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# Changes in Membrane Fatty Acids Composition of Microbial Cells Induced by Addiction of Thymol, Carvacrol, Limonene, Cinnamaldehyde, and Eugenol in the Growing Media

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Major active compounds from essential oils are well-known to possess antimicrobial activity against both pathogen and spoilage microorganisms. The aim of this work was to determine the alteration of the membrane fatty acid profile as an adaptive mechanism of the cells in the presence of a sublethal concentration of antimicrobial compound in response to a stress condition. Methanolic solutions of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol were added into growth media of *Escherichia coli* O157:H7, *Salmonella enterica* serovar typhimurium, *Pseudomonas fluorescens*, *Brochothrix thermosphacta*, and *Staphylococcus aureus* strains. Fatty acid extraction and gas chromatographic analysis were performed to assess changes in membrane fatty acid composition. Substantial changes were observed on the long chain unsaturated fatty acids when the *E. coli* and *Salmonella* strains grew in the presence of limonene and cinnamaldehyde and carvacrol and eugenol, respectively. All compounds influenced the fatty acid profile of *B. thermosphacta*, while *Pseudomonas* and *S. aureus* strains did not show substantial changes in their fatty acid compositions.

**KEYWORDS: Thymol; carvacrol; limonene; cinnamaldehyde; eugenol; fatty acids**

## INTRODUCTION

In the last 20 years, much interest has been focused on understanding the mechanisms involved in adaptation of microbial cells to environmental conditions (*1*). Because of concern of the increased bacterial resistance in the clinical sector and pharmaceutical and food-processing environments, there is a need to understand these mechanisms and to evaluate the potential for the development of microbial resistance in these areas. It is well-known that sublethal exposure to particular environmental conditions and antimicrobial substances may result in development of increased resistance and promotion of cross-resistance to antimicrobial compounds (*2*−*4*).

This work is exclusively focused on some foodborne pathogens and spoilage microorganisms and their ability to adapt the cytoplasmic membrane to sublethal environmental stress. Many of these microorganisms are capable of undertaking an adaptive response to sublethal stresses, enabling them to tolerate and survive subsequent exposure to normally lethal levels of the same stress or even a different type of stress (*5*).

The major adaptive response of the cells is to keep the fluidity of their membranes at a constant value, irrespective of actual environmental conditions. Such stabilization of membrane fluidity, known as homeoviscous adaptation, constitutes the predominant response of bacteria to membrane-active substances or changing environmental conditions (*6*), preventing the loss of the mechanical and chemical properties of the lipid bilayer (*7*). If disturbance of membrane integrity occurs, then its functions as a barrier, as a matrix for enzymes, and as an energy transducer are compromised (*6*).

To decrease the effects of environmental changes on the membrane, the cell regulates its fluidity by changing the proportion of iso- and anteiso-branched fatty acids, isomerization of cis unsaturated fatty acids (UFAs) to corresponding trans isomers, and altering the average fatty acid chain length, protein content, and fatty acid composition (*5*).

Lipids have many biological functions in microbial cells, and consequently, numerous researches were dedicated to lipids and their role in cell physiology (*7*−*11*). Variations in temperature, pH, ethanol concentration and external osmolarity, the presence of substances able to affect the microbial growth, and transition to the stationary phase lead to the alteration of fatty acid content to control membrane viscosity (*1*, *2*).

The increase of unsaturation, as a consequence of the reduction of growth temperature, has been described for several microbes (*12*, *13*) and can be regarded as a universally conserved adaptation response (*14*). In particular, the way in which the fatty acid composition of membrane lipids is altered in response

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**Table 1.** MSC of Antimicrobial Compounds

| strains | compounds[a] (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
| *E. coli* O157:H7 (nontoxigenic) ATCC 43888 | 0.77 a | 8.40 a | 3.18 a | 0.78 a | 2.10 a |
| *S. enterica* serovar typhimurium ATCC 14028 | 0.96 a | 8.40 a | 3.18 a | 0.97 a | 3.15 b |
| *P. fluorescens* NCIMB 10586 | 2.88 b | 8.40 a | 2.12 b | 1.84 b | 3.15 b |
| *B. thermosphacta* NCTC 10822 | 0.58 c | 1.68 b | 2.12 b | 0.78 a | 0.84 c |
| *S. aureus* NCTC 6571 | 0.96 a | 1.68 b | 2.12 b | 0.97 a | 2.10 a |

[a] Values are means of duplicate determinations. Means followed by different letters in the same column differ ($P < 0.05$).

to growth temperature appears to depend on the mechanism of UFA synthesis utilized (*14*). In fact, the incorporation of more UFAs into the membrane tends to increase membrane fluidity (*15*). Recent work suggests that monounsaturated membrane fatty acids are necessary for the maintenance of a $\Delta$pH across the membrane (*16*, *17*) and an increase in fatty acid length is another important membrane alteration to increase survival in acidic environments (*16*).

