

## Supporting Information for

### **Coupling Solid Phase Microextraction to complementary separation platforms for metabotyping of *E. coli* metabolome in response to natural antibacterial agents.**

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### ***Section 1.1- Chemical and materials and Metabolite Standard Mixture Preparation***

LC-MS grade solvents and LC-MS grade formic acid (1 mL glass ampules) were obtained from Fisher Scientific (Ottawa, Canada). Polypropylene deep 96-well plates (Nunc) and easily modified polystyrene–divinylbenzene (Macherey-Nagel) particles were purchased from VWR International (Mississauga, Canada). All metabolites, peptone, yeast extract, NaCl, clove bud oil, eugenol, eugenyl acetate,  $\beta$ -caryophyllene, and n-alkane mixture (C<sub>8</sub>–C<sub>40</sub>) were purchased from Supelco (Bellefonte, PA, USA). *E.coli* BL21 strain was kindly donated by Professor John Brennan's laboratory at McMaster University (Hamilton, Ontario, Canada). The Concept 96-SPME-blade unit and robotic Concept 96 autosampler were purchased from Professional Analytical Systems (PAS) Technology (Magdala, Germany) for SPME sample preparation. Commercial SPME fiber assemblies in 23-gauge needle sizes and automated formats, 50/30  $\mu$ m DVB/Car/PDMS (stableflex), were purchased from Supelco (Oakville, Canada). Automation of the SPME protocol was achieved using a Gerstel MPS 2 autosampler. For LC analyses, a standard mixture of metabolites with a wide range of polarities, such as amino acids, amines, organic acids, sugars, nucleosides, and small peptides (Table SI.1), was prepared for optimization of SPME conditions using multivariate analysis. Stock standard solutions, prepared fresh weekly, were prepared in water/methanol/ethanol, kept frozen (-30°C), and protected from light. Extractions were conducted from a spiked standard 1 $\mu$ g/mL stock solution and added to Lysogeny broth (LB) media. Organic solvent content for all extraction standards was maintained at 1% (v/v). For instrument calibration, working standard solutions with known concentrations of metabolites were prepared by dilution of the stock standard with a desorption solvent. For GC analyses, compounds were tentatively identified by matching mass spectra with library data and Kovats retention indexes.

### ***Section 1.2- Coating preparation, multivariate optimization of the 96-blade SPME method, and HS-SPME sampling conditions***

When utilizing the 96-blade SPME-LC/MS, the SPME method development procedure needs to be conducted efficiently, as the effectiveness of the resulting analyte pre-concentration depends on many parameters, such as coating type, choice of stationary phase for the 96-blade system, extraction conditions, desorption conditions, and wash conditions.

The 96-blade SPME platform is comprised of four different steps: preconditioning, extraction, wash, and desorption. A triangular design and a central composite design (CCD) were applied for variable optimization. Triangular design was used for desorption solvent optimization, while CCD was used for investigation of optimum extraction and desorption times, and in the case of the targeted metabolites listed in Table SI.3, for optimization of wash time for each step of the 96-blade SPME method. Sums of peak areas were investigated as inputs (dependent variables) for optimization. Data obtained from the multivariate experimental matrix designs was analyzed using Statistica 8.0 (StatSoft 2007 Edition, Tulsa, USA). In order to achieve the most efficient desorption of analytes with a wide range of polarities and physical-chemical properties from the surface of the coating, the optimum desorption solvent was investigated. A triangular design was carried out to determine the influence of different desorption solvents and their interactions on the recovery of the 96-blade SPME method. Acetonitrile, water, methanol, and their combination (100% acetonitrile, 100% water, 100% methanol, acetonitrile:water 1:1 (v/v), acetonitrile:methanol 1:1 (v/v), methanol:water 1:1 (v/v), , and acetonitrile:methanol:water 1:1:1 (v/v/v)) were applied using the triangular design. Figure SI.1 demonstrates the results of this triangular design process. An evaluation of results indicated that based on the average and sum of the detector responses to each metabolite, acetonitrile:water 1:1 (v/v) was the optimum desorption solvent composition for the study. Finally, 1 mL of acetonitrile:water 1:1 (v/v) at 1500 rpm was chosen as the optimized desorption solvent in this study for the extraction of targeted metabolites with logP from -7 to +7 spiked in LB media. To optimize the time for each stage of the 96-blade SPME procedure, CCD was applied to obtain the optimum time that provided the highest extraction recovery. Minimum and maximum values of time for each step were arbitrarily selected to cover the wide range of experimental conditions. The experimental variables that were evaluated included extraction time (15–120 min), wash time (0–120 s) and desorption time (15–120 min), all using the optimized desorption solvent. A CCD with a central point (17 runs in total) was performed to determine the optimized time for each step of the extraction, wash, and desorption, and their interactions on the 96-blade SPME system. The CCD matrix and the surface response are presented in Table SI.3 and Figure SI.2, respectively. The sensitivity of the method was significantly improved by applying the method at optimum times for each step. The optimized conditions obtained from utilization of the CCD matrix and response surface methodology indicated that a 120 min extraction time, 30 seconds as wash time, and 90 min desorption time provided the highest extraction recovery for all targeted metabolites. At optimized conditions, fibers were pre-conditioned and sterilized for 1 hour in 1 mL ethanol:water 70:30 (v/v). Next, extractions were conducted from 1 mL culture (control and antibacterial-

agent treated) set in sterile 96-well plates for 2 h (1000 rpm agitation speed, 2.5 mm amplitude). A 20s wash step in water was performed after the extraction step so as to remove proteins, salt, and particulates from the coating surfaces. Afterwards, desorption was carried out for 90 min in 1 mL of acetonitrile/water 1:1 (v/v). Final extracts were kept at -80°C until analysis.

