA, USA) was used for chromatographic separation.

The gas chromatograph was connected to a triple quadrupole mass spectrometer Varian 300-MS TQ MS (Varian Inc., Walnut Creek, CA, USA) by an inert transfer line heated at 280 °C. The source and manifold (QqQ) temperatures were kept at 250 and 40 °C respectively. Electron impact ionization (EI) was operated at 70 eV. A filament current of 50 µA and a multiplier voltage of 1300 V were used in MS mode. Specific SIM ions were recorded for each compound analyzed (167 ms acquisition time for each ion). A filament multiplier delay of 2.3 min was fixed in order to prevent detector overload/saturation. The mass spectrometer was calibrated as needed with perfluorotributylamine (PFTBA). Varian WorkStation software (version 6.9) was used for automated analysis and data acquisition and Varian MS Data Review was used for data processing. Olives and olive oil samples were analyzed by an automated HS-GC-MS method described elsewhere (Gilbert-López, Robles-Molina, García-Reyes and Molina-Díaz, 2010). The analytical parameters of the methods used are described in the Supplementary data (**Tables S1 and S2**).

2.2.2. Head-space conditions

Olives samples. 5 g of olive paste (from 500 g of olives crushed in a mill) were weighed into a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar. Then 10 mL of milli-Q water plus, and 1.5 g of KCl were added and the vial was immediately sealed with a PTFE-silicone septum. Samples were analyzed by triplicate.

Extra virgin olive oil samples obtained from Abencor system. 12.5 g of olive oil (*ca*. 15 mL) were weighed into a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar and was immediately sealed with a PTFE-silicone septum. Samples were analyzed by triplicate. The HS vial was placed in the CombiPal heated module for heating with mechanical stirring (700 rpm) for 30 minutes at 90 °C in order to ensure the equilibration between gas-phase and sample. An aliquot of the (gas-phase) vial headspace (1 mL) was automatically injected in the GC-MS system. During GC-MS analysis specific SIM ions were recorded for each target compound.

2.3. Lab-scale experiment for BTEXS absorption in olive oil under saturated fuel atmosphere

Experiments were carried out introducing inside a desiccator a 1000 mL beaker containing 600 mL of virgin olive oil together with a 100 mL beaker where 50 mL of gasoline or gasoil were placed. It is supposed that the saturation of the volume of air inside the desiccator (10 L approx.) by volatile compounds coming from fuel is reached in a short period of time. Then, 9 aliquots of olive oil of 30 mL each one were taken during a period of 72 hours, 15 mL were put in a 20 mL head space vial for its prompt analysis by HS-GC-MS and the other 15 mL were kept for its further use if necessary. BTEXS concentration in gasoil and gasoline were estimated using standard addition method by spiking 15 mL of olive oil with fuel to a final proportion of fuel of 0.01% (v/v). The peak area obtained for each compound was interpolated in a matrix-matched standard calibration curve prepared at 50, 100, 200, 500 and 1000 $\mu g \cdot kg^{-1}$. The HS-GC-MS analysis of these samples was accomplished using the same experimental conditions previously described in section 2.2.

2.4. Study of processing factors: Transfer from (spiked) olives to olive oil produced by a lab-scale Abencor system.

To study the processing factors, the first step is spiking the milled samples, since there is no significant amount of BTEXS in them. Owing to this fact, 3.3 kg of homogenized olives mass were spiked adding a suitable volume of BTEXS mixture up to a final concentration of 50 $\mu g \cdot kg^{-1}$. Later, the resulting mixture was gently stirred at room temperature for 60 minutes to foster analyte homogenization and allow solvent evaporation. Finally, it was kept at +4 °C overnight.

