



Machine olfaction discrimination of Spanish-style green olives inoculated with spoilage mold species

Ramiro Sánchez^a, Francisco Pérez-Nevaldo^{b,c,*}, Sara Martillanes^{d,e}, Ismael Montero-Fernández^d, Jesús Lozano^f, Daniel Martín-Vertedor^a

^a Technological Institute of Food and Agriculture CICYTEX-INTAEX, Junta of Extremadura, Avda. Adolfo Suárez s/n, 06007, Badajoz, Spain

^b Área de Nutrición y Bromatología, Departamento de Producción Animal y Ciencia de los Alimentos, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Avda. Adolfo Suárez s/n, 06007, Badajoz, Spain

^c Research Institute of Agricultural Resources (INURA), Avda. de la Investigación s/n, Campus Universitario, 06071, Badajoz, Spain

^d Department of Agricultural and Forestry Engineering, Escuela de Ingenierías Agrarias, Universidad de Extremadura, vda. Adolfo Suárez s/n, 06007, Badajoz, Spain

^e Mediterranean Institute for Agriculture, Environment and Development (MED), Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal

^f Industrial Engineering School, University of Extremadura, 06006, Badajoz, Spain

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ABSTRACT

As an alternative to traditional sensory analysis evaluation, machine olfaction represents a valuable tool for detecting incipient defects caused by microorganisms. For this purpose, different strains of spoilage molds were inoculated into Spanish-style table olives and the effect on their sensory quality and volatile profile were analyzed using an electronic nose (E-nose). The main defects obtained for the different inoculated microorganisms associated with abnormal fermentation were mold and humidity. A total of 36 volatile compounds were identified and classified into phenolics, alcohols and carboxylic acids, while minor ones included derivatives of acids and oxygenated compounds. In general, a decrease in the concentrations of acetic acid, phenolic compounds and creosol was observed in olives inoculated with mold strains. However, propanoic acid, 2-methoxyphenol, 2,4-dimethyl-heptane, among others, increased after mold inoculation. Sensorial and volatile compound analysis showed the table olives inoculated with the strains of *Aspergillus flavus* A.F.18 and *Penicillium expansum* P.E.20 to be the most altered. The E-nose data were able to classify the inoculated olives into different categories and distinguish them from the control treatment, regardless the intensity of the defect. The principal component analysis (PCA) of table olives inoculated showed that 70.23% of the total variance of data was explained by PC1 and 23.27% by PC. The sum of the elements of the diagonal of the confusion matrix gives the percentage of success in discrimination of 95.5%. Therefore, these results show the capacity and precision of E-nose to discriminate between the alterations caused by different mold strains.

1. Introduction

Green table olives are a popular food worldwide, with their green color being an indication of freshness (Gandul-Rojas et al., 2016). Spanish-style green olives are of significant economic importance, especially in the Mediterranean countries (Papadaki & Mantzouridou, 2016). According to the International Olive Council (IOC) (2020), the annual production in Mediterranean countries exceeds three million tons, and 50% of that market is represented by the Spanish-style green olive. This consumption is increasing throughout the world, due to its pleasant sensory characteristics and nutritional benefits (Ramírez et al.,

2017). During the fermentation process of green table olives a wide variety of microorganisms develop; some lactic acid bacteria and yeasts are considered to be of interest in obtaining a quality product (Arroyo-López et al., 2012; Hernández et al., 2007; Hurtado et al., 2012; Schaide et al., 2019; Zago et al., 2013). However, spoilage of table olives is of great concern and is mainly due to microorganisms that cause visual deterioration and affect their sensorial properties such as smell, taste and texture (De Castro et al., 2022; Franzetti et al., 2011). Molds are considered spoilage microorganisms responsible for alterations such as softening, negative flavor characteristics and bad appearance. Also, these microorganisms are producers of mycotoxins, a group of

* Corresponding author. Área de Nutrición y Bromatología, Departamento de Producción Animal y Ciencia de los Alimentos, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Avda. Adolfo Suárez s/n, 06007, Badajoz, Spain.

E-mail address: fpen@unex.es (F. Pérez-Nevaldo).

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secondary metabolites considered a relevant health threat (Bavaro et al., 2017). Within fungi, *Aspergillus* and *Penicillium* are the most representative genera in table olives and cause major spoilage problems in this product (Bavaro et al., 2017). An International Olive Council (IOC, 2020) regulation established a recommendation to classify table olives into different categories according to sensory analysis. A tasting panel analyzes table olives according to olfactory defects such as putrid, zapateria, butyric, musty, rancid, or vinegary sensations (Lanza & Amoruso, 2016; Martín-Vertedor et al., 2021). Uncontrolled industrial practices could facilitate the development of the fermentation process that could cause these alterations.

Alterations in table olives are currently detected using gas chromatography or spectroscopic methods such as mass spectrometry, ion mobility or infrared spectrometry. However, their analysis processes are slow, complex, and voluminous (Seesaerd et al., 2022). For this reason, multisensory analysis systems such as VE-tongue and E-nose are used as an alternative method. Unlike traditional methodologies, these multisensory analysis systems are fast, reliable, inexpensive and can be used in complex environments without the need for highly qualified personnel (Wilson, 2018). The E-nose is an advance in bioengineering based on a system of sensors that allows the detection of Volatile Organic Compounds (VOCs). This is a powerful tool to quickly discriminate samples that release VOCs in a non-destructive way (Loulrier et al., 2020). For example, Sánchez et al. (2021) used an E-nose to discriminate anomalous fermentations in Spanish-style table olives. Therefore, the objective of this work is to discriminate defective olives that were inoculated with different mold strains by using an E-nose and validating the results obtained with the sensory and gas chromatography analysis.

