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Assessment of sensory properties and *in vitro* antimicrobial activity of monofloral Sicilian honey

Nunziatina Russo [a,b,1], Ambra Rita Di Rosa [c,1], Alessandra Pino [a,b,d,*], Gaetana Mazzeo [a], Luigi Liotta [c], Cinzia Caggia [a,b,d], Cinzia Lucia Randazzo [a,b,d,**]

[a] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, 95123, Catania, Italy
[b] ProBioEtna srl, Spin-off of University of Catania, Via S. Sofia, 100, 95123, Catania, Italy
[c] Department of Veterinary Sciences, University of Messina, Polo Universitario Annunziata, 98168, Messina, Italy
[d] CERNUT (Interdepartmental Research Centre in Nutraceuticals and Health Products), University of Catania, Via le A. Doria 6, 95125, Catania, Italy

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ABSTRACT

The antimicrobial and antioxidant properties of honey are well-established and recently the potential of honey for biotechnological and food preservative applications appeared promising. According to that, the present study evaluated the sensory profile and the antimicrobial potential of honey samples produced in different phyto-geographical zones, in the Sicilian areas, and obtained from five different floral sources (chestnut, eucalyptus, sulla, thyme, and citrus). The specific sensory fingerprint of the analyzed samples was assessed by artificial senses (E-Eye, E-Nose, and E-Tongue). In addition, the antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 19114, *Bacillus subtilis* DSM 10, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* Typhimurium ATCC 14026, and *Candida albicans* ATCC 10231 was evaluated. Artificial senses allowed to confirm the impact of climate factors and genetic features on honey composition suggesting implications for antimicrobial activity. A broad spectrum of antagonistic activity was displayed by the analyzed honey samples in both undiluted and diluted formulations. In particular, thyme honey showed interesting antagonistic features. In addition, a greater inhibitory effect of amber-colored honey than light ones was observed.

1. Introduction

The need to find natural preservatives for food as well as the necessity of alternative agents able to control infections, in view of the increasing prevalence of antimicrobial resistance, has prompted renewed scientific interest in complex natural products with antimicrobial activity, like honey (Bouzo et al., 2020). Honey is a complex natural sweet substance used, since ancient times, to treat infections. It is well-known that honey owns the capability to kill or suppress the growth and proliferation of a broad spectrum of microorganisms, including multi-drug resistant pathogens (Cilia et al., 2020; Zhang et al., 2021). Botanical and geographical origin as well as chemical composition strongly influence the compositional variety of honey, especially color, aroma, and flavor as well as antimicrobial features. In this context, "artificial senses", such as E-Nose, E-Tongue, and E-Eye, are widely used to assess sensory profile and distinguish the declared botanical origin of the honey (de Sousa et al., 2016; Di Rosa et al., 2019; Di Rosa & Leone, 2018; Tan & Xu, 2020; Veloso et al., 2018). Likewise, the antimicrobial potential of honey is affected not only by its composition, rather variable and mainly dependent on the floral source, but also by season and environment as well as processing, manipulation, packaging, and storage conditions (Maddocks & Jenkins, 2013; Da Silva et al., 2016; Combarros-Fuertes et al., 2019; 2020). The antimicrobial potential of honey is related to several parameters, such as the low water content, the high viscosity, acidity, and H_2O_2 content along with the presence of various compounds, including phytochemicals, peptides, non-peroxidase glycopeptides, and proteins (Cebrero et al., 2020; Machado et al., 2020; Almasaudi, 2021). However, hydrogen peroxide (H_2O_2), methylglyoxal (MGO), and polyphenolic compounds are recognized as the main compounds influencing the antimicrobial

* Corresponding author. Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, 95123, Catania, Italy.
** Corresponding author. Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, 95123, Catania, Italy.
E-mail addresses: alessandra.pino@unict.it (A. Pino), cranda@unict.it (C.L. Randazzo).
[1] These authors have contributed equally to this work and share first authorship.

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activity of honey (Nolan et al., 2019). In addition, polyphenols/-flavonoids synergistically act with biologically active compounds derived from the metabolism of the microbiota harbored in honey, bees, and nectar (Bucekova et al., 2018; Farkasovska et al., 2019), including those (Brudzynski, 2021). The mechanism of action of honey against bacteria differs among Gram-positive and Gram-negative microorganisms, and some studies hypothesized that, at least, some cellular targets might be broadly specific for each class of bacteria (Maddocks & Jenkins, 2013). Previous studies have identified several biological processes in bacteria that may be affected by the action of honey, including cell division, motility, quorum sensing, protein synthesis, and responses to oxidative stress (Bouzo et al., 2020; Fidaleo et al., 2015; Truchado et al., 2009). However, given the complexity of the matrix, there are probably several mechanisms that are not completely understood (Combarros-Fuertes et al., 2020). In this context, a detailed analysis of the chemical composition of honey may provide insight into the mechanism of action of these synergistic components, sharing light on the most effective and broad-spectrum honey types with antagonistic activity against a variety of bacteria (Almasaudi, 2021; Costa et al., 2019; Patrignani et al., 2018).

According to that, the present study aimed to evaluate the sensory profile and the antimicrobial potential of honey samples collected in different phytogeographical zones, in the Sicilian area. In detail, the sensory profile was assessed by using artificial senses whereas the antimicrobial potential was evaluated against Gram-positive, Gram-negative bacteria and yeasts.

2. Materials and methods

2.1. Honeys samples

Honey samples from chestnut (C), eucalyptus (E), sulla (S), thyme (T), and citrus (Z) floral sources were kindly provided by both individual producers and associations of beekeepers located in different part of the Sicily Island (Italy). Samples were stored at 21 °C in the dark until the use. Detailed information about floral source, city, and year of production are listed in Table 1.

Table 1
General information about the analyzed honey samples.

Honey samples	Geographical origin	Collection date	Headquarters of the packaging factory
Chestnut (*Castanea sativa* Mill., Fagaceae)			
C1	Raccuja (ME)	August 2020	Floridia (SR)
C2	Mount Etna	July–August 2020	Zafferana Etnea (CT)
C3	Peloritani Mountains (ME)	July 2020	San Giovanni La Punta (CT)
Eucalyptus (*Eucalyptus camaldulensis* Dehnh., Myrtaceae)			
E1	Lercara Friddi (PA)	July 2020	Sant'Alfio (CT)
E2	Nicosia (EN)	July 2020	Zafferana Etnea (CT)
E3	Contrada Vaccarizzo (CT)	July 2020	Donnalucata (RG)
Sulla (*Sulla coronaria* (L.) B.H.Choi & H.Ohashi, Fabaceae)			
S1	Nicosia (EN)	June 2020	Zafferana Etnea (CT)
S2	Plain of Catania (CT)	April 2020	Paternò (CT)
S3	Castellana Sicula (PA)	May 2020	Sant'Alfio (CT)
Thime (Thymbra capitata *(L.) Cav., Lamiaceae)*			
T1	Floridia (SR)	August 2020	Floridia (SR)
T2	Mount Etna	July 2020	Zafferana Etnea (CT)
T3	Vendicari Reserve (SR)	July 2020	Syracuse province
Citrus (*Citrus* spp., Rutaceae)			
Z1	Palagonia (CT)	April–May 2020	Sant'Alfio (CT)
Z2	Paternò (CT)	May 2020	Zafferana Etnea (CT)
Z3	Paternò (CT)	May 2020	Donnalucata (RG)

2.2. Artificial sensory analysis

2.2.1. E-Tongue

The electronic tongue (E-Tongue, αAstree, Alpha M.O.S., Toulouse, France) was used to evaluate the quality of honeys, according to botanical origins, as previously reported (Di Rosa et al., 2018a). In detail, 5 g of honey were dissolved in 25 mL of bi-distilled water and tested thirty times. Only the last ten acquisitions (with more stable signals) were used for data processing. Each measurement lasted 120 s and data were collected every 1 s. Measurement data, obtained for each solution, was taken as the average of the last 20 s. Prior to each sample, the sensors were conditioned and calibrated with a solution very close to the samples, as requested by the manufacturer.

