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Origanum vulgare ssp. *hirtum* Essential Oil as a Natural Intrinsic Hurdle against Common Spoilage and Pathogenic Microbes of Concern in Tomato Juice

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Abstract: The aim of the present study was to assess the commercial potential of the *Origanum vulgare* ssp. *hirtum* essential oil (OEO) as a natural intrinsic hurdle against common spoilage and pathogenic microbes in tomato juice. The main volatile compounds of the OEO identified by gas chromatography mass spectrometry (GC/MS) analysis were thymol and carvacrol, accounting for approximately 48% and 27%, respectively. Its activity against common food spoilage and pathogenic microbes was confirmed and the minimum inhibitory concentration (MIC), non-inhibitory concentration (NIC), and minimum lethal concentration (MLC) values were determined. OEO effectiveness was further validated in commercial tomato juice. Supplementation of tomato juice with OEO at concentrations lower than the MIC (350 ppm) resulted in significant delay of food spoilage and extension of the product's shelf-life, as well as in inhibition of *Listeria monocytogenes*, *Clostridium difficile*, *Saccharomyces cerevisiae*, and *Aspergillus niger* growth after deliberate inoculation in both room and refrigerated temperatures. In conclusion, the results suggested that OEO may be used as an efficient intrinsic inhibitor of food spoilage and growth of pathogenic microbes in tomato juice.

Keywords: food spoilage; pathogens; *Origanum* essential oil; tomato juice; biopreservatives; antimicrobials; hurdles

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1. Introduction

Currently, there is a strong debate regarding the safety aspects of chemical preservatives added widely in most manufactured foods to prevent the growth of spoilage and pathogenic microbes. Hence, an increased interest in essential oils from various plant origins as alternative antimicrobial natural supplements to harmful synthetic additives is occurring [1,2].

The antimicrobial activity of essential oils isolated from plants of the *Lamiaceae* family has been well established [3–5]. Among the *Lamiaceae* herbs, *Origanum vulgare* ssp. *hirtum*, commonly known as oregano, is wildly grown and also cultivated in Mediterranean countries, and the essential oil has been previously tested for its antimicrobial properties [6,7]. However, the effectiveness of the oil may vary, depending on when the plant is harvested, the cultivar, and environmental and climate factors, etc. [6,8]. Its properties are basically attributed to the main constituents of the essential oil, thymol and carvacrol [9].

A properly designed strategy for incorporation of essential oils into foods (formulation strategies, efficiency, and organoleptic quality issues) is a key factor in the development of novel products. Their insertion into a food matrix is considered an additional inherent

factor for the growth prevention of pathogens or delay of spoilage onset. In this context, several attempts have been focused on the successful addition of essential oils and plant extracts on various foods [10,11], but only a very limited number of products are available in the market, mainly due to incompatibility with food taste, ineffectiveness owing to interaction of bioactive substances with food components, and intense aroma [12].

Tomato juice spoilage and pathogenicity is mainly due to growth and activity of several microbial species, including, among others, *Listeria monocytogenes, Saccharomyces* sp., and *Aspergillus niger* [13,14]. *L. monocytogenes* is a tenacious microorganism that is ubiquitous in the environment. This Gram-positive opportunistic bacterium is of very high public health concern, as it is the cause of a life-threatening disease called listeriosis [14]. *Saccharomyces* sp. are unicellular microorganisms that are frequently involved in food spoilage, especially beverages and processed fruit juices [15]. *A. niger* is a fungus capable of producing ochratoxin, which is considered a potent hazard to human health [16]. Additionally, incidences of *Clostridium difficile* infection have been increased lately, as different food types may act as vectors for *C. difficile* strains. Considering that *C. difficile* spores are able to survive at temperatures up to 85 °C and thus the pasteurization process, it is not unreasonable to suggest *C. difficile* as a "foodborne pathogen" [17,18].

The aim of the present study was to assess the industrial potential of *O. vulgare* ssp. *hirtum* essential oil (OEO) as a natural intrinsic hurdle against common spoilage and pathogenic microbes in tomato juice. Data suggesting extension of the product's self-life and repression of microbial growth after deliberate inoculation are presented.

2. Materials and Methods

2.1. Extraction of OEO by Hydrodistillation

Dried leaves of OEO were obtained from the Institute of Plant Breeding and Genetic Resources (Hellenic Agricultural Organization-DEMETER, Athens, Greece). The plant was collected during the maximum blooming period from regions of North Greece, dried, and screened by hand. A voucher sample of the material has been kept at the Laboratory of Organic Synthesis of the Vioryl Chemical and Agricultural Industry, Research S.A. (Vioryl). Hydrodistillation took place at Vioryl facilities in Eleonas, Thiva, Greece, in an own-constructed apparatus. The dried leaves (9.6 kg) were mixed with 150 L water into a glass enamel vessel (400 L), equipped with mechanical stirring and a two-stage glass condenser. Saturated steam of 2 bars was introduced into the vessel. After hydrodistillation for 16 h, the resulting essential oil (OEO) was separated from water, dried over anhydrous sodium sulfate, and stored at 4 °C (412 g OEO was isolated, yield 4.29%).