The second step was extracting the olive oil from the spiked olives, by means of an Abencor system (MC2 Ingeniería y sistemas, S.L., Seville, Spain), simulating commercial oil-extraction systems. Five portions (250 g each one) of the spiked mass (3.3 kg) were selected and introduced in the malaxater with a thermometer (model TB-100), where it was shaking for 30 minutes at temperatures lower than 20.6 °C. Malaxation temperature was kept at low values to avoid possible losses of BTEXS by evaporation. Afterwards, the paste was centrifuged for 1 min at 3500 rpm in the Abencor centrifuge (model CF-100) for phase separation (olive oil is separated from solids and water). Virgin olive oils obtained (n = 5) were weighted and stored in opaque glass bottles until they were analyzed by HS-GC-MS. The BTEXS concentration levels of the spiked olives and their respective virgin olive oils were used to calculate a processing factor for each analyte.

2.5. Determination of BTEXS levels in olives samples

Sampling was done according to the Spanish sampling regulation for pesticide residues monitoring in foodstuffs (Spanish regulation, 2003). Briefly, one kilogram of representative whole olives was picked, at the same height of the view and around the four cardinal points. They were frozen at -20 °C, to preserve their composition until the moment in which the analysis was carried out. The day of the analysis, approximately 500 g of olives (including the kernel) were first crushed by means of a mill manufactured by Talleres Lopera (Priego de Córdoba, Córdoba, Spain) and designed especially for crushing up olives (*molino triturador-reductor (M-R)*, 45 cm (length) x 51.5 cm (high) x 35 mm (width), 40 kg (weight)). The mill consisted in a hopper that led the olives to a worm gear connected to a rotor (1.1 kW). This rotor rips the olives and olive kernel, then obligating them to pass through a sieve of small orifices (5.0 mm i.d.). As a result, a homogenized paste is obtained and collected in an appropriate food-container.

2.5.1 Distribution study of BTEXS in olive groves from Andalusia

Four provinces of Andalucía region were selected to do the sampling, Jaén, Málaga, Granada, and Córdoba, together with Badajoz, which is located in Extremadura community. A total number 40 of olives samples was picked during the season 2011-2012. The places selected can be seen in **Figure 1.a**. The samples have been differentiated depending on their proximity to the possible contamination sources; therefore we can analyze two different groups: the samples next to roads or fuel stations and those located inside of the olive groves (> 500 m far from a possible contamination source). Detailed information about the samples is included in **Table S7** (Supplementary data).

< **Figure 1** >

2.5.2 Distribution of BTEXS in olive samples from an olive grove plot close to a contamination source (road)

First, thirty samples were selected from a plot of olive grove located near to Jódar (Jaén). The geographical coordinates are latitude 37° 52’ 1.31” N and longitude 3° 21’ 12.41” W, with an area of 1813 ha of surface and 186 olives trees. A satellite view of the plot can be seen in **Figure 1.b**.

Samples were collected during the season 2012-2013 and divided in two groups for the statistical study. The first group (identified with red circles) corresponds to samples collected near the road A-401, on the top of **Figure 1.b**. The second group (identified with black circles) corresponds to samples collected from inside of the plot (around 50-70 m far from the road). Their respective features and nomenclature are detailed in **Table S3** and **Figure S1.** (Supplementary data).