2.2.2. E-Nose

The olfactory profile of the honey samples was evaluated by using the electronic nose (E-Nose) able to mimic the olfactory system of humans. The FOX 4000 (Alpha M.O.S., Toulouse, France) equipped with 18 MOS (Metal-oxide semiconductor) gas sensors (P10/1, P10/2, T30/1, P40/1, PA/2, T70/2, TA/2, T40/1, T40/2, P30/2, P40/2, P30/1, LY2/G, LY2/gCT, LY2/GH, LY2/LG, LY2/AA, LY2/gCTL) and combined with an automatic headspace sampler HS100 was used. The sensors array has partial specificity and among theme, sensor PA/2 is sensitive to nitrogen compounds, T30/1 is sensitive to organic solvents, LY2/G is sensitive to amines, ammonia, alcohols, and ketones, and P10/2 is sensitive to methane and propane and aliphatic nonpolar molecules. To perform the analysis, 3 g of each honey sample were weighed in 10 ml headspace vials and positioned in the auto-sampler racket. In order to develop volatile compounds, all samples were incubated at 55 °C for 120 s under agitation (500 rpm). The measurement sequence started with the following parameters: injection volume 500 μl, injection speed 500 μl/s, gas flow 150 ml/min (Di Rosa et al., 2018b). All samples were tested ten times. Before every cycle of analysis, a blank was acquired.

2.2.3. E-Eye (CVS)

The color of honey samples was assessed using a computer vision system (CVS), Iris Visual Analyzer 400 (Alpha M.O.S., Toulouse, France). The used instrument consists of a closable measurement chamber of large dimensions, that guarantees controlled light conditions, and a CCD (Charge-coupled device) camera, with 16 million colors, for high-resolution data acquisition. Image analysis is a fundamental step for the distinction of the objects from the background and the production of quantitative information. To perform the analysis, each honey sample was divided in ten aliquots, into ten different cylindrical plastic containers (2 cm diameter and 3 cm height) and positioned in the available surface of the measurement chamber. Then, a picture for each honey sample was taken, with a white background and top lighting only. The color of any pixel was registered and expressed in terms of R (red), G (green), and B (blue) amounts. Finally, the RGB code, corresponding to each color extracted from the different images, was obtained and used as input for the statistical analysis.

2.3. Microbiological analysis of honey samples

The microbiological quality of the tested honey samples was evaluated by plate count in accordance with the current legislation on microbiological criteria applicable to foodstuffs (EC Regulation no. 2073/05). In detail, 25 g of each sample was homogenized with 225 ml of sterile saline solution (0.9% w/v), ten-fold diluted and plated on specific media as reported by the ISO method indications. In detail, total mesophilic count was performed using the Plate Count Agar (PCA) medium, incubated aerobically at 30 °C for 72 h; sulfite-reducing bacteria were cultivated on Sulphite Polymyxin Sulphadiazine (SPS) agar and incubated anaerobically at 37 °C for 24–48 h; presumptive *Bacillus cereus* was plated on Bacillus cereus agar base medium, incubated at 30 °C for 24–48 h. In addition, Enterobacteriaceae were enumerated on

Violet Red Bile Glucose Agar (VRBGA) medium anaerobically incubated at 37 °C for 24 h; yeasts and molds were cultivated on Sabouraud Dextrose Agar (SDA) medium and incubated at 25 ± 1 °C for 72 h; fecal coliforms were enumerated on RAPID' *E. coli* 2 Medium (Biorad, Italy) and incubated at 44 °C for 24 h; the presence of *Salmonella* spp. was evaluated using the differential Hoektoen medium following the pre-enrichment and enrichment steps using the Rappaport Vassialidis broth (Milani et al., 2020; Pino et al., 2018; Randazzo et al., 2009). All media were purchased from Liofilchem (Roseto degli Abruzzi, Italy). Microbial count was performed in triplicate.

2.4. Reference strains and culture conditions

Staphylococcus aureus ATCC 29213, *Listeria monocytogenes* ATCC 19114, *Bacillus subtilis* DSM 10, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* Typhimurium ATCC 14026, and *Candida albicans* ATCC 10231 were used in the antimicrobial assay. Each reference strain was cultured using the media and conditions suggested by ATCC or DSM. In detail, *S. aureus* and *E. coli*, were grown in Trypticase Soy Broth (TSB) medium, while *L. monocytogenes* and *E. faecalis* in Brain Heart Infusion Broth (BHI). *B. subtilis, P. aeruginosa* and *S.* Typhimurium were cultivated in Nutrient broth. All reference strains were aerobically incubated at 37 °C for 24 h. Finally, *C. albicans* was grown in YPD medium, incubated at 24–26 °C for 1–2 days. All media were purchased from Liofilchem (Roseto degli Abruzzi, Italy).

2.5. Antimicrobial activity by agar spot test

Honey samples were tested for the ability to antagonize the aforementioned reference strains by using the agar spot method, according to the criteria established by the Clinical Laboratory Standard Institute (CLSI, 2018). In detail, after overnight growth, each reference strain was standardized to 0.5 McFarland (~10^8 cfu/ml) and distributed, using a sterile cotton swab, on Petri dishes containing the BHI agar medium (Liofilchem, Italy). 20 μl of honey, heated at 75 °C for about 5 min or solubilized in PBS buffer (Phosphate Buffered Saline, 1X Solution, pH 7.4) at 75%, 50%, 25%, 12.5% and 6.25% (w/v), were spotted. After incubation for 24–48 h at the optimal growth temperature of the reference strain, the appearance of inhibition zones, around the honey's spot, was visually detected and the diameter size was measured with a ruler (Pino et al., 2019, 2021, 2022). Results were expressed as millimeters (mm) of the inhibition zone. The analysis was carried out in triplicate.

2.6. Antimicrobial activity by microdilution assay

Based on the results of the agar spot test, the antimicrobial activity of the analyzed honey samples was evaluated by microdilution assay. Serial dilutions of honey samples were freshly prepared in BHI broth following the method reported by Taormina et al. (2001). In detail, 96-well plates were filled with 100 μl of honey samples at different concentration (from 100% to 25% w/v) and 100 μl of the reference pathogen strains standardized to 0.5 McFarland (Pino, Mazza, et al., 2022). After 24 h of incubation, at the optimal growth temperature for each target microorganism, tenfold dilutions were made, using 0.9% (w/v) of physiological water (Merck KGaA, Germany), and plated on the specific agar media previously described. The inhibition rate (Inh%) exhibited by both undiluted and diluted honey samples was calculated after counting viable cells (Pino, Mazza, et al., 2022). Each assay was performed in triplicate.

2.7. Statistical analysis

Results from the 7 E-Tongue sensors, the 18 E-Nose sensors, and the 60 color codes, present with a frequency greater than 1% from E-Eye were acquired. The native instrumental software (AlphaSoft, v14.1) was used to build the sensorial fingerprint of the analyzed sample, taking into account, for each instrument, only the sensors with the greatest discrimination power.

Microbiological data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test for means separation using the SPSS software (version 27, IBM Statistics, Armonk, NY, USA). Differences were considered statically significant at $p < 0.05$.

To evaluate the relationship between the selected sensors and antibacterial activity, the results were subjected to Principal Component Analysis (PCA), using the XLSTAT statistical package.