The lipophilic character of the compounds used in this study suggests interaction with bacterial membranes; in fact, the hydrophobicity of these molecules enables them to partition in the lipids of the bacterial cell membrane, disturbing the structure and rendering it more permeable (*18*). Little is known about physiological changes in foodborne pathogens and spoilage bacteria exposed to a sublethal concentration of natural antimicrobial compounds.

The European Commission has registered a number of essential oil components for use as flavorings in foodstuffs. The registered flavorings include among others carvacrol, carvone, cinnamaldehyde, citral, *p*-cymene, eugenol, limonene, menthol, and thymol (*18*). However, the prospect of using natural antimicrobial compounds as preservatives in food raises the need for more information on the behavior of spoilage and pathogenic microorganisms that may adapt to otherwise lethal concentrations of these compounds and subsequently exhibit altered survival or growth behavior and increased resistance to other environmental stresses imposed by traditional preservation technologies. The aim of this study was to investigate alterations in the cellular fatty acid composition, an adaptive stress mechanism induced by sublethal concentrations of active compounds from essential oils.

## MATERIALS AND METHODS

**Bacterial Strains.** *Escherichia coli* O157:H7 ATCC 43888 (nontoxigenic), *Salmonella enterica* serovar typhimurium ATCC 14028, *Pseudomonas fluorescens* NCIMB 10586, *Brochothrix thermosphacta* NCTC 10822, and *Staphylococcus aureus* NCTC 6571 were grown in tryptone soy broth (Oxoid) supplemented with 0.5% yeast extract (Oxoid) (TSBYE) at optimal growth temperatures for 18−20 h.

**Antimicrobial Compounds.** All components were purchased from Sigma; they were (*R*)-(+)-limonene (97%), thymol (99%), carvacrol (98%), cinnamaldehyde (98%), and eugenol (98%) and are referred to below as essential oil components. Each component was prepared as a 1 M solution in methanol.

**Maximum Sublethal Concentration (MSC) Determination and Culture Conditions.** Known quantities of antimicrobial solutions were added to TSBYE in order to obtain broths with different concentrations of each antimicrobial compound. The MSC was defined as the highest concentration of antimicrobial compound allowing the growth at the optimal temperature. Each strain was cultivated in TSBYE without (control) and with antimicrobial compound at the MSC for 18−20 h at the optimal growth temperature.

**Total Lipid Extraction.** The culture broths of each microorganism were centrifuged for 10 min at 5000*g*, and the cell pellet was harvested and submitted for membrane fatty acid extraction. Extraction of fatty acid from cellular materials was carried out as described by Evans et al. (*19*). Lipid samples were transmethylated for analysis of their acyl groups as fatty acid methyl esters (FAME). The samples of total lipid extract were evaporated to dryness in a round-bottom flask using a boiling water bath. To the dried samples, heated under a reflux condenser (20−30 cm), were added 10 mL of KOH in methanol (0.2 M) and 1 mL of heptane. After 10 min, 5 mL of boron trifluoride ($BF_3$) was added followed, after 2 min, by 4 mL of heptane. After waiting for 1 min, the samples were cooled. A saturated solution of $Na_2SO_4$ was added to the samples, and after settling into a two-phase system, the upper layer was taken and transferred into a vial.

**Analysis of the Fatty Acid Composition.** Analytical gas chromatography (GC) was carried out on a Perkin-Elmer Auto System XL Gas Chromatograph, equipped with a flame ionization detector and a capillary column BPX 70 (50 m × 0.32 mm, i.d.) (SGE Ltd, Milton Keynes); 1 $\mu$L of sample was injected, using the following operating conditions: carrier gas helium flow, 1 mL $min^{-1}$; injector temperature, 220 °C; detector temperature, 240 °C; initial oven temperature, 70 °C for 3 min; ramp 1, 22 °C $min^{-1}$ to 180 °C; ramp 2, 2 °C $min^{-1}$ to 190 °C, hold for 10 min; and ramp 3, 2.0 °C $min^{-1}$ to 220 °C.