2.2. Microbial Strains

Listeria monocytogenes NCTC 10527 (serotype 4b) and *Clostridium difficile* (kindly provided by the Laboratoty of Clinical Microbiology of Sismanoglio Hospital, Athens, Greece), *Saccharomyces cerevisiae* uvaferm NEM (Lallemand, Montreal, QC, Canada), and *Aspergillus niger* 19111 (kindly provided by Prof. G.J.E Nychas, Agricultural University of Athens) were used in the present study.

Both bacterial strains were grown in brain heart infusion (BHI) broth (LabM, Heywood, UK) at 37 °C for 24 h, whereas YPD broth (yeast extract 10 g/L, peptone 20 g/L, and dextrose 20 g/L) or malt extract agar (LabM) were used for the growth of *S. cerevisiae* or *A. niger* at 28 °C for 3 days or at 37 °C for 7 days, respectively.

2.3. OEO Emulsion Preparation

Water emulsions containing OEO were prepared by stirring the 2 phases (water phase (79.645% *w*/*w*): 0.25% *w*/*w* citric acid, 10.0% *w*/*w Quillaia* extract (Ingredion Inc., Westchester, IL, USA); oil phase (20.355% *w*/*w*): 10% *w*/*w* OEO, 0.005% *w*/*w* tocopherol mix (Biotecnologías Aplicadas, S.L. Madrid, Spain)) at 5000 rpm for 20 min followed by homogenization using an APV-2000 homogenizer (SPX Flow Technology Rosista GmbH, Unna, Germany) as follows: first phase 50 bar, second phase 280 bar. For comparison,

emulsions containing medium-chain triglycerides (Sternchemie, Hamburg, Germany) instead of OEO were also prepared and used as controls.

2.4. Tomato Juice Supplementation with OEO

The OEO emulsion was added in commercial tomato juice with no preservatives (Pummarò Passata, Unilever) in order to achieve a final concentration of 350 ppm. For comparison reasons, tomato juice containing emulsions with medium-chain triglycerides instead of OEO were also prepared and used as controls. The pH of the tomato juice samples was approximately 4.90. The mixtures were homogenized at 8000 rpm for 15 min, pasteurized at 90 °C for 30 min, and then either stored in glass containers at room or low temperature (4 °C) or deliberately spiked with spoilage or pathogenic microorganisms, as described below in Section of Antimicrobial Activity of OEO in Tomato Juice.

2.5. Analytical Procedures

2.5.1. GC/MS Analysis

Gas chromatography mass spectrometry (GC/MS) analysis was carried out by a GC/MS system (GC: 6890A, Agilent Technologies, USA; MSD: 5973, Agilent Technologies, Santa Clara, CA, USA) using a Factor Four VF 1 ms column (25 m, 0.2 mm i.d., 0.33 μm film thickness, Agilent Technologies), as described recently [19]. Identification was carried out by comparing the retention times and mass spectra of volatiles to Willey/NIST05 and in-house-created libraries using authentic compounds (kindly provided by Vioryl) and by determining linear retention indexes (LRI) and comparing them with those reported in the literature (Nist Mass Spectral Search Program for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library, Version 2.3, build 4 May 2017).

2.5.2. Antimicrobial Assays

Screening of OEO Antimicrobial Activity by the Disc Diffusion Assay

The antibacterial activity of the OEO was initially tested using the disk diffusion assay using BHI agar plates, as described previously [19]. Gentamycin (10 μg) (Oxoid Ltd, Hampshire, UK) and metronidazole (5 μg) (Oxoid) were used as positive inhibitory controls for *L. monocytogenes* and *C. difficile*, respectively, whereas sterile water was used as negative control [19].

The same procedure was also followed for screening the activity against *S. cerevisiae* and *A. niger* using YPD and malt agar plates, respectively [19]. Voriconazole (1 μg) (BioRad Laboratories Inc., Hercules, CA, USA) and sterile water were used as positive inhibitory and negative controls, respectively [19].

Determination of Minimum Inhibitory Concentration (MIC), Non-Inhibitory Concentration (NIC), and Minimum Lethal Concentration (MLC)

Determination of minimum inhibitory (MIC) and non-inhibitory (NIC) concentrations was carried out as recently described [19,20]. Briefly, BHI broths supplemented with various OEO concentrations (ranging from 22 to 4628 mg/L) were inoculated with *L. monocytogenes* or *C. difficile* and incubated at 37 °C for 24 h. Changes in optical density were monitored using a microplate reader (Molecular Devices, VERSAmax, Hayward, CA, USA, Softmaxprov.5.0 software). BHI broths supplemented with gentamycin (ranging from 2 to 20 mg/L) and metronidazole (ranging from 0.012 to 8 mg/L) were used as positive inhibitory controls for *L. monocytogenes* and *C. difficile*, respectively. BHI broths with no inoculum and inoculated BHI broths with no OEO were used as negative controls [19]. The calculation of MIC and NIC values was based on the Lambert-Pearson model (LPM) [21,22].

