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Article

Influence of lipids and proteins amounts and pH values on the inhibitory effects of *Origanum vulgare* L. essential oil against *Escherichia coli* and *Salmonella* Typhimurium

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Origanum vulgare L. (OVEO) essential oil has been considered a candidate antimicrobial for use in food conservation systems. However, studies on the influence of concomitant variations of different food components or physicochemical parameters on the antibacterial properties of OVEO are scarce. This study assessed the influence of concomitant variations in amounts of proteins - PTN (4.0, 6.0 or 8.0 g/100 mL) and lipids - LIP (3.75, 5.0 or 6.25 g/100 mL) and pH values (5.0, 5.5 or 6.0) in cultivation medium on the inhibitory effects of OVEO against *Escherichia coli* (EC) and *Salmonella* Typhimurium (ST). Lowest minimum inhibitory concentration values of OVEO against EC and ST were observed in media with the highest LIP amounts regardless the PTN amount and pH value. In absorbance based microtiter plate assay (MPA), for both EC and ST, OVEO caused the lowest Grmax values in medium containing the highest LIP and PTN amounts and lowest pH value. Highest Grmax values for EC and ST were observed in medium containing the lowest LIP and PTN amount and highest pH value. Grmax values estimated from viable counts of EC and ST in tested media with OVEO confirmed bacterial growth behavior similar to that observed in MPA. Overall, the LIP amount in media was as the most influential factor to enhance the antibacterial effects of OVEO. These results indicate that the concomitant influence of LIP and PTN amounts and pH values on the antibacterial effects of OVEO should be considered for optimizing its antimicrobial efficacy in foods.

Keywords: Oregano. Antibacterial effects. Influential factors. Foodborne pathogens.

INTRODUCTION

Increased consumer awareness and concern with the use of synthetic preservatives to control microbial growth in foods have stimulated the food industry and researchers to investigate natural antimicrobial substances (de Souza, 2016). Essential oils (EOs) have been considered "green" antimicrobials to reach food safety and stability because of their strong and wide spectrum antimicrobial properties (Smith-Palmer, Stewart, Fyfe, 2001; Carvalho *et al.*, 2015; Barbosa *et al.*, 2016). Additionally, EOs are classified as "Generally Recognized as Safe" (GRAS) by the Food and Drug Administration for use as flavoring substances in foods (USFDA, 2015).

The essential oil from *Origanum vulgare* L. (oregano) has shown active to inhibit a variety of food contaminant bacteria (Barbosa *et al.*, 2016), being considered a candidate for use in food conservation systems (Calo *et al.*, 2015; Sarikurkcu *et al.*, 2015).

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Origanum vulgare essential oil (OVEO) is a rich source of terpenes and terpenoids, in which carvacrol, thymol, γ-terpinene and *p*-cymene are typically identified as the majority constituents (Barbosa *et al*., 2016; Melo *et al*., 2017). The *in vitro* antibacterial properties of OVEO against potentially pathogenic or pathogenic bacteria, e.g., *Escherichia coli* and *Salmonella enterica* Serovar Typhimurium, respectively, have been confirmed in a variety of food matrices (Carvalho *et al*., 2015; Melo *et al*., 2017).

Differences in antibacterial effects of EOs observed in distinct food matrices have been tentatively associated with the influence of a number of possible interfering food intrinsic factors, such as fat and protein content, enzymes, water activity, pH, redox potential and structure (Calo *et al*., 2015). Although some previous studies have evaluated the influence of food components or physicochemical parameters separately on the antimicrobial efficacy of EOs (Gutierrez, Barry-Ryan, Bourke, 2008; Gutierrez, Barry-Ryan, Bourke, 2009), there is a lack of studies on the influence of concomitant variations of two or more factors on the antimicrobial effects of EOs on bacteria of concern in foods.

This study assessed the influence of concurrent variations in amounts of food components, namely PTN and LIP, and pH values in medium on the inhibitory effects of OVEO against *E. coli* and *S.* Typhimurium. The minimum inhibitory concentration (MIC) of OVEO and the growth kinetics of the target bacteria when exposed to OVEO in media with different PTN and LIP amounts and pH values were determined.

