

1 **Ginger and parsley essential oils: chemical composition, antimicrobial activity, and**  
2 **evaluation of their application in cheese preservation**

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21 Running head: Essential oils of parsley and ginger for cheese preservation.

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## 31 **Abstract**

32 Chemical composition and *in vitro* antimicrobial activity of parsley [*Petroselinum crispum* (Mill.) Fuss]  
33 and ginger (*Zingiber officinale* Roscoe) essential oils from store-bought parsley plants and ginger  
34 rhizomes were evaluated. A sensory analysis was performed on aged cheese slices treated with a mix of  
35 each essential oil and seed oil. Parsley essential oil was selected as the best compromise between sensory  
36 influence and antimicrobial activity and tested to evaluate its antifungal effects on cheese slices  
37 experimentally contaminated with *Aspergillus flavus*. Monoterpenes and  $\alpha$ -zingiberene were the most  
38 abundant compounds in parsley and ginger essential oil, respectively. Both essential oils showed *in vitro*  
39 antimicrobial activity against different fungal species while only a bacterial strain (*Enterococcus*  
40 *faecalis*) was sensible to ginger essential oil. Only the cheese slices treated with parsley essential oil  
41 were appreciated by the panellists and its use has been effective in preventing the *A. flavus* growth in  
42 cheese.

43

44 **Keywords:** Natural compounds, Moulds, Fungi, Sensory analysis, GC-MS, GC-FID

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## 46 **Supplementary materials**

## 47 **Experimental**

### 48 *Extraction of the Essential oils*

49 The essential oils (EOs) tested were obtained from the aerial parts of parsley [*Petroselinum*  
50 *crispum* (Mill.) Fuss] and rhizomes of ginger (*Zingiber officinale* Roscoe) purchased in a local  
51 supermarket in Milan, Italy. Parsley plants were cultivated in a private garden in Verceia  
52 (Sondrio, Italy) whereas ginger plants were cultivated in a greenhouse in the Azienda Agricola  
53 Salera (Cividate al Piano, Bergamo, Italy). The samples were identified by the botanist and  
54 coauthor Sara Vitalini (Department of Food, Environmental and Nutritional Sciences,  
55 University of Milan). Samples were deposited in the Herbarium of the Department of  
56 Agricultural and Environmental Sciences, University of Milan (Milan, Italy), under the voucher  
57 numbers ZOCPBG124 (ginger) and PCVSO126 (parsley). At the time of sampling, the products  
58 were kept in plastic crates and maintained at room temperature. Once purchased, they were  
59 brought inside clean plastic bags to the “Laboratory of the Inspection Unit of Food of Animal  
60 Origin” of the “Department of Health, Animal Science and Food Safety”, University of Milan  
61 (Milan, Italy). According to Vitalini et al. (2022), around 24 g of air-dried samples were steam-  
62 distilled for 3 h in a Clevenger-type apparatus. The distillate was saturated with NaCl (Carlo

63 Erba, Milan, Italy), extracted with freshly distilled Et<sub>2</sub>O (3 × 100 mL) (Sigma-Aldrich,  
64 Missouri, USA), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> (Sigma-Aldrich, Missouri, USA) and  
65 concentrated with a rotary evaporator at 30 °C.

#### 66 *Chemical characterization of the Essential oils*

67 The identification of components has been carried out at the “Department of Biomedical and  
68 Dental Sciences and Morphofunctional Imaging”, University of Messina (Messina, Italy) by  
69 using three analytical tools: *i*) mass spectral matching with reference spectra from dedicated  
70 databases; *ii*) co-injection with reference standard compounds; *iii*) Retention Index matching  
71 of experimental with published values (Costa et al. 2007). Each EO sample has been run in  
72 triplicate, in order to assess the method’s precision, expressed as coefficient of variation. The  
73 details of each performed analysis are reported below.

#### 74 *Gas chromatography-mass spectrometry (GC-MS) analysis*

75 GC-MS analyses were performed on a GCMS-TQ8030 system (Shimadzu, Milan, Italy),  
76 equipped with a Zebron-5ms (Phenomenex, California, USA) column, 30 m × 0.25 mm i.d. ×  
77 0.25 μm df. Oven program: from 50 °C (held 2 min) to 250 °C (10 min) at 3 °C/min. Injector:  
78 split (1:50), 250 °C. Carrier gas (He) linear velocity: 30 cm/s. MS parameters: source (EI) 200  
79 °C, 0.95 kV, interface 250 °C, scan speed 1666 u/sec, scan mass range 40-350 m/z, mass  
80 spectral libraries “Flavors and Fragrances of Natural and Synthetic Compounds 2” (FFNSC;  
81 Shimadzu, Milan, Italy), Adams 4th edition, Wiley 9, NIST11, and other homemade databases.  
82 Data handling was by GCMSsolution software (Shimadzu, Milan, Italy). Qualitative analysis  
83 was supported by the Retention Indices, measured in real samples acquisitions through  
84 concurrent injection of n-paraffins, and compared to published data (FFNSC 2, NIST webbook,  
85 Adams). All the samples were run in triplicate for precision evaluation. Before injection, the  
86 samples of essential oil of parsley (EO-P) were diluted at 1:10 v/v in chloroform while the  
87 samples of essential oil of ginger (EO-G) were diluted at 1:10 v/v in n-hexane. In both cases,  
88 injection volume was 1.0 μL. For the measurement of Retention Indices, an aliquot (1.0 μL) of  
89 a mixture of n-paraffins (C7-C30, 100 ppm in n-hexane, Sigma-Aldrich, Missouri, USA) was  
90 preliminarily injected.

