



Identification of Specific Odour Markers in Oil from Diseased Olive Fruits Using an Electronic Nose

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This study applied an electronic nose (e-nose) to identify specific odour markers in oil extracted from diseased olive fruits. Virgin olive oil (VOO) is a valuable vegetable oil extracted from fresh and healthy olive fruits. Anthracnose, the most important fungal disease of olives worldwide, results in damage to mature olive fruits. The HERACLES II e-nose (Alpha MOS, Toulouse, France) is based on dual fast gas chromatography technology (two columns of different polarities in parallel coupled to two flame ionization detectors). Therefore, two chromatograms are obtained simultaneously, providing a fingerprint of the product to allow an easy comparison of the overall odour profiles of a batch with the target profile. E-nose analysis made it possible to pre-screen specific odour compounds. Comparing the chromatograms showed important differences in the concentrations of volatile compounds between VOO and the oil extracted from diseased fruits. Twelve peaks were highly specific for oil from the diseased fruits. To allow the e-nose to determine the minimum limit of detection of the unique odour compounds, olive oil from healthy fruit was mixed with oil from diseased fruits at different concentrations. Alpha MOS's AroChemBase library was used to investigate the nature of the unique odour compounds in the oils based on the retention times of the main peaks. Almost all of these compounds were associated with off-odours of oil from diseased olive fruits. These results have shown that specific odour markers in oil extracted from diseased olive fruits can be identified using the e-nose.

1. Introduction

In the globalized agri-food supply chain, high standards of food quality, safety assurance and certification are some of the most important goals. The market for imported, premium-priced olive oil has increased dramatically over recent years because olive oil is a main component of the traditional Mediterranean diet which has been associated with a reduced risk of cardiovascular mortality as well as overall mortality (D' Alessandro and De Pergola, 2015). Olive oil is rich in monounsaturated fatty acids such as oleic acid, which has several therapeutic and health benefits (Rahmani et al., 2014). Virgin olive oil (VOO) is a valuable vegetable oil extracted from fresh and healthy olive (*Olea europaea* L.) fruits only by mechanical treatment. Extra VOO, the highest quality grade of olive oil, is defined as having a free fatty acidity of 0.8 % or less and peroxides at less than 20 mq/kg with storage properties that comply with standards established by the International Olive Council (IOC, 2015). Therefore, above all, a producer needing to guarantee the best quality olive oil must avoid contaminating olive fruits with hazardous microorganisms. In particular, anthracnose of olive, caused by a complex fungal species, is the most destructive disease of olive fruits and occurs widely in the humid olive-growing areas of many countries (Martin and Garcia-Figueres, 1999; Cacciola et al., 2012). Olive anthracnose causes heavy losses in yield and lowers the oil quality by affecting parameters such as acidity, peroxide value, the spectrophotometric indices K230 and K270, and the content of phenolic compounds (Moral et al., 2014). Contamination with diseased fruits must be avoided during the production of olive oil so producers need a detection system which can rapidly assess oil quality to prevent the possibility of contamination. Sensory evaluation plays a central role in characterizing and classifying olive oil (EU 796/2002; EU 640/2008). Instrumental analysis can contribute useful information for characterizing olive oil (Azizian et al., 2015; Diraman and Dibeklioglu, 2009; Gouvinhas et al., 2015; Mendes et al., 2015; Sinelli et al., 2010). The HERACLES electronic nose (e-nose) (Alpha MOS) is

based on the technology of ultra-fast chromatography. It features two metal capillary columns of different polarities mounted in parallel and coupled to two flame ionization detectors. Therefore, two chromatograms are obtained simultaneously, allowing the sharper identification of chemical compounds. This allows the pre-screening of chemical compounds and offers a sensory feature by directly clicking on the chromatogram's peaks generated by the e-nose. It has been practically demonstrated that the odour profiles obtained by the HERACLES e-nose could explain the sensory attributes of olive oil (Melucci et al., 2016; Kishimoto et al., 2017). The objective of this study is to identify the specific odour markers in oil from diseased olive fruits using an e-nose and to determine the lower detection limit of this oil.

2. Materials and methods

2.1 Materials

Olive (*Olea europaea* L.) fruits and fruits exhibiting anthracnose disease of the *Mission* cultivar and the fruits of the *Lucca* cultivar were harvested from *Olive-no-Mori* farm on Shodoshima in Kagawa prefecture, Japan.

2.2 Olive oil samples

A sample size of approximately 1 kg was used for the production of each olive oil sample. Each sample of olives was manually processed to olive paste then malaxed at 120 rpm for 40 min at 24 °C in a blender. Olive juice was obtained by pressing the malaxed olive paste in a juicer then centrifuging at 6,574 x g for 10 min at 20 °C in a SORVALL ST8R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). The top layer of the olive oil was collected then filtered through a paper filter. The oil was stored in amber bottles until analysis.