As the LPM model was not applicable to *S. cerevisiae* and *A. niger* due to yeast cell sedimentation and conidia flotation, the standard protocols described by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied for MIC determination [23,24], using voriconazole (ranging from 0.12 to 4 mg/L) as positive inhibitory control [25,26].

Minimum lethal concentration (MLC) was defined as the lowest concentration, resulting in a negative subculture or presence of only 1 colony-forming unit after incubation (99.9% of the inoculum was killed). It was determined by subculturing 100 μL from each well at which no growth was observed onto BHI (for *L. monocytogenes* and *C. difficile*) and YPD or malt (for *S. cerevisiae* or *A. niger*) agar plates [19].

Antimicrobial Activity of OEO in Tomato Juice

Spoilage of tomato juice supplemented with OEO or without OEO both at room temperature (18–20 °C) and 4 °C was monitored by determining bacteria on BHI agar (LabM) and yeast/mold counts on malt extract agar (LabM) after media incubation at 37 or 30 °C, respectively, for 2–5 days. Cell counts below 1 log colony forming units/mL were determined by pour plating 1 mL of undiluted tomato juice directly to the culture medium. Of note, all juices were pasteurized prior treatments, as described above.

Both tomato juice containing OEO and tomato juice without OEO were also deliberately inoculated with *L. monocytogenes* (10^4 cfu/mL), *C. difficile* (10^4 cfu/mL), *S. cerevisiae* (10^4 cfu/mL), or *A. niger* (100 spores/mL), separately, and microbial counts were monitored both at room temperature (18–20 °C) and 4 °C. At various intervals, samples were collected to monitor the levels of the inoculated strains. *L. monocytogenes* or *C. difficile* counts were measured in BHI agar (LabM) and further verified in Palcam agar (LABM) supplemented with Palcam *Listeria* selective supplement (LABM) or *C. difficile* agar (Oxoid) with selective supplement (Oxoid) and egg yolk emulsion (Oxoid), respectively, after incubation at 37 °C for 24–48 h (anaerobic conditions provided by Genbox atmospheric generators (BioMerieux, Athens, Greece) were applied for *C. difficile*). *S. cerevisiae* counts were determined in YPD agar after incubation at 28 °C for 3 days. Numbers of *A. niger* spores/mL were determined by microscopic enumeration with a cell-counting hematocytometer (Neubauer chamber).

2.6. Preliminary Sensory Evaluation and Spoilage Determination

To determine the optimum amount of OEO that leads to acceptable organoleptic characteristics, we conducted preliminary sensory analysis of tomato juice samples by 11 not trained consumers using locally approved protocols.

Spoilage was determined macroscopically and by sensory tests. Specifically, the following scoring scale was applied: (i) class 1 for products corresponding to high quality with no off odors or off flavors, (ii) class 2 for products corresponding to slight off odors or off flavors but still acceptable, and (iii) class 3 for products corresponding to unacceptable quality. Estimation of the shelf-life limit was based on definition of the time point at which 50% of the panelists rejected the tomato juice samples.

2.7. Statistical Analysis

All experiments were performed in 4 replicates and the mean values are presented.

Fig. P.2.1 software (Fig.P Software Incorporated, Hamilton, ON, Canada) was used to calculate standard deviation for MIC and NIC values.

The results were analyzed with analysis of variance (ANOVA) using Duncan's multiple range test to determine significant differences ($p < 0.05$) among results (coefficients, ANOVA tables, and significance ($p < 0.05$) were computed using Statistica v.10.0).

3. Results and Discussion

3.1. GC/MS Analysis of OEO

GC/MS analysis provided data about the percentage content of the volatile compounds and not their actual concentration. In total, 51 compounds were identified (Table 1). The main compounds detected were thymol and carvacrol, accounting for approximately 48% and 27% of the total area, respectively.

Table 1. Gas chromatography mass spectrometry (GC/MS) analysis of *Origanum vulgare* ssp. *hirtum* essential oil (OEO).

LRI [1]	Compounds	% Area [2]
595	ethyl acetate	0.010
741	methyl isovalerate	0.047
743	methyl-2-methyl-butyrate	Tr
820	methyl tiglate	0.022
821	*cis*-3-hexenol	Tr
852	ethyl isobutylketone	0.015
919	thujene	0.250
926	*α*-pinene	0.263
937	camphene	0.058
959	oct-1-en-3-ol	0.607
975	myrcene	1.026
988	*a*-phellandrene	0.125
994	unknown alkane (C_nH_{2n+2})	0.026
996	*δ*-3-carene	0.053
1001	*a*-terpinene	1.103
1005	*p*-cymene	6.502
1012	*β*-phellandrene	0.132
1013	limonene	0.172
1020	*cis*-ocimene	0.022
1032	*trans*-ocimene	0.053
1045	*γ*-terpinene	9.014
1048	*cis*-sabinene hydrate	0.065
1059	unknown	0.120
1075	terpinolene	0.079
1082	linalol	0.129
1097	unknown alkane (C_nH_{2n+2})	0.024
1115	unknown	0.130
1147	borneol	0.260
1158	terpin-4-ol	0.316
1169	*α*-terpineol	0.100
1194	unknown alkane (C_nH_{2n+2})	0.087
1212	citronellol	0.101
1213	thymyl methyl ether	0.033
1256	citronellyl formiate	0.027
1262	indol	0.020
1283	thymol	48.120
1294	carvacrol	27.190
1315	unknown	0.150
1388	unknown alkane (C_nH_{2n+2})	0.032
1398	unknown alkane (C_nH_{2n+2})	0.059
1413	caryophellene	1.060
1423	unknown	0.100
1433	unknown sesquiterpene	0.050
1446	humulene	0.120
1488	υnknown sesquiterpene	0.070
1500	*β*-bisabolene	1.720