MATERIAL AND METHODS

Material

OVEO

OVEO (batch SZB1206; density at 20 °C: 0.90; refractive index at 20 °C: 1.47), obtained by steam distillation, was purchased from Laszlo Ind. Com. Ltda. (Minas Gerais, Brazil). Emulsions of OVEO were prepared in brain heart infusion (BHI) broth (HiMedia, Mumbai, India) in a range of concentrations (40 - 0.312 µL/mL) using Tween 80 (1 mL/100 mL; Sigma-Aldrich, St. Louis, USA) as an emulsifier (Rodrigues *et al*., 2017). Tween 80 at the highest assayed concentration (1 g/100 mL) presented no inhibitory effects against the tested bacterial strains.

Strains

Salmonella Typhimurium phage type (PT) 4 (*Salmonella* Typhimurium PT4) isolated from chicken meat involved in outbreak occurred in the South of Brazil (Kottwitz *et al*., 2011) and *Escherichia coli* UFPEDA 224 (originally ATCC 25922, a surrogate for *E. coli* O157:H7; Kim, Harrison, 2009) were used as test strains. Stocks were maintained in BHI broth containing glycerol (20 mL/100 mL) at -20 °C in. For inoculum preparation of both strains, a 3-mL aliquot from an overnight culture grown in BHI broth at 37 °C for 18–24 h (to reach stationary growth phase) was harvested (4,500 x *g*, 15 min, 4 °C), washed twice and re-suspended in phosphate buffer solution (50 mM K_2HPO_4/KH_2PO_4; pH 7.4; Sigma-Aldrich, St. Louis, USA) to obtain standard cell suspensions. Optical density (OD) reading at 625 nm (OD_{625}) of these suspensions was 0.13, which provided viable counts of approximately 8 log colony forming units per milliliter (CFU/mL) when pour-plated on BHI agar (HiMedia, Mumbai, India).

Preparation of cultivation media

Inhibitory effects of OVEO on the tested bacterial strains were evaluated in media prepared with different amounts of proteins (PTN), lipids (LIP) and pH values. These media were used to simulate environmental conditions found by microorganisms in different foods, including low-acidic to neutral pH (5, 5.5 and 6), low to medium amounts of LIP (3.75, 5 and 6.25 mL/100 mL of sunflower oil - 99 g/100 mL LIP, Liza®, Brazil Ltda) or distinct PTN contents (4, 6 and 8 g/100 mL of beef extract - 75 g/100 g protein, Sigma Aldrich, St. Louis, USA) (Gutierrez *et al*., 2008). The combination of the different LIP and PTN amounts and pH values comprised 12 distinct media, as presented in Table I.

To obtain the different media, BHI broth was firstly added of beef extract (BE) at the desired amount and sterilized (121 °C, 1.1 atm, 15 min). Subsequently, the corresponding amounts of sunflower oil previously sterilized (121 °C, 1.1 atm, 15 min) were incorporated into the BHI broth-BE media, vortexed for 15 s and the pH value adjusted using HCl 1 mol/L (Gutierrez *et al*., 2008). Cultivation media were prepared in the same day of the experiments.

TABLE I - Minimum inhibitory concentration (MIC) of *Origanum vulgare* L. essential oil against *Escherichia* coli UFPEDA 224 and *Salmonella* Typhimurium PT4 in media with different amounts of proteins, lipids and pH values

Media	PTN amount (g/100 mL)	LIP amount (g/mL)	pH value	MIC (µL/mL)	
				E. coli UFPEDA 224	*S.* Typhimurium PT4
1	4.0	3.75	5.0	9.6	9.6
2	8.0	3.75	5.0	4.8	4.8
3	4.0	6.25	5.0	9.6	4.8
4	8.0	6.25	5.0	4.8	4.8
5	4.0	3.75	6.0	4.8	4.8
6	8.0	3.75	6.0	4.8	4.8
7	4.0	6.25	6.0	4.8	4.8
8	8.0	6.25	6.0	4.8	4.8
9	6.0	5.00	5.5	9.6	9.6
10	6.0	5.00	5.5	4.8	4.8
11	6.0	5.00	5.5	4.8	4.8
12	6.0	5.00	6.0	9.6	4.8

PTN: proteins; LIP: lipids.