2.3 Analytical procedures

The free acidity, peroxide value (PV), total phenolic content and K270 of the oil samples were measured using an OxiTester (CDR, Ginestra Fiorentina, Italy) (Kamvissis et al., 2008). The free acidity and PV determined using the OxiTester method have been preliminarily confirmed against the official method of analysis using oil samples over a wide range of values (Gucci et al., 2012). An aliquot of oil sample (2.5 µL for measuring free acidity and PV and 10 µL for measuring total phenolic content and K270) was added to a prefilled cuvette. The vitamin A (retinol equivalent) and vitamin E (α -tocopherol) contents were measured by Japan Food Research Laboratories (Tokyo, Japan).

2.4 Flash gas chromatography electronic nose analysis

The headspace of the oil samples was analyzed using the HERACLES II electronic nose (Alpha MOS). The HERACLES II was equipped with two identical gas chromatography columns working in parallel mode: a non-polar column (MXT-5: 10-m length and 180-µm diameter) and a polar column (MXT-WAX: 10-m length and 180-µm diameter) that produced two chromatograms simultaneously. It was also equipped with an HS100 auto-sampler (CTC Analytics AG, Zwingen, Switzerland) to automate sample incubation and injection. For calibration, an alkane mix (from *n*-heptane to *n*-hexadecane) was used to convert retention times into Kovats indices. The analytical conditions were as follows: an aliquot of oil sample (2.0 g) was placed in a 20-mL vial and sealed with a magnetic cap. The vial was placed in the auto-sampler, which was placed in the HERACLES's shaker oven where it incubated for 15 min at 60 °C, while shaken at 500 rpm. A syringe sampled 5 mL of the headspace and then injected it into the gas chromatograph. The thermal program started at 40 °C (held for 10 sec) then increased to 250 °C at 1.5 °C/s. The final temperature was held for 60 s. The total separation time was 120 s. The data were acquired and processed using AlphaSoft software v14 (Alpha MOS). The AroChemBase module was used to identify the volatile compounds.

2.5 Statistical analyses

The data are presented as mean \pm standard deviation from three replicates. The Student's *t*-test was performed using Microsoft Excel 2013. Values of $p < 0.05$ were considered statistically significant ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$).

3. Results and discussion

3.1 Effect of the olive anthracnose on olive oil quality

The effect of olive anthracnose infecting the fruits on olive oil quality is shown in Table 1. The acidity and PV were very low and well within the limits set by EC regulation 1989/2003 (European Union, 2003) for categorising VOOs. The acidity, PV and K270 of oil extracted from the diseased fruits were much higher than those of VOO extracted from the healthy *Mission* fruits. These parameters were outside the limits for VOO classification, suggesting that the olive oil quality had been affected by the olive anthracnose. These results were consistent

with oil properties described previously (Iannotta et al., 1999; Moral et al., 2014). Phenolic compounds play an important role in evaluating the quality of VOO, because these compounds exhibit antioxidant activity and contribute to the oxidation stability of VOO (Lerma-Garcia et al., 2009). A great decrease in antioxidants (vitamins A, E, and phenolic compounds) was also observed in oil from the diseased fruits. Fungal species causing anthracnose are known to produce a range of hydrolytic, proteolytic and cellulolytic enzymes and metabolites which promote the penetration and colonization of host tissues (Agrios, 2005), which may result in decreasing the olive oil quality (Chliyah et al., 2014).

Table 1: Effect of olive anthracnose on oil quality.

Olive oils	Acidity (%)	PV (meqO ₂ /kg)	K270	Total phenolic content (mg/kg tyrosol)	Vitamin A content (µg/100 g)	Vitamin E content (mg/100 g)
VOO	0.05	8.38	0.117	318.7	6	18.5
Oil extracted from diseased fruits	3.07	33.70	0.464	Not detected	Not detected	5.4

3.2 Screening specific odour markers for oil from diseased fruits using an e-nose

To find specific markers for oil from diseased fruits, an e-nose analysis was performed. A comparison of the chromatograms from the two columns (MXT-5 and MXT-WAX) showed important differences in terms of the concentration of volatile compounds between oil extracted from diseased fruits and VOO from *Mission* fruits (Figure 1a vs. 1b and 1d vs. 1e). Twelve peaks were found to be highly specific for oil from diseased fruits. This specificity was observed regardless of the olive cultivar (Figure 1a vs. 1c and 1d vs. 1f). Screening specific odour markers for oil from diseased fruits was therefore successfully achieved using an e-nose.