3. Results

3.1. Artificial sensory profile of different monofloral honey samples

The sensory profile of the honey samples was evaluated by using the three instruments E-Eye, E-Nose, and E-Tongue. Only the sensors with the greatest discrimination power, obtained for each instrument, were selected for pattern recognition, classification, and quantitative purposes.

Concerning the color profile, all honey samples were acquired by the E-Eye and all colors with a frequency greater than 1% were considered. The color profile of the different types of honey was built using 60 color codes, from 1057 to 2985. As reported in Fig. 1, based on the 10 color codes with the greatest discrimination power, the chestnut (C) and eucalyptus (E) honey samples, with colors between 1584 and 2420, were classified as dark, whereas the sulla (S) and citrus (Z) honey samples, showing colors from 2709 to 2966, were categorized as light honey. The thyme honey sample (T) showed colors between 2147 and 2710 (Fig. 1).

The same procedure was applied to define the volatile profile of the honey samples. The responses of the 18 E-Nose sensors were evaluated and the 8 sensors (LY2/AA, LY2/GH, LY2/gCTL, P10/1, T70/2, PA/2, P30/2, TA/2), with the greatest discrimination power, were selected. As shown in Fig. 2, all the selected sensors showed the greatest intensity of response only towards chestnut honey (C) while variable responses were obtained from the remaining honeys. In detail, LY2/GH, LY2/gCTL, and PA/2, immediately after chestnut, showed the highest response to sulla (S) and citrus (Z) honey, respectively, whereas the sensor P10/1 mostly responded to thyme (T) and eucalyptus (E) honey (Fig. 2).

Concerning the taste profile, the responses of all 7 sensors were evaluated. As reported in Fig. 3, all the sensors responded strongly for sulla (S) and chestnut (C) samples, followed by the eucalyptus (E) one, whereas a less extent response was registered for the thyme (T) honey sample. All the sensors responded completely differently, with negative intensity, to the citrus (Z) sample.

All data from the three instruments (E-Eye, E-Nose, and E-Tongue) were used to build the sensory profiles of the analyzed honey samples. Fig. 4 shows the sensory fingerprint of all samples, divided by category. For each type of honey (sulla, citrus, chestnut, eucalyptus, thyme), from left to right, the colorimetric, odour, and taste profile are shown in the form of radar plot, whose vertices are represented by the color codes as regards the CVS and by all considered sensors, as regards the E-Nose and the E-Tongue. A specific fingerprint was revealed for each type of analyzed honey with high variability within the same floral variety (Fig. 4).

3.2. Microbiological analysis of honey samples

Microbiological data are reported in Table 2. Overall, sulfite-reducing anaerobic bacteria, total coliforms, and *Salmonella* spp. were never detected in the analyzed samples (data not shown). In accordance with the current legislation on microbiological criteria applicable to foodstuffs (EC Regulation no. 2073/05), all analyzed samples fulfilled the European standard for all tested microbial groups (Table 1). Although Bacillus cereus was detected in eucalyptus (E) and citrus (Z)

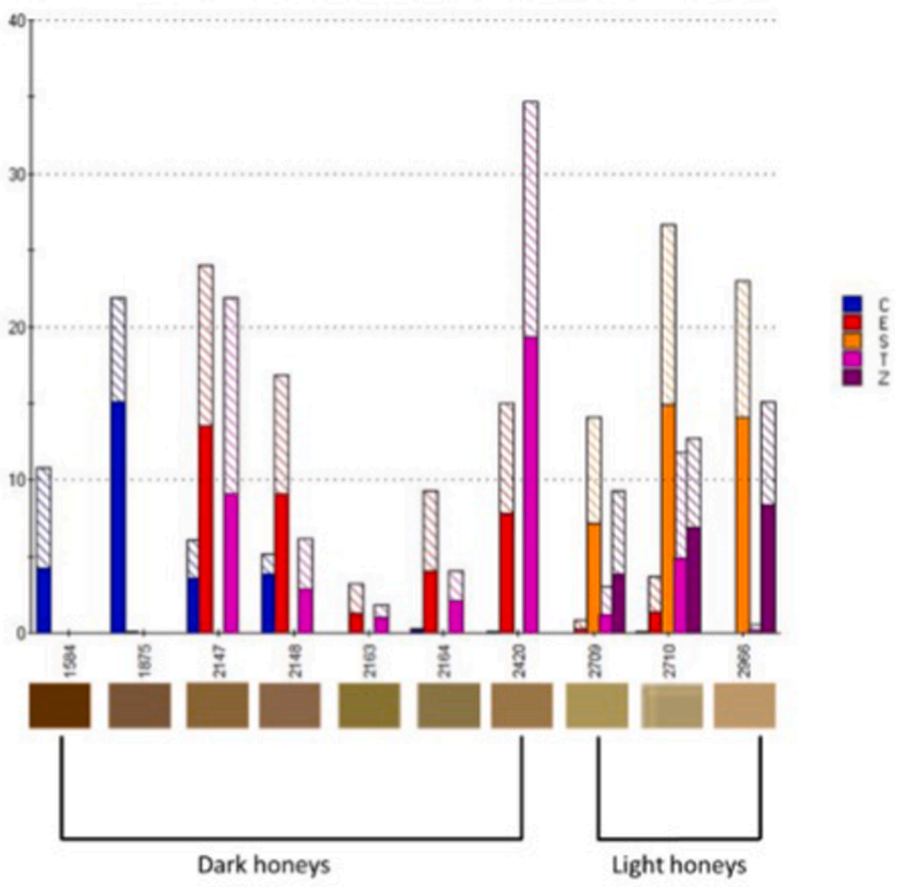

Fig. 1. CVS: bar graph of 10 selected colors with high discrimitation power for all samples (Mean and SD). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

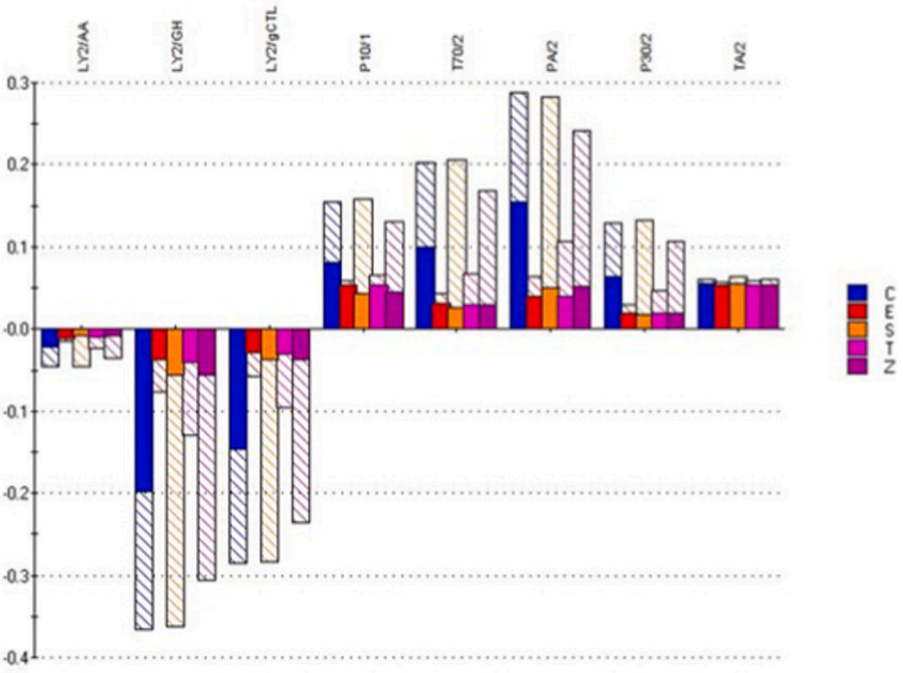

Fig. 2. E-Nose: bar graph of 8 selected sensors with high discrimination power for all samples (Mean and SD).