HS-SPME analysis of the culture media was carried out prior to GC analysis as follows: a DVB/Car/PDMS coating was exposed for 30 minutes to the headspace of culture media kept at 37°C under agitation conditions (500 rpm). The extraction time used was selected in order to maximize the amount of volatiles extracted and in the meantime avoid saturation of both the SPME coating and capillary columns by the most abundant compounds present in the headspace sampled. In order to maintain unaltered the growing conditions of the bacteria culture during the extraction an extraction temperature of 37°C was used. The SPME coating used for GC analysis, DVB/Car/PDMS, was selected according to previous results reported in literature, reporting its superior performances, compared to other GC-compatible coatings, in terms of metabolite coverage and extraction efficiency<sup>1</sup>. The desorption of the coating was carried out at 270°C for 15 minutes in the gas-chromatograph injector port. Blanks of the coating carried out after extraction/desorption cycles from culture media did not reveal carryover effects.

### ***Section 1.3 UHPLC-ESI-MS, GC-IT-MS and GCxGC-ToF-MS conditions***

LC separation was carried out on a Kinetex pentafluorophenyl coreshell column (1.7 µm, 2.1 mm × 10 mm) (Phenomenex, Torrance, CA, USA) with a guard filter (SecurityGuard ULTRA Cartridges UHPLC PFP for 2.1 mm). The column temperature was maintained at 25°C, and gradient mobile phase conditions were composed of phase A (water containing 0.1% formic acid) and phase B (acetonitrile with 0.1% formic acid) set to the following conditions: 0-1 min 90% A; 1-9 min 90-10% A; 9-12 min 10% A; 12-16 min 10-90% A. Total run time was 16 min per sample. The injection volume for this method was 10 µL. Samples were stored under refrigeration (4°C) in the autosampler while waiting for injection, and injected in a randomized fashion. A QC sample was run periodically to verify instrument performance. Injection of control samples and antibacterial-treated samples was done alternatively. Blank and QC samples were injected after every set of 15 injections so as to avoid cross contamination as well as verify instrument performance. The used QC sample was prepared by mixing 10 µL of each sample into one combined sample. The MS system was operated using an accurate mass Exactive benchtop Orbitrap system (Thermo, San Jose, California, USA) in both negative and positive electrospray ionization (ESI) modes and 100-1000 m/z mass range. Optimum values for sheath gas (arbitrary

units), auxiliary gas (arbitrary units), ESI voltage (kV), capillary voltage (V), capillary temperature (°C), and tube lens voltage (V) were set at 40, 25, 4.0, 27.5, 275, and 100 for positive ESI mode, and 50, 25, -2.7, -67.5, 325, and -85 for negative ESI mode, respectively. External instrument mass calibrations were performed every 24 h, and found to be within 2 ppm for all ions. Separated metabolites were analyzed using Xcalibur software version 2.1 (Thermo, San Jose, California, USA) by isolating the extracted ion chromatograms (XIC), using a 5 ppm window around the accurate mass. Regarding the analysis performed by gas-chromatography: Full factorial design optimization experiments were performed in a Varian CP-3800 GC system coupled to a Varian 4000 Ion-Trap Mass Spectrometer (IT/MS). Tentative compound identification was performed using the National Institute of Standards and Technology (NIST, version 2.05) mass spectral library. For analyses on control and treated samples, separation and detection of VOCs produced by *E.Coli* were performed by a GCxGC-ToF/MS Pegasus 4D (LECO Corp., St Joseph, MI, USA). The trapping and refocusing of compounds eluting from the first dimension (<sup>1</sup>D) to the second dimension (<sup>2</sup>D) column was performed by a quad-jet modulator consisting of two cold nitrogen jets and two hot-air jets. An Agilent 7890 GC oven was used as a chromatographic system, containing the secondary oven and the modulator. Liquid nitrogen was used to create the cold jets. The column configuration included an Rtx®-5SilMS (30 m x 0.25 mm x 0.25 µm) (Restek Corp., Bellefonte, PA, USA) capillary column in the first dimension (<sup>1</sup>D) and a BP-20 (1 m x 0.1 mm x 0.1 µm) (SGE, Austin, TX, USA) in the second dimension (<sup>2</sup>D), according to a method previously developed for metabolomics applications<sup>1</sup>. The two columns were connected using a universal glass press tight connector (Restek Corp.). A modulation period of 5 s was used, with hot pulse duration of 0.8 s and a cool time of 1.70 s. Ultra high purity helium (99.999%) was used as carrier gas with a constant flow rate of 1.5 ml/min. Desorption of analytes from the SPME coating was performed in splitless mode at 270 °C for 15 min. Evaluation of carryover of analytes onto the coating was performed by re-desorbing the same coating immediately after analysis. Chromatographic separation of analytes was achieved using the following temperature program in the primary oven: an initial temperature of 35 °C was held for 5 min; a ramp of 6°C/min was then used to reach a temperature of 250 °C, which was then held for 10 min, for a total run time of 50.83 min. The offset for the secondary oven temperature was set at +10 °C above the primary oven temperature. The modulator offset was set at 30 °C. The transfer line and ion source temperatures were 250 °C and 200 °C, respectively. A solvent delay of 60 s was used. Electron impact ionization was performed at 70 eV; ions ranging from 35-600 m/z were acquired.

### ***Section 2.1-Effect of eugenol on the fatty acid Profile of the E. coli cell membrane***

Cellular responses to stress include adjustments in the membrane system, modifications of the membrane architecture, and changes in cell cycles and cell division. The cell membrane is the first target of antibacterial agents in this study, as these compounds can change cell permeability and the functions of proteins embedded inside. The antibacterial candidates under study in the present research are characterized by significant hydrophobicity, which facilitates their diffusion across the cell membrane, leading to the disruption of the membrane via alteration of the fatty acid balances. In order to survive, cells adapt to the presence of essential oils through changes in the ratios of iso to anteiso-branched fatty acids, unsaturated fatty acids to saturated fatty acids, and also by changing their fatty acid composition <sup>2</sup>. Changes in the degree of saturation of fatty acids are caused by the desaturase enzyme, as it adds or removes two hydrogens to or from the acid's double bond <sup>3</sup>.