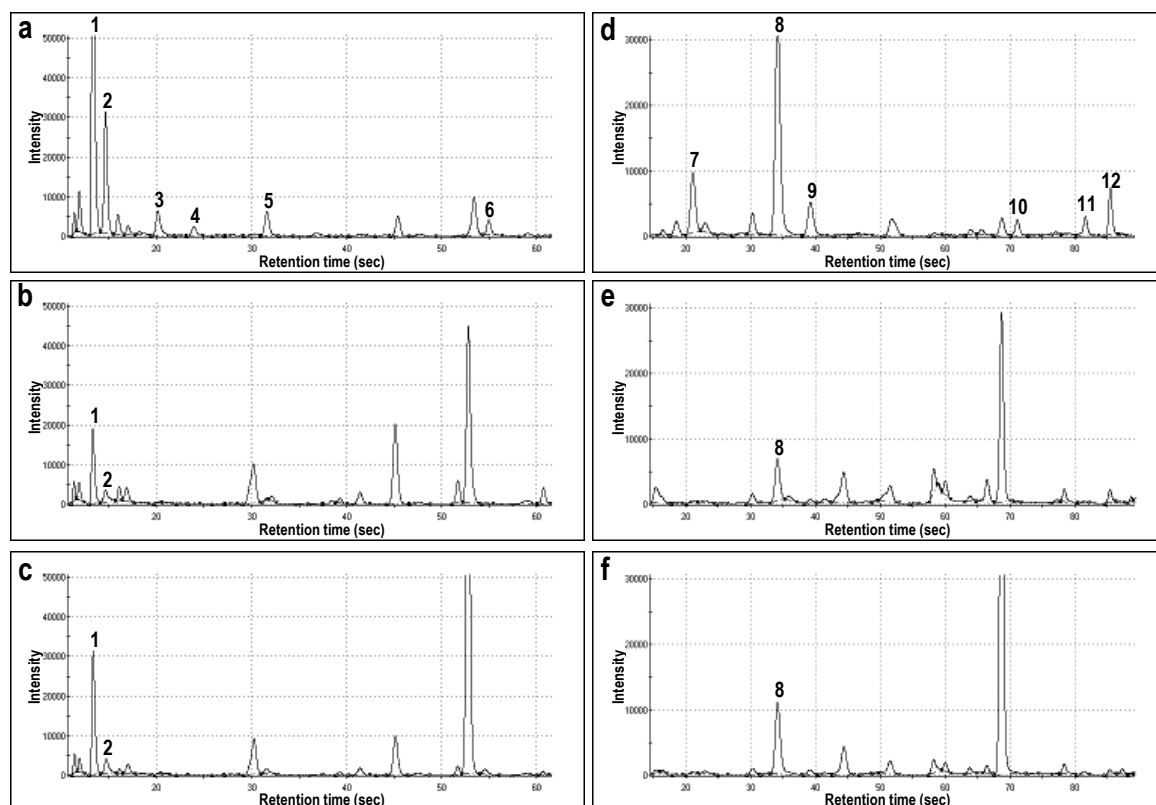


Figure 1: Raw e-nose data of volatile compounds in oils: a, d) oil from diseased fruits; b, e) oil from *Mission* fruits; c, f) oil from *Lucca* fruits. The panels on the left show chromatograms from the MXT-5 column and those on the right show chromatograms from the MXT-WAX column. The numbers show the peak numbers which were highly specific for oil from diseased fruits.

3.3 Evaluation of specific odour markers

To evaluate the twelve peaks shown in Figure 1, the relative ratios of areas of the specific peaks for oil from diseased fruits to VOO were compared statistically (Figure 2). All peak areas were significantly different, indicating that these peaks can be used as specific odour markers for oil from diseased olive fruits.

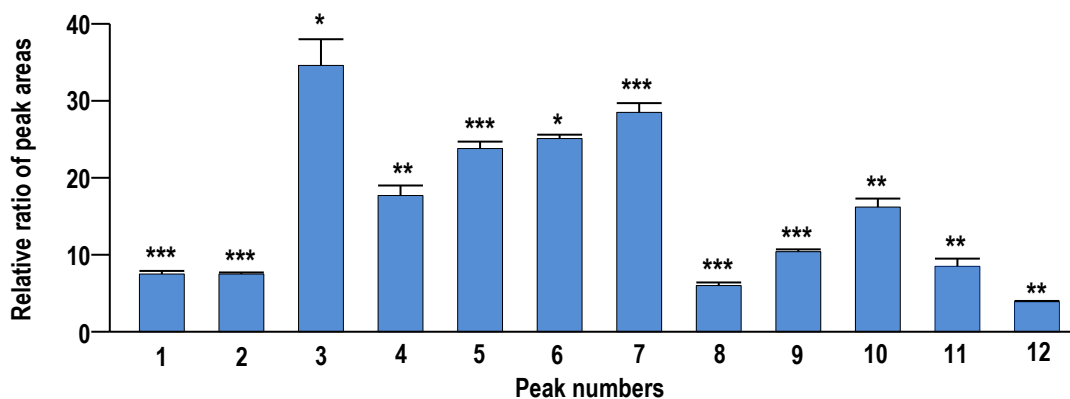


Figure 2: Relative ratio of peak area of oil from diseased fruits to VOO from Mission fruits. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The lower detection limit of these specific markers was also determined. VOO mixed with oil from diseased fruits at different concentrations (0 % to 10 %) were subjected to e-nose analysis. Figure 3 shows that the increase in the intensity of peak 1 depended on the concentration of added oil from diseased fruit, indicating that a level of 1 % contamination with oil from diseased fruits could be detected by the e-nose.