[1] Linear retention indices, [2] Tr: traces (<0.010%).

3.2. Antimicrobial Assays

Considering that composition and thus effectiveness of essential oils depends greatly upon a number of cultivation, environmental, and climate factors [6,27,28], the antimicrobial activity of OEO against spoilage and pathogenic microbes of concern for tomato juice (*L. monocytogenes*, *C. difficile*, *S. cerevisiae*, and *A. niger*) was initially confirmed by the disk diffusion method (data not shown). Subsequently, MIC, NIC, and MLC values were assessed, as their precise determination is crucial for the food industry, in order to regulate the optimum amount of the antimicrobial agent to secure microbial safety. The

effective growth inhibition of OEO against all microorganisms tested (Table 2) was verified, although MIC, NIC, and MLC values were significantly ($p < 0.05$) higher compared to gentamycin [19], metronidazole, and voricanozole [20], which were used as positive inhibitory controls, since antibiotics have been used as food additives in the past [29]. Although the antimicrobial activity of OEO is well documented [4–7,30–32], MIC, NIC, and MLC values should be always determined, since chemical composition may vary greatly due to environmental conditions, climate, and time of harvesting [6,8].

Table 2. Minimum inhibitory concentration (MIC), non-inhibitory concentration (NIC), and minimum lethal concentration (MLC) (mg/L) of *Origanum vulgare* ssp. *hirtum* essential oil (OEO) against *Listeria monocytogenes*, *Clostridium difficile*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Gentamycin, metronidazole, and voriconazole were used as positive inhibitory controls.

Microbial Species	Essential Oil			Metronidazole			Gentamycin			Voriconazole		
	MIC	NIC	MLC [a]	MIC	NIC	MLC [a]	MIC	NIC	MLC [a]	MIC [a]	NIC	MLC [a]
L. monocytogenes	1148 ± 27	528 ± 37	5738	–	–	–	3.121 ± 0.002	3.001 ± 0.002	16.0	–	–	–
C. difficile	1314 ± 27	204 ± 27	7404	0.064 ± 0.001	0.032 ± 0.001	4.0	–	–	–	–	–	–
S. cerevisiae	1647 [a]	-	6590	–	–	–	–	–	–	0.25	–	1.00
A. niger	2197 [a]	-	8786	–	–	–	–	–	–	0.50		2.00

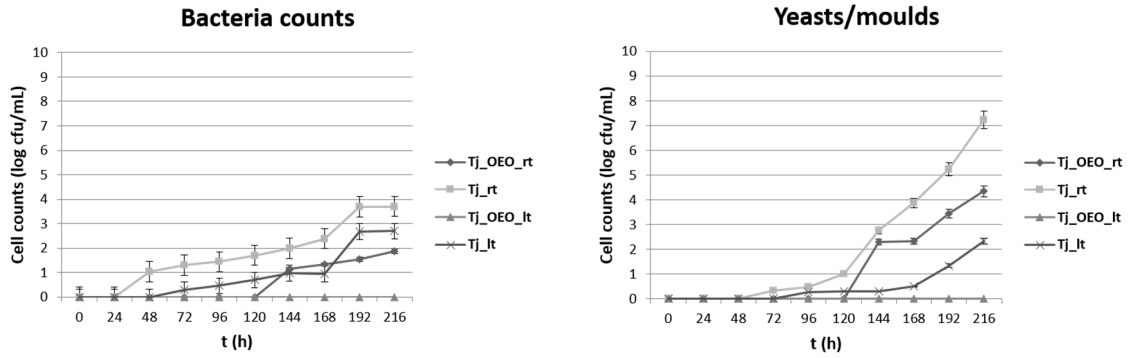
[a] Standard deviation ranged in zero values.

3.3. Antimicrobial Activity in Tomato Juice Supplemented with OEO

Primarily, the effect of OEO on the extension of the preservation time was investigated. OEO was introduced in tomato juice in an emulsion form to facilitate homogeneous dilution at a concentration (350 ppm) lower than the MIC, as higher levels resulted in sensory faults. However, at the concentration applied, no off flavors were detected according to the preliminary sensory evaluation as the choice of an emulsifier depends greatly on the properties of the component to be dissolved, the properties of the emulsifier and the food matrix, *Quillaia* extract, an acid-stable and effective natural emulsifier, was chosen [33] in order to achieve micelle stability and to replace synthetic surfactants usually applied in foods and beverages [34]. Citric acid was added as a stabilizer at the emulsion (0.003 ppm final concentration at the tomato juice), but at the pH of the juice (4.90), it is highly unlikely to have any antimicrobial effect [35].