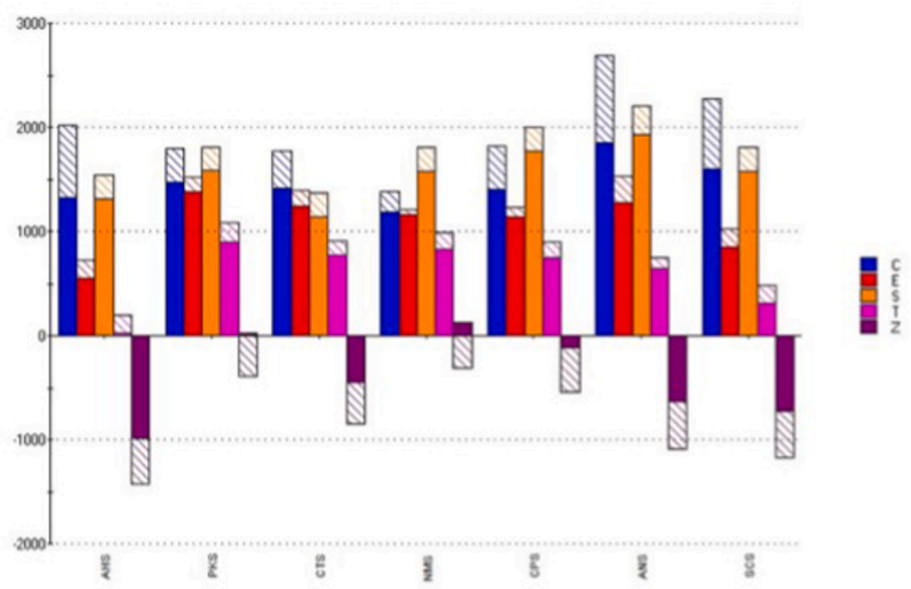

Fig. 3. E-Tongue: bar graph of 7 sensors for all samples (Mean and SD).

honey samples, the cell density returned to the acceptable limits suggested by the EC Regulation no. 2073/05. Concerning the remaining microbial groups, Enterobacteriaceae were found only in three samples (Z1, Z3, and C2), reaching the highest cell density in the Z3 sample. Yeasts were detected in Z1, Z3, C3, and T3 samples whereas the presence of staphylococci, at the density of 2.00 $\log_{10}$ CFU/g, was observed in S2, T1, E1, E2, and E3 samples.

3.3. Antimicrobial activity by agar spot test

Results of antagonistic activity exhibited by the tested honey samples and expressed as diameter (mm) of the inhibition zone are reported in Table 3. Although no antimicrobial activity was detected for honey samples diluted to 12.5% and 6.25%, a broad range of antagonistic activity against the tested pathogen strains was exhibited by honey samples undiluted and diluted to the concentrations of 75%, 50%, and 25%. In detail, S3, Z3, T1, T2, E1, E3, C2, and C3 honey samples showed antagonistic activity against *Escherichia coli* ATCC 25922 in both the undiluted and diluted formulations (Table 3). A marked inhibitory effect against *Listeria monocytogenes* ATCC 19114 was displayed by all the analyzed honey samples with the exception of Z2, Z3, T1, and E3 samples at the concentration of 25%. All the tested honey samples, in both the undiluted and diluted formulations, with the exception of C1 at a concentration of 25%, were able to antagonize *Pseudomons aeruginosa* ATCC 9027 showing inhibition halos with diameters ranging from 7 mm to 28 mm. The same behaviour was observed against *Salmonella* Typhimurium ATCC 14026. In fact, only the C2 honey, diluted at a concentration of 25%, did not show antagonistic activity. Concerning *Staphylococcus aureus* ATCC 29213, only the C2 sample exhibited antagonistic activity in both undiluted and diluted formulations, generating inhibition halos of about 15 mm, whereas only Z2 and Z3 samples did not show antimicrobial activity. For the remaining honey samples, variable antagonistic activity was observed. In fact, S1, E1, and E2 samples showed antagonistic activity only in the undiluted formulation; Z1, T1, T2, and E3 honey exhibited inhibition halos when tested in both undiluted formulation and at the concentration of 75%; S2, S3, T3, C1, and C3 samples were able to antagonize *Staphylococcus aureus* ATCC 29213 in the range of concentrations from 100% to 50% (Table 3). Antagonistic activity against *Enterococcus faecalis* ATCC 29212 was displayed only by the Z3 sample, undiluted and diluted to 75% and 50%, whereas only the E1 sample in the undiluted formulation was able to antagonize *Candida albicans* ATCC 10231 (data not shown). No antagonistic activity was observed against *Bacillus subtilis* DSM 10.

3.4. Antimicrobial activity by microdilution assay

Results of the antimicrobial activity expressed as inhibition rate (Inh %) and obtained through the microdilution assay are shown in Table 4. Overall, all honey samples, with few exceptions for some diluted formulations, were effective against *L. monocytogenes* ATCC 19114, *P. aeruginosa* ATCC 9027, and *S.* Typhimurium ATCC 14026. A high inhibition rate was also registered against *E. coli* ATCC 25922. In particular, the S3, Z3, T1, T2, E1, E3, C2, and C3 samples displayed inhibition activity when tested in both undiluted and diluted formulations. Among the remaining samples, Z2 was able to antagonize the aforementioned pathogen only in the undiluted form, showing an inhibition rate of 40.95%, the Z1 and E1 samples exerted inhibition activity till the dilution of 75% whereas the T3 and C1 honey were able to inhibit the growth of *E. coli* ATCC 25922 till the dilution of 50%. Only S1 and S2 honey samples, in both undiluted and diluted formulations, did not show antagonistic activity against the aforementioned pathogen. All the analyzed samples showed inhibition activity against *S. aureus* ATCC 29213 when tested in the undiluted formulation. In particular, E3 and C2 samples were able to antagonize *S. aureus* ATCC 29213 in both undiluted and diluted formulations showing an inhibition rate higher than 50% (Table 4). Concerning the inhibition activity against *B. subtilis* DSM 10, the analyzed samples were able to exert antagonistic activity mainly when tested in the undiluted formulation whit the exception of the E3 sample, able to antagonize the *B. subtilis* DSM 10 at all the tested concentrations. Similar behavior was observed against *E. faecalis* ATCC 29212. Inhibition rate generally lower than 20% was displayed by the undiluted formulation of the tested samples with the exception of Z2 and E3 samples.