#### 91 *Gas Chromatography/Flame Ionization Detector (GC-FID) analysis*

92 For quantification, samples of EO-P and EO-G were injected in a GC-FID system  
93 (MDGC/GCMS, Shimadzu, Milan, Italy), operated in standby mode. Samples were injected in  
94 GC-1, provided with a split/splitless injection port. The analytical column (1D) was a Zebron-  
95 5ms, 30 m × 0.25 mm i.d. × 0.25 µm df. Temperature programs were: from 50 °C (held 2 min)  
96 to 280 °C (10 min) at 4 °C/min. Injection occurred in split mode (1:50), at 250 °C. Sample  
97 volume, 1.0 µL of the same sample solutions, as per GC-MS analyses. Carrier gas (He) linear  
98 velocity: 25 cm/s. FID: 300 °C, gases H<sub>2</sub> (40 mL/min), N<sub>2</sub> (80 mL/min), air (400 mL/min).

### 99 *In vitro Antimicrobial activity*

#### 100 *Preparation of the strains and essential oils*

101 The antimicrobial activity of the EO-P and EO-G was tested against several different wild  
102 strains of moulds (*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus*  
103 *ochraceus*, *Candida albicans*, *Cladosporium herbarum*, *Fusarium graminearum*, *Geotrichum*  
104 *candidum*, *Mucor circinelloides*, *Penicillium camemberti*, *Penicillium roqueforti*) and Gram-  
105 positive (*Enterococcus faecalis*, *Listeria innocua*, *Staphylococcus aureus*) and Gram-negative  
106 (*Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium*) bacteria. All the strains were  
107 previously isolated from different food matrices and stored at -80 °C. The tested strains were  
108 prepared by inoculating a loopful from a frozen stock into Tryptone Soy Agar (TSA, Biolife,  
109 Milan, Italy) for the bacteria and Sabouraud Dextrose Agar (SDA; Biolife, Milan, Italy) for the  
110 moulds and incubated overnight at 37 °C for 24 h and 25 °C for 72 h, respectively. A fresh  
111 colony of each strain was grown in Tryptone Soy Broth (TSB; Biolife, Milan, Italy) at 37 °C  
112 for 24 h and in Malt Extract Broth (MEB; Biolife, Milan, Italy) at 25 °C for 72 h, respectively  
113 for bacteria and moulds. The EOs were diluted (2 %, 1 %, 0.5 %) in sterilized ultrapure water  
114 mixed with Tween 20 (Biolife, Milan, Italy) at a concentration of 0.5 % in order to ease their  
115 diffusion in the culture media during in vitro analyses.

#### 116 *Agar disc diffusion assay*

117 The antimicrobial activity of the EOs against the different strains of bacteria and moulds was  
118 preliminarily tested by the agar diffusion method according to a modified protocol of Mazzarino  
119 et al. (2015). A total of 1 mL from each broth culture with a concentration of 10<sup>8</sup> CFU/mL  
120 measured with a spectrophotometer (OD600; Biosigma, Cona, Italy) was inoculated by  
121 swabbing on TSA plates for the bacteria and SDA plates for the moulds. A drop of 10 µL of

122 each EO concentration (2 %, 1 %, 0.5 %) was deposited on a cellulose disc filter (6 mm in  
123 diameter; Biolife, Monza, Italy) placed individually on a proper plate. Then plates were  
124 incubated at an appropriate temperature and time depending on the microorganism tested. A  
125 drop of 10  $\mu$ L of Tween 20 at a concentration of 0.5 % was used as a negative control, while  
126 sodium hypochlorite (Sigma-Aldrich, Missouri, USA) was used as a reference test. The  
127 diameter of the inhibition zones was measured with a Vernier calliper with a minimum  
128 resolution of 0.005 mm and expressed as the mean  $\pm$  standard deviation of three replicates. The  
129 antibacterial activity was classified into three levels based on the diameter of the inhibition  
130 zone: weak (inhibition zone  $\leq$  12.0 mm), intermediate (12.1 mm  $\leq$  inhibition zone  $\leq$  20.0 mm)  
131 and marked (inhibition zone  $\geq$  20.1 mm).

### 132 *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

133 The MIC and MBC of the EO-P and EO-G were assessed in those microorganisms that were  
134 sensible to the activity of the EOs in the previous agar disc diffusion assay. The MIC was  
135 evaluated by the broth microdilution method as follows (Kowalska-Krochmal and Dudek-  
136 Wicher 2021). A 96-microwell plate was filled with decreasing concentrations of each EO (6  
137 %, 4 %, 2 %, 1 %, 0.5 %, 0.25 %, 0.125 %, 0.0625 %, 0.0125 %) prepared in 100  $\mu$ L of TSB  
138 and MEB for bacteria and moulds, respectively, with the addition of 0.5 % of Tween 20. A  
139 fresh inoculum of each bacteria and mold was grown in TSB at 37 °C for 24 h and in MEB at  
140 25 °C for 72 h, respectively. Subsequently, a microbial suspension from each broth culture was  
141 inoculated in the microwell plates obtaining a final concentration of 10<sup>4</sup> CFU/mL in each well.  
142 The microwell plates were incubated a 37 °C for 24 h and 25 °C for 72 h respectively for  
143 bacteria and moulds. The positive control consisted of broth media without EO inoculated with  
144 microbial suspensions while uninoculated broth media with the EOs served as the negative  
145 control. Once incubation was concluded, the lowest concentration of each EO in which there  
146 was no visible growth was considered to be the MIC. The MBC was determined by inoculating  
147 the suspensions from each microwell on TSA plates incubated at 37 °C for 24 h and on SDA at  
148 25 °C for 72 h for bacteria and moulds, respectively. The lowest concentration of each EO in  
149 which there was no microbial growth was considered to be the MBC.