Methods

Determination of minimum inhibitory concentration (MIC)

MIC of OVEO against the tested strains was determined using a microdilution in broth procedure (CLSI, 2012), with minor modifications related to the cultivation media and inoculum size. Initially, 50 µL of the different formulated media containing the OVEO emulsion in concentrations of 20 to 0.312 µL/mL were dispensed into each well of a 96-well microplate. Subsequently, 50 µL of bacterial suspension (approximately 8 log CFU/mL) were added to each well. The microplate with the lid was loosely wrapped with cling wrap to prevent OVEO volatilization. Each plate included controls without OVEO. The systems were incubated at 37 °C for 24 h. MIC was defined as the lowest OVEO concentration that was capable of inhibiting the visible growth of the test strain.

Assessing the maximum specific growth rate (Grmax) of the test strains in absorbance based microtiter plate assay

Grmax, as a growth kinetic parameter, of the test bacterial strains was estimated in media containing sub-MICs of OVEO using an absorbance based microtiter plate assay (MPA). Initially, 50 µL of the tested bacterial suspension (approximately 8 log CFU/mL) were added to each well of a 96-well microplate containing 50 µL of the respective media. The bacterial growth (absorbance

at 625 nm optical density) was monitored in a microplate reader/incubator (EON, BioTek, USA) at 37 °C each 2 h-intervals during 24 h. Positive controls comprised the respective media inoculated with the test strains without OVEO, and negative controls comprised the media containing OVEO without inoculation of the test strain. The Grmax values (log CFU/h) were estimated from the analysis of the growth curves using the EON-Gen5 software (EON, BioTek, USA) (Gutierrez *et al.*, 2009).

Enumerating the viable counts and modeling the growth kinetics of the test strains during exposure to OVEO in selected media

Viable counts of the test bacterial strains were determined during 24 h of incubation at 37 °C in media that provided the highest and the lowest Grmax values in turbidity assays. Viable counts of *E. coli* and *S.* Typhimurium were monitored in media 4 and 5 containing a sub-MIC of OVEO. Initially, 20 µL of each bacterial suspension (approximately 8 log CFU/mL) were inoculated into 3480 µL of the selected cultivation media containing OVEO. The mixtures (final viable cell counts of approximately 6 log CFU/mL) were gently hand-shaken for 30 s and subsequently incubated at 37 °C (de Souza *et al*., 2016). At intervals of 0 (just after homogenization), 2, 4, 6, 8, 12, 16 and 24 h of cultivation, a 100 µL-aliquot of each medium was serially diluted in sterile saline solution, inoculated on BHI agar and incubated at 37 °C for 24 h (Carvalho *et al*., 2018). Control media without OVEO were similarly assayed.

Data from viable cell counts (CFU/mL) were modeled with primary models describing the growth kinetics over time. Microbial population densities were log-transformed and raw growth data were recorded in an Excel spreadsheet along with the time. The primary growth model of Baranyi and Roberts (Baranyi, Roberts, 1994) was fitted to the raw growth data by DMFit, a Microsoft Excel Add-In developed by the Institute of Food Research (Norwich, UK), which calculated the estimates of the Grmax (expressed in log CFU/h).

Statistical analysis

All assays were performed in triplicate in three independent experiments and the results were expressed as an average of the obtained data. For MIC determination assays, the results were expressed as modal values because the MIC values did not vary in the independent experiments. For the Grmax, statistical analyses were performed to determine significant differences ($p \leq 0.05$) using ANOVA followed by Tukey's test or Student *t* test. All analyses were performed using the Statistica software version 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

MIC of OVEO against *E. coli* UFPEDA 224 and *S.* Typhimurium PT4 was 4.8 or 9.6 µL/mL in all the assayed media. In most cases, the highest MIC (9.6 µL/mL) of OVEO was observed in media (1, 9 and 12) that contained the lowest tested LIP amounts (3.75 or 5.0 g/100 mL) despite of the PTN amount and pH value (Table I). MIC values of OVEO against the test strains in assayed media were higher than those previously observed to OVEO against *E. coli* and *S.* Typhimurium in laboratorial media (Carvalho *et al.*, 2015). MIC determination using laboratorial media has been commonly applied as a primary test to evaluate the efficacy of EOs to inhibit food-related pathogens (de Souza, 2016); however, our results suggest that MIC determination of EOs in media that mimic the food environment could be more appropriate for screening the antimicrobial efficacy of EOs intended for use in foods.