3.5. PCA analysis

The relationship between the selected sensors and the antimicrobial activity was evaluated by Principal Component Analysis (PCA). As shown in Fig. 5, Principal component 1 (PC1) expressed 35.53% of the variance whereas Principal component 2 (PC2) explained 28.78% of the variance. The loadings of groups of sensors on the PCA explicitly showed that the different honey samples were mainly grouped according to

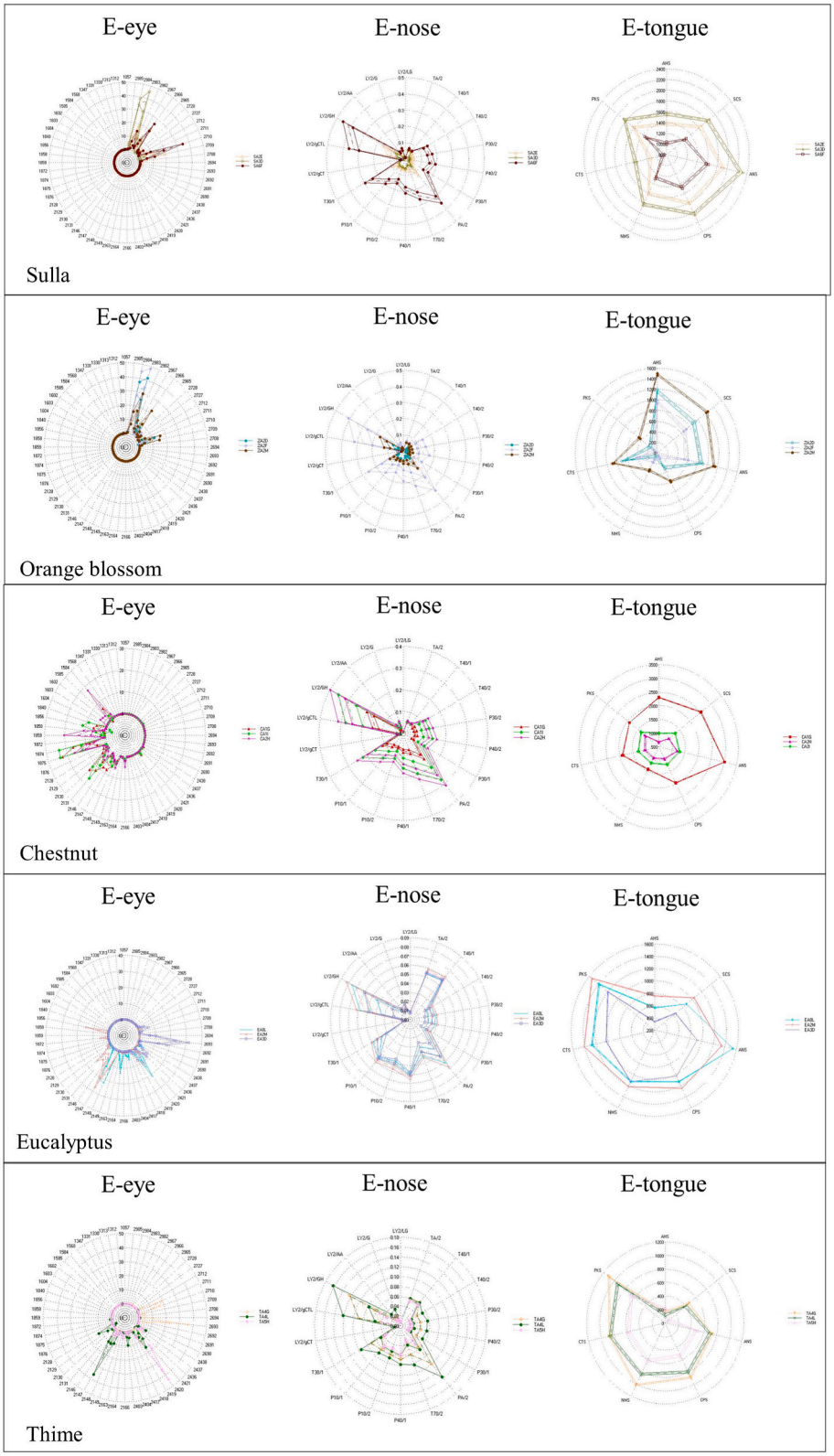

Fig. 4. Artificial sensory fingerprint of the 5 types of honey.

certain sensory profiles and specific antimicrobial activity. The PCA results indicated that the honey samples did not occupy relatively independent spaces in the distribution map, but based on the country of origin, the five kinds of honey could be completely distinguished. In detail, the score values of the first two principal components allowed to establish a relatively good separation among samples. For light honey S3, Z1, and C2, the observed discrimination capacity was mainly explained by 3 P sensors (P10/1, P30/2, and PA/2) and 2 T sensors (TA/2 and T70/2). For these honey samples, low antimicrobial activity was observed against *E. coli*, *L. monocytogenes*, *P. aeruginosa* and *S. aureus*. Conversely, for the intermediate-colored honey samples, L sensors showed intense signals in all E honey, as well as in T2, T3, and S1

Table 2
Microbiological quality of the analyzed honey samples.

Honey samples[a]	Microbial groups				
	Mesophilic aerobic bacteria	Presumptive *B. cereus*	Enterobacteriaceae	Yeasts	*Staphylococcus* spp.
C1	$<1^{b}$	$<1^{e}$	$<1^{d}$	$<1^{e}$	$<1^{b}$
C2	2.00 ± 0.02^{a}	$<1^{e}$	2.00 ± 0.00^{c}	$<1^{e}$	$<1^{b}$
C3	$<1^{b}$	$<1^{e}$	$<1^{d}$	2.30 ± 0.02^{c}	$<1^{b}$
E1	$<1^{b}$	2.00 ± 0.04^{d}	$<1^{d}$	$<1^{e}$	2.00 ± 0.01^{a}
E2	$<1^{b}$	3.00 ± 0.02^{ab}	$<1^{d}$	$<1^{e}$	2.00 ± 0.07^{a}
E3	2.00 ± 0.06^{a}	3.11 ± 0.12^{a}	$<1^{d}$	$<1^{e}$	2.00 ± 0.00^{a}
S1	$<1^{b}$	$<1^{e}$	$<1^{d}$	$<1^{e}$	$<1^{b}$
S2	2.00 ± 0.00^{a}	$<1^{e}$	$<1^{d}$	$<1^{e}$	2.00 ± 0.09^{a}
S3	$<1^{b}$	$<1^{e}$	$<1^{d}$	$<1^{e}$	$<1^{b}$
T1	2.00 ± 0.03^{a}	$<1^{e}$	$<1^{d}$	$<1^{e}$	2.00 ± 0.04^{a}
T2	$<1^{b}$	$<1^{e}$	$<1^{d}$	$<1^{e}$	$<1^{b}$
T3	$<1^{b}$	$<1^{e}$	$<1^{d}$	2.00 ± 0.06^{d}	$<1^{b}$
Z1	2.11 ± 0.03^{a}	2.00 ± 0.00^{d}	5.99 ± 0.03^{b}	6.24 ± 0.07^{a}	$<1^{b}$
Z2	$<1^{b}$	2.30 ± 0.10^{c}	$<1^{d}$	$<1^{e}$	$<1^{b}$
Z3	2.00 ± 0.10^{a}	2.95 ± 0.05^{b}	6.08 ± 0.08^{a}	6.08 ± 0.09^{b}	$<1^{b}$

Data are shown as log mean ± standard deviation based on 3 replicates. Different superscript letters within the same column indicate significant differences at $p < 0.05$.

[a] C, chestnut; E, eucalyptus; S, sulla; T, thyme; Z, citrus.

samples, which showed high antagonistic activity against the aforementioned pathogens. With respect to E-Tongue, AHS, CTS, NMS, ANS, and SCS sensors, positively correlated with all C samples and S2 honey, as well as for T1, Z2, and Z3 samples, showing only a positive correlation with the antimicrobial activity against Salmonella and with no correlation with sensory descriptors.

4. Discussion

Honey is a complex sweet natural product with well-established antimicrobial and antioxidant properties as well as a long history of use for the treatment of surface wounds, burns, and inflammation (Almasaudi, 2021; Nolan et al., 2019). Nowadays, the honey potential for food, cosmetic, and biotechnological applications was widely explored with promising evidence (Krushna et al., 2007; Malik & Sharma, 2010; Scepankova et al., 2021).

In the present study, the antimicrobial potential and the sensory profile of honey samples produced in different phytogeographical zones, in the Sicilian areas, were in-depth evaluated.