### 150 *Sensory analysis on cheese slices treated with essential oils*

151 Cheese samples were experimentally treated with a solution of sunflower seed oil enriched with  
152 EO-P and EO-G in order to test the sensory influence of both EOs on a food matrix. The cheese

153 selected for the survey was a typical Italian semi-hard pasta filata cheese called Provola  
154 Ragusana. It is a full-fat and raw cow's milk cheese, marketed in a typical pear-like shape,  
155 characterized by a smooth, shiny, thin, yellowish rind while the interior is straw-coloured and  
156 compact. Several 1-mo.-aged Provole Ragusane, weighing ~ 300 g each, were collected in a  
157 commercial factory and brought, into a clean plastic bag, to the "Laboratory of Microbiology  
158 of Food of Animal Origin" at the "Department of Veterinary Sciences" of the University of  
159 Messina (Messina, Italy). The rind and a few mm of the edible portion were removed from each  
160 cheese which was then cut with a knife to obtain square slices. Each cheese slice was ~ 1 cm  
161 thick and was trimmed to fit into 9 cm diameter plastic Petri dishes. The slices were immersed  
162 for 15 min in a solution of commercial sunflower seed oil (Carapelli-Giglio Oro, Firenze, Italy)  
163 enriched with EO-P or EO-G at different concentrations (0 %, 1 % and 2 %) then left to drain  
164 for 5 min and placed in the plates. The solutions to be tested were previously prepared by mixing  
165 the sunflower seed oil and EOs using a magnetic stirrer at room temperature.

166 Cheese slices were served at room temperature, just after their preparation, under normal light  
167 conditions in white porcelain trays coded with random digit numbers. Colour, flavour, odour,  
168 aspect and texture of the cheese slices were evaluated by a panel of twenty-one untrained  
169 random people of both genders selected among the staff and internal students of the unit of  
170 "Inspection of Food of animal origin" of the Department of Veterinary Sciences, University of  
171 Messina (Italy) using a 5-points scale: 1 (Not acceptable), 2 (fair acceptable), 3 (acceptable), 4  
172 (very acceptable), 5 (highly acceptable). A general acceptability value for the differently treated  
173 slices was established from the average of the scores of all the parameters. A total of 10 cheese  
174 slices per treatment were evaluated by each panellist under separate conditions using untreated  
175 slices as the reference sample. It is important to underline that the sensory evaluation herein  
176 performed was based on a hedonistic analysis that requires a much greater number of  
177 participants than those involved in this study. Therefore, the present assessment has had only  
178 an exploratory purpose, and more extensive and in-depth analyses are necessary to understand  
179 how the EOs herein tested influence the sensory profile of the cheese tested.

### 180 *In situ evaluation of the activity of the essential oils against fungal growth on experimentally* 181 *contaminated cheese*

182 This survey was carried out considering the results of the preliminary sensory evaluation testing  
183 the EO which was most appreciated by the panellist. Cheese slices obtained as described above  
184 were exposed to UV light for 60 min on both sides before being immersed in the solutions of

185 sunflower seed oil and EO (EO-P) at different concentrations (1 % and 2 %). Once drained and  
186 placed in the plates, 30 µl of fungal suspension ( $10^6$  spores/mL) was inoculated onto the slices'  
187 surface and distributed using sterile L-shaped spreaders. The fungal strain tested (*A. flavus*) was  
188 chosen based on the antifungal activity of the EO observed in vitro and considering their hazard  
189 to humans and the ability to spoil the cheese. In this regard, pH (pHmeter HI90023CW, Hanna  
190 In-struments, Woonsocket, USA – electrode InLab 427, Mettler-Toledo, Milan, Italy) and  $a_w$   
191 (Aqualab 4TE, METER Group, Pullman, WA, USA) of the cheese was previously determined  
192 to ascertain if it was a suitable medium for supporting fungal growth.

193 The slices were stored at room temperature (~20 °C) and under refrigeration (~4 °C) and  
194 checked every 5 d for 30 d to evaluate the fungal growth according to Bedoya-Serna et al.  
195 (2018). In detail, the antifungal activity of the EO was evaluated by measuring the area  
196 contaminated by fungal growth on the surface of each slice through image processing software  
197 (Adobe Photoshop 2020, Mountain View, California, USA). For each slice examined, a specific  
198 script was designed capable of determining the number of pixels whose colours were different  
199 from the colour range detected in the slice before being incubated. The percentage of  
200 contamination of each slice was calculated through the ratio between the number of pixels of  
201 the contaminated portion and the total inoculated surface expressed as a percentage.

202 Each trial was carried out in triplicate using slices treated only with sunflower seed oil as control  
203 (CTL) and untreated slices (without EO-P and sunflower seed oil) as positive control.

#### 204 *Statistical analysis*

205 The normality of data distribution was tested by D'Agostino-Pearson test and, if necessary,  
206 proper transformation was also performed. One-way analysis of variance (ANOVA) was used  
207 to evaluate any significant differences in the antimicrobial efficacy between the different  
208 concentrations used for each EO tested. The scores obtained for each sensory parameter were  
209 compared between and within the different treatments. The post hoc Tukey's test was  
210 performed for the multiple comparisons within the obtained ANOVA data. The critical  
211 significance level ( $p$ ) was set at 5 % (0.05), and all tests were performed two-tailed. All the  
212 statistical analyses were carried out by Graph Pad Prism 9 software (San Diego, CA, USA).