2. Material and methods

2.1. Experimental design

The ‘Carrasqueña’ variety of Spanish-style table olives was used throughout this study. The olives were fermented, pitted, and submerged in a brine by a company in the southwest of Extremadura (Spain). The olives were transported to the laboratory and stored refrigerated for a maximum of 24 h until use in the experiment. Prior to the inoculation, the olive brine was diluted with distilled water to achieve a final salt concentration of 3.75%, and the pH was adjusted to 4.5 to promote the growth of the inoculated microorganisms. Next, 50 g of pitted olives and 70 g of the modified brine were placed into glass jars and pasteurized at 80 °C for 20 min to eliminate microorganisms present that could interfere with the study.

Pasteurized olives with brine were inoculated with nine spoilage mold strains: 1 strain of *Galactomyces* (*G. geotrichum*, G.G.2). 4 strains of *Penicillium* (3 *P. expansum*, P.E.3, P.E.4 and P.E.20; and 1 *P. glabrum* P. G.19). 3 strains of *Aspergillus* (*A. flavus* A.F.9, A.F.18 and A.F.21). 1 of *Fusarium* (*F. solani* F.S.11). These microorganisms had been isolated, identified and characterized during the fermentation process from table

olives (Pérez-Nevado et al., 2011). Prior to the inoculation, mold strains were grown in YPD agar (Condalab, Madrid, Spain) at 25 °C for 10 days. Mold spores were then collected in distilled water and the spore number was determined using a Neubauer improved chamber. The spore suspensions were diluted properly to inoculate a concentration of 10^5 spores/mL in the olives with brine. Uninoculated control olives were carried out. All treatments were performed in triplicate.

After the inoculation, the olives were incubated for 30 days at room temperature (18 °C). Volatile compound analysis, sensory analysis and headspace measurements of the samples using an E-nose were performed at the end of the incubation. A diagram of the experiment is shown in Fig. 1.

2.2. Analyses

Three analyses were carried out on the table olives inoculated with the different mold strains and on the control, non-inoculated olives. Specifically, sensory analysis, chromatographic analysis of volatile compounds and E-nose measurements were performed.

2.2.1. Sensory analysis

Table olives were evaluated by a sensory panel composed of eight experts from the CICYTEX research center (Extremadura, Spain) and the University of Extremadura, trained according to IOC recommendations (IOC, 2011). The sensory evaluation was carried out in an individual testing cabin inside one of the tasting rooms (ISO 8589). The intensity and type of off-odor perceived by the tasters was assessed on a structured scale from 0 to 10 to evaluate positive and negative attributes. Sensory evaluation outcomes were expressed as average values. Values were considered valid when the coefficient of variation was less than 20.

2.2.2. Volatile compound analysis

Volatile compounds were determined using a Bruker Scion 456-GC triple quadrupole gas chromatograph with a DB WAXETR capillary column (60 m × 0.25 mm; ID: 0.25 mm) following the same procedure as reported in previous literature (López-López et al., 2019; Sánchez et al., 2021). Briefly, approximately 10 g of pitted olives were homogenized. Aliquots of 2.0 g of paste were placed in a 15 mL glass vial with 7.0 mL of 30% (w/v) NaCl. A polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 µm, Supelco) was manually inserted into the sample vial for headspace extraction of the sample at 40 °C for 30 min. After extraction, the fiber was inserted into the injection port of the GC for desorption at 250 °C for 15 min and the analysis was performed. The identification of the compound was based on the coincidence of mass spectra with the NIST standard reference database.

2.2.3. E-nose

The E-nose was designed by the University of Extremadura (Spain) (Arroyo et al., 2020). It consisted of an array of 11 metal oxide (MOX) sensors spread over four chips: BME680 from Bosch, SGP30 from Sensirion and CCS811 and iAQ-Core from ScioSense. The sensors of the

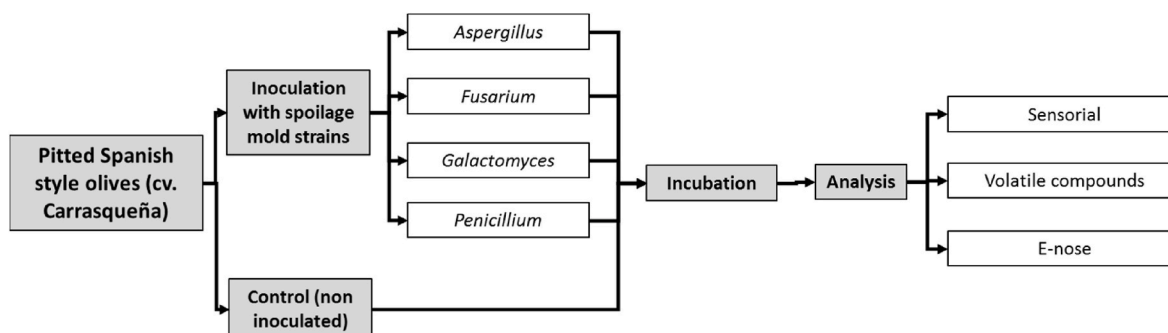


Fig. 1. Diagram of the experimental design.

portable E-nose (39 mm × 33 mm) measured the headspace of the samples and sent data to a smartphone via Bluetooth.