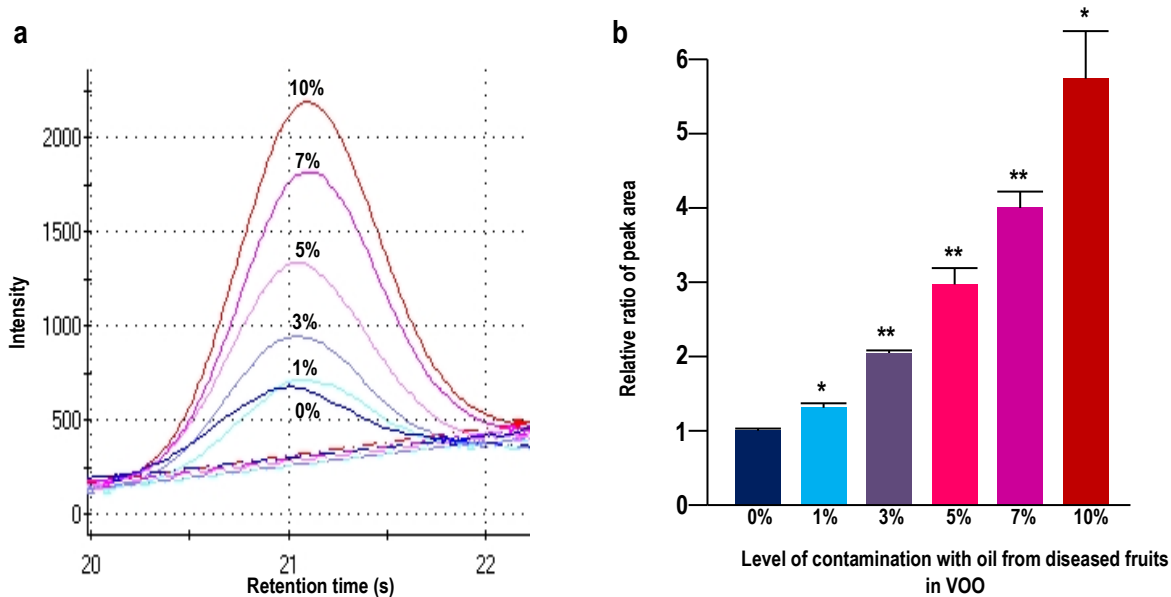


Figure 3: Determining the lower detection limit for contamination by oil from diseased olive fruits: a) the increase in intensity of peak 1 from the MXT-WAX column; b) relative ratio of area of peak 1 of oil mixtures to VOO. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.4 Identification of specific odour markers using the AroChemBase library

To identify specific odour markers, a cross search was made using two retention indices obtained on different columns. In the present study, the chemical composition could be determined using the AroChemBase and the Kovats indices from the MXT-5 and MXT-WAX columns. Table 2 shows the possible volatile compounds in oils from diseased fruit based on the retention time of the main peaks. Of the compounds identified, almost all were associated with oil off-odours. This could explain why oil produced from olives infected with fungi exhibits off-flavours (Cacciola et al., 2012).

Table 2: Possible volatile compounds identified in the oil from diseased olive fruits.

Retention time in MXT-5 (s)	Retention time in MXT-WAX (s)	Possible matching compounds	Odour descriptor
13.3	-	-	-
14.6	34.2	Ethanol	alcoholic
31.7	71.2	Methylthiocyanate	sulphurous
31.7	81.7	Acetoin	tallowy
31.7	85.6	Methylglycolate	-
20.2	21.1	2-Propanethiol	septic, garlic
20.2	39.3	2-Buten-2,3-dione	alcoholic
55.0	81.7	Hexanol	green, tallowy

4. Conclusions

The results of the present study have demonstrated that e-nose analysis can detect odours in oils extracted from diseased olive fruits. Twelve odour peaks found in oil from diseased fruits were significantly higher than those found in VOO. A level of 1 % contamination with oil from diseased fruits could be detected through these specific odour markers. These markers were identified as specific odour markers associated with oil off-odours. These results have shown that the e-nose can be an efficient means of characterizing olive oil. Combining rapid analysis using the e-nose system with a chemical library will be a powerful tool for the characterizing unknown olive oils and for the quality control of samples during routine analysis.

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