The results are presented in Figure 1. The OEO resulted in significantly ($p < 0.05$) lower counts for bacteria and yeasts/molds compared to the control samples (without OEO), suggesting an important extension of the product's shelf-life, up to 48 h, at room temperature. Importantly, no spoilage was noted in tomato juice with OEO at 4 °C.

Subsequently, the resistance of tomato juice supplemented with OEO against growth of pathogenic microbes was further investigated. In this vein, tomato juice containing OEO and tomato juice with no OEO were deliberately inoculated with *L. monocytogenes*, *C. difficile*, *S. cerevisiae*, or *A. niger*, separately, and microbial growth was monitored both at room temperature or at low temperature, specifically at 4 °C (Figure 2a–d). A relatively high inoculum was tested in order to investigate the effectiveness of the oil in high microbial counts. The results showed that in all cases microbial counts were significantly ($p < 0.05$) lower in tomato juice containing OEO.



(**a**) (**b**)

Figure 1. Effect of *O. vulgare* ssp. *hirtum* essential oil (OEO) and storage temperature on tomato juice spoilage. (**a**) Bacteria counts, (**b**) counts of yeasts/molds. Tj_OEO_rt: tomato juice containing OEO (350 ppm) stored at room temperature (18–20 °C); Tj_rt: tomato juice with no OEO (medium chain triglycerides were used instead) stored at room temperature (18–20 °C) (control); Tj_OEO_lt: tomato juice containing OEO (350 ppm) stored at low temperature (4 °C); Tj_lt: tomato juice with no OEO (medium chain triglycerides were used instead) stored at low temperature (4 °C) (control).

Supplementation of the juice with OEO at concentration lower than NIC or only slightly higher than the NIC determined for *Clostridium difficile* was sufficient to suppress the growth of the deliberately inoculated microbes, suggesting a clear synergistic effect of the oil and the refrigeration at 4 °C. Microorganisms are very often unable to overcome the combination of several food preservation systems and hence the multiple hurdles can result in a stable and safe product without any sensory quality deterioration [36]. The use of essential oils can be thus considered an additional intrinsic determinant (hurdle) to ensure food safety, although the limitations associated to their strong smell at effective doses and the decrease in their effectiveness when added to complicated food ecosystems compared with microbiological media [37].

Several efforts on extending the preservation time of tomato juice included the use of natural volatile compounds (methyl jasmonate, ethanol, their combination, tea tree oil, and garlic oil) [38]; investigation of antimicrobial activity of food-compatible plant extracts and chitosan against naturally occurring micro-organisms in tomato juice [39]; dipping treatment in 2% ascorbic acid, citric acid, and calcium lactate solutions [40]; dipping treatment in calcium chloride and packaging in biocompostable materials or in conventional plastics [41]; and introduction of fresh-cut tomato in nisin or in nisin in combination with sodium citrate or sodium acetate solutions [17]. However, to the best of the authors' knowledge, the present study reports for the first time the effectiveness of OEO in suppressing microbial growth in deliberately spiked tomato juice.