Headspace measurements of the samples were performed for sensory analysis by a table olive tasting panel, as recommended by the International Olive Council (IOC, 2011). Standard tasting glasses with four olives and 5 mL of brine were placed on a heating block at 25 °C and covered with a watch glass. The E-nose data collection was divided into two phases: i) adsorption phase, where the volatile compounds were put in contact with the sensors for 60 s; and ii) desorption phase, where sensors were put in contact with the air in an empty cup for 30 s. The E-nose recorded data at 1 s intervals and the system took a reading of the resistive value supplied by each sensor. Eight measurements were taken for each sample of table olives.

2.3. Statistical analysis

One-way ANOVA was used followed by Tukey's multiple range test in sensorial analysis to establish statistically significant differences between different thermal treatments within each of the table olive varieties. Significance was set at $p < 0.05$. SPSS 18.0 software was used for statistical analysis (SPSS Inc. Chicago, IL, USA). Data were expressed as means and standard deviations (SD).

The data obtained by the E-nose were processed by MATLAB provide source. These data were treated by an unsupervised exploratory principal component analysis (PCA) (Abdi & Williams, 2010). This analysis was used to show how the values of the olive samples altered with different molds were grouped according to their volatile compounds profile and also to try to discriminate between the strains of the two most frequent mold genera in table olives (*Aspergillus* and *Penicillium*). By performing a PCA, a reduction in the dimension of the input variables could be carried out, thereby obtaining principal components that were linear combinations of original response vectors. Since the study variables were measured according to different units, the original variables were auto scaled.

Next, a supervised classification analysis by the name of partial least squares discriminant analysis (PLS-DA) was applied (Barker & Rayens, 2003). This analysis applies an algorithm that identifies the components or latent variables (LV) that discriminated between groups of different samples. It examined the X matrix developed from the data according to the maximum covariance with a target class defined in the Y matrix. From this analysis we could obtain the confusion matrix. Several PLS-DA models were built with different classification objectives. Specifically, one model was developed to discriminate between olive samples altered by mold strains of different species, and another to discriminate between olive samples altered by mold strains of the same species. A confusion matrix was constructed to derive the cross-validation predictions. The proportion of correct predictions was calculated from the sum of the diagonal elements found in the confusion matrices.

Table 1

Predominantly perceived sensory defects (mean ± standard deviation) of Spanish-style table olive inoculated with different mold strains (*Galactomyces geotrichum* G. G.2, *Penicillium expansum* P.E.3, P.E.4 and P.E.20; *Aspergillus flavus* A.F.9, A.F.18 and A.F.21; *Fusarium solani* F.S.11; and *Penicillium glabrum* P.G.19), and uninoculated (C). Different small letters indicate significant statistical differences according to the microorganisms inoculated (Tukey's Test, $p < 0.05$).

Attribute	C	G.G.2	P.E.3	P.E.4	P.E.20	A.F.9	A.F.18	A.F.21	F.S.11	P.G.19
Positive attribute										
Fruity	4.0 ± 0.1d	2.0 ± 0.3b	2.0 ± 0.4b	2.0 ± 0.2b	2.0 ± 0.4b	2.0 ± 0.4b	1.5 ± 0.6a	1.5 ± 0.5a	2.5 ± 0.6c	2.5 ± 0.4c
Fermented	4.0 ± 0.2d	2.0 ± 0.5b	2.0 ± 0.3b	2.0 ± 0.1b	2.0 ± 0.3b	2.0 ± 0.5b	1.5 ± 0.4a	1.5 ± 0.4a	2.5 ± 0.4c	2.5 ± 0.4c
Vinegar	3.0 ± 0.3b	1.5 ± 0.2a	1.5 ± 0.3a	1.5 ± 0.2a	1.5 ± 0.2a	1.5 ± 0.4a	1.5 ± 0.3a	1.5 ± 0.3a	1.5 ± 0.3a	1.5 ± 0.3a
Sweet	n.d	2.0 ± 0.6b	n.d	n.d	n.d	n.d	1.0 ± 0.5a	n.d	n.d	n.d
Negative attribute										
Mold	n.d	n.d	3.0 ± 0.3c	3.0 ± 0.1c	3.0 ± 0.4c	3.5 ± 0.3d	4.5 ± 0.3e	3.0 ± 0.3b	2.0 ± 0.4a	3.0 ± 0.4b
Humidity	n.d	1.0 ± 0.2a	2.0 ± 0.2c	1.0 ± 0.6a	2.0 ± 0.5c	1.5 ± 0.3b	4.0 ± 0.2f	2.5 ± 0.4d	2.0 ± 0.3c	3.0 ± 0.4e
Woody	n.d	2.0 ± 0.3a	2.0 ± 0.5a	3.0 ± 0.5b	3.0 ± 0.2b	2.0 ± 0.4a	4.0 ± 0.2c	3.0 ± 0.5b	2.0 ± 0.2a	3.0 ± 0.2b
Leather	n.d	n.d	2.0 ± 0.6a	3.0 ± 0.4b	3.5 ± 0.3c	4.0 ± 0.6d	3.5 ± 0.3c	2.0 ± 0.6a	2.0 ± 0.3a	3.0 ± 0.4b
Toasted	n.d	1.0 ± 0.2a	1.5 ± 0.2b	1.5 ± 0.3b	2.0 ± 0.4c	2.0 ± 0.3c	4.0 ± 0.2e	2.0 ± 0.4c	1.0 ± 0.4a	3.0 ± 0.6d
Rancid	n.d	n.d	n.d	1.5 ± 0.3b	1.5 ± 0.6b	2.5 ± 0.3d	1.0 ± 0.4a	3.0 ± 0.4e	2.0 ± 0.4c	2.5 ± 0.7d
Chemical	n.d	n.d	n.d	1.0 ± 0.2a	3.0 ± 0.3d	n.d	1.5 ± 0.5b	2.5 ± 0.4c	1.0 ± 0.4a	n.d

3. Results and discussion

3.1. Sensory aroma of inoculated olives

Table 1 shows the sensory evaluation of the Spanish table olives inoculated with molds according to the predominantly perceived defect (PPD). Some significant differences were obtained for the various mold strains inoculated for each sensory attribute. The control treatment (C), olives not inoculated with molds, showed positive attributes, with a high intensity of fruity, fermented, and vinegary flavors. Tasters did not detect any negative attributes.