Grmax is an important parameter in modelling microbial growth, representing a specific characteristic of a microorganism or strain growing in a particular environment (Baranyi *et al.*, 1995; Baranyi, Tamplin, 2004). Grmax values estimated to *E. coli* and *S.* Typhimurium in MPA are shown in Table II and Table III, respectively. *E. coli* Grmax in media containing 2.4 or 1.2 µL/mL OVEO ranged from -2.39±0.24 to 1.77±0.05 and -1.85±0.15 to 1.95±0.04 CFU/h, respectively; and for *S.* Typhimurium PT4 ranged from -2.52±0.20 to 1.60±0.11 and -1.71±0.13 to 1.95±0.05 log CFU/h, respectively. Grmax of *E. coli* and *S.* Typhimurium in media without OVEO ranged from 1.03±0.26 to 2.64±0.15 and 0.98±0.28 to 2.49±0.01 log CFU/h, respectively. Grmax values in media without OVEO were higher ($p \leq 0.05$) than those observed in media with OVEO. These data indicate that OVEO in both tested concentrations was

capable of inhibiting the bacterial growth regardless the cultivation media characteristic.

For both *E. coli* and *S.* Typhimurium, 2.4 and 1.2 µL/mL OVEO caused the lowest Grmax values (-2.39±0.24 and -1.85±0.15, and -2.52±0.20 and -1.71 log CFU/h, respectively) in medium 4, which contained the highest LIP (6.25 mL/100 mL) and PTN amount (8.0 g/100 mL) and the lowest pH value (5.0). In contrast, the highest Grmax values were observed in medium 5, which contained the lowest LIP (3.75 mL/100 mL) and PTN (4.0 g/100 mL) amount and the highest pH value (6.0). Overall, considering the growth kinetics estimates under the conditions tested in this study, the highest LIP amount in media enhanced the inhibitory effects of OVEO against the target bacteria regardless the tested PTN amount and pH value. Grmax in media without OVEO (control) was not affected by added LIP amounts. These results are in accordance with those obtained in MIC determination assays when the lowest MIC values were found in media with the highest LIP amounts.

TABLE II - Maximum specific growth rate (Grmax, log CFU/h) estimates for *Escherichia coli* UFPEDA 224 in media with different amounts of proteins, lipids and pH values and 2.4 or 1.2 µL/mL *Origanum vulgare* L. essential oil (OVEO). Results of Grmax values are expressed as the average ± standard deviation (n=9). Grmax values were measured by EON-Gen5 software (EON, BioTek, USA) considering the results of absorbance based microtiter plate assay

Media	Characteristics			Positive control*	2.4 µL/mL OVEO	1.2 µL/mL OVEO
	PTN amount (g/100 mL)	LIP amount (g/100 mL)	pH value	Grmax (log CFU/h)	Grmax (log CFU/h)	Grmax (log CFU/h)
1	4.0	3.75	5.0	1.23 ± 0.22^{Ba}	-0.56 ± 0.20^{Cc}	0.33 ± 0.09^{Db}
2	8.0	3.75	5.0	1.06 ± 0.17^{Ba}	0.82 ± 0.13^{Bb}	0.96 ± 0.16^{Cb}
3	4.0	6.25	5.0	1.25 ± 0.19^{Ba}	-1.71 ± 0.20^{Dc}	-1.26 ± 0.12^{Gb}
4	8.0	6.25	5.0	1.41 ± 0.12^{Ba}	-2.39 ± 0.24^{Ec}	-1.85 ± 0.15^{Fb}
5	4.0	3.75	6.0	2.11 ± 0.14^{Aa}	1.77 ± 0.05^{Ab}	1.95 ± 0.04^{Ab}
6	8.0	3.75	6.0	1.03 ± 0.26^{Ba}	-0.33 ± 0.06^{Dc}	0.13 ± 0.03^{Eb}
7	4.0	6.25	6.0	2.64 ± 0.15^{Aa}	-0.82 ± 0.18^{Cc}	1.39 ± 0.11^{Bb}
8	8.0	6.25	6.0	1.35 ± 0.24^{Ba}	-2.22 ± 0.12^{Eb}	1.21 ± 0.20^{Ba}
9	6.0	5.00	5.5	2.30 ± 0.23^{Aa}	0.81 ± 0.20^{Bc}	1.25 ± 0.11^{Bb}
10	4.0	6.25	5.5	2.52 ± 0.53^{Aa}	-1.61 ± 0.09^{Dc}	-1.44 ± 0.25^{Fb}