FAME peaks were identified by comparison of their retention times with those of a standard solution (SUPELCO 37 Component FAME Mix).

**Statistical Analysis.** *T*-test and standard deviation (SD) calculations were performed using Systat software for Macintosh version 5.2.1 in order to evaluate, although the difference between the means is statistically significant.

## RESULTS

**MSC of Antimicrobial Compounds. Table 1** reports the MSC of antimicrobial compounds added to the growth media. *E. coli* O157:H7, *P. fluorescens*, and *Salmonella* typhimurium strains were able to grow in the presence of high concentrations of all tested compounds, showing more resistance than the other microorganisms.

**Changes in Fatty Acid Composition.** Membrane lipid composition changes were investigated by using GC analysis. **Table 2** shows the concentrations of the UFAs and the variation of their values ($\Delta$UFAs) following exposure to the stress. The results showed that the UFAs are always present at a higher amount than SFAs in the total lipid profile of the microorganisms used in this research. The fatty acids and their concentrations found in the lipid extracts of control and treated cells of each microorganism are reported in **Tables 3−7**.

The lipid profile of *E. coli* O157:H7 ATCC 43888 was affected by the presence of almost all antimicrobial compounds, as reported in **Table 2**. However, the UFA concentrations increased more when the cells grew in the presence of limonene and cinnamaldehyde. In both cases, the net increase of UFAs resulted from a decrease of palmitic acid (C16) concentration and an increase in linoleaidic (C18:2 trans), docosanoic (C22), and eicosapentaenoic (C20:5 cis) acid concentrations. Additionally, cinnamaldehyde also resulted in an increase in concentration of palmitoleic acid (C16:1 cis). It is important to highlight

**Table 2.** Percentage of Total UFAs

| | *E. coli* O157:H7 | | *S. typhimurium* | | *P. fluorescens* | | *B. thermosphacta* | | *S. aureus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| compounds | UFA[a] | ΔUFA[b] | UFA | ΔUFA | UFA | ΔUFA | UFA | ΔUFA | UFA | ΔUFA |
| control | 55.85 a | | 59.56 a | | 67.57 a | | 58.05 a | | 71.00 a | |
| thymol | 62.61 b | 6.76 | 57.93 b | –1.63 | 54.91 b | –12.66 | 61.26 b | 3.21 | 72.31 a | 1.31 |
| limonene | 68.57 b | 12.72 | 64.98 b | 5.42 | 64.54 b | –3.03 | 81.74 b | 23.69 | 78.55 b | 7.55 |
| eugenol | 56.15 a | 10.31 | 70.20 b | 10.63 | 65.96 a | –1.61 | 66.21 b | 8.16 | 70.01 a | –1.00 |
| carvacrol | 57.57 b | 1.72 | 71.49 b | 11.93 | 70.75 b | 3.18 | 69.42 b | 11.37 | 66.66 b | –4.35 |
| cinnamaldehyde | 71.01 b | 15.16 | 61.78 a | 2.22 | 66.69 a | –0.88 | 70.19 b | 12.15 | 53.76 b | –17.25 |

[a] Values are means of duplicate determinations. [b] UFAs control – UFAs-treated cells. Means in the same column followed by a letter other than control value differ from this ($P < 0.05$).

**Table 3.** Changes in Fatty Acid Profiles of Cell Membranes of *E. coli* O157:H7 ATCC 43888 as Induced by the Addition of Antimicrobial Compounds