3. Results and discussion

3.1. BTEXS Absorption experiment in a enclosed fuel-saturated environment

Due to their relatively high lipophilicity BTEXS absorption in olive oil may occur directly by fuel vapor exposure, as suggested by Biedermann et al. (Biedermann, Grob & Morchio, 1996). We performed a lab-scale experiment to study the absorption kinetics of BTEXS in olive oil by direct exposition to fuel vapors. As was previously indicated in the experimental section, 600 mL of olive oil were exposed to a saturated atmosphere of gasoline and gasoil separately. Results of these experiences by means of absorption curves (µg compound Vs. sampling time) of each BTEXS in gasoline atmosphere are shown in **Figure 2**. As can be seen, the absorption of these compounds in olive oil under the experimental conditions follows a linear trend. Saturation of detector is reached before the end of the experiment (only the linear region of calibration curve is shown in the graphics) for all compounds except for styrene, which is by far the least concentrated BTEXS in gasoline. Benzene is also present at low concentrations in gasoline although it displays the highest vapor pressure within the group (**Table S4**, Supplementary data). In the case of gasoil atmosphere experiment, it was not possible to construct the absorption curves since the instrument gave saturated signals for most of compounds with as little as one hour of exposure. These results are consistent with the higher concentration of BTEXS found in gasoil (**Table S5,** Supplementary data)). These results confirm the hypothesis of high affinity of these compounds for lipid matrices and highlight the importance of prevent the exposition of olives and olive oil to BTEXS sources such as for instance fuel-based engines or fuel tanks that should be kept away.

< **Figure 2**>

3.2. Lab-scale study of processing factors of BTEXS during olive oil production

The olive oils obtained after Abencor extraction, were weighted and the yields were calculated. The results are shown in **Table S6** (Supplementary data). Processing factors can be used to predict the concentration of BTEXS in the extra virgin olive oil from the concentration of BTEXS analyzed in the olives used as starting raw material. Additionally, processing factors are a useful tool to establish Maximum Residue Levels (MRLs) in processed foods. In fact, current EU regulations on pesticide residues in food consider MRLs from the raw material due to the lack of information about processing factors. The calculation of the processing factor for each analyte was based on a mass balance using the BTEXS concentrations found in the olives milled together with those found in the olive oils extracted from them, and the yield of each extraction. Thus, processing factor (F) was calculated according to the equation III:

Equation III: (BTEXS concentration in olive oil/BTEXS concentration in olives sample)*Yield(%)

The respective BTEXS concentration levels are displayed in **Table 1**, as well as the corresponding processing factor (F) for each analyte, expressed in percentage of transference from olives to olive oil. For ethylbenzene and styrene, a value over 100% is obtained; therefore they are completely transferred from the olives to the olive oil. However, toluene and xylenes are partially transferred, with processing factors ranging 20-50%. Finally, the lowest transference factor is for benzene, obtaining a value close to 15%. This is consistent with the higher water solubility of this compound compared to the other species (**Table S4**, Supplementary data). Considering an average yield of 10.6% and a minimum transference of 14.6%, the analytes are more concentrated in the olive oil than in the olives. However, it should be noted that in the present study processing factors have been examined at relatively low temperature (20 °C) to prevent BTEXS losses by evaporation, whereas in the industrial production of extra virgin olive oil the olive paste is usually heated up to 25-35 °C during the malaxation step. The higher temperature used in the industrial process can favor BTEXS evaporation and therefore a lower transfer efficiency to the final product. On the other hand, the higher malaxation temperature and the occasional use of technological additives as talcum increase the yield (industrial yield for olive oil production is in the range 15-20%), modifying thus the concentration factor of BTEXS in the factory-scale produced virgin olive oil.

< Table 1>

3.3 Determination of BTEXS in olives collected in different olive groves in Andalusia and within an olive grove close to a road

Concentrations of BTEXS found in the samples are summarized in **Figure 3**, where average concentration of BTEXS is represented, together with the interval between the minimum and the maximum values found in each group of studied samples. It is observed that samples close to a possible contamination source showed higher concentration of BTEXS, but in any case they were within the range considered as background concentrations (up to 10 µg·kg^{-1} of benzene and 250 µg·kg^{-1} for the sum of benzene, toluene, and the C$_2$-benzenes), (Biedermann, Grob & Morchio, 1996).