It is well known that honey is able to antagonize both Gram-positive and Gram-negative microorganisms, including *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *L. monocytogenes* as well as their multidrug-resistant counterparts. Although the antimicrobial activity of honey is complex and not fully understood, high sugar content, low pH, hydrogen peroxide, and polyphenolic compounds are recognized as key factors synergistically contributing to the antagonistic activity against pathogens (Balázs et al., 2021; Mundo et al., 2004; Nolan et al., 2019). In addition, previous studies reported that the inhibitory effect is related to both botanical and geographical origins (Anand et al., 2019; Gür et al., 2020; Majkutet al., 2021). According to that, a broad spectrum of antagonistic activity was exhibited by the honey samples analyzed in the present study. Statistical analysis showed that, besides honey concentration, the main influence on activity, albeit with some exceptions, depended by the floral origin. In fact, thyme honey showed better antagonistic features, emphasizing as climate factors, genetic composition of plant, and bee species affect the honey composition and, in turn, its properties such as the antimicrobial potential (Gür et al., 2020; Osés et al., 2016). Up to now, the antimicrobial potential of honey was mainly evaluated on samples produced in Australia and New Zealand and few information is now available about honey produced in Europe (Escuredo et al., 2012; Fidaleo et al., 2011; Mavric et al., 2008; Patton et al., 2006; Voidarou et al., 2011). In addition, the vast majority of the conducted studies focused the attention on Gram-positive rather than Gram-negative bacteria. In this regard, our results highlighted appreciable antagonistic activity also against Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, and *S.* Typhimurium. The use of artificial sense allowed us to highlight a strict correlation between antimicrobial activity and specific sensor characteristics. In particular, the antagonistic activity exhibited by sulla honey against *E. coli* and by chestnut, sulla, and citrus honey against *L. monocytogenes* significantly correlated with P10/1, T70/2, PA/2, P30/2, and TA/2 sensors, which mainly respond to organic and aromatic compounds, such as methylbenzene, xylene, toluene, hydrocarbons, and ethanol (Xu et al., 2016). In citrus honey, the inhibitory effect against *P. aeruginosa* and *S.* Typhimurium was correlated with LY2/AA, LY2/GH, and LY2/gCTL sensors, which mainly respond to organic and carbon-oxygen compounds as well as to alcohol and aldehydes. It is well known that specific compounds, such as linalool, ß-terpineol, dihydrocitronellol, ß-citronellol, tetrahydrogeraniol, and cavacrol are active against a wide range of microorganisms, including Gram-negative and Gram-positive bacteria, along with viruses and fungi (Castro-Várquez et al., 2006; Inouye et al., 2001; Peña et al., 2004). In addition, benzene acetaldehyde, benzaldehyde, 1-phenylbutane-2, 3-diol, 1, 4-dihydroxybenzene, and benzene ethanol, dominant in some honey types, are categorized as interesting antibacterial compounds (D'Arcy et al., 1997; Viuda-Martos et al., 2010). Our study, corroborated previously reported data concerning the influence of the color of honey on the observed antimicrobial activity (Alvarez-Suarez et al., 2010; Marić et al., 2021). According to that higher antimicrobial activity was observed in amber honey compared to light colored and transparent ones.

Further studies will be conducted in order to deepen the specific sensory fingerprint of honey and to characterize both the molecules and the mechanism of action responsible for the observed antibacterial activity.

5. Conclusion

In the present study, the use of artificial senses allowed us to demonstrate how climate factors, genetic composition of plants and bee species are able to affect honey composition, and thereby such properties as antibacterial activity. The obtained widespread antimicrobial activity pattern, against both Gram-negative and Gram-positive bacteria, and yeasts, suggested that the tested honey samples may have a relevant role as natural antibacterial products and food preservative. More attention should be deserved to thyme honey as a powerful natural antibacterial agent. Further studies will be done to deep the specific sensory fingerprint of honey and to characterize both the molecules and the mechanism of action responsible of the observed antibacterial

Table 3
Antimicrobial activity, as inhibition halos (mm), of the tested honey samples.