#### 213 **Results and discussions**

##### 214 *Chemical characterization of parsley and ginger essential oils*

215 The chemical compositions of EO-P and EO-G are detailed in Table 1S and Table 2S while their  
216 chromatographic profiles are shown in Figure 4A-S and Figure 4B-S, respectively. Volatile  
217 fingerprint of EO-P was dominated by the monoterpene fraction, with a major contribution  
218 provided by  $\alpha$ - and  $\beta$ -pinene (~31 % and ~19 %, respectively), followed by limonene (~13 %),  
219 terpinolene (~5 %), and *p*-cymenene (~5 %). Noteworthy is the presence of myristicin (~9 %),  
220 elemicin (~2 %) and congeners that, if on one hand represent typical contributors of the parsley  
221 volatile fraction, on the other fall within the group of anti-nutritional factors according to the  
222 neuro-effects observed when these substances are ingested after their isolation and  
223 concentration (Rahman et al. 2015; Wang et al. 2019). Nonetheless, myristicin and elemicin are  
224 ubiquitous in vegetables and in common culinary herbs and spices such as nutmeg, dill, carrot,  
225 celery, and parsnip suggesting that the amount taken with a conventional diet should not be  
226 toxic (Martinez-Sena et al. 2017). Regarding the EO-G, the distinct predominance of  $\alpha$ -  
227 zingiberene (~27 %) and the abundant sesquiterpene fraction, mostly represented by  $\gamma$ -  
228 cuprenene (~11 %),  $\beta$ -sesquiphellandrene (~8 %) and  $\alpha$ -curcumene (~7 %), appear highly  
229 characteristic of *Zingiber* spp. as well as the unusual composition of the monoterpene fraction  
230 (Mahboubi 2019). In fact, compared to the common monoterpenes found in EOs, especially  
231 from the genus citrus, the analysed EO-G showed a high content of camphene (~7 %). The  
232 presence of many terpenoids such as limonene, characteristic constituents of citrus oils, gives  
233 reason to some sensory descriptors of lemon. Overall, the chemical compositions of the EOs  
234 are affected by geographical origin, harvesting time and extraction methods (Mutlu-Ingok et al.  
235 2020). In the present study, the EOs were obtained from store-bought parsley and ginger;  
236 therefore, it would be speculative to carry out a characterization based on their origin. The EOs  
237 were obtained by hydrodistillation that, on one hand, has the advantage of removing part of the  
238 compounds responsible for their intrinsic flavour, concentrating polyphenols responsible for  
239 their main biological effects (Hinneburg et al. 2006). On the other hand, the use of distillation  
240 can lead to losses of some volatiles and degradation of unsaturated or ester compounds due to  
241 thermal effects (Reyes-Jurado et al. 2015). Regardless of the extraction method as well as the  
242 place and time of the harvest, the chemical composition of the EO-P and EO-G herein observed  
243 agrees with those reported by previous Authors for both EOs (Sivasothy et al. 2011; Fusani et  
244 al., 2017). However, despite these similarities in the constituent compounds, the antimicrobial  
245 efficacy observed is highly variable between studies. Several factors can affect the effectiveness  
246 of an EO against microorganisms and the biological activities of their constituents as well as  
247 their ability to reach molecular targets certainly play a key role (Lv et al. 2011). It could be

248 assumed that the main constituent compounds of each EO are responsible for their antimicrobial  
249 activities. In this regard, the results observed for EO-P could be related to the  $\alpha$ - and  $\beta$ -pinene,  
250 for which different biological activities were described including fungicidal and bactericidal  
251 effects (Salehi et al. 2019). In the same way, antimicrobial and antifungal properties were  
252 observed for EOs-G in which  $\alpha$ -zingiberene was the most represented compound (Al-Dhahli et  
253 al. 2020). In contrast, several studies have shown how the EOs usually have a stronger  
254 antimicrobial effect than a mixture of its main components (Punoševac et al. 2021). Reports on  
255 greater antimicrobial activity of crude EOs compared to blends of their major individual  
256 components suggests that trace components in the crude EOs are critical for their activity and  
257 may have a synergistic effect (Requena et al. 2019). It should also be considered that, regardless  
258 of the amount present, the effectiveness of a compound also depends on the ability to reach its  
259 molecular targets. This is related to both intrinsic factors, such as its solubility, and extrinsic  
260 factors, such as the pH of the medium, the times and temperatures of exposure as well as the  
261 availability of oxygen (Seow et al. 2014). Ultimately, it is not surprising that biological  
262 activities vary between studies as they are conditioned by many factors not strictly dependent  
263 on the EO.

#### 264 ***In vitro antimicrobial activity***

265 The biological activities of EO-P and EO-G have long been investigated and there is numerous  
266 evidence of their fungicidal and bactericidal activity (Linde et al. 2016; Beristain-Bauza et al.  
267 2019). Overall, greater in vitro efficacy of the EOs was observed against moulds rather than  
268 bacteria in the present study (Figure 5S). On one hand, results obtained with the agar disc  
269 diffusion assay showed that the EO-P had no antibacterial activity against all the bacteria strains  
270 tested while the EO-G showed a weak antibacterial activity only against *E. faecalis* both at 2 %  
271 and 1 %. On the other hand, in vitro antifungal activity against spoilage and pathogenic fungi  
272 as well as against virtuous fungal species was observed for both EOs. Inhibitory effects were  
273 observed on the mycelium growth of several fungal species belonging to the genus *Aspergillus*  
274 spp. whose hazard to public health and the ability to spoil food and feed has long been known  
275 (Mousavi et al. 2016). In particular, antifungal activity for both EOs was observed against *A.*  
276 *flavus* and *A. ochraceus*, cosmopolitan moulds known for their ability to contaminate and spoil  
277 cereals, legumes, coffee and dairy products, and to produce mycotoxins whose toxic effects  
278 raise concerns for human and animal health (da Rocha et al. 2014; Taniwaki et al. 2018). The  
279 EO-P showed an intermediate antifungal activity at 2 % and weak both at 1 % and 0.5 % against