< Figure 3 >

Some differences can be seen between the comparative study among different olive groves from Andalusia (Figure 3a) and the comparative study of samples within the same olive grove plot (Figure 3b). In the first study, toluene was only found in samples close to a potential source of contamination, while it was found in samples of the inner part of the olive grove plot of the second study. These results are consistent because in both cases the olive samples were harvested at a distance less than 500 m far from the contaminating source. On the contrary, o-xylene was found in the first study in samples far (> 500 m) from a potential source of contamination, while in the second study it was only found in samples collected in the samples closer to the road in the olive grove plot. Benzene was not detected in any of the studied samples, perhaps due to its high vapor pressure and the relatively high water solubility, so that it may eventually be removed from the olives surface by the rainfall. Detailed information about BTEXS concentrations found in different samples of Andalusia is given in the supplementary information (**Table S7**).

Distribution of BTEXS within an olive grove plot. Concentrations of BTEXS found in different samples from the same olive grove plot are shown in **Table 2**.

< Table 2 >

With the aim to find out if significant differences exist among the BTEXS concentrations of the samples closer to the contamination sources compared with those far from them, a statistical study using the Student's t-test (Miller & Miller, 1988) has been accomplished to compare the concentration averages from the two groups.

The F-test of equality of variances (Miller et al., 1988) showed a significant difference between the variances of the two groups of olives samples in the case of all compounds, except for styrene. The equations used for the degrees of freedom and the t-experimental are detailed in Supplementary data section along with the detailed results from the statistical study (**Table S8**).

Differences were calculated between the t-experimental and the corresponding t-tabulated for a two-tailed test and a probability of 95% (P=0.05). Results are shown in **Table 3**. Only benzene and styrene did not yield significant differences between the two groups. Concentrations of the rest of studied compounds showed significant differences between the samples close to the road and the samples located inside the plot. The highest difference was found for o-xylene, which appeared in olives samples close to the road, but not in the samples located inside the plot. Actually, the distinctly higher values shown by samples 2x4 and 2x6 for toluene, ethylbenzene and xylenes can be attributed to the position of these olive trees, near to the two roads and, more interestingly, besides the spot where trucks and tractors frequently pull off, which would support the initial hypothesis of the experiment.

< Table 3 >

4. Conclusions

The calculated processing factors demonstrated that the different compounds tested were not transferred in the same extent from olives to virgin olive oil. Only ethylbenzene and styrene were completely transferred, while for instance benzene was hardly transferred to olive oil due to its relatively high water solubility, so it would be mostly diverted to the aqueous-phase byproduct generated during virgin olive oil production. Besides, it should be kept in mind that an addition that prior to olive oil production steps (malaxation and centrifugations), olives are subjected to a washing step, which may remove partially these compounds. This step was not included in the current study, which was focused on studying the actual behavior during the processing steps.

As regards to the study of the source of BTEXS in olives, low concentration levels were detected in olives samples from the South Spain using HS-GC-MS. The results were within the range considered as background concentrations (up to 10 $\mu g \cdot kg^{-1}$ of benzene and 250 $\mu g \cdot kg^{-1}$ for the sum of benzene, toluene, and the C_2-benzenes) by other authors. Nevertheless, higher BTEXS concentrations were found in olive samples closer to contamination sources (road or gas stations) compared with samples located far from them. Thus, environmental pollution is evident, but it happens at such very low levels that it does not pose a risk for olive oil safety. Actually, the contribution of this source of contamination is minor if compared with the values of total BTEXS concentration found in virgin olive oil by previous studies. This fact suggests that there may be other relevant sources yet to evaluate apart from that examined in the present article.

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Figure captions

Figure 1. a) Location of olives samples harvested throughout Andalusia region. **b)** Aerial view of the olive grove, in which the olive trees closer to the secondary road (A-401) selected for the sampling are marked with red color, while the black circles refers to the trees selected to collect the olives inside the plot.

Figure 2. Absorption curves of BTEXS in olive oil under gasoline saturated atmosphere. (t: exposition time (hours)).

Figure 3. (a) Concentration ranges of BTEXS in the two groups of studied samples, far (> 500 m) and close (< 500 m) to a possible contamination source from different parts of Andalusia; **(b)** Concentration ranges of BTEXS in olives samples from the inner part of a plot and samples close to a road. The number of samples analyzed in each group is indicated in the parenthesis.