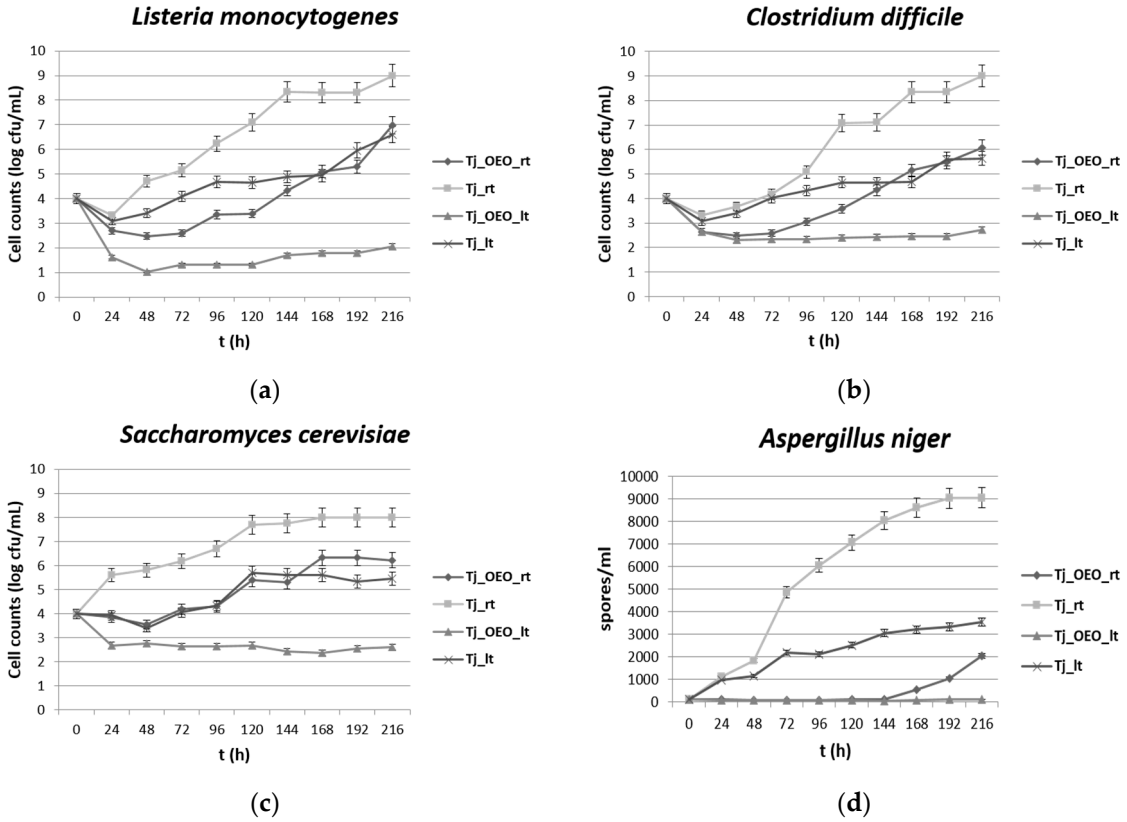


Figure 2. Effect of *O. vulgare* ssp. *hirtum* essential oil (OEO) and storage temperature on *Listeria monocytogenes* (**a**), *Clostridium difficile* (**b**), and *Saccharomyces cerevisiae* (**c**) counts and *Aspergillus niger* (**d**) spores in deliberately spiked tomato juice. Tj_OEO_rt: tomato juice containing OEO (350 ppm) stored at room temperature (18–20 °C); Tj_rt: tomato juice with no OEO (medium chain triglycerides were used instead) stored at room temperature (18–20 °C) (control); Tj_OEO_lt: tomato juice containing OEO (350 ppm) stored at low temperature (4 °C); Tj_lt: tomato juice with no OEO (medium chain triglycerides were used instead) stored at low temperature (4 °C) (control).

4. Conclusions

The results revealed that OEO may be used as an efficient intrinsic inhibitor (hurdle) of food spoilage and as a protection shield against food pathogens, since fortification of tomato juice with OEO, even at concentration below MIC, resulted in significant suppression of *L. monocytogenes*, *C. difficile*, *S. cerevisiae*, and *A. niger* growth after deliberate inoculation. Thus, its use as a biopreservative by the food industry is of utmost importance since the demand for replacement of chemical preservatives with natural alternatives is increasing.