In this current research, when antibacterial agents such as  $\beta$ -caryophyllene and eugenyl acetate were applied under minimum inhibitory concentration (MIC) to the bacteria culture, the percentage of unsaturated fatty acids increased to enhance cell membrane fluidity, whereas in experiments where the antibacterial agents were applied above the MIC (clove oil and eugenol), an increase in the amount of saturated fatty acids caused loss of membrane fluidity, therefore enhancing its rigidity. As demonstrated in Table 1, the levels of the unsaturated fatty acids octadecadienoic acid, 9-hydroperoxy-10E,12-octadecadienoic acid, dodecadienoic acid, tridecadienoic acid, 2Z-dodecenedioic acid, decatetraenedioic acid, tridecadienoic acid, nonadienoic acid, and 4,7,10-hexadecatrienoic acid were observed to decrease, while the level of myristic acid alkyne, as a saturated fatty acid, was observed to increase. Short and medium length organic acids levels such as adipic and pimelic acids also increased.

On the other hand, when bacteria culture was treated with  $\beta$ -caryophyllene and eugenyl acetate, the levels of unsaturated fatty acids such as 2-methyl-dodecanedioic acid, 12S-hydroxy-5Z, 8E,10E-heptadecatrienoic acid, myristoleic acid, tetradecadienoic acid, tridecadienoic acid, and 9-oxo-2,4,5,7-decatetraenoic acid increased.

In agreement with our findings, Di Pasque et al. perceived that treatment of *E. coli O157:H7 ATCC 43888* with sub-lethal concentrations of thymol, limonene, eugenol, carvacrol, and cinnamaldehyde increased the levels of saturated fatty acids such as myristic (C14), palmitic (C16), and stearic (C18) acids, while levels of unsaturated acids such as unsaturated oleic (C18:1 cis), linoleaidic (C18:2 trans), and linolenic (C18:3 cis) acids were observed to decrease as a result of treatment <sup>3</sup>. Phospholipids, sterols, and sphingolipids, considered as secondary metabolites and signaling molecules, or part of lipoproteins, are fatty acids precursors <sup>4</sup>. Any

alterations in the saturation grade of phospholipids within the cellular membrane can change its fluidity <sup>4</sup>. Regarding the remarkable variation in the lipidomic profile of *E. coli*, it is obvious that employing eugenol influences lipid biosynthesis pathways. Depending on the agent concentration applied to the *E. coli* media, this can either facilitate the adaptation of *E. coli* to a new environment, or lead to cell disruption.

### ***Section 2.2-Action of eugenol on proteins***

Eugenol and clove oil also target proteins by binding them to the hydroxyl group present in the structure of eugenol, preventing them from functioning as enzymes <sup>5</sup>. Up-regulation or down-regulation of various metabolites involved in specific metabolic pathways in the treated sample demonstrated the changes observed in the microorganism were caused by changes in enzymatic function. For example, down regulation of cadaverine, one of the amines produced by biodegradative decarboxylases <sup>4</sup>, accompanied by up-regulation of lysine in a sample treated with clove oil and eugenol, demonstrated the inhibition of lysine decarboxylase <sup>6</sup>. Wendakoon et al. observed that for crude extracts of *Enterobacter aerogenes*, eugenol and cinnamaldehyde, as the most effective species with inhibitory action towards the activity of the amino acid decarboxylase, prevented the production of metabolites such as cadaverine, histamine, putrescine, and tryamine <sup>7</sup>. In addition, the down-regulation of glucose in treated samples was established to be a consequence of amylase down-regulation. Amylase catalyzes the hydrolysis of long chain carbohydrates into simple sugars such as glucose. Thoroski et al. observed that the production of enzymes such as alpha-amylase and protease was inhibited or reduced in *Bacillus subtilis* when the concentration of eugenol was increased from 0.0 to 0.04% v/v <sup>8</sup>. This phenomenon may delay glucose utilization in the glycolysis pathway; for that reason, clove oil has been suggested as a cancer care drug. Based on the Warburg effect, glycolysis is enhanced in most cancer cells to generate ATP as a main source of energy production. Consequently, targeting the glycolytic pathway may kill malignant cells by starvation, and the investigation of new molecules that can act as glycolytic inhibitors as novel class of anticancer agents could have a several therapeutic applications <sup>9</sup>. In addition, one of the most important pathways influenced in response to eugenol addition in *E. coli* growing media is the guanine and guanosine salvage pathway. The observed guanosine level enhancement and corresponding decrease in guanine could be attributed to enzymes involved in the above-mentioned pathways, such as guanosine phosphorylation or guanine phosphoribosyltransferase.

### ***Section 2.3-Anti-quorum sensing activity***

When the number of bacteria reaches a particular threshold during growth, intercellular

communication, called quorum sensing (QS), will organize the interaction among bacteria. Genes involved in the expression of QS produce the prompt for chemical-signaling molecules called autoinducers. Autoinducers are produced until the number of bacteria reaches their maximum threshold. QS controls virulence factor expression, bioluminescence, sporulation, biofilm formation, and mating <sup>10</sup>. Researchers have introduced essential oils as nontoxic inhibitors of QS without strain resistance <sup>11</sup>. Various mechanisms are involved in QS inhibition, such as the inhibition of acyl homoserine lactones as autoinducers, inhibition of acyl homoserine lactone transport, and inhibition of targets downstream of the acyl homoserine lactone receptor binding <sup>12</sup>. In the present study, the level of homoserine lactone decreased in samples treated with clove oil and eugenol, demonstrating that these compounds are potential QS inhibitors that prevent biofilm formation. Previously, Khan et al. investigated the anti-QS activity of clove oil and other essential oils against *C. violaceum* and *Ps. Aeruginosa* <sup>13</sup>. Selection of these compounds for study as potential drugs was determined based on their established antipathogenic activity. The findings of this study suggested antipathogenic activity could be achieved through inhibition of signal biosynthesis or inhibition enzymes involved in N-acyl-homoserine lactones production.