280 *A. flavus* while only at 2 % EO-G was weakly effective against this strain. Instead, a similar  
281 efficacy for both EOs was observed against *A. ochraceus* with an intermediate antifungal  
282 activity at 2 % and weak both at 1 % and 0.5 %. A weak effect of EO-G at 2 % was observed  
283 also against *A. niger* which is among the main spoilage organisms of fruit and vegetables  
284 (Gautam et al. 2011). Also other potential pathogenic fungi were sensitive to the activity of the  
285 EOs tested. Mycelium growth of *A. alternata*, a species often associated with mould-induced  
286 respiratory allergies in humans (Kustrzeba-Wójcicka et al. 2014), and *C. albicans*, the most  
287 common agent of human invasive mycotic disease (Lee et al. 2020), was inhibited by EO-G.  
288 Also *M. circinelloides*, the causal agent of mucormycosis, an uncommon but frequently lethal  
289 fungal infection of humans, was sensitive to EO-P activity (Lee et al. 2013). Antifungal activity  
290 of both EOs was also detected against *G. candidum* and *P. roqueforti*, which, if on one hand  
291 are used as starter cultures in many blue, semi-fresh, soft and semi-hard cheeses, on the other,  
292 they can also spoil food and feed (Flórez et al. 2007). The occurrence of *G. candidum* in certain  
293 dairy products such as butter, cream and fresh cheese is undesirable and can determine spoilage  
294 (Koňuchová and Valík 2021) as well as certain strains of *P. roqueforti* which can contaminate  
295 silages (Boysen et al. 2000). In detail, against *P. roqueforti*, EO-P showed intermediate  
296 antifungal activity at 2 % and weak at 1 % while EO-G had an intermediate antifungal activity  
297 at 2 % and weak both at 1 % and 0.5 %. Against *G. candidum*, EO-P showed an intermediate  
298 antifungal activity at 2 % and weak both at 1 % and 0.5 % while only at 2 % EO-G showed an  
299 intermediate antifungal activity. Results of the agar disc diffusion assay showed statistically  
300 significant differences in the effectiveness of the concentrations tested for each EO. Antifungal  
301 activity observed for both the EOs was significantly greater at 2 % than at 0.5 % ( $p = 0.0096$   
302 for the EO-P and  $p = 0.0012$  for the EO-G) while only for EO-G was observed a significant  
303 difference between 2 % and 1 % ( $p = 0.0189$ ). No difference for both EOs was instead observed  
304 between 1 % and 0.5 % ( $p > 0.05$ ). Different sensitivity of the microorganisms towards the EOs  
305 was detected through the micro dilution assay. In this regard, the values of MICs and MBCs  
306 obtained for both the EOs are detailed in Table 3S. Comparing our results with those of other  
307 studies, the antimicrobial effectiveness against a strain appears highly variable from one  
308 experiment to another. On one hand, the results of the in vitro analysis herein observed for  
309 bacteria are confirmed by previous studies that do not report an antibacterial effect both for EO-  
310 P against various species of Gram-positive (e.g., *B. cereus*, *L. innocua*, *S. aureus*, *Lactobacillus*  
311 *brevis*) and Gram-negative (e.g., *E. coli*, *S. enterica*, *P. fluorescens*) (Gutierrez et al. 2008;  
312 Viuda-Martos et al. 2011) and for EO-G against bacteria of high interest in the food and clinical

313 sectors such as *S. typhimurium* and *E. coli* (Mesomo et al. 2013). On the other hand, several  
314 studies report high in vitro antibacterial effects against numerous and different species (Linde  
315 et al. 2016; Noori et al. 2018; Wang et al. 2020). In addition to chemical composition, also the  
316 microorganism tested plays a key role in the effectiveness of the EOs. Recently, Van de Vel et  
317 al. (2019), reviewing the influencing factors on the MIC of EOs, have pointed out how fungi  
318 have a lower median MIC ( $2.0 * 10^3 \mu\text{M}$ ) than bacteria ( $4.4 * 10^3 \mu\text{M}$ ) mainly due to differences  
319 in the cell wall charge and/or composition. Basically, our findings agree with the data reported  
320 by Van de Vel et al. (2019), as at a concentration of 2 % both EO-P and EO-G showed inhibitory  
321 activity against certain fungi strains while no significant activity against bacteria was observed.  
322 The negative charge of the cell wall of bacteria, rich in phosphatidylglycerol and cardiolipin,  
323 compared to the neutral one of the fungi, characterized by high chitin and phosphatidylcholine  
324 content, would explain why fungi repelled less the hydrophobic compounds of the EOs. The  
325 reason could also lie in other more specific mechanisms such as the presence in bacteria of  
326 more efficient multidrug efflux systems or due to the specific target of EOs such as cell wall-  
327 related enzymes present only in fungi (Almeida et al. 2020; Pereira da Cruz et al. 2020).  
328 Furthermore, it is noteworthy that all the microorganisms tested were wild strains previously  
329 isolated from food matrices. Interesting studies evidenced how certain bacterial strains that  
330 experienced stress, such as the stringent environmental conditions of food, can develop  
331 inheritable or transient defence systems able to increase their resistance or tolerance and so their  
332 ability to survive bactericidal stress such as exposure to bactericidal concentrations of EOs  
333 (Brauner et al. 2016).

#### 334 **Disclosure statement**

335 No potential conflict of interest was reported by the authors.

336

#### 337 **References**

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451

452 **Table 1S.** Gas chromatographic fingerprint of essential oil of parsley [*Petroselinum*  
 453 *crispum* (Mill.) Fuss] extracted from the aerial parts of the plant. Peak number: see  
 454 Figure 4A-S for peak identity. CV %: coefficient of variation. RI<sub>exp</sub>: experimental  
 455 Retention Index measured on a Zebron-5ms stationary phase. RI<sub>lit</sub>: Retention Index  
 456 reported by FFNSC 2 (Flavors and Fragrances of Natural and Synthetic Compounds)  
 457 and Adams 4<sup>th</sup> edn. mass spectral databases.