Reference strains	Honey concentration (%)	Diameters of the inhibition zone (mm)													
		C1	C2	C3	E1	E2	E3	S1	S2	S3	T1	T2	T3	Z1	Z2	Z3
E. coli	100	27 ± 0.00^{ab}	26 ± 1.00^{abc}	25 ± 1.00^{bc}	10 ± 0.58^{e}	10 ± 0.60^{e}	10 ± 1.00^{e}	0 ± 0.00^{f}	0 ± 0.00^{f}	18 ± 1.00^{d}	27 ± 1.00^{ab}	28 ± 1.00^{a}	28 ± 0.58^{a}	9 ± 0.00^{e}	9 ± 1.00^{e}	26 ± 2.08^{abc}
	75	24 ± 0.58^{bcd}	24 ± 0.58^{cd}	23 ± 0.58^{d}	9 ± 0.00^{f}	10 ± 0.00^{f}	9 ± 0.00^{f}	0 ± 0.00^{g}	0 ± 0.00^{g}	17 ± 1.00^{e}	25 ± 0.00^{abcd}	27 ± 1.00^{a}	27 ± 0.58^{a}	9 ± 2.08^{f}	0 ± 0.00^{g}	26 ± 1.00^{ab}
	50	18 ± 0.58^{d}	22 ± 0.00^{c}	21 ± 0.60^{c}	8 ± 0.58^{f}	0 ± 0.00^{g}	8 ± 0.58^{f}	0 ± 0.00^{g}	0 ± 0.00^{g}	16 ± 1.00^{e}	26 ± 0.00^{a}	24 ± 1.15^{c}	25 ± 1.53^{b}	0 ± 0.00^{g}	0 ± 0.00^{g}	24 ± 1.15^{c}
	25	0 ± 0.00^{h}	15 ± 0.58^{c}	18 ± 0.58^{b}	7 ± 0.00^{g}	0 ± 0.00^{h}	8 ± 0.58^{fg}	0 ± 0.00^{h}	0 ± 0.00^{h}	13 ± 1.53^{d}	24 ± 1.53^{a}	11 ± 0.58^{e}	0 ± 0.00^{h}	0 ± 0.00^{h}	0 ± 0.00^{h}	10 ± 1.00^{ef}
L. monocytogenes	100	10 ± 0.00^{g}	20 ± 0.58^{de}	22 ± 0.58^{c}	19 ± 1.15^{b}	27 ± 0.00^{a}	24 ± 1.53^{abc}	20 ± 2.10^{de}	22 ± 1.50^{cd}	27 ± 2.08^{ab}	24 ± 0.00^{bc}	28 ± 0.58^{a}	28 ± 1.53^{a}	28 ± 1.00^{a}	17 ± 2.08^{fg}	18 ± 0.58^{efg}
	75	10 ± 0.58^{l}	19 ± 0.58^{efg}	21 ± 0.00^{de}	18 ± 1.00b^{c}	23 ± 1.53^{bcd}	18 ± 1.00^{gh}	17 ± 1.73^{ghi}	20 ± 2.00^{def}	27 ± 2.10^{ab}	17 ± 0.58^{ghi}	28 ± 1.00^{a}	27 ± 0.58^{ab}	27 ± 1.73^{ab}	17 ± 1.15^{ghi}	16 ± 1.73^{hi}
	50	8 ± 0.60^{i}	17 ± 0.60^{e}	19 ± 0.60^{d}	17 ± 1.15^{e}	21 ± 0.58^{bc}	16 ± 1.53^{ef}	10 ± 0.58^{h}	21 ± 3.06^{bc}	25 ± 2.00^{a}	15 ± 1.00^{g}	19 ± 0.00^{d}	26 ± 0.58^{a}	25 ± 1.53^{ab}	16 ± 1.00^{ef}	16 ± 2.30^{ef}
	25	7 ± 0.00^{g}	10 ± 0.60^{e}	16 ± 0.60^{c}	15 ± 0.00^{d}	8 ± 0.58^{fg}	0 ± 0.00^{h}	9 ± 1.73^{f}	16 ± 1.00^{c}	22 ± 2.00^{a}	0 ± 0.00^{h}	9 ± 0.00^{f}	10 ± 0.00^{e}	18 ± 0.58^{b}	0 ± 0.00^{h}	0 ± 0.00^{h}
P. aeruginosa	100	20 ± 0.58^{cde}	17 ± 0.58^{def}	19 ± 0.58^{de}	11 ± 0.00^{gh}	10 ± 0.58g^{hi}	12 ± 0.58^{g}	23 ± 1.00^{bc}	21 ± 3.00^{cd}	23 ± 2.00^{bc}	23 ± 1.00^{bc}	24 ± 1.15^{ab}	21 ± 1.00^{cd}	18 ± 0.00^{bcd}	28 ± 1.53^{a}	28 ± 2.08^{a}
	75	18 ± 0.00^{bcd}	17 ± 1.00^{bcd}	18 ± 0.00^{bcd}	11 ± 0.58^{f}	10 ± 1.00^{f}	11 ± 1.00^{f}	23 ± 2.00^{a}	20 ± 2.65^{bc}	23 ± 0.58^{a}	20 ± 0.00^{bc}	23 ± 2.08^{a}	21 ± 0.58^{ab}	16 ± 2.00^{e}	17 ± 1.53^{bcde}	15 ± 1.00^{de}
	50	17 ± 0.58^{cd}	16 ± 0.58^{cd}	17 ± 0.00^{cd}	10 ± 0.00^{cd}	8 ± 1.00^{f}	9 ± 0.00^{g}	21 ± 3.06^{ab}	19 ± 1.52^{bcd}	21 ± 0.58^{ab}	19 ± 1.15^{bc}	17 ± 0.58^{cd}	17 ± 0.58^{cd}	16 ± 1.00^{de}	17 ± 1.53^{cd}	13 ± 1.00^{ef}
	25	0 ± 0.00^{e}	16 ± 0.00^{a}	17 ± 0.00^{a}	9 ± 1.00^{d}	8 ± 1.15^{d}	7 ± 1.00^{d}	18 ± 1.00^{a}	15 ± 3.05^{ab}	16 ± 1.15^{a}	15 ± 0.58^{ab}	12 ± 0.58^{bc}	15 ± 0.00^{ab}	16 ± 0.00^{a}	9 ± 1.73^{d}	9 ± 0.00^{d}
S. aureus	100	18 ± 0.00^{d}	16 ± 0.58^{d}	25 ± 0.00^{abc}	17 ± 1.00^{de}	10 ± 0.00^{d}	11 ± 0.60^{e}	17 ± 1.70^{de}	27 ± 1.63^{ab}	28 ± 1.00^{a}	24 ± 0.00^{bc}	22 ± 1.15^{c}	23 ± 1.73^{c}	18 ± 1.00^{d}	0 ± 0.00^{f}	0 ± 0.00^{f}
	75	18 ± 0.58^{e}	16 ± 1.00^{g}	10 ± 0.00^{h}	0 ± 0.00^{i}	0 ± 0.00^{i}	9 ± 0.58^{h}	0 ± 0.00^{i}	26 ± 0.58^{b}	28 ± 1.53^{a}	20 ± 1.00^{d}	20 ± 0.00^{d}	22 ± 2.00^{c}	17 ± 1.00^{f}	0 ± 0.00^{i}	0 ± 0.00^{i}
	50	18 ± 0.58^{d}	15 ± 0.58^{e}	9 ± 0.58^{f}	0 ± 0.00^{g}	0 ± 0.00^{g}	0 ± 0.00^{g}	0 ± 0.00^{g}	23 ± 2.08^{b}	27 ± 1.25^{a}	0 ± 0.00^{g}	0 ± 0.00^{g}	19 ± 0.58^{c}	0 ± 0.00^{g}	0 ± 0.00^{g}	0 ± 0.00^{g}
	25	0 ± 0.00^{b}	15 ± 0.58^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}
***S.* Typhimurium**	100	27 ± 1.15^{bc}	18 ± 0.58^{ef}	26 ± 1.15^{bc}	20 ± 0.58^{de}	25 ± 0.58^{c}	26 ± 0.58^{bc}	28 ± 1.00^{ab}	17 ± 1.00^{g}	27 ± 1.53^{bc}	30 ± 0.00^{a}	28 ± 1.00^{ab}	28 ± 0.58^{ab}	23 ± 0.00^{d}	27 ± 1.53^{bc}	28 ± 0.00^{ab}
	75	27 ± 0.00^{b}	16 ± 1.00^{fgh}	25 ± 0.00^{c}	18 ± 1.00^{ef}	20 ± 0.00^{de}	25 ± 0.58^{c}	27 ± 0.58^{b}	16 ± 1.00^{fgh}	27 ± 1.00^{b}	27 ± 1.53^{b}	27 ± 1.53^{b}	28 ± 1.15^{a}	17 ± 1.15^{ef}	22 ± 2.00^{d}	18 ± 1.15^{efg}
	50	17 ± 0.58^{efg}	15 ± 0.58^{h}	18 ± 0.58^{def}	16 ± 0.58^{de}	19 ± 0.58^{de}	21 ± 0.00^{d}	24 ± 1.73^{bc}	14 ± 1.15^{i}	25 ± 1.53^{ab}	19 ± 1.00^{de}	21 ± 0.58^{d}	27 ± 0.00^{a}	16 ± 2.00^{gh}	15 ± 0.58^{h}	17 ± 0.58^{de}
	25	15 ± 0.00^{ab}	0 ± 0.00^{h}	16 ± 0.00^{ab}	8 ± 0.58^{ef}	10 ± 0.00^{de}	9 ± 1.00^{ef}	14 ± 2.08^{bc}	12 ± 1.00^{cd}	17 ± 2.08^{a}	11 ± 0.00^{de}	9 ± 1.15^{ef}	10 ± 1.00^{de}	8 ± 0.00^{ef}	7 ± 1.73^{g}	8 ± 0.58^{e}

Data are presented as average diameter ± standard deviations, based on 3 parallel measurements. Different superscript letters within the same line indicate significant differences at $p < 0.05$.

Table 4
Inhibition rate (Inh%) of the reference strains after exposure to different concentration of each honey sample.