## Tables

Table SI-1. Physicochemical properties of metabolites included in standard metabolite mixture for SPME-LC analyses.

Analyte	Formula	Molecular Weight	pKa	Log P
3-hydroxybutyric acid (HBA)	C <sub>4</sub> H <sub>8</sub> O <sub>3</sub>	104.1	4.41	-0.47
Adenine	C <sub>5</sub> H <sub>5</sub> N <sub>5</sub>	135.1	4.15	-0.09
Adenosine	C <sub>10</sub> H <sub>13</sub> N <sub>5</sub> O <sub>4</sub>	267.2	NA	-1.05
Adenosine diphosphate (ADP)	C <sub>10</sub> H <sub>15</sub> N <sub>5</sub> O <sub>10</sub> P <sub>2</sub>	427.2	NA	-2.64
Adenosine monophosphate (AMP)	C <sub>10</sub> H <sub>14</sub> N <sub>5</sub> O <sub>7</sub> P	347.2	NA	-1.68
Adenosine triphosphate (ATP)	C <sub>10</sub> H <sub>16</sub> N <sub>5</sub> O <sub>13</sub> P <sub>3</sub>	507.2	NA	-3.61
β-Estradiol	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	272.4	NA	4.01
β-NAD	C <sub>21</sub> H <sub>27</sub> N <sub>7</sub> O <sub>14</sub> P <sub>2</sub>	663.4	NA	-3.68
Cholic acid	C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>	408.6	4.98	2.02
Choline	C <sub>5</sub> H <sub>14</sub> NO <sup>+</sup>	104.1	NA	-5.16
Citric acid	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	192.1	2.79	-1.64
Fructose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.2	12.1	-1.55
Fumaric acid	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	116.1	3.03	0.46
Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.2	12.9	-3.24
Glucose 6-phosphate	C <sub>6</sub> H <sub>13</sub> O <sub>9</sub> P	260.1	1.11	-3.79
Glutamic acid	C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>	147.1	2.23	-3.69
Glutathione (oxidized)	C <sub>20</sub> H <sub>32</sub> N <sub>6</sub> O <sub>12</sub> S <sub>2</sub>	612.6	NA	-7.89
Glutathione (reduced)	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	307.3	NA	-5.41
Histamine	C <sub>5</sub> H <sub>9</sub> N <sub>3</sub>	111.1	9.8	-0.7
Histidine	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	155.2	2.76	-3.32
Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.4	4.77	7.05
Lysine	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	146.2	3.12	-3.05
Maleic acid	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	116.1	1.83	-0.48
Nicotinamide	C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O	122.1	3.35	-0.37
Phenylalanine	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	165.2	1.24	-1.38
Protoporphyrin IX	C <sub>34</sub> H <sub>34</sub> N <sub>4</sub> O <sub>4</sub>	562.7	NA	7.43
Pyruvic acid	C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>	88.1	2.45	-1.24
Riboflavin	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	376.4	10.2	-1.46
Ribose-5-phosphate	C <sub>5</sub> H <sub>11</sub> O <sub>8</sub> P	230.1	NA	-2.65
Sucrose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342.3	12.6	-3.7
Taurocholic acid	C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S	515.7	NA	0.01
Tryptophan	C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	204.2	7.38	-1.06
Uridine diphosphate glucose (UDPG)	C <sub>15</sub> H <sub>24</sub> N <sub>2</sub> O <sub>17</sub> P <sub>2</sub>	566.3	NA	-5.8

Table SI.2 Full factorial design matrix for investigation of the effects of different clove oil constituents on *E.coli* metabolic profile (Eugenol: 8  $\mu$ L, eugenyl acetate: 1  $\mu$ L and  $\beta$ -caryophyllene: 0.6  $\mu$ L Carryophelene).

Exp#	Eugenol	Eugenyl acetate	$\beta$ -Caryophyllene
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

Table SI.3. Central composite design matrix used to obtain optimum extraction, wash, and desorption times for targeted metabolites (Table SI 1), using the coating PS-DVB-WAX:HLB 50:50 (w:w).

Experiment #	Extraction time (min)	Wash time (s)	Desorption time (min)
1	36.3	24.3	36.3
2	36.3	95.7	98.8
3	98.8	95.7	36.3
4	98.8	24.3	98.8
5	67.5	60.0	67.5
6	36.3	96.7	36.3
7	36.3	24.3	98.8
8	98.8	24.3	36.3
9	98.8	95.7	98.8
10	67.5	60.0	67.5
11	15.2	60.0	67.5
12	119.7	60.0	67.5
13	67.5	60.0	15.2
14	67.5	60.0	119.7
15	67.5	0	67.5
16	67.5	119.7	67.5
17	67.5	60.0	67.5

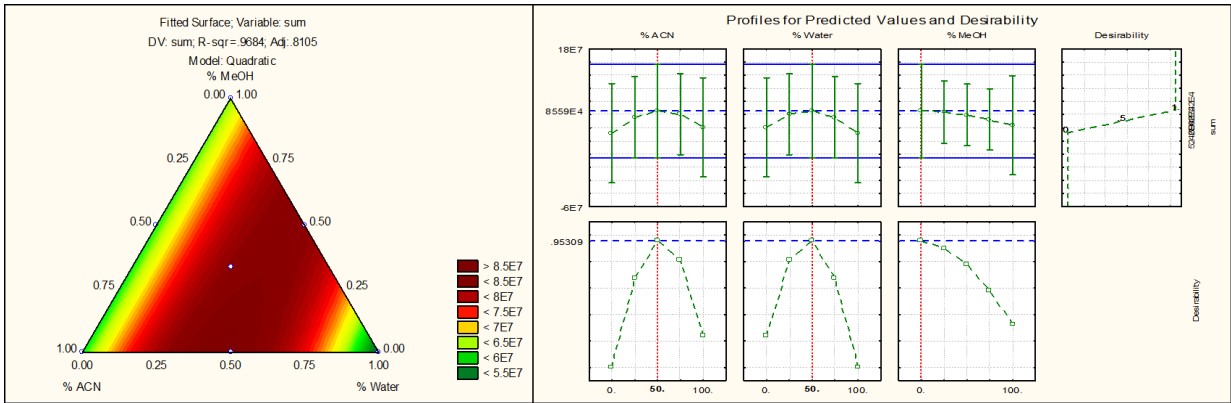


Figure SI.1 Triangular design and desirability plots for desorption solvent optimization applying sum of analytical signal vs. desorption solvents. Each angle is related to one desorption solvent, while the middle of the triangle side is the mixture of desorption solvent at the angle of each side. The mixture of all three solvents represents the center of triangle.