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References

1. Bakkali, F.; Averbeck, S.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef] [PubMed]
2. Swamy, M.K.; Akhtar, M.S.; Sinniah, U.R. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evid. Based Complement. Altern. Med.* **2016**, *2016*, 1–21. [CrossRef]
3. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef] [PubMed]
4. Chorianopoulos, N.G.; Evergetis, E.T.; Aligiannis, N.; Mitakou, S.; Nychas, G.-J.E.; Haroutounian, S.A. Correlation between Chemical Composition of Greek Essential Oils and their Antibacterial Activity against Food-borne Pathogens. *Nat. Prod. Commun.* **2007**, *2*, 419–426. [CrossRef]
5. Nychas, G.J.E.; Skandamis, P.N.; Tassou, C. Antimicrobials from herbs and spices. In *Natural Antimicrobials for the Minimal Processing of Foods*; Elsevier BV: Amsterdam, The Netherlands, 2003; pp. 176–200.
6. Chorianopoulos, N.; Kalpoutzakis, E.; Aligiannis, N.; Mitaku, S.; Nychas, G.-J.; Haroutounian, S.A. Essential Oils of Satureja, Origanum, and Thymus Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. *J. Agric. Food Chem.* **2004**, *52*, 8261–8267. [CrossRef]
7. Şahin, F.; Güllüce, M.; Daferera, D.; Sökmen, A.; Sökmen, M.; Polissiou, M.; Agar, G.; Özer, H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. *Food Control.* **2004**, *15*, 549–557. [CrossRef]
8. Baranauskienė, R.; Venskutonis, P.R.; Dambrauskienė, E.; Viškelis, P. Harvesting time influences the yield and oil composition of *Origanum vulgare* L. ssp. vulgare and ssp. hirtum. *Ind. Crop. Prod.* **2013**, *49*, 43–51. [CrossRef]
9. De Martino, L.; De Feo, V.; Formisano, C.; Mignola, E.; Senatore, F. Chemical Composition and Antimicrobial Activity of the Essential Oils from Three Chemotypes of *Origanum vulgare* L. ssp. hirtum (Link) Ietswaart Growing Wild in Campania (Southern Italy). *Molecules* **2009**, *14*, 2735–2746. [CrossRef]
10. Chorianopoulos, N.G.; Skandamis, P.N.; Nychas, G.-J.E.; Haroutounian, S.A. Agricultural University of Athens Iera odos Athens Greece Use of essential oil treatments to control the *Listeria monocytogenes* growth in various food commodities. *Planta Med.* **2008**, *74*, PI31. [CrossRef]
11. Tsimogiannis, D.; Choulitoudi, E.; Bimpilas, A.; Mitropoulou, G.; Kourkoutas, Y.; Oreopoulou, V. Exploitation of the biological potential of Satureja thymbra essential oil and distillation by-products. *J. Appl. Res. Med. Aromat. Plants* **2017**, *4*, 12–20. [CrossRef]
12. Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components. *Front. Microbiol.* **2012**, *3*, 12. [CrossRef] [PubMed]
13. Oviasogie, F.E.; Ogofure, A.G.; Obatusin, S.V.; Beshiru, A.; Onwuaduegbo, J. Microbial spoilage of canned tomato sold in Benin city. *J. Adv. Sci. Res.* **2015**, *6*, 6–9.
14. Oladunjoye, A.O.; Singh, S.; Ijabadeniyi, O.A. Inactivation of *Listeria monocytogenes* ATCC 7644 on fresh-cut tomato using nisin in combinations with organic salts. *Braz. J. Microbiol.* **2016**, *47*, 757–763. [CrossRef]
15. Turtoi, M. Inactivation of *Saccharomyces cerevisiae* using nonconventional treatments. A review. *Rom. Biotechnol. Lett.* **2014**, *19*, 8901–8909.
16. Samuel, O.; Orji, M.U. Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria. *Univers. J. Microbiol. Res.* **2015**, *3*, 11–16. [CrossRef]
17. Metcalf, D.; Costa, M.C.; Dew, W.; Weese, J. Clostridium difficile in vegetables, Canada. *Lett. Appl. Microbiol.* **2010**, *51*, 600–602. [CrossRef] [PubMed]
18. Bakri, M.M.; Brown, D.; Butcher, J.P.; Sutherland, A. Clostridium difficilein Ready-to-Eat Salads, Scotland. *Emerg. Infect. Dis.* **2009**, *15*, 817–818. [CrossRef]
19. Mitropoulou, G.; Fitsiou, E.; Spyridopoulou, K.; Tiptiri-Kourpeti, A.; Bardouki, H.; Vamvakias, M.; Panas, P.; Chlichlia, K.; Pappa, A.; Kourkoutas, Y. Citrus medica essential oil exhibits significant antimicrobial and antiproliferative activity. *LWT* **2017**, *84*, 344–352. [CrossRef]
20. Mitropoulou, G.; Sidira, M.; Skitsa, M.; Tsochantaridis, I.; Pappa, A.; Dimtsoudis, C.; Proestos, C.; Kourkoutas, Y. Assessment of the Antimicrobial, Antioxidant, and Antiproliferative Potential of *Sideritis raeseri* subps raeseri Essential Oil. *Foods* **2020**, *9*, 860. [CrossRef] [PubMed]
21. Chorianopoulos, N.; Lambert, R.; Skandamis, P.; Evergetis, E.; Haroutounian, S.; Nychas, G.-J.E. A newly developed assay to study the minimum inhibitory concentration of Satureja spinosa essential oil. *J. Appl. Microbiol.* **2006**, *100*, 778–786. [CrossRef]
22. Lambert, R. A model for the efficacy of combined inhibitors. *J. Appl. Microbiol.* **2003**, *95*, 734–743. [CrossRef] [PubMed]