Figures

Figure 1

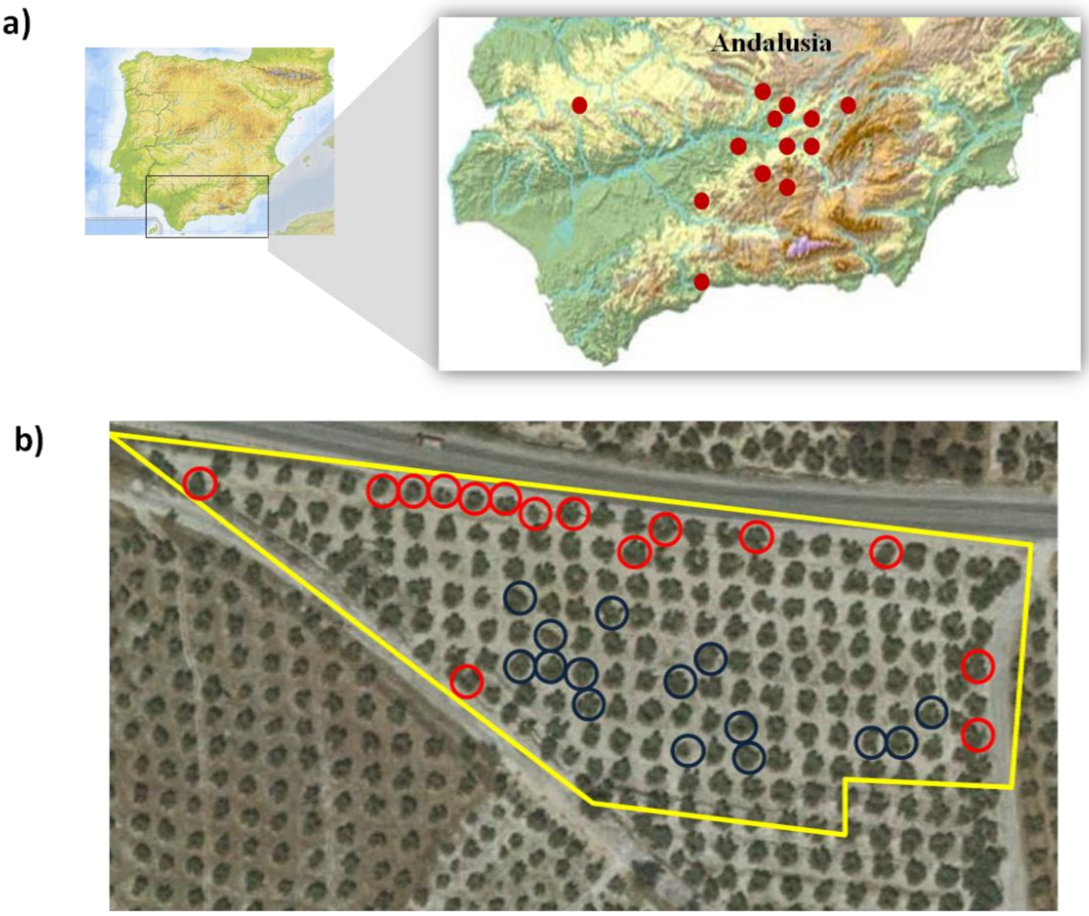


Figure 2