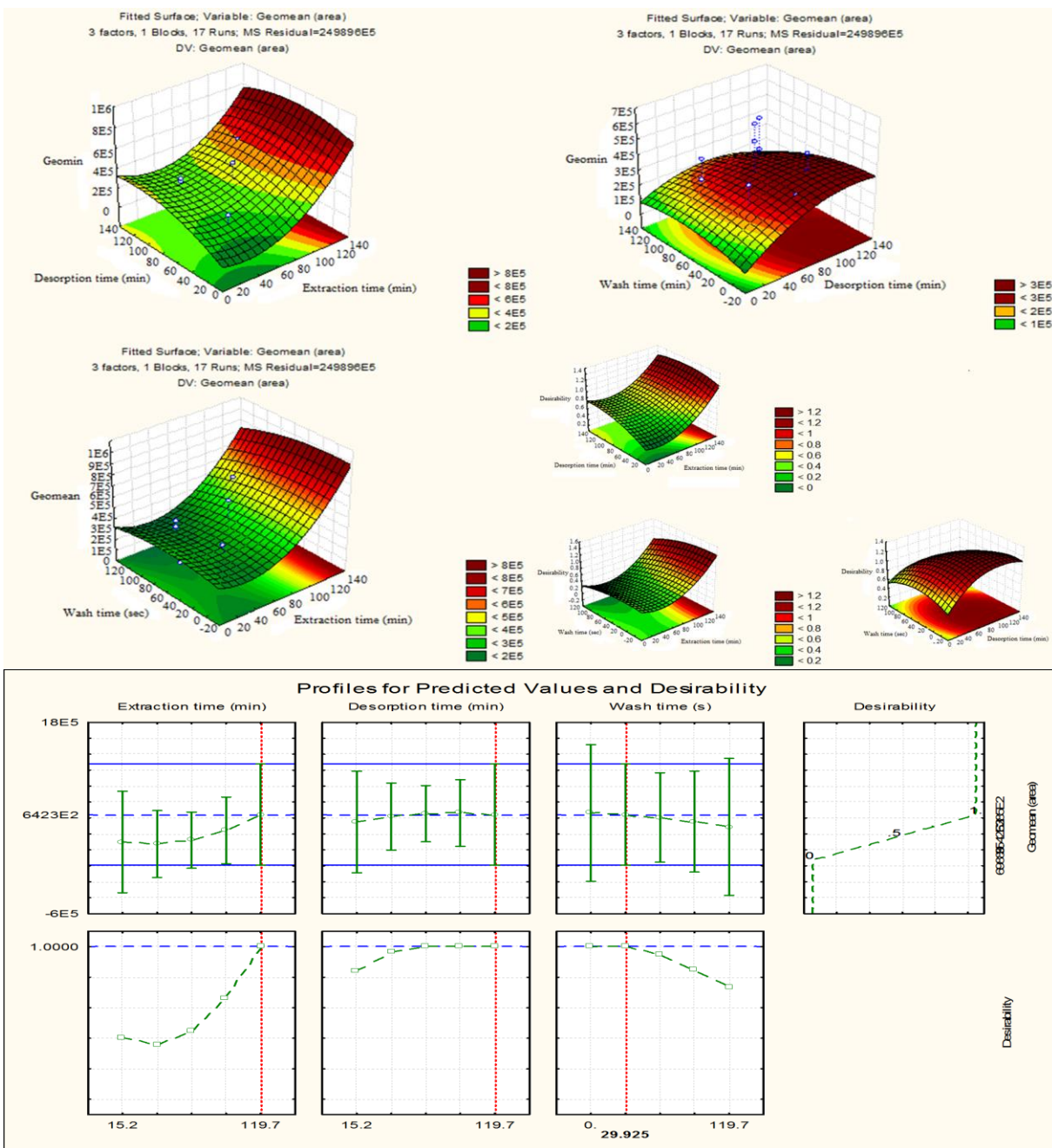


Figure SI.2 Response surface and desirability plots for analytical signal vs. extraction time, desorption time, and wash time (min) for extraction from spiked analytes in LB media ( $100 \mu\text{g mL}^{-1}$ ) resulted from central composite design in order to obtain optimum time for extraction, wash, and desorption steps in 96-blade SPME.