However, table olives inoculated with different mold strains showed some sensory defects associated with abnormal fermentation. The main defects found in olives inoculated with altering microorganisms were mold and humidity ranging from 0 to 4.5 points, although the values of the moldy attribute were greater than those of the humidity. Further outstanding attributes were woody and leathery odors. These defects were detected in olives in less quantity. Table olives inoculated with *Aspergillus flavus* A.F.18 presented the highest score of these attributes, while the lowest was assigned in *Galactomyces geotrichum* G.G.2. The sensory toasted attribute was also detected in all the inoculated olives with microorganisms. Rancid and chemical attributes were detected in lower concentrations in some of the inoculated olive samples. In general, the greatest negative sensory defects were attributed to the olives inoculated with *A. flavus* A.F.18 and A.F.20; and *Penicillium expansum* P. E.20; while the lowest values were assigned to *G. geotrichum* G.G.2, *P. expansum* P.E.3 and *Fusarium solani* F.S.11. These microorganisms were also responsible for the decrease in the intensity of the fruity, fermented, and vinegary attributes of the olives after the fermentation process. In the same way, Marx et al. (2017) classified table olives and brine solution according to sensory attributes including mold alterations.

In accordance with the legislation (IOC, 2011), and taking into account only sensory attributes, olives inoculated with *A. flavus* A.F.18 and A.F.9 could be classified into the second or standard category as the PPD was higher than 3.5 and less than or equal to 6.0. The rest of the inoculated olives could be classified into the first category ($2 < \text{PPD} \leq 3.5$). Thus, all these olives could be legally marketed despite the significant defects. This result has interesting consequences for table olive producers who are required to carry out necessary quality controls to avoid the development of undesirable microorganisms in olives during the fermentation process.

3.2. Volatile compounds of inoculated olives

Fig. 2 shows the distribution of volatile compounds classified according to different families in table olives inoculated with different mold strains. There are statistically significant differences among the

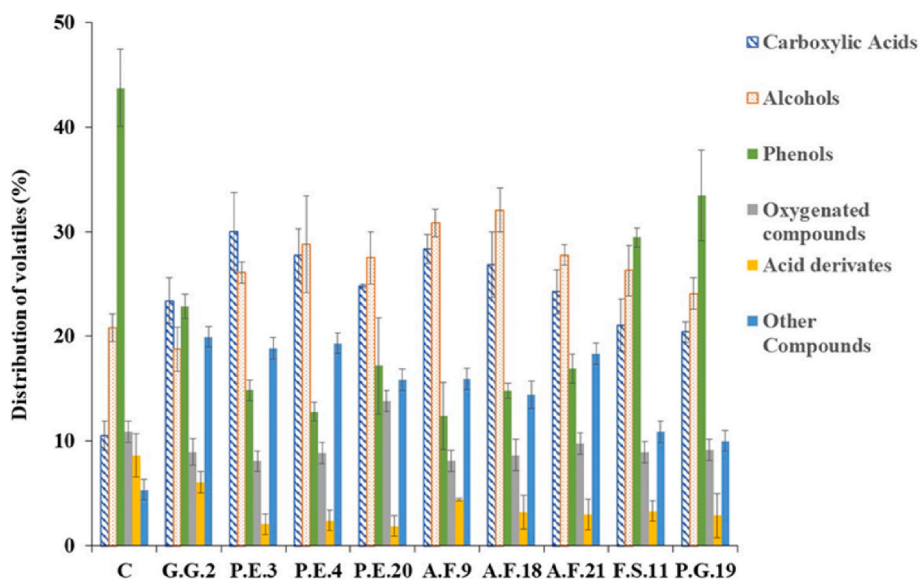


Fig. 2. Chemical distribution of volatile compounds in control olives and olives inoculated with different mold strains. The bars of each column show standard deviation (SD).

different families of volatile compounds analyzed in olives inoculated with different strains. Most of the volatile compounds were phenolics, alcohols and carboxylic acids, while the minor ones were derivatives of acids, oxygenated and other compounds. Different researchers have indicated that volatile compounds are mainly formed through microbial fermentation reaction instead of fatty acids, sugar fermentations or amino acid conversions metabolism (Üçüncüoğlu & Sivry-Ozay, 2020).