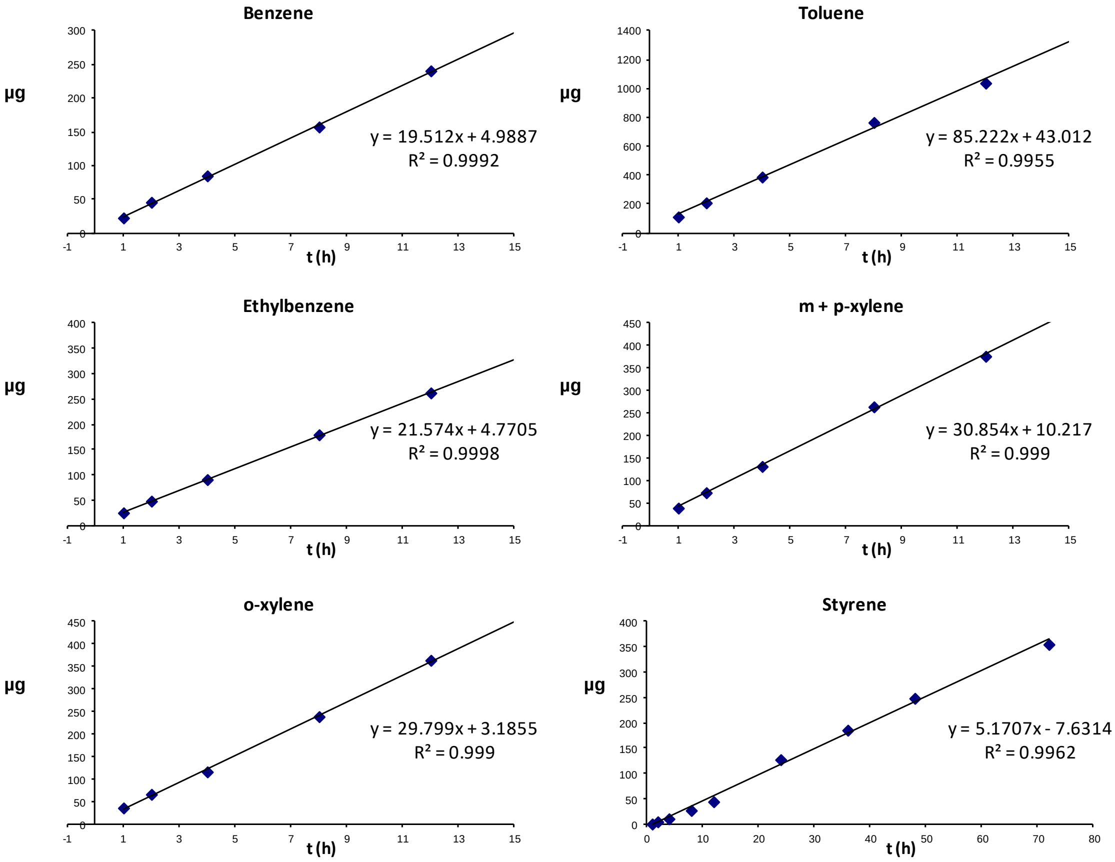
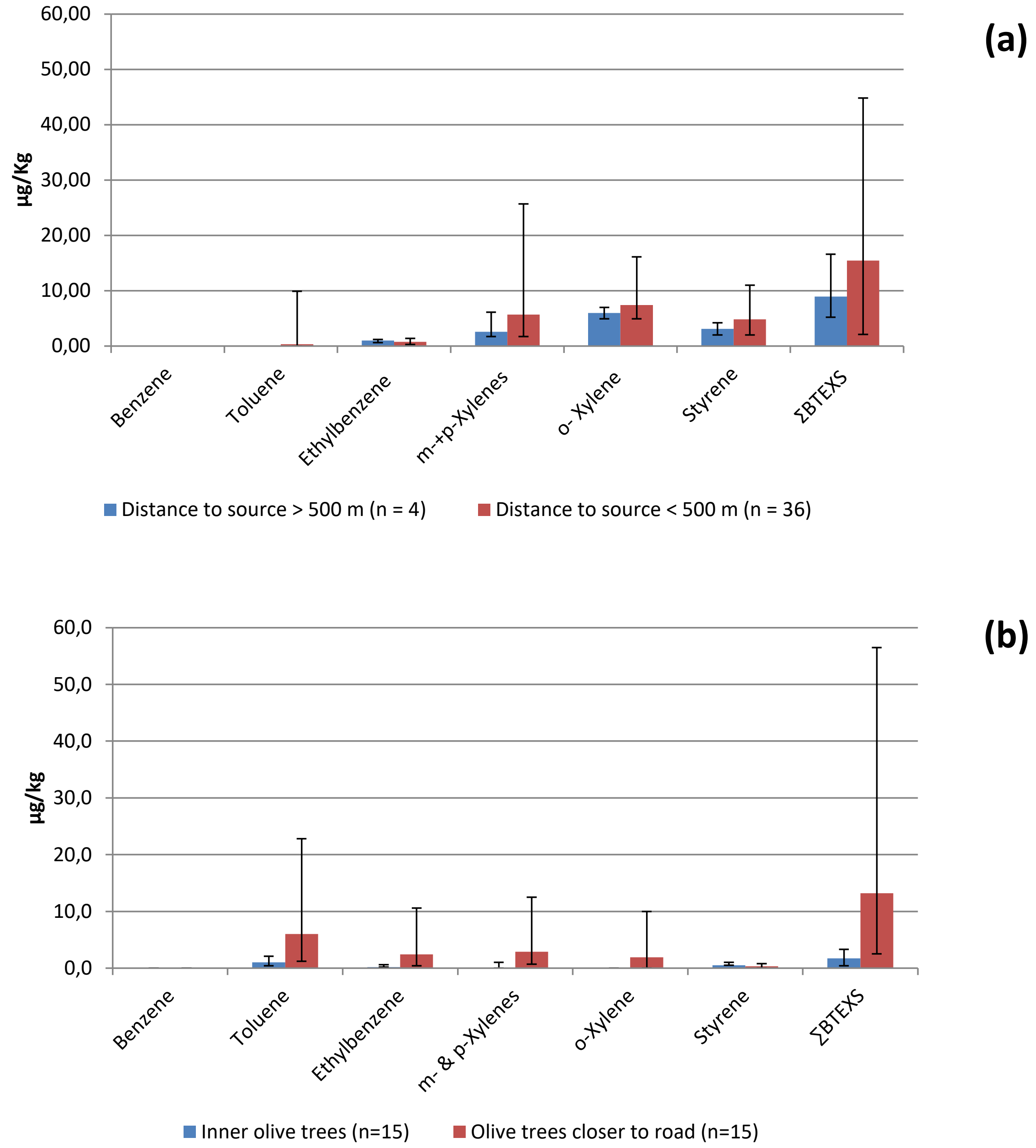