| FAME | control | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
|---|---|---|---|---|---|---|
| C16:0 | 18.47 ± 2.67[a] | 12.42 ± 6.81 | 9.84 ± 3.58 | 15.91 ± 5.97 | 22.42 ± 11.04 | 8.39 ± 2.64 |
| C16:1cis | 1.61 ± 1.03 | 2.59 ± 2.35 | 0.41 ± 0.58 | 2.27 ± 3.21 | 4.07 ± 1.96 | 8.58 ± 1.88 |
| C18:1cis | 7.07 ± 4.33 | 12.09 ± 4.57 | 6.83 ± 7.83 | 36.72 ± 8.39 | 7.63 ± 6.36 | 5.30 ± 4.13 |
| C18:2trans | 0.62 ± 0.87 | 0.62 ± 0.87 | 9.82 ± 6.33 | 1.08 ± 1.52 | 8.83 ± 12.18 | 10.88 ± 3.56 |
| C22:0 | 0.85 ± 1.21 | 8.03 ± 2.50 | 7.02 ± 2.55 | 1.08 ± 1.53 | 1.11 ± 0.21 | 5.68 ± 2.17 |
| C20:5cis | 0.63 ± 0.89 | 5.63 ± 3.26 | 5.33 ± 4.07 | ND[b] | 0.12 ± 0.17 | 5.31 ± 1.67 |

[a] Percentage values, means of duplicate determinations ± SD. [b] Not detected.

**Table 4.** Changes in Fatty Acid Profiles of Cell Membranes of *S. enterica* Serovar Typhimurium ATCC 14028 as Induced by the Addition of Antimicrobial Compounds

| FAME | control | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
|---|---|---|---|---|---|---|
| C15:1cis | 4.00 ± 2.60[a] | 6.66 ± 2.01 | 6.32 ± 2.95 | 7.50 ± 1.59 | 9.11 ± 1.27 | 5.58 ± 0.81 |
| C16:0 | 25.54 ± 3.92 | 18.80 ± 1.93 | 15.10 ± 3.02 | 11.64 ± 1.06 | 11.69 ± 2.37 | 18.06 ± 1.98 |
| C18:2trans | 13.12 ± 4.29 | 14.54 ± 4.81 | 17.02 ± 8.15 | 20.43 ± 4.48 | 19.25 ± 4.55 | 13.73 ± 3.55 |
| C18:3cis | 10.42 ± 3.09 | 11.94 ± 5.56 | 14.78 ± 4.15 | 15.83 ± 3.47 | 18.68 ± 1.94 | 10.56 ± 3.22 |

[a] Percentage values, means of duplicate determinations ± SD.

**Table 5.** Changes in Fatty Acid Profiles of Cell Membranes of *P. fluorescens* NCIMB 10586 as Induced by the Addition of Antimicrobial Compounds

| FAME | control | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
|---|---|---|---|---|---|---|
| C14:0 | 1.59 ± 0.80[a] | 4.09 ± 0.29 | 1.63 ± 1.03 | 2.98 ± 0.41 | 1.80 ± 0.60 | 3.19 ± 1.13 |
| C16:0 | 11.45 ± 2.50 | 19.55 ± 1.58 | 17.45 ± 1.55 | 10.20 ± 1.22 | 8.17 ± 0.08 | 10.39 ± 2.11 |
| C18:0 | 3.25 ± 1.37 | 9.54 ± 0.98 | 2.48 ± 1.06 | 5.39 ± 1.40 | 3.70 ± 1.69 | 5.89 ± 2.51 |
| C18:1cis | 25.19 ± 2.89 | 16.95 ± 0.42 | 21.56 ± 1.07 | 22.38 ± 1.40 | 25.19 ± 1.92 | 22.99 ± 0.96 |
| C18:2trans | 3.09 ± 0.30 | ND | 0.06 ± 0.09 | 0.92 ± 1.30 | ND[b] | 0.05 ± 0.08 |
| C18:3cis | 18.49 ± 1.05 | 12.94 ± 2.90 | 16.56 ± 0.93 | 14.38 ± 2.84 | 16.55 ± 1.72 | 17.36 ± 1.39 |

[a] Percentage values, means of duplicate determinations ± SD. [b] Not detected.

the increase in oleic acid (C18:1 cis) in the lipid extract of *E. coli* cells grown in the presence of sublethal concentrations of eugenol.

For the control cells of *Salmonella* typhimurium ATCC 14028, approximately 60% of the fatty acids were UFAs. This percentage increased when carvacrol and eugenol were added to the growth media. In contrast, in the broth supplemented with thymol, the UFAs slightly decreased (**Table 2**). The increase of UFAs observed in the presence of eugenol and carvacrol is due to a balanced variation of the two classes of fatty acids. In fact, as observed in **Table 4**, the unsaturated *cis*-10-pentadecanoic (C15:1 cis), linoleaidic (C18:2 trans), and linolenic (C18:3 cis) acids underwent a greater increase than the other, while palmitic acid (C16), among the SFAs, showed a relative decrease.