(continuing)

TABLE II - Maximum specific growth rate (Grmax, log CFU/h) estimates for *Escherichia coli* UFPEDA 224 in media with different amounts of proteins, lipids and pH values and 2.4 or 1.2 µL/mL *Origanum vulgare* L. essential oil (OVEO). Results of Grmax values are expressed as the average ± standard deviation (n=9). Grmax values were measured by EON-Gen5 software (EON, BioTek, USA) considering the results of absorbance based microtiter plate assay

Media	Characteristics			Positive control*	2.4 µL/mL OVEO	1.2 µL/mL OVEO
	PTN amount (g/100 mL)	LIP amount (g/100 mL)	pH value	Grmax (log CFU/h)	Grmax (log CFU/h)	Grmax (log CFU/h)
11	6.0	6.25	5.5	1.98 ± 0.24^{Aa}	-2.11 ± 0.05^{Ec}	-1.65 ± 0.33^{Fb}
12	6.0	5.00	6.0	2.09 ± 0.35^{Aa}	1.42 ± 0.44^{Ab}	1.55 ± 0.29^{Bb}

* Cultivation media without OVEO.
$^{A-G}$ Different superscript capital letters in the same column denote differences ($p \leq 0.05$) in maximum specific growth rates in different media, based on Tukey test.
$^{a-c}$ Different superscript small letters in the same raw denote differences ($p \leq 0.05$) in maximum specific growth rate in the same media without OVEO or containing OVEO at 2.4 or 1.25 µL/mL, based on Tukey test.

TABLE III - Maximum specific growth rate (Grmax, log CFU/h) estimates for *Salmonella* Typhimurium PT4 in media with different amounts of proteins, lipids and pH values and 2.4 or 1.2 µL/mL *Origanum vulgare* L. essential oil (OVEO). Results of Grmax values are expressed as the average ± standard deviation (n=9). The Grmax values were measured by EON-Gen5 software (EON, BioTek, USA) considering the results of absorbance based microtiter plate assay

Media	Characteristics			Positive control	2.4 µL/mL OVEO	1.2 µL/mL OVEO
	PTN amount (g/100 mL)	LIP amount (g/100 mL)	pH value	Grmax (log CFU/h)	Grmax (log CFU/h)	Grmax (log CFU/h)
1	4	3.75	5.0	1.02 ± 0.09^{BCa}	0.36 ± 0.28^{Cc}	0.60 ± 0.28^{Db}
2	8	3.75	5.0	0.98 ± 0.28^{Ca}	-1.09 ± 0.22^{Fc}	-0.42 ± 0.14^{Eb}
3	4	6.25	5.0	2.30 ± 0.25^{Aa}	0.92 ± 0.13^{Bb}	0.94 ± 0.09^{Cc}
4	8	6.25	5.0	1.17 ± 0.19^{BCa}	-2.52 ± 0.20^{Ec}	-1.71 ± 0.13^{Fb}
5	4	3.75	6.0	2.40 ± 0.13^{Aa}	1.60 ± 0.11^{Ab}	1.95 ± 0.05^{Ab}

(continuing)