Benzene
µg
t (h)
y = 19.512x + 4.9887
R² = 0.9992
Toluene
µg
t (h)
y = 85.222x + 43.012
R² = 0.9955
Ethylbenzene
µg
t (h)
y = 21.574x + 4.7705
R² = 0.9998
m + p-xylene
µg
t (h)
y = 30.854x + 10.217
R² = 0.999
o-xylene
µg
t (h)
y = 29.799x + 3.1855
R² = 0.999
Styrene
µg
t (h)
y = 5.1707x - 7.6314
R² = 0.9962

Figure 3


(a)
µg/Kg
60,00
50,00
40,00
30,00
20,00
10,00
0,00
Benzene
Toluene
Ethylbenzene
m-+p-Xylenes
o- Xylene
Styrene
ΣBTEXS
🟦 Distance to source > 500 m (n = 4)
🟥 Distance to source < 500 m (n = 36)
(b)
µg/kg
60,0
50,0
40,0
30,0
20,0
10,0
0,0
Benzene
Toluene
Ethylbenzene
m- & p-Xylenes
o-Xylene
Styrene
ΣBTEXS
🟦 Inner olive trees (n=15)
🟥 Olive trees closer to road (n=15)

Tables

Table 1. BTEXS concentrations in olives and their corresponding virgin olive oil samples. Study of processing factor.