Carboxylic acids increased significantly for the different olives inoculated. Olives inoculated with *P. expansum* P.E.3 (30.02%) and *A. flavus* A.F.9 (28.33%) presented 66% more of these compounds than olives without inoculation (C). The alcohols increased slightly in relation to the control (20.86%), except for the *G. geotrichum* G.G.2 sample (18.78%). This increase in alcohol concentrations is associated with natural fermentation processes (Bleve et al., 2014). The highest concentrations of phenolic compounds were found in C. However, olives inoculated with different mold strains showed a decrease in phenol content. The highest concentrations of these compounds were found in *P. glabrum* P.G.19 and *F. solani* F.S.11, but the concentrations represented around 23% less than C. The olives inoculated with *Penicillium expansum* (P.E.4, P.E.3 and P.E.20), and *Aspergillus flavus* (A.F.9, A.F.18 and A.F.21) were those with the lowest values, 70% less than C. The decrease in phenols in the inoculated olives may be due to the microbial enzymatic activity of the mold strains (Benicasa et al., 2015). Authors such as Fernández et al. (2020) and Othman et al. (2009) pointed out that there is a decrease in phenolic compounds through fermentation. Many of these phenolic compounds come from glycosides found in olives such as oleuropein, ligustroside and dimethyloleuropein and are transformed by the action of oxidative enzymes (Landa et al., 2019). Certain mold species such as *Aspergillus*, naturally found in olive fruits, produce lipase and lipoxygenase. These two enzymes contribute to the biogenesis of volatile aromatic compounds and could affect the organoleptic properties of table olives (Fakas et al., 2010). Furthermore, Smid and Kleerebezem (2014) remarked that the primary metabolites that exist in brine solution are aromatic compounds precursors produced by specific mold strains. Microorganisms have a different way of metabolizing the different substrates to produce volatile compounds (Ricci et al., 2018).

The presence of oxygenated compounds (aldehydes, ketones, and ethers) does not show a differentiated trend. The olives inoculated with *P. expansum* P.E.20 showed the highest concentrations. Acid derivatives decreased when the olives were inoculated with the different mold strains. Finally, another group of volatile compounds such as

hydrocarbons and secondary metabolites increased in olives inoculated with *G. geotrichum* G.G.2 (19.95%), *P. expansum* P.E.4 (18.88%) and *A. flavus* A.F.21 (18.32%).

Table 2 shows the volatile compounds profile of green table olives inoculated with different mold strains (*G. candidum*, *P. expansum*, *A. flavus*, *F. solani* and *P. glabrum*). In total, 36 volatile compounds were identified and grouped into the families of volatile compounds (Fig. 2). The main volatile compounds were propanoic acid, 3-methyl-butan-1-ol, 1-propanol and creosol, and the minor ones were 1-methoxy (Z)-3-hexene and 4-hexen-1-ol-acetate.

The carboxylic acids showed the lowest concentrations in the altered olives in the different mold strains. This may be due to the fact that some volatile compounds of this family increased or decreased in the inoculated samples (Table 2). Acetic acid decreased in some of the inoculated olives; these altering microorganisms could have consumed the acids in the medium. Other relevant carboxylic acids responsible for differences between olives were propanoic acid, with higher concentrations in *P. expansum* P.E.3 (13.06%) and P.E.4 (12.04%), followed by butanoic acid, with higher concentrations in *A. flavus* A.F.9 (11.12%) and A.F.18 (10.74%). It should be noted that butanoic acid is responsible for the cheesy, sharp, and dairy-like odor and therefore contributes negatively to the brine sensorial properties (Montaño et al., 2021).

On the other hand, the results showed that some higher alcohols were found in the olives inoculated with molds. Certain volatiles produce unpleasant odors such as 1-propanol, which gives sensory properties to olives such as alcoholic, abnormal fermentation and a musty smell (Montaño et al., 2021). Furthermore, 3-methyl-butan-1-ol produces a woody, whiskey and sweet smell (Sánchez et al., 2021). These negative volatile compounds increased in the inoculated olives with molds. The highest concentrations of these undesirable compounds are shown in *A. flavus* A.F.9 and A.F.18, representing 70% more than the treatment without inoculation. On the contrary, we also found volatile compounds with positive sensory implications with floral or fruity aromas such as (E)-3-hexen-1-ol and benzyl alcohol, and these decreased with different mold strains. It should be noted that creosol, the main phenolic compound in this type of olive, decreased when the olives were inoculated with these spoilage microorganisms. This is responsible for smoky, sweet, and spicy odors in olives (Sánchez et al., 2018). Its concentration decreased after microbial growth. However, the volatile compounds that provide unpleasant aromas such as 2-methoxy-phenol, increased their concentration with the development of spoilage molds.

Table 2

Content of volatile compounds (mean %, n = 3) obtained from table olives inoculated with different mold strains (*Galactomyces geotricum* G.G.2, *Penicillium expansum* P.E.3, P.E.4 and P.E.20; *Aspergillus flavus* A.F.9, A.F.18 and A.F.21; *Fusarium solani* F.S.11; and *Penicillium glabrum* P.G.19), and uninoculated (C). RT = retention time.