TABLE III - Maximum specific growth rate (Grmax, log CFU/h) estimates for *Salmonella* Typhimurium PT4 in media with different amounts of proteins, lipids and pH values and 2.4 or 1.2 µL/mL *Origanum vulgare* L. essential oil (OVEO). Results of Grmax values are expressed as the average ± standard deviation (n=9). The Grmax values were measured by EON-Gen5 software (EON, BioTek, USA) considering the results of absorbance based microtiter plate assay

Media	Characteristics			Positive control	2.4 µL/mL OVEO	1.2 µL/mL OVEO
	PTN amount (g/100 mL)	LIP amount (g/100 mL)	pH value	Grmax (log CFU/h)	Grmax (log CFU/h)	Grmax (log CFU/h)
6	8	3.75	6.0	1.38 ± 0.14^{BCa}	0.84 ± 0.07^{Bb}	0.97 ± 0.05^{Bb}
7	4	6.25	6.0	2.49 ± 0.04^{Aa}	-1.76 ± 0.17^{Gc}	1.04 ± 0.17^{Bb}
8	8	6.25	6.0	1.34 ± 0.16^{BCa}	-1.50 ± 0.28^{Gc}	1.09 ± 0.13^{Bb}
9	6	5.00	5.5	1.13 ± 0.15^{BCa}	-0.91 ± 0.24^{Fc}	0.99 ± 0.06^{Bb}
10	4	6.25	5.5	2.21 ± 0.21^{Aa}	-0.94 ± 0.18^{Fc}	0.95 ± 0.09^{Bb}
11	6	6.25	5.5	2.06 ± 0.38^{Aa}	-0.88 ± 0.16^{Ec}	0.85 ± 0.08^{Cb}
12	6	5.00	6.0	1.56 ± 0.21^{Ba}	-0.35 ± 0.09^{Dc}	1.21 ± 0.18^{Bb}

***** Cultivation media without OVEO.

$^{A-G}$ Different superscript capital letters in the same column denote differences ($p \leq 0.05$) in maximum specific growth rates in different media, based on Tukey test.

$^{a-c}$ Different superscript small letters in the same raw denote differences ($p \leq 0.05$) in maximum specific growth rates in the same media without OVEO or containing OVEO at 2.4 or 1.25 µL/mL, based on Tukey test.

Since the estimates of bacterial growth from turbidity assays demonstrated that the lowest and highest Grmax values for *E. coli* and *S.* Typhimurium occurred in media 4 and 5, respectively, these media were selected for assessing the bacterial viable counts over time when exposed to 2.4 µL/mL OVEO and modelling the growth kinetics from these data. The counts of *E. coli* and *S.* Typhimurium in medium 4 with 2.4 µL/mL OVEO after 24 h (5.86±0.28 and 6.32±0.32 log CFU/mL, respectively) did not differ ($p > 0.05$) from the initial counts (5.87±0.43 and 6.15±0.26 log CFU/mL, respectively), indicating a bacteriostatic effect of OVEO in this medium. In turn, the counts of *E. coli* and *S.* Typhimurium in medium 5 with 2.4 µL/mL OVEO after 24 h (7.32±0.32 and 6.96±0.31 log CFU/mL, respectively) differed ($p \leq 0.05$) from the initial counts (5.92±0.29 and 5.96±0.5 log CFU/mL, respectively). Counts of these bacteria after 24 h in medium 5 with 2.4 µL/mL OVEO were higher ($p \leq 0.05$) than those observed in medium 4. Viable counts in media 4 and 5 with 2.4 µL/mL OVEO over 24 h were lower ($p \leq 0.05$) than those observed in media without OVEO (8.1±0.52 – 8.5±0.46 log CFU/mL).

As seen in Table IV, Grmax values estimated by DMFit for *E. coli* and *S.* Typhimurium from viable counts in media 4 and 5 during 24 h were higher than those measured from data of bacterial growth in MPA. However, in accordance with the estimates made in MPA, *E. coli* and *S.* Typhimurium presented lower ($p \leq 0.05$) Grmax values in medium 4 (0.026±0.001 and 0.166±0.003 log CFU/h, respectively) compared to medium 5 (0.061±0.002 and 0.035±0.001 log CFU/h, respectively), confirming that the highest LIP (6.25 mL/100 mL) and PTN (8.0 g/100 mL) amounts and the lowest pH value (5.0) enhanced the inhibitory effects of OVEO against the target bacteria.