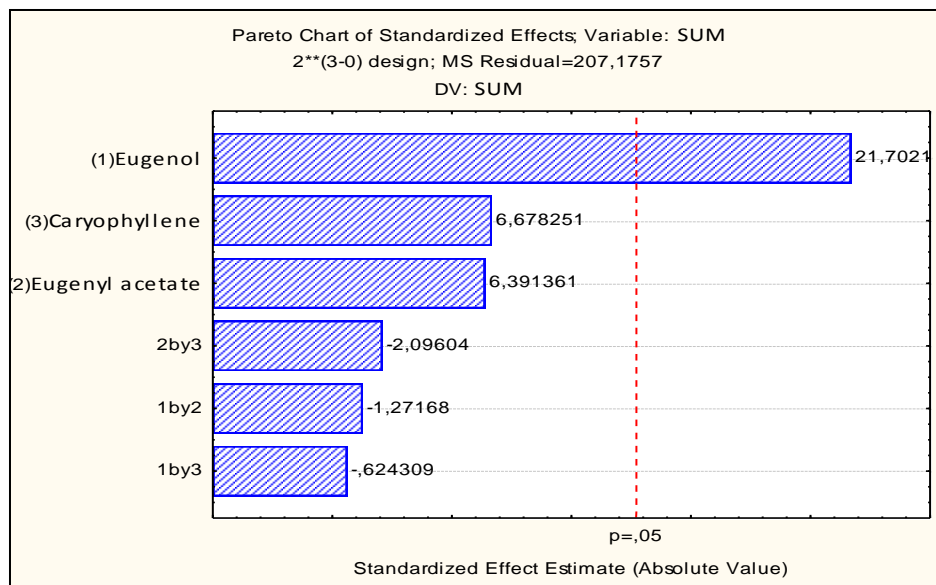


Figure SI.3 Pareto chart plots based on the sum of peak areas obtained from 96-blade SPME coupled to LC-MS related to metabolites whose peak areas were increased by addition of antibacterial agents, demonstrated as up-regulated metabolites in Table 1 (Group A)

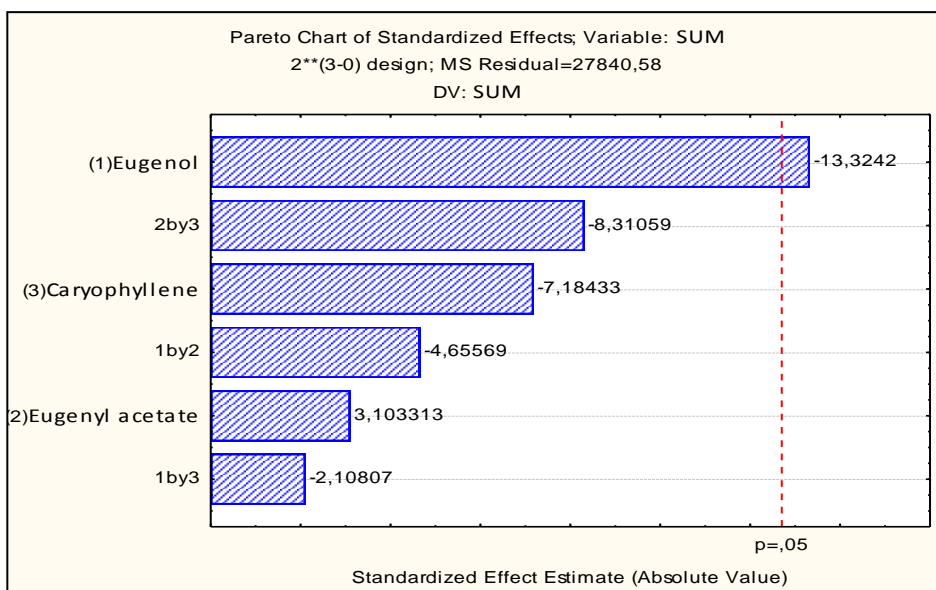


Figure SI.4 Pareto chart plots based on the sum of peak areas obtained from 96-blade SPME coupled to LC-MS related to metabolites whose peak areas were decreased by addition of antibacterial agents, demonstrated as down-regulated metabolites in Table 1 (Group B)