Peak number	Compound	Area% (CV%)	RI <sub>exp</sub>	RI <sub>lit</sub>
1	tricyclene	0.01 (1.01)	925	925
2	α-thujene	0.19 (0.15)	927	927
3	α-pinene	30.81 (1.96)	932	933
4	camphene	0.15 (0.5)	955	954
5	sabinene	0.83 (0.05)	975	972
6	β-pinene	19.43 (2.29)	976	978
7	myrcene	4.05 (0.33)	993	991
8	α-phellandrene	1.11 (0.15)	1010	1007
9	octanal	0.91 (0.25)	1011	1007
10	p-cymene	0.37 (0.22)	1027	1025
11	Limonene+β-phellandrene	13.33 (0.2)	1032	1030
12	(Z)-β-ocimene	0.03 (1.87)	1036	1032
13	(E)-β-ocimene	0.11 (2.41)	1047	1046
14	terpinolene	5.35 (0.21)	1086	1086
15	p-cymenene	4.94 (0.2)	1089	1090
16	fenchyl alcohol	0.08 (0.72)	1120	1123
17	α-campholenal	0.06 (0.97)	1123	1126
18	β-elemene	0.11 (0.6)	1386	1389
19	(E)-α-ionone	0.21 (0.32)	1419	1421
20	(E)-caryophyllene	0.45 (0.16)	1422	1424
21	β-duprezianene	0.09 (0.73)	1428	1427
22	trans-α-bergamotene	0.14 (0.34)	1430	1432
23	cis-thujopsene	0.06 (0.58)	1432	1433
24	3-phenylpropylpropionate	0.05 (1.32)	1465	1469
25	9-epi-(E)-caryophyllene	0.15 (0.38)	1466	1464
26	cabreuva oxide C	0.10 (0.64)	1467	1463
27	myristicin	9.45 (0.18)	1522	1520
28	(E)-γ-bisabolene	0.05 (2.14)	1524	1528
29	elemicin	1.79 (0.14)	1548	1551
30	germacrene B	0.22 (2.76)	1554	1557
31	6-methoxyelemicin	1.07 (0.14)	1592	1595
32	carotol	0.49 (0.61)	1594	1594
33	apiole	0.41 (0.22)	1675	1677
TOTAL		96.61		

458 Peak number: see Figure 4A-S for peak identity.  
 459 CV%: coefficient of variation.  
 460 RI<sub>exp</sub>: experimental Retention Index measured on a Zebron-5ms stationary phase.  
 461 RI<sub>lit</sub>: Retention Index reported by FFNSC 2 and Adams 4th edn. mass spectral databases.

463 **Table 2S.** Gas chromatographic fingerprint of essential oil of ginger (*Zingiber officinale*  
 464 Roscoe) extracted from the rhizomes of ginger. Peak number: see Figure 4B-S for peak  
 465 identity. CV %: coefficient of variation. RI<sub>exp</sub>: experimental Retention Index measured  
 466 on a Zebron-5ms stationary phase. RI<sub>lit</sub>: Retention Index reported by FFNSC 2 (Flavors  
 467 and Fragrances of Natural and Synthetic Compounds) and Adams 4<sup>th</sup> edn. mass spectral  
 468 databases.

Peak number	Compound	Area% (CV%)	RI <sub>exp</sub>	RI <sub>lit</sub>
1	tricyclene	0.13 (1.24)	925	925
2	$\alpha$ -pinene	2.70 (0.18)	933	933
3	camphene	7.16 (0.03)	954	953
4	sabinene	0.14 (1.07)	973	972
5	$\beta$ -pinene	0.31 (0.94)	978	978
6	6-methyl-5-hepten-2-one	0.19 (0.73)	987	986
7	myrcene	0.83 (0.17)	992	991
8	$\alpha$ -terpinene	0.46 (0.57)	1019	1018
9	p-cymene	0.18 (0.71)	1026	1025
10	limonene + $\beta$ -phellandrene	4.60 (0.17)	1030	1030
11	1,8-cineole	4.32 (0.07)	1033	1032
12	$\gamma$ -terpinene	0.02 (4.18)	1062	1060
13	trans-sabinene hydrate	0.13 (0.87)	1098	1096
14	linalool	0.04 (2.37)	1101	1100
15	nonanal	0.61 (0.58)	1102	1101
16	camphor	0.27 (0.41)	1152	1150
17	citronellal	0.28 (0.41)	1154	1152
18	borneol	1.09 (0.24)	1168	1165
19	terpinen-4-ol	0.38 (0.72)	1176	1175
20	$\alpha$ -terpineol	0.66 (0.61)	1198	1195
21	nerol	0.84 (0.14)	1235	1232
22	neral	0.62 (0.74)	1240	1238
23	geraniol	0.38 (0.61)	1261	1259
24	geranial	0.93 (0.38)	1270	1268
25	$\alpha$ -elemene	0.39 (0.58)	1336	1335
26	neryl acetate	0.64 (0.48)	1361	1361
27	$\alpha$ -copaene	0.68 (0.44)	1383	1378
28	geranyl acetate	0.92 (0.25)	1382	1380
29	$\beta$ -elemene	0.49 (0.59)	1392	1389
30	$\gamma$ -elemene	0.60 (0.49)	1436	1434
31	(E)- $\beta$ -farnesene	0.63 (0.30)	1455	1453
32	$\alpha$ -guaiene	0.48 (0.79)	1440	1438
33	$\gamma$ -gurjunene	0.51 (0.52)	1478	1476
34	ar-curcumene	7.63 (0.08)	1481	1479
35	germacrene D	1.16 (0.23)	1488	1486
36	$\alpha$ -zingiberene	27.12 (0.91)	1498	1496

37	(E,E)- $\alpha$ -farnesene	4.79 (0.19)	1505	1504
38	$\beta$ -sesquiphellandrene	8.19 (0.14)	1525	1523
39	$\delta$ -cadinene	0.50 (0.29)	1529	1526
40	$\gamma$ -cuprenene	11.14 (0.13)	1532	1532
41	(E)- $\alpha$ -bisabolene	0.64 (0.17)	1541	1540
42	$\alpha$ -elemol	0.65 (0.40)	1548	1549
43	(E)-nerolidol	0.34 (0.39)	1562	1561
44	germacrene-D-4-ol	0.30 (0.96)	1579	1576
45	caryophyllene oxide	0.44 (0.36)	1589	1587
46	2,3-dihydrofarnesol	0.53 (0.95)	1603	1602
47	dodecyl acetate	0.43 (0.74)	1611	1610
TOTAL		96.48		