The lipid profile of *P. fluorescens* NCIMB 10586 cultured in unsupplemented broth contained about 67% UFAs. All antimicrobial substances, with the exception of thymol, slightly affected the ratio S/UFAs. A relative net decrease of UFAs was induced by the presence of thymol in the growth media. It resulted principally from an increase in saturated myristic (C14), palmitic (C16), and stearic (C18) acids and a decrease in unsaturated oleic (C18:1 cis), linoleaidic (C18:2 trans), and linolenic (C18:3 cis) acids (**Table 5**).

The results reported in **Tables 2** and **6** showed that the fatty acid composition of *B. thermosphacta* NCTC 10822 was strongly influenced by the presence of all antimicrobial compounds with the exception of thymol. In the cases of eugenol and carvacrol, the net increase in UFAs was due to minor variations in the concentration of many acids. Limonene and cimmaldehyde increased the concentration of unsaturated *cis*-10-pentadecanoic (C15:1 cis) and linolenic (C18:3 cis) acids and decreased the concentration of palmitic acid (C16). These variations were particularly evident when limonene was added to the culture media.

*S. aureus* NCTC 6571 had the highest concentration (71%) of UFAs when it was cultured in the unsupplemented medium. No substantial change in lipid composition of the extracts was

**Table 6.** Changes in Fatty Acid Profiles of Cell Membranes of *B. thermosphacta* NCTC 10822 as Induced by the Addition of Antimicrobial Compounds

| FAME | control | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
|---|---|---|---|---|---|---|
| C15:1cis | 5.81 ± 2.20[a] | 8.65 ± 0.46 | 13.37 ± 1.42 | 7.30 ± 1.48 | 8.82 ± 2.19 | 9.45 ± 1.70 |
| C16:0 | 16.41 ± 2.60 | 10.39 ± 1.19 | 4.49 ± 2.47 | 12.21 ± 2.86 | 11.34 ± 1.45 | 9.56 ± 0.97 |
| C18:3cis | 12.67 ± 3.36 | 15.11 ± 3.56 | 22.60 ± 3.05 | 15.46 ± 2.91 | 18.53 ± 3.12 | 17.09 ± 3.93 |

[a] Percentage values, means of duplicate determinations ± SD.

**Table 7.** Changes in Fatty Acid Profiles of Cell Membranes of *S. aureus* NCTC 6571 as Induced by the Addition of Antimicrobial Compounds

| FAME | control | thymol | limonene | eugenol | carvacrol | cinnamaldehyde |
|---|---|---|---|---|---|---|
| C14:1cis | 13.19 ± 2.74[a] | 10.79 ± 0.61 | 11.09 ± 0.21 | 5.64 ± 0.69 | 6.40 ± 7.57 | 5.93 ± 0.38 |
| C16:0 | 3.59 ± 0.94 | 2.84 ± 0.88 | 3.46 ± 0.63 | 1.11 ± 0.37 | 4.12 ± 0.96 | 17.77 ± 0.79 |
| C18:0 | 4.04 ± 0.66 | 2.05 ± 0.25 | 1.70 ± 0.63 | 2.43 ± 0.84 | 3.75 ± 0.94 | 13.73 ± 0.85 |
| C18:1cis | 22.74 ± 1.17 | 21.38 ± 0.64 | 26.37 ± 2.11 | 21.50 ± 1.34 | 20.01 ± 0.49 | 17.91 ± 0.48 |

[a] Percentage values, means of duplicate determinations ± SD.

observed in the presence of antimicrobial substances, except when the broth was supplemented with a sublethal concentration of cinnamaldehyde. In this case, the 17% UFAs decrease was mainly due to a decrease in myristoleic (C14:1 cis) and oleic (C18:1 cis) acids and by an increase in the palmitic (C16) and stearic (C18) acids (**Table 7**).