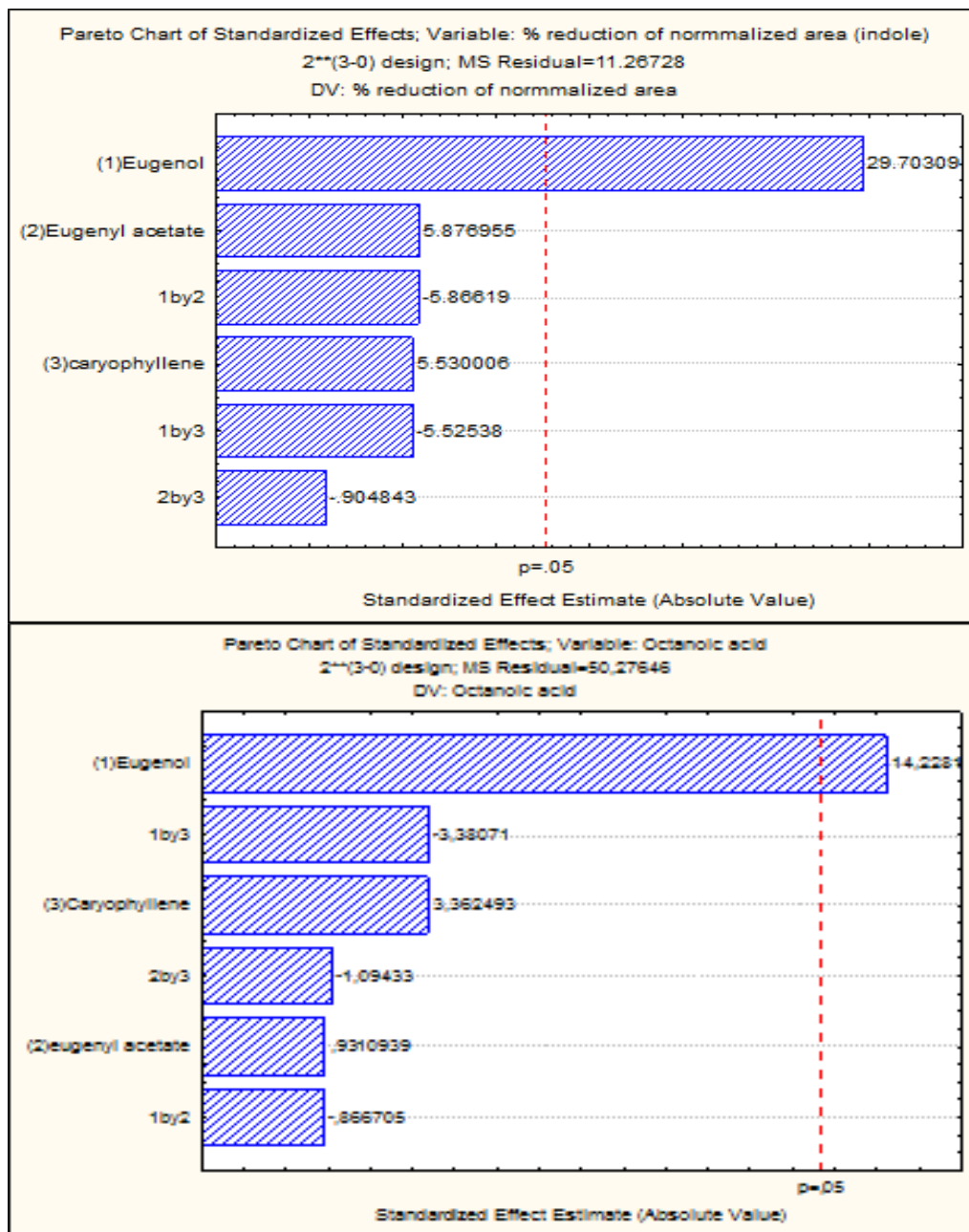


Figure SI.5 Pareto chart plots based on the sum of peak areas obtained from HS-SPME coupled to GC-IT/MS related to metabolites whose peak areas have undergone statistically significant changes by addition of antibacterial agents.

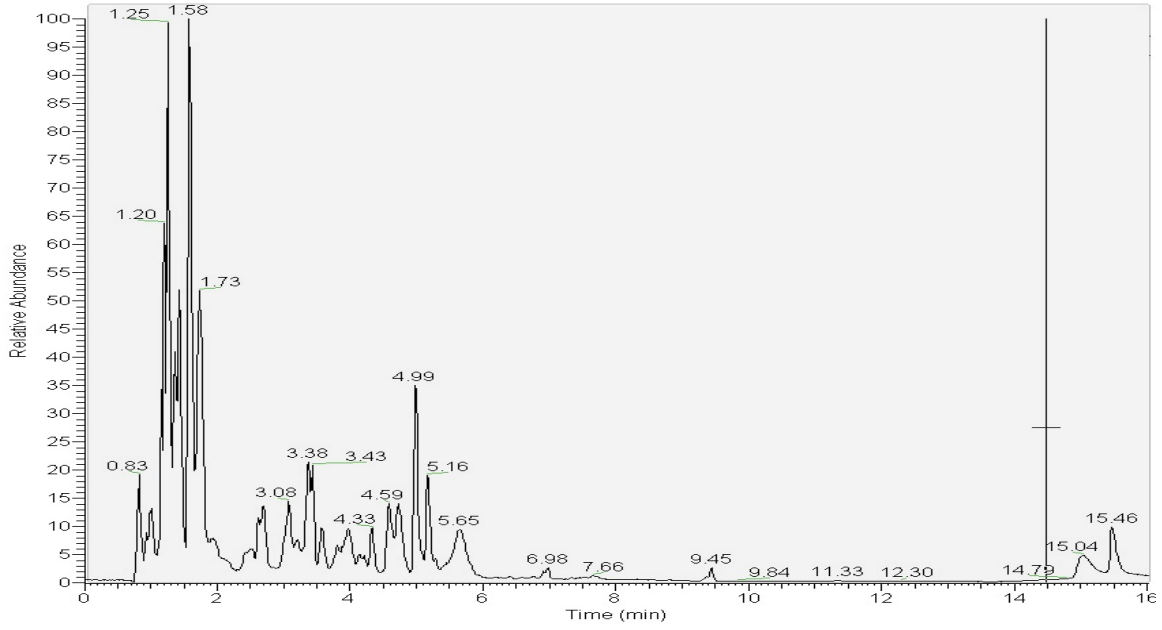


Figure SI.3 Chromatogram of *E.coli* extract by PS-DVB-WAX:HLB 50:50 (w:w) – positive ionization mode.

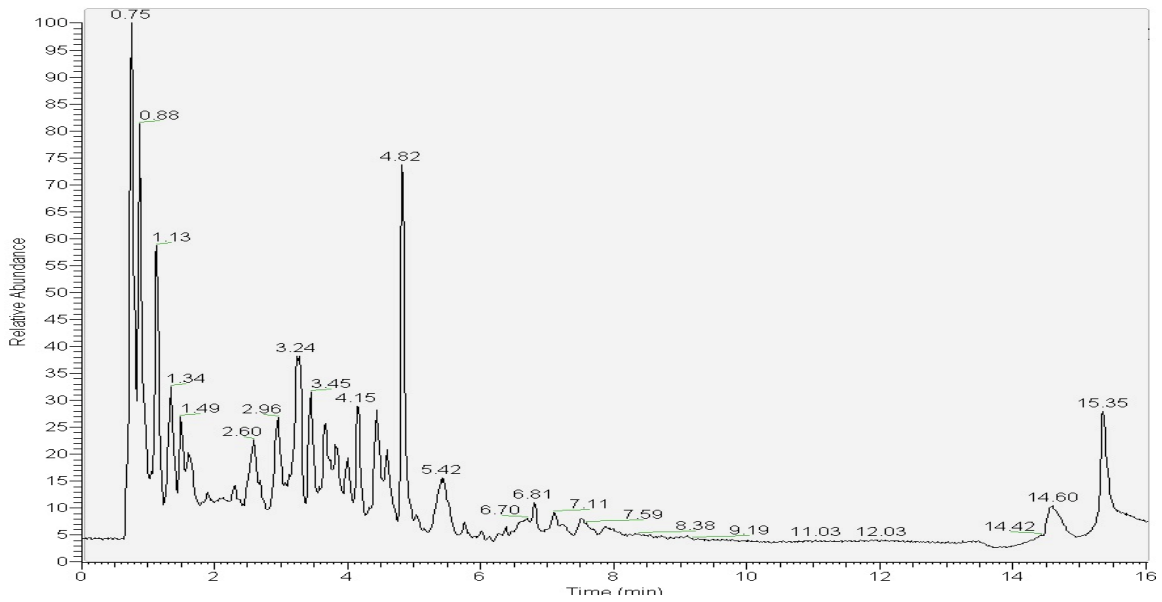


Figure SI.7 Chromatogram of *E.coli* extract by PS-DVB-WAX:HLB 50:50 (w:w) – negative ionization mode.