469 Peak number: see Figure 4B-S for peak identity.

470 CV%: coefficient of variation.

471 RI<sub>exp</sub>: experimental Retention Index measured on a Zebron-5ms stationary phase.

472 RI<sub>lit</sub>: Retention Index reported by FFNSC 2 and Adams 4th edn. mass spectral databases.

473

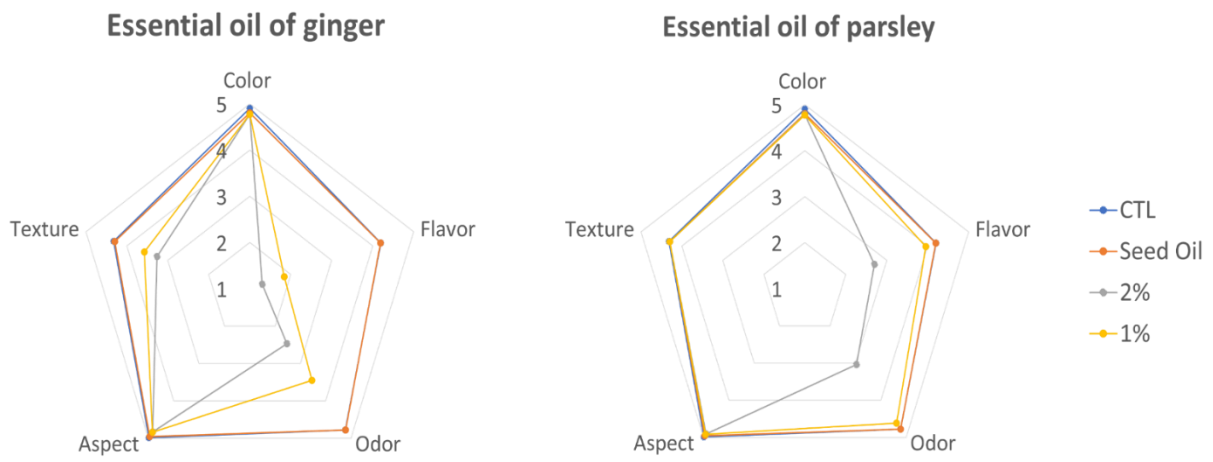
474 **Table 3S.** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations  
 475 (MBC) of essential oils extracted from the aerial parts of parsley [*Petroselinum crispum* (Mill.) Fuss]  
 476 and rhizomes of ginger (*Zingiber officinale* Roscoe). Results are expressed as volume fraction given  
 477 as a percentage.

	Strain	Essential oil of parsley		Essential oil of ginger	
		MIC	MBC	MIC	MBC
Fungus	<i>Alternaria alternata</i>	-	-	0.25 %	0.5 %
	<i>Aspergillus flavus</i>	0.25 %	0.5 %	2 %	4 %
	<i>Aspergillus niger</i>	-	-	2 %	4 %
	<i>Aspergillus ochraceus</i>	0.125 %	0.5 %	0.25 %	0.5 %
	<i>Candida albicans</i>	-	-	2 %	4 %
	<i>Geotrichum candidum</i>	0.25%	0.5 %	2 %	2 %
	<i>Mucor circinelloides</i>	2 %	4 %	-	-
	<i>Penicillium roqueforti</i>	1 %	2 %	1 %	2 %
Bacterium	<i>Enterococcus faecalis</i>	-	-	1 %	1 %

478-): No antimicrobial activity detected.

479

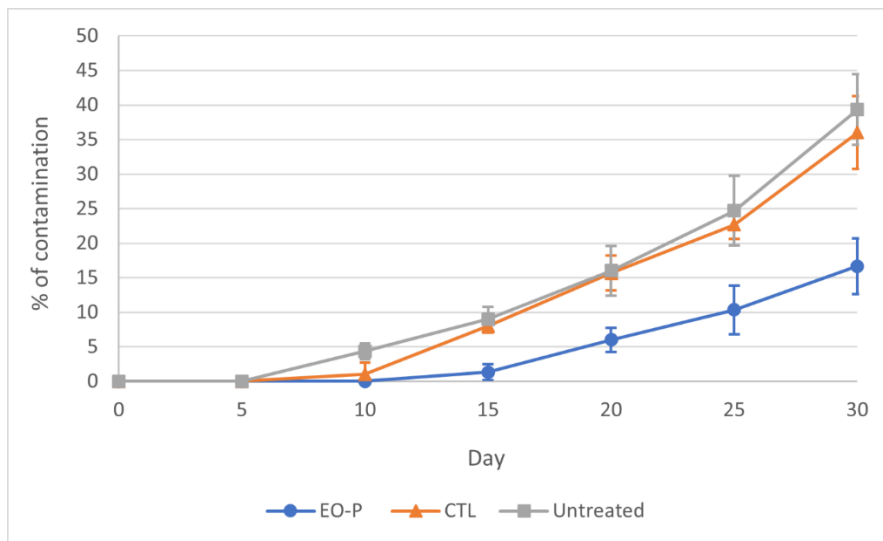
480 **Figure 1S**



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483 **Figure 2S**



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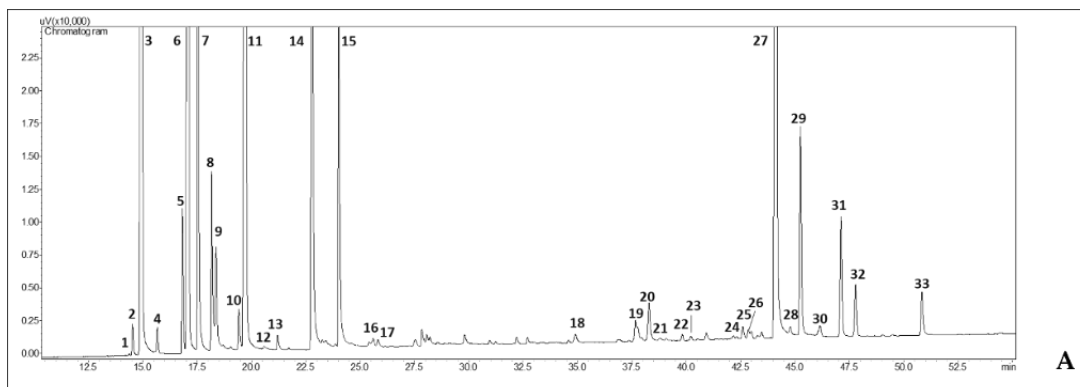
486 **Figure 3S**