Honey samples[a]	Honey concentration	Inhibition rate (Inh%)							
		E. coli	*L. monocytogenes*	*P. aeruginosa*	*S. aureus*	*S. Typhimurium*	*B. subtilis*	*E. faecalis*	*C. albicans*
C1	100%	96.58	36.19	71.52	64.30	96.82	61.61	0.49	0.00
	75%	85.82	35.94	64.43	64.30	96.58	0.00	0.00	0.00
	50%	64.30	28.61	60.76	64.06	60.76	0.00	0.00	0.00
	25%	0.00	25.06	0.00	0.00	53.67	0.00	0.00	0.00
C2	100%	98.04	75.55	64.06	60.51	67.85	62.71	26.77	3.06
	75%	90.46	71.64	63.81	60.51	60.51	0.00	0.00	0.00
	50%	83.01	64.06	60.39	56.72	56.72	0.00	0.00	0.00
	25%	56.60	37.65	60.27	56.60	0.00	0.00	0.00	0.00
C3	100%	97.80	86.06	77.26	97.80	99.27	37.16	0.49	0.00
	75%	89.98	82.15	73.23	39.12	99.02	0.00	0.00	0.00
	50%	82.15	74.33	69.07	35.21	68.70	0.00	0.00	0.00
	25%	70.42	62.59	68.83	0.00	61.00	0.00	0.00	0.00
E1	100%	45.60	86.55	55.75	77.63	91.20	63.81	0.24	0.00
	75%	40.95	82.03	50.12	0.00	82.03	0.00	0.00	0.00
	50%	36.43	77.51	45.60	0.00	72.98	0.00	0.00	0.00
	25%	31.91	68.34	40.95	0.00	36.43	0.00	0.00	0.00
E2	100%	45.60	97.92	36.31	41.93	90.71	59.17	1.47	5.50
	75%	45.60	83.25	36.31	0.00	90.46	52.57	0.00	0.00
	50%	0.00	76.16	28.97	0.00	76.16	0.00	0.00	0.00
	25%	0.00	28.97	28.97	0.00	36.31	0.00	0.00	0.00
E3	100%	58.44	97.80	70.05	65.04	98.53	52.57	56.02	51.22
	75%	52.57	73.35	64.30	52.57	97.80	50.57	54.05	0.00
	50%	46.70	65.16	52.57	50.13	85.57	6.36	8.80	0.00
	25%	46.45	0.00	40.95	50.07	36.67	5.31	8.80	0.00
S1	100%	0.00	91.24	97.85	77.54	99.07	0.00	0.00	17.00
	75%	0.00	77.54	96.26	0.00	97.85	0.00	0.00	0.00
	50%	0.00	45.25	94.54	0.00	77.54	0.00	0.00	0.00
	25%	0.00	36.69	82.07	0.00	63.84	0.00	0.00	0.00
S2	100%	0.00	97.85	94.18	97.85	77.54	0.00	0.00	5.99
	75%	0.00	95.40	85.62	97.60	73.02	0.00	0.00	0.00
	50%	0.00	85.62	79.50	85.62	63.84	0.00	0.00	0.00
	25%	0.00	72.16	68.37	0.00	53.82	0.00	0.00	0.00
S3	100%	32.66	97.85	97.85	100	98.46	8.56	0.00	20.91
	75%	24.46	96.62	97.85	97.85	97.85	0.00	0.00	0.00
	50%	12.23	77.30	94.79	97.60	89.53	0.00	0.00	0.00
	25%	7.95	68.00	72.90	0.00	76.44	0.00	0.00	0.00
T1	100%	97.80	97.80	100.24	97.56	97.92	74.57	0.00	16.75
	75%	97.80	77.51	91.20	91.20	88.02	0.00	0.00	0.00
	50%	89.85	68.34	86.55	0.00	61.98	0.00	0.00	0.00
	25%	85.57	0.00	68.46	0.00	35.94	0.00	0.00	0.00
T2	100%	87.41	84.23	13.69	84.23	99.14	74.33	75.43	12.22
	75%	87.16	75.18	13.69	76.53	95.60	1.10	13.45	0.00
	50%	86.19	73.35	9.66	0.00	74.33	0.00	0.00	0.00
	25%	50.12	40.95	6.85	0.00	31.91	0.00	0.00	0.00
T3	100%	99.02	98.04	95.72	60.64	99.14	73.59	47.43	9.05
	75%	98.41	97.80	95.72	57.95	99.14	0.00	0.00	0.00
	50%	96.45	95.72	77.51	50.12	97.80	0.00	0.00	0.00
	25%	0.00	45.60	54.65	0.00	35.33	0.00	0.00	0.00
Z1	100%	40.97	98.70	82.07	73.38	97.85	25.93	6.85	17.00
	75%	40.36	97.85	72.90	72.04	77.54	0.00	0.00	0.00
	50%	0.00	92.95	72.65	0.00	72.90	0.00	0.00	0.00
	25%	0.00	82.03	72.13	0.00	36.43	0.00	0.00	0.00
Z2	100%	40.95	67.11	97.80	9.66	97.80	70.66	91.93	88.75
	75%	0.00	63.94	67.24	0.00	85.82	0.00	0.00	0.00
	50%	0.00	60.27	61.12	0.00	68.34	0.00	0.00	0.00
	25%	0.00	0.00	41.08	0.00	31.91	0.00	0.00	0.00
Z3	100%	97.80	82.03	100	9.05	97.80	0.00	0.00	20.17
	75%	97.80	72.98	68.34	0.00	82.03	0.00	0.00	0.00
	50%	96.58	72.13	59.29	0.00	77.63	0.00	0.00	0.00
	25%	45.60	0.00	41.08	0.00	36.43	0.00	0.00	0.00

Data are presented as inhibition rate (%) calculated after counting viable cells.
[a] C, chestnut; E, eucalyptus; S, sulla; T, thyme; Z, citrus.

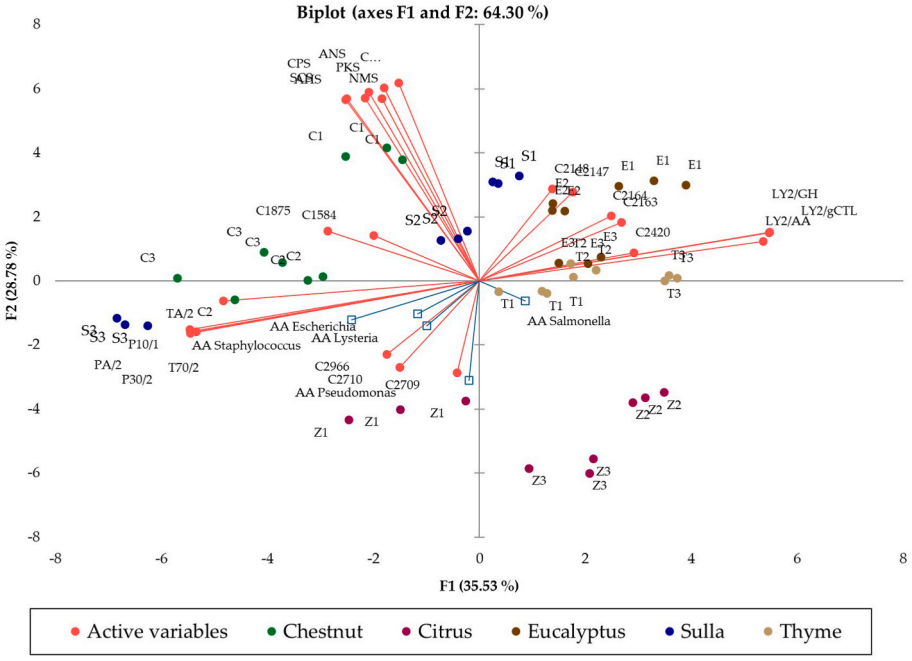

Fig. 5. PCA plot obtained for the different honey varieties.

activity.

Author statement

Nunziatina Russo: formal analysis; writing—original draft preparation; data curation.

Ambra Rita Di Rosa: methodology; formal analysis; writing—original draft preparation; data curation.

Alessandra Pino: data curation; writing - review & editing; supervision.

Gaetana Mazzeo: methodology.

Luigi Liotta: conceptualization.

Cinzia Caggia: conceptualization.

Cinzia Lucia Randazzo: conceptualization; methodology; writing - review & editing; supervision.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Nunziatina Russo, Alessandra Pino, Cinzia Caggia, and Cinzia Lucia Randazzo declare that they are members of ProBioEtna, a spinoff of the University of Catania, Italy.

The authors declare that they do not have any personal, financial, professional, political, or legal interests with a significant chance of interfering with the performance of their ethical or legal duties.

Data availability

Data will be made available on request.

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