Volatile Organic Compound	R.T. (min)	C	G.G.2	P.E.3	P.E.4	P.E.20	A.F.9	A.F.18	A.F.21	F.S.11	P.G.19
Carboxylic Acids											
Acetic acid	2,4	8,74	5,80	5,68	5,87	6,79	5,37	5,84	6,67	8,86	9,10
Propanoic acid	3,9	1,08	9,50	13,06	12,04	10,33	11,54	9,86	8,53	5,43	5,35
Butanoic acid	8,2	n.d.	8,10	10,67	9,29	7,17	11,12	10,74	8,36	6,03	5,32
2-methyl-butanoic acid	10,2	0,72	n.d.	0,62	0,59	0,50	0,31	0,40	0,70	0,76	0,65
Alcohols											
1-propanol	2,0	5,24	5,60	9,88	10,29	9,21	11,63	12,15	12,37	9,92	7,10
3-methyl-butan-1-ol	4,5	1,66	7,80	10,80	9,59	7,88	12,83	12,61	8,95	9,35	9,37
(E)-3-hexen-1-ol	9,3	4,94	1,30	0,98	2,90	2,66	n.d.	0,67	0,75	0,78	0,77
Benzyl alcohol	18,9	7,44	3,00	3,50	3,49	3,13	5,32	5,85	5,03	5,68	6,13
Farnesol	22,6	1,56	1,08	0,95	2,53	4,61	1,06	0,77	0,68	0,56	0,72
Phenols											
2-methoxy-phenol	21,3	2,82	6,39	3,08	3,25	3,38	2,81	2,79	3,34	2,81	2,69
Phenylethyl alcohol	22,8	4,20	5,72	4,76	4,05	5,40	1,53	4,38	4,24	6,44	6,50
Creosol	27,0	36,70	10,74	7,01	5,50	8,42	8,05	7,61	9,30	20,19	24,26
Oxygenated compounds											
1-methoxy-hexane	7,7	n.d.	0,22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-methoxy-(Z)-3-Hexene	7,9	0,18	0,42	0,19	0,22	0,21	0,24	0,18	0,25	0,20	0,22
6-methyl-5-hepten-2-one	16,0	5,72	2,35	2,22	4,62	5,83	5,35	5,22	5,38	5,41	5,54
Octanal	17,1	1,23	n.d.	0,87	0,72	1,19	0,45	0,69	1,01	0,63	0,91
Nonanal	22,3	1,70	3,74	3,34	1,38	2,27	0,38	0,41	1,49	0,83	1,17
(E)-2-decenal	29,9	2,07	2,24	1,48	1,93	3,27	1,70	2,17	1,64	1,89	1,31
Acids derivates											
n-Propyl acetate	3,8	1,30	n.d.	1,60	1,84	1,47	2,06	1,28	1,04	1,14	0,60
Methyl isovalerate	5,7	n.d.	1,97	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propanoic acid, 2-hydroxy-, ethyl ester	7,2	0,27	n.d.	0,31	0,34	0,25	0,26	0,29	0,28	0,28	0,29
2-Butenoic acid, 3-methyl-, methyl ester	8,7	n.d.	0,40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-methylbutyl acetate	10,3	3,55	0,98	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hexanoic acid, methyl ester	12,8	n.d.	0,35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hexanoic acid, ethyl ester	16,8	n.d.	0,77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(Z)-3-Hexen-1-ol, acetate	17,1	3,36	1,59	n.d.	n.d.	n.d.	1,91	1,51	1,49	1,66	1,71
4-Hexen-1-ol, acetate	17,5	0,17	n.d.	0,14	0,24	0,17	0,17	0,15	0,19	0,25	0,29
Other compounds											
2,4-dimethyl-hexane	6,5	0,26	0,65	0,22	0,36	0,41	0,43	0,29	0,55	0,19	0,27
2,4-dimethyl-heptane	7,0	0,52	8,81	11,03	10,52	8,07	9,52	9,17	8,17	6,13	5,11
Octyl-cyclohexane	8,0	n.d.	n.d.	n.d.	n.d.	0,19	n.d.	0,14	0,27	0,16	0,17
Ethyl-cyclohexane	8,0	0,18	0,31	0,12	0,25	0,18	0,13	0,12	0,23	0,15	0,16
2,4-dimethyl-1-heptene	8,3	0,11	0,42	0,12	0,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-ethyl-hexane	9,4	1,07	1,98	1,01	1,36	1,36	1,46	1,26	2,63	1,06	1,63
4-terpinenyl acetate	28,3	0,80	1,74	0,83	0,84	0,88	0,79	0,80	1,67	0,81	0,68
Copaene	34,8	2,07	5,30	5,31	5,48	4,26	3,21	2,34	4,36	2,11	1,76
α -muurolene	40,0	0,34	0,74	0,24	0,39	0,51	0,38	0,32	0,43	0,30	0,24

The higher concentrations of this altering compound were produced by *G. geotricum* G.G.2, while *P. glabrum* P.G.19 and *F. solani* F.S.11 presented the highest creosol content. The concentration of phenylethyl alcohol, responsible for the mild rose odor, was not significant in the altering molds, except for *A. flavus* A.F.9.

Acid derivatives constitute another group that influences the organoleptic properties of the aroma, albeit to a lesser extent. This study identified a total of nine esters, the predominant being ethyl and methyl esters. Many were detected in C but not in the inoculated olives. The main esters were n-propyl acetate, which were found in higher concentration in *A. flavus* A.F.9 and P.E.4, and ethyl ester 2-hydroxypropionic acid, with a higher amount detected in *P. expansum* P.E.4 (0.34%) and P.E.3 (0.31%). The decrease in ester concentrations can be attributed to hydrolytic reactions since the microorganisms present in the brines use dissolved oxygen in their own metabolic reactions (Sánchez et al., 2018).

Other volatile compounds could affect the sensory characteristic of the final product such as the hydrocarbon 2,4-dimethyl-heptane. This compound is characterized by its strong, pungent plastic smell, similar to the odor of recycled resin (Fuller et al., 2020). This hydrocarbon was found in major concentrations in olives inoculated with *P. expansum* P.E.3. and P.E.4 strains. Sesquiterpenes such as copaenes also stand out, which positively influence the organoleptic properties with a woody and honey odor (Yao et al., 2021). The highest concentrations of this compound were found for the *P. expansum* P.E.3. and P.E.4 strains. Finally,

α -muurolene, which presents an herbal aroma and odor of truffle (Gioacchini et al., 2008), offered higher contents in certain olives inoculated with the mold strains, showing higher concentrations for *P. expansum* P.E.20 and *A. flavus* A.F.21.