	Olives		Oil		F (%)
	Average [BTEXS] μg·kg^{-1}	RSD (%)	Average [BTEXS] μg·kg^{-1}	RSD (%)	
Benzene	68.82	7.32	96.05	16.69	**14.64 ± 1.60**
Toluene	59.05	9.07	247.62	13.68	**44.20 ± 5.05**
Ethylbenzene	104.19	11.69	1081.10	10.50	**110.35 ± 16.28**
m-, p-Xylene	53.55	5.70	256.57	10.09	**50.61 ± 5.97**
o-Xylene	45.10	5.30	88.50	11.17	**20.70 ± 2.43**
Styrene	143.95	10.29	1561.66	9.03	**115.39 ± 17.05**

Table 2. BTEXS concentrations obtained from the analyses of olives from trees closer to the road (contamination source) and from the inner of the olive plot.

	BTEXS concentration (µg·kg^{-1})						
	Sample code	**Benzene**	**Toluene**	**Ethylbenzene**	**m-, p-Xylene**	**o-Xylene**	**Styrene**
Olive samples from trees closer to road (A-401)	2x4	N.D.	21.3	8.1	9.4	7.2	0.8
	2x6	N.D.	22.9	10.6	12.5	10.0	0.6
	5x1	N.D.	2.8	0.8	1.1	N.D.	N.D.
	9x1	N.D.	3.7	1.2	1.7	N.D.	N.D.
	12x1	N.D.	5.3	2.2	2.7	2.0	0.5
	13x2	N.D.	5.4	1.7	2.2	1.8	0.6
	15x1	N.D.	3.8	1.3	1.9	1.7	0.5
	16x1	N.D.	6.1	2.8	3.4	2.6	N.D.
	17x1	N.D.	2.9	1.0	1.4	N.D.	N.D.
	18x1	N.D.	4.4	1.8	2.5	1.9	N.D.
	19x1	N.D.	3.3	1.2	1.5	1.4	0.5
	19x7	N.D.	1.2	0.4	0.7	N.D.	0.5
	20x1	N.D.	2.5	0.9	1.2	N.D.	N.D.
	21x1	N.D.	2.8	1.2	1.5	N.D.	N.D.
	27x1	N.D.	1.2	0.5	0.8	N.D.	N.D.
	Average (x_1)	N.D.	6.0 **(*)**	2.4 **(*)**	3.0 **(*)**	1.9 **(*)**	0.3
Inner olives samples	4x6	N.D.	0.9	0.4	N.D.	N.D.	N.D.
	5x7	N.D.	1.5	0.2	1.0	N.D.	N.D.
	6x7	N.D.	0.5	N.D.	N.D.	N.D.	0.5
	10x7	N.D.	2.1	N.D.	N.D.	N.D.	0.8
	10x8	N.D.	0.9	N.D.	N.D.	N.D.	0.5
	11x5	N.D.	2.1	0.5	N.D.	N.D.	0.7
	12x6	N.D.	1.2	0.6	N.D.	N.D.	0.7
	12x8	N.D.	1.0	0.3	N.D.	N.D.	0.5
	14x4	N.D.	0.7	N.D.	N.D.	N.D.	0.5
	15x6	N.D.	0.6	N.D.	N.D.	N.D.	0.5
	15x7	N.D.	0.6	N.D.	N.D.	N.D.	N.D.
	16x5	N.D.	0.4	N.D.	N.D.	N.D.	N.D.
	16x6	N.D.	1.2	0.4	N.D.	N.D.	0.7
	17x4	N.D.	0.6	N.D.	N.D.	N.D.	0.5
	17x6	N.D.	1.0	0.6	N.D.	N.D.	1.0
	Average (x_2)	0.0	1.0 **(*)**	0.2 **(*)**	0.1 **(*)**	0.0 **(*)**	0.5

N.D. Not detected.

(*) Differences between the two groups were statistically significant.