## DISCUSSION

As reported by Russell (*2*), the mechanisms used by bacterial cells to alter the unsaturation ratio of membrane fatty acids depend on the mechanism of fatty acid synthesis. The author described two distinct and mutually exclusive UFA synthetic pathways in bacteria: the anaerobic and the aerobic pathway. The former, used by anaerobes and some facultative aerobes, produces UFAs by de novo synthesis through the action of a fatty acid synthetase (*2*, *20*). The latter produces only saturated fatty acids, employing the multicomponent membrane desaturase enzymes (*2*, *21*). The only way for these bacteria to produce UFAs, most commonly palmitoleic and oleic acids, is by the action of the desaturase enzyme, which creates a double bond in the saturated acyl chains by removing two hydrogen atoms and transferring them to oxygen (*2*, *22*).

The raised concentration of some UFAs detected in *E. coli* O157:H7, *Salmonella*, and *B. thermosphacta* was not supported by a relative decrease in the same length SFAs. It is hypothesized that these microorganisms regulate their UFA synthesis by the anaerobic biosynthetic pathway. The findings of this study confirmed those of Chiou et al. (*23*), who found that the presence of a sublethal concentration of ethanol during growth of *E. coli* O157:H7 resulted in extensive synthesis of lipids containing high amounts of UFAs. It seemed that, in both cases, the addition of a sublethal concentration of the antimicrobial compounds resulted in the cells using a similar adaptation mechanism to maintain membrane structure and function. Therefore, the cells responded to a sublethal concentration of antimicrobials by increasing the UFAs resulting in membrane fluidity changes. In fact, it is well-known that UFAs give the membrane a high degree of fluidity, whereas those composed predominantly of saturated fatty acids tend to be relatively rigid (*24*). Preliminary experiments showed no influence of methanol on the microbial growth (*25*) or on cell lipid profiles (data not shown) when tested at the same concentrations used to dissolve the antimicrobial compounds in the growing media.

Yuk and Marshall (*4*) and Sampathkumar et al. (*23*) found that adaptation to pH in *E. coli* O157:H7 and *S. enterica* resulted in a decrease of UFA concentration, suggesting that the adaptation mechanism depends on the type of stress factor to which the cells have been exposed. Fozo et al. (*16*) demonstrated that increased fatty acid length is another important membrane alteration to increase survival in acidic environments; the same adaptation mechanism probably occurs when the cells grow in the presence of antimicrobial compounds. The results showed that *E. coli* O157:H7 short−medium chain fatty acids (C4−C14) and long chain fatty acids (C20−C22) were either absent or present in low concentrations under control conditions. It is interesting to observe an increase of the C20−C22 level when the cells were grown in broths supplemented with the antimicrobials used in this study (data not shown).

The same effect was found as an adaptation mechanism to a rise in growth temperature, where an increase in the proportion of long chain and saturated fatty acids within the membrane was observed (*1*). Russell (*2*) found that when bacteria anaerobically synthesize UFAs at low temperatures, they also synthesize longer chain fatty acids. However, the fluidizing effects of the UFAs chains dominate, over those due to change in chain length.

*P. fluorescens* and *S. aureus* showed generally small variations in their fatty acid composition when grown in the presence of the tested antimicrobials. It is probable that the high resistance of *P. fluorescens* and *S. aureus* to the tested compounds explains the unchanged composition of fatty acid profile. The findings discussed in this paper on *P. fluorescens* are in agreement with those found by other authors (*6*, *26*, *27*) who reported a high resistance of this microorganism to several antimicrobials.

The biosynthetic pathway by which *Pseudomonas* synthesizes UFAs is through the action of the desaturase enzyme. The presence of antimicrobial compounds during growth presumably affects the action of this enzyme. However, the overall effect of the variations in lipid composition on the membrane fluidity is determined by the sum of the effect of every single lipid. A small change in a particular lipid could have a greater impact than a larger change in another lipid (*28*).

The results of this study show the effect of an environmental stress on membrane fatty acids composition. However, further studies are necessary to confirm the behavior observed also considering that only one strain for each species was tested.

It is believed that this is the first report on the effect that sublethal concentrations of antimicrobial compounds from essential oils have on the fatty acid profile of foodborne spoilage and pathogen microorganisms. Understanding how the cells

adapt their functional structures upon exposure to antimicrobial compounds will enhance the understanding of the prevention of resistance mechanisms. However, further studies are necessary to see whether the modifications in membrane composition demonstrated in this research really lead to an increased resistance or are just part of a general adaptive response. These findings provide interesting information to any studies bridging the gap between mechanisms evaluated at the molecular level and observations at the organism level.

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