3.3. Discrimination of table olive spoiled with different mold species with E-nose

The E-nose data from table olives inoculated with different mold species showed multiple variables. To determine the relationships between the E-nose measured variables, the first step was to reduce the variables using a principal component analysis (PCA). The PCA plots visually represent how similar data are grouped together. Fig. 3 shows a first separation between C olives and those altered by molds.

The PCA results showed that 70.23% of the total variance of data was explained by PC1 and 23.27% by PC2. The model based on the first two components showed a clear differentiation of the samples according to the volatile compounds profile and was able to separate healthy olives from those altered with the different molds.

A classification analysis was then performed using the PLS-DA and leave-one-out cross-validation. Thus, the classification results as a confusion matrix are shown in Table 3. The sum of the diagonal elements of the confusion matrix gives the percentage of success in the classification; in this case, 95.5% was obtained. These results prove the ability and accuracy of the E-nose to discriminate between different

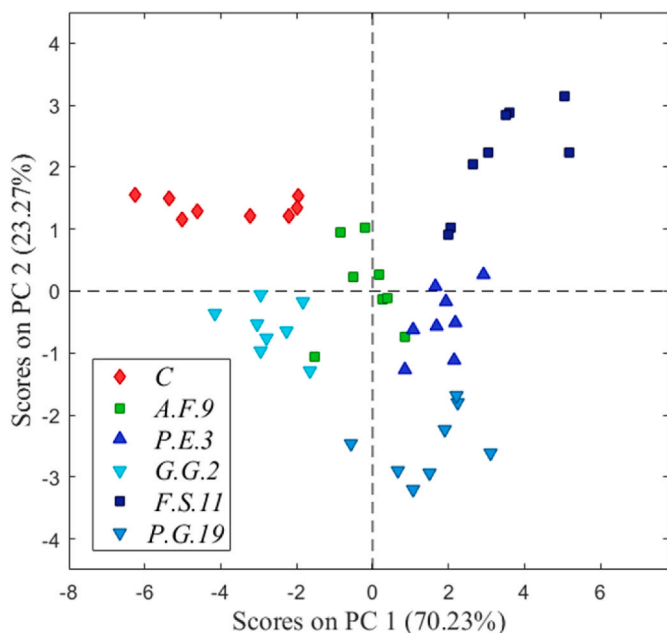


Fig. 3. Score plot of the PCA analysis for healthy olives (control) and olives altered with the molds *Aspergillus flavus* A.F.9, *Penicillium expansum* P.E.3, *Galactomyces geotrichum* G.G.2, *Fusarium solani* F.S.11 and *Penicillium glabrum* P.G.19.

Table 3

Confusion matrix obtained through PLS-DA for discrimination between control (C) and olives inoculated with mold strains (*Aspergillus flavus* A.F.9; *Penicillium expansum* P.E.3; *Galactomyces geotrichum* G.G.2; *Fusarium solani* F.S.11; and *Penicillium glabrum* P.G.19). Values are expressed in percentage.

Real Class	Predicted Class					
	C	A.F.9	P.E.3	G.G.2	F.S.11	P.G.19
Control	16.6	0	0	0	0	0
A.F.9	0	12.5	0	0	0	0
P.E.3	0	2.1	16.6	0	0	0
G.C.2	0	0	0	16.6	0	0
F.S.11	0	2.1	0	0	16.6	0
P.G.19	0	0	0	0	0	16.6

alterations caused by molds (*A. flavus* A.F.9, *P. expansum* P.E.3, *G. geotrichum* G.G.2, *F. solani* F.S.11 and *P. glabrum* P.G.19) and compare them with healthy control olives.

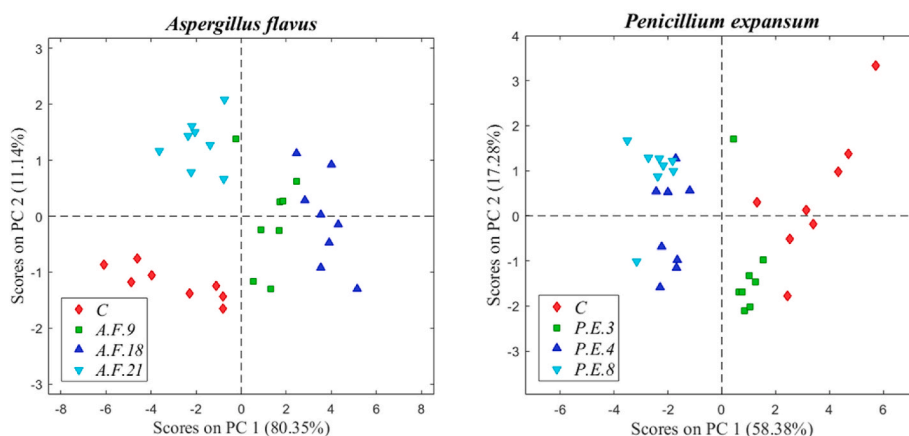


Fig. 4. Score plot of the PCA for healthy olives (control) and molds of species *Aspergillus flavus* (left) and score plot of the PCA for healthy olives (control) and mold strains of *Penicillium expansum* (right).