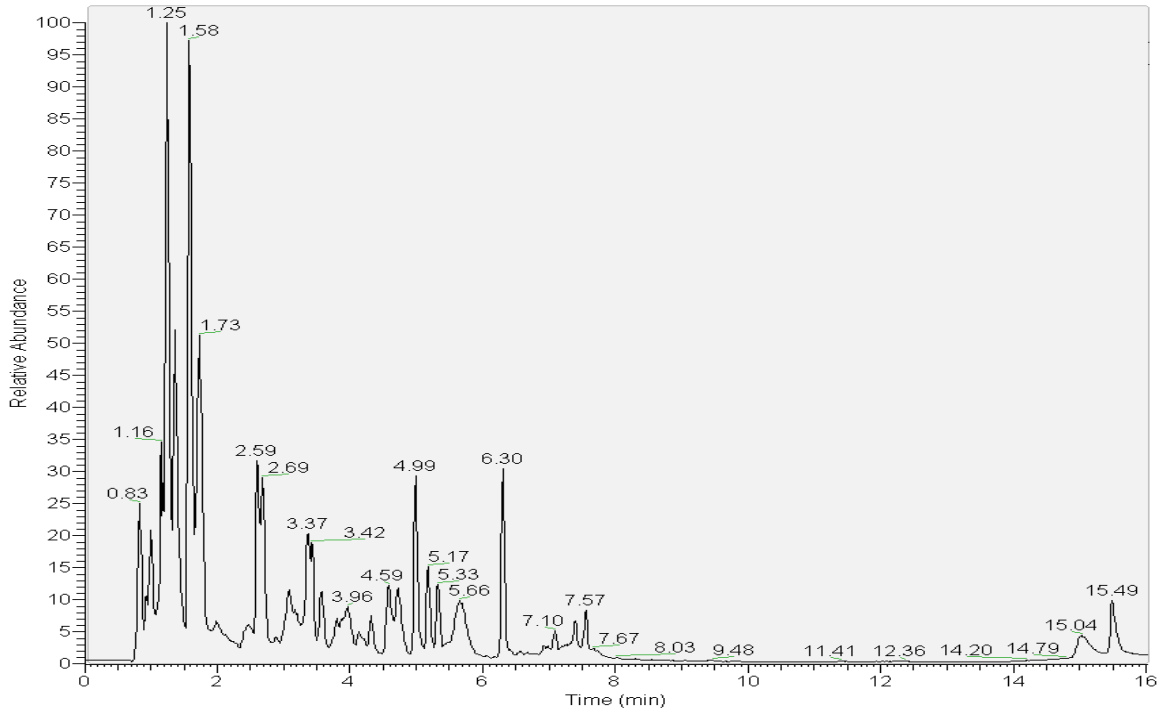


Figure SI.8 Chromatogram of extract from *E.coli* treated by clove oil by PS-DVB-WAX:HLB 50:50 (w:w) – positive ionization mode.

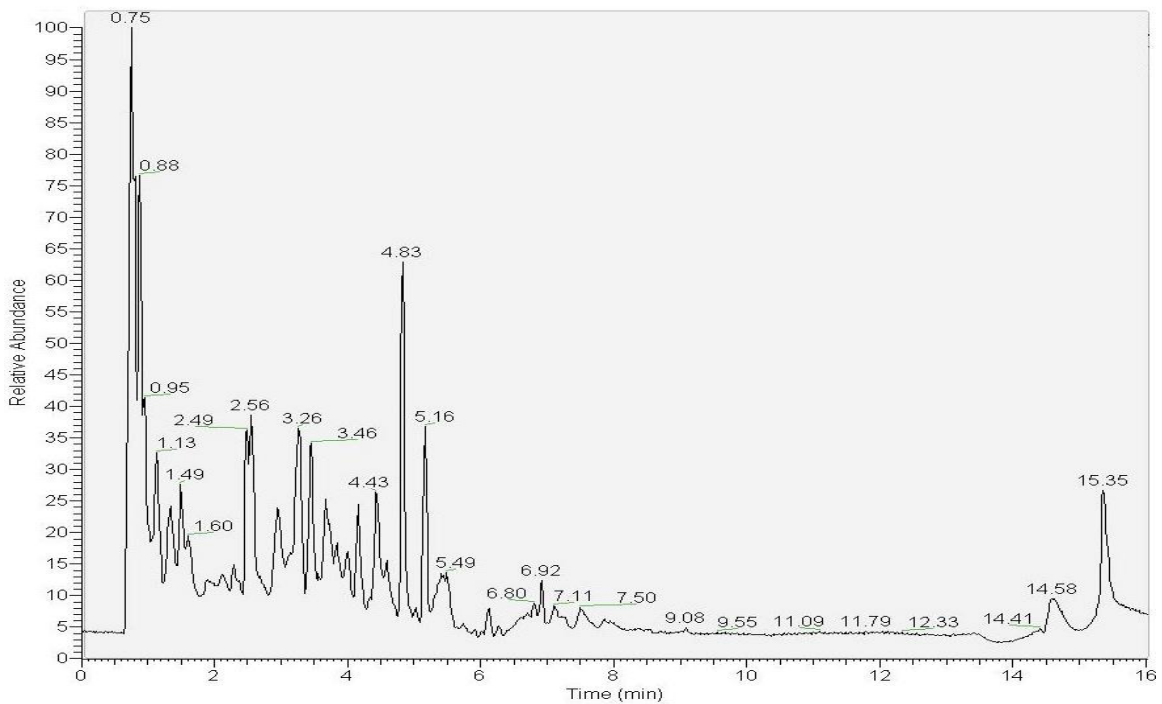


Figure SI.9 Chromatogram of extract from *E.coli* treated by clove oil by PS-DVB-WAX:HLB 50:50 (w:w) – negative ionization mode.