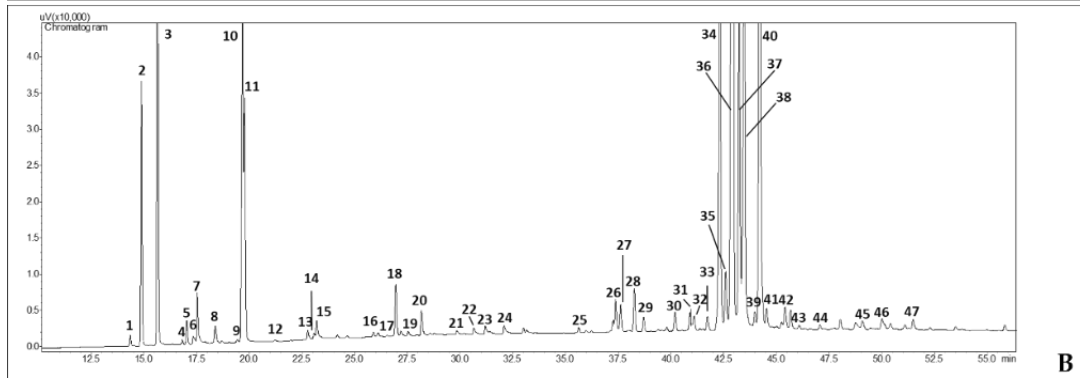
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489 **Figure 4S**



**A**

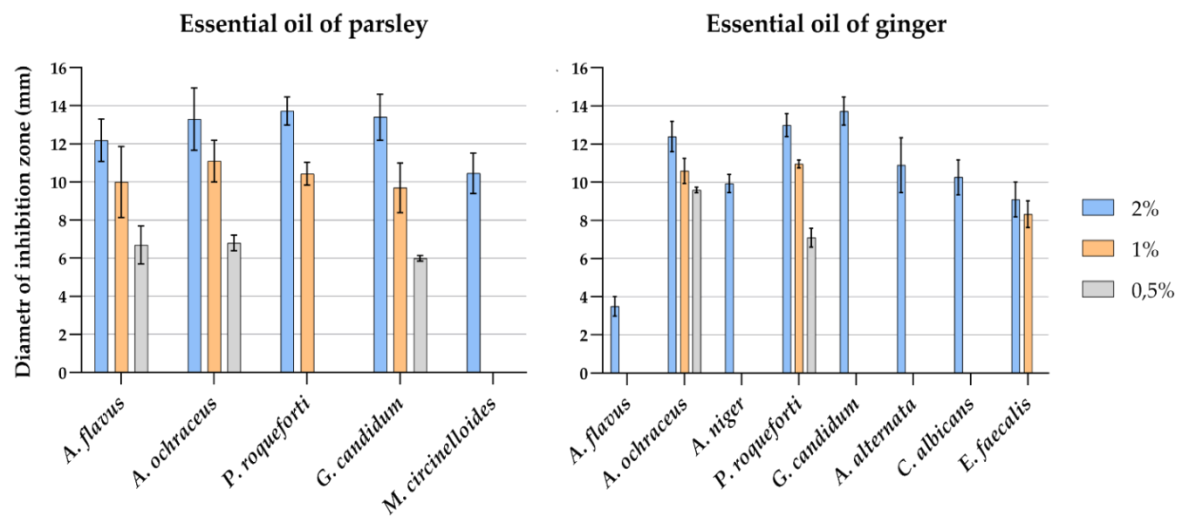


**B**

490

491

492 **Figure 5S**



493

494

495 **Figure captions**

496 **Figure 1S.** Results of the sensory analysis of aged cheese slices experimentally treated with a  
497 solution of sunflower seed oil enriched with essential oils extracted from the aerial parts of  
498 parsley [*Petroselinum crispum* (Mill.) Fuss] and rhizomes of ginger (*Zingiber officinale* Roscoe)  
499 purchased in a local supermarket in Italy.

500 **Figure 2S.** Percentage of contamination over times of the surface of aged cheese slices  
501 experimentally contaminated with *Aspergillus flavus* treated with a mix of sunflower seed oil  
502 enriched with 1 % of essential oil of parsley [*Petroselinum crispum* (Mill.) Fuss] (EO-P). Slices  
503 treated only with sunflower seed oil was used as control (CTL) and untreated slices (without  
504 EO-P and sunflower seed oil) as positive control. The graph shows the means and standard  
505 deviations of the results of three samples analyzed for each time points. The samples were  
506 maintained at room temperature.

507 **Figure 3S.** Aged cheese slices experimentally contaminated with *Aspergillus flavus* after 30 d  
508 at room temperature.

509 **Figure 4S.** GC-FID chromatogram of essential oils extracted from (A) the aerial parts of parsley  
510 [*Petroselinum crispum* (Mill.) Fuss] and (B) rhizomes of ginger (*Zingiber officinale* Roscoe)  
511 purchased in a local supermarket in Italy. See Table 1S and Table 2S for peak number identity  
512 for parsley and ginger essential oils, respectively.

513 **Figure 5S.** Results of the agar disc diffusion assay for the antimicrobial activity of essential  
514 oils extracted from the aerial parts of parsley [*Petroselinum crispum* (Mill.) Fuss] and rhizomes of  
515 ginger (*Zingiber officinale* Roscoe) purchased in a local supermarket in Italy. The diameters of  
516 inhibition zone are shown as means and standard deviations of three replicates excluding the  
517 diameter of the paper disc (6 mm).