23. Arendrup, M.C.; Meletiadis, J.; Mouton, J.W.; Lagrou, K.; Hamal, P.; Guinea, J. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing. EUCAST Definitive Document EDEF 9.3.2 Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Anti-fungal Agents for Conidia Forming Moulds. 2020. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9.3.2_Mould_testing_definitive_revised_2020 (accessed on 7 April 2020).
24. Arendrup, M.C.; Meletiadis, J.; Mouton, J.W.; Lagrou, K.; Hamal, P.; Guinea, J. Subcommittee on Antifungal Susceptibil-ity Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing. EUCAST Definitive Document EDEF 7.3.2 Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Anti-fungal Agents for Yeasts. 2020. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_7.3.2_Yeast_testing_definitive_revised_2020 (accessed on 8 April 2020).
25. Balkan, C.; Ercan, I.; Isik, E.; Akdeniz, E.S.; Balcioglu, O.; Kodedová, M.; Zimmermannová, O.; Dundar, M.; Sychrová, H.; Koc, A. Genomewide Elucidation of Drug Resistance Mechanisms for Systemically Used Antifungal Drugs Amphotericin B, Caspofungin, and Voriconazole in the Budding Yeast. *Antimicrob. Agents Chemother.* **2019**, *63*. [CrossRef]
26. Mandras, N.; Roana, J.; Scalas, D.; Fucale, G.; Allizond, V.; Banche, G.; Barbui, A.; Vigni, N.L.; A Newell, V.; Cuffini, A.M.; et al. In vitro antifungal activity of fluconazole and voriconazole against non-Candida yeasts and yeast-like fungi clinical isolates. *New Microbiol.* **2015**, *38*, 583–587.
27. Aboukhalid, K.; Al Faiz, C.; Douaik, A.; Bakha, M.; Kursa, K.; Agacka-Mołdoch, M.; Machon, N.; Tomi, F.; Lamiri, A. Influence of Environmental Factors on Essential Oil Variability in *Origanum compactum* Benth. Growing Wild in Morocco. *Chem. Biodivers.* **2017**, *14*, e1700158. [CrossRef]
28. Shams, M.; Esfahan, S.Z.; Esfahan, E.Z.; Dashtaki, H.N.; Dursun, A.; Yildirim, E. Effects of Climatic Factors on the Quantity of Essential Oil and Dry Matter Yield of Coriander (*Coriandrum sativum* L.). *Indian J. Sci. Technol.* **2016**, *9*, 6. [CrossRef]
29. *Code of Federal Regulations (Annual Edition) AE 2.106/3:9/ Title 9—Animals and Animal Products. Chapter I—Animal and Plant Health Inspection Service, Department of Agriculture Subchapter E—Viruses, Serums, Toxins, and Analogous Products; Organisms and Vectors Part 114–Production Requirements for Biological Products. Section 114.10–Antibiotics as Preservatives. 9 CFR § 114.10. 1 January 2011, 39 FR 16869, May 10, 1974, as Amended at 56 FR 66784*; U.S. Government Publishing Office: Washington, DC, USA, 1991.
30. Barbosa, L.N.; Rall, V.L.M.; Fernandes, A.A.H.; Ushimaru, P.I.; Probst, I.D.S.; Júnior, A.F. Essential Oils Against Foodborne Pathogens and Spoilage Bacteria in Minced Meat. *Foodborne Pathog. Dis.* **2009**, *6*, 725–728. [CrossRef] [PubMed]
31. Chami, F.; Chami, N.; Bennis, S.; Bouchikhi, T.; Remmal, A. Oregano and clove essential oils induce surface alteration of *Saccharomyces cerevisiae*. *Phytother. Res.* **2005**, *19*, 405–408. [CrossRef]
32. Pawar, V.C.; Thaker, V.S. In vitro efficacy of 75 essential oils against Aspergillus niger. *Mycoses* **2006**, *49*, 316–323. [CrossRef]
33. Klingenberg, A. Emulsion. US Patent 20130089644, 2013. Available online: https://patents.justia.com/inventor/andreas-klingenber (accessed on 16 April 2020).
34. Yang, Y.; Leser, M.E.; Sher, A.A.; McClements, D.J. Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). *Food Hydrocoll.* **2013**, *30*, 589–596. [CrossRef]
35. Hawkins, S.G. Antimicrobial Activity of Cinnamic Acid, Citric Acid, Cinnamaldehyde, and Levulinic Acid against Foodborne Pathogens. Chancellor's Honors Program Projects. University of Tennessee, Knoxville Trace: Tennessee Research and Creative, Knoxville, TN, USA, 2014. Available online: http://trace.tennessee.edu/utk_chanhonoproj (accessed on 1 May 2014).
36. Leistner, L.; Gorris, L.G. Food preservation by hurdle technology. *Trends Food Sci. Technol.* **1995**, *6*, 41–46. [CrossRef]
37. Skandamis, P.N.; Nychas, G.-J.E. Development and Evaluation of a Model Predicting the Survival of Escherichia coli O157:H7 NCTC 12900 in Homemade Eggplant Salad at Various Temperatures, pHs, and Oregano Essential Oil Concentrations. *Appl. Environ. Microbiol.* **2000**, *66*, 1646–1653. [CrossRef] [PubMed]
38. Ayala-Zavala, J.F.; Oms-Oliu, G.; Odriozola-Serrano, I.; González-Aguilar, G.A.; Alvarez-Parrilla, E.; Martín-Belloso, O. Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *Eur. Food Res. Technol.* **2007**, *226*, 1047–1055. [CrossRef]
39. Giner, M.J.; Vegara, S.; Funes, L.; Martí, N.; Saura, D.; Micol, V.; Valero, M. Antimicrobial activity of food-compatible plant extracts and chitosan against naturally occurring micro-organisms in tomato juice. *J. Sci. Food Agric.* **2012**, *92*, 1917–1923. [CrossRef] [PubMed]
40. Antunes, M.D.; Rodrigues, D.; Pantazis, V.; Cavaco, A.M.; Siomos, A.S.; Miguel, M.G. Nutritional quality changes of fresh-cut tomato during shelf life. *Food Sci. Biotechnol.* **2013**, *22*, 1–8. [CrossRef]
41. Patanè, C.; Malvuccio, A.; Saita, A.; Rizzarelli, P.; Rapisarda, M.; Rizzo, V.; Muratore, G. Quality aspects of fresh-cut 'long-storage tomato' as affected by package, calcium chloride and storage time. *Int. J. Food Sci. Technol.* **2017**, *53*, 819–827. [CrossRef]