For most cases, the average R^2-values for growth curves of tested strains in media 4 and 5 were ≥0.90, indicating a good fit of the data and that the general trend of the bacterial growth was well represented by the primary model predictions (Sant'Ana, Franco, Schaffner, 2012). Even with differences in Grmax values, the results of viable counts indicate that the growth kinetics estimates in MPA were representative of the influence of higher LIP amount in medium to enhance the antibacterial effects of OVEO. The differences in Grmax values could be related to the fact that the viable cell count method identifies the growth of viable cells (viable colony), which could even recover some injury during the cultivation period in a rich laboratory medium under adequate environmental condition, while the MPA identifies the increase in cell mass in broth (Brock *et al.*, 1994).

Although no previous study had assessed the effects of concurrent variations in food components and pH values on the antibacterial effects of EOs, one study that assessed the influence of food components or pH separately have reported negative impacts of increased LIP amounts in medium on the antimicrobial properties of EOs (Gutierrez *et al.*, 2008). Similarly, the available literature commonly reports that low pH values may enhance the antibacterial effects of EOs (Gutierrez *et al.*, 2008). In turn, there has been no consensus regarding the influence of PTN amounts in media on the antimicrobial effects of EOs (Smith-Palmer, Stewart, Fyfe, 2001; Gutierrez *et al.*, 2008).

The results of this study showed that LIP amounts in medium was the driving tested factor to enhance the inhibitory effects of OVEO against *E. coli* and *Salmonella typhimurium*. Probably, the highest LIP amounts in media creating a more amphipathic environment (as the presence of fatty acids typically does) could increase the interactions of the OVEO constituents with target bacteria membranes (Turina *et al.*, 2006). Antibacterial effects of constituents commonly found in OVEO have been tentatively associated with their ability to disturb bacterial plasma membrane lipid fraction, resulting in alterations of membrane permeability and leakage of intracellular materials (de Souza, 2016). In addition to the physicochemical characteristics of OVEO constituents, the environmental characteristics that facilitate these compounds to reach bacterial membranes could be key factors to establish their antimicrobial effects. EOs constituents have to cross the cell membranes and penetrate into the cell interior where interact with intracellular sites critical for antibacterial activity (Turina *et al.*, 2006; Cristani *et al.*, 2007).

Only a previous investigation assessed the influence of concomitant variations of LIP and PTN amounts and pH values in medium on the antibacterial effects of carvacrol. The antibacterial effects of carvacrol were similarly enhanced by highest LIP amounts in media (Carvalho *et al.*, 2018). Carvacrol was previously identified as the majority constituent (69%) in OVEO tested in this our study, followed by thymol (14.12%), γ-terpinene (3.71%) and *p*-cymene (3.67%) (de Souza *et al.*, 2016). Another study observed that carvacrol failed to inhibit *Salmonella* growth in low-LIP peanut (<5%) (Chen *et al.*, 2015). These results indicate that the influence of food components or physicochemical parameters when tested separately on the antibacterial effects of EOs or their individual constituents might differ from those obtained when variations of these factors are simultaneously tested, which could simulate more realistically the distinct conditions found in real foods.

CONCLUSION

The obtained results showed that, under the conditions tested in this study, the highest inhibitory effects of OVEO against *E. coli* and *S.* Typhimurium were observed in media with the highest LIP and PTN amounts and the lowest pH. However, the set of the obtained growth kinetics data indicated LIP amount in media as the most influential factor to enhance the inhibitory effects of OVEO against *E. coli* and *S.* Typhimurium. Overall, the lowest Grmax values were observed in media containing the highest LIP amounts regardless the PTN amounts and pH values. These findings indicate that the concomitant influence of different food components, particularly LIP and PTN amounts, and pH values on the antibacterial effects

of OVEO should be considered for optimizing its use as an antimicrobial in food conservation systems. Further studies assessing the antimicrobial efficacy of EOs using real foods with different compositions could be considered to decrease the risk of possible failure on the expected microbial control exerted by these substances in foods.

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