Thus, the outcomes show that the E-nose can discriminate olives according to their health status. These findings are consistent with those obtained with the sensorial (Table 1) and volatile profile analysis (Fig. 2 and Table 2), differentiating between olives with different mold strains, and confirming the suitability of the E-nose for distinguishing between different alterations during the table olives process. This is an interesting result given that this tool could be useful at industrial level in detecting incipient alterations during the tank fermentation in Spanish-style table olives. Therefore, the olive quality would be increased in the table olives sector.

3.4. Discrimination of table olive spoiled with mold strains of *Aspergillus* and *Penicillium*

The response of the E-nose to volatile aromatic compounds resulting from the inoculation of different strains of the two mold genera most frequently found in table olives (*Aspergillus* and *Penicillium*) was further studied. The data were first analyzed by the PCA. The score plots of the first two principal components for the sample's discrimination of the inoculated mold species are shown in Fig. 4. The PCA based on E-nose data was able to differentiate table olives altered with different mold strains of the same species. The first and second principal components (PC1 and PC2) were enough to visualize the clustering of data and explained 91% and 75% of the total variance of the mold of the species *A. flavus* and *P. expansum*, respectively. These data are interesting since these strains of microorganisms produced different alterations regardless of the strain of mold for each of the microorganisms studied.

Subsequently, the PLS-DA was applied to build the classification model and the corresponding confusion matrix (leave-one-out cross-validation) for both species (Table 4). The results obtained (90.5 and 87.3% correct predictions) showed that the samples analysis of the table olives altered by molds of the same species showed a clear discrimination using this electronic tool. To our knowledge, not much literature exists on the discrimination of mold-altered table olives with electronic devices. However, a recent study by Sánchez et al. (2021) described the PCA discrimination of table olive defects by different microorganisms that produce sensory alteration such as zapateria, butiric, putrid and mold during the fermentation period of green table olives. Therefore, these results highlight the feasibility of these devices as a rapid analytical tool to control the table olive processing.

The E-nose offers better discrimination results than those obtained by the tasting panel. The sensory analysis can clearly differentiate the healthy control olives from the samples altered by the different molds. However, it showed similar values for table olives altered by the different mold strains. This similarity of the sensory values is even greater when it comes to comparing strains of the same mold species.

Table 4

Confusion matrix obtained through PLS-DA for discrimination between control and olives inoculated with mold strains (*Aspergillus flavus* A.F.9, A.F.18 and A.F.21; *Penicillium expansum* P.E.3, P.E.4 and P.E.8). Values are expressed in percentage.

Predicted Class (%)		<i>Aspergillus flavus</i>					<i>Penicillium expansum</i>				
Real Class	C	A.F.9	A.F.18	A.F.21	Real Class	C	P.E.3	P.E.4	P.E.8		
C	25.0	0	0	0	C	21.8	3.1	0	0		
A.F.9	0	18.7	3.1	0	P.E.3	3.1	18.7	0	0		
A.F.18	0	3.1	21.8	0	P.E.4	0	3.1	25.0	3.1		
A.F.21	0	3.1	0	25.0	P.E.8	0	0	0	21.8		

Thus, we can confirm that the discrimination power of the E-nose is consistent with the different volatile compound's profiles found in the different altered samples. Authors such as Lanza and Amoruso (2020), used chemometric techniques to separate characterization features of green olives in the tasting panel and authors such as Marx et al. (2017), used the electronic tongue as sensors to classify the presence or intensity of negative defects in olives and classify them according to their commercial categories.

4. Conclusions

In general, the greatest negative characteristics were attributed to the olives inoculated with the strains *A. flavus* A.F.18, and *P. expansum* P.E.20. These strains showed a high score on several attributes in sensorial analysis and produced higher concentrations of different volatile compounds such as oxygenated compounds, acid compounds such as butanoic acid, or higher alcohols related with negative sensorial properties. The E-nose proved to be a powerful tool with analytical capacity to discriminate VOCs derived from the fermentation of Spanish-style table olives inoculated with different mold strains, even of the same species. The classification provided with this device coincides with the results provided by the tasting panel and with the VOC profile. This proves that, combined with chemometric tools, and as an auxiliary tool in the tasting panel, the E-nose represents a fast, simple, reliable, and low-cost method suitable for use in the table olive industry as a quality control tool in detecting the presence of different molds in the table olives process.

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Compliance with ethics requirements

This research does not include any experiment using animal and/or human subjects.

CRedit authorship contribution statement

Ramiro Sánchez: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, and, Investigation, All authors have revised, read, and approved the submitted version of the manuscript. **Francisco Pérez-Navado:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Funding acquisition, Project administration, Investigation, Supervision, and, Writing – review & editing, All authors have revised, read, and approved the submitted version of the manuscript. **Sara Martillanes:** Data curation, Formal analysis, Investigation, and, Visualization, All authors have revised, read, and approved the submitted version of the manuscript. **Ismael Montero-Fernández:** Methodology, Data curation, Formal analysis, Investigation, Visualization, and, Writing – review & editing, All authors have revised, read, and approved the submitted version of the manuscript. **Jesús Lozano:** Methodology, Data curation, Formal analysis, All

authors have revised, read, and approved the submitted version of the manuscript. **Daniel Martín-Vertedor:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Funding acquisition, Project administration, Investigation, Supervision, and, Writing – review & editing, All authors have revised, read, and approved the submitted version of the manuscript.

Declaration of competing interest

The authors state that they have no conflicts of interest.

Data availability

Data will be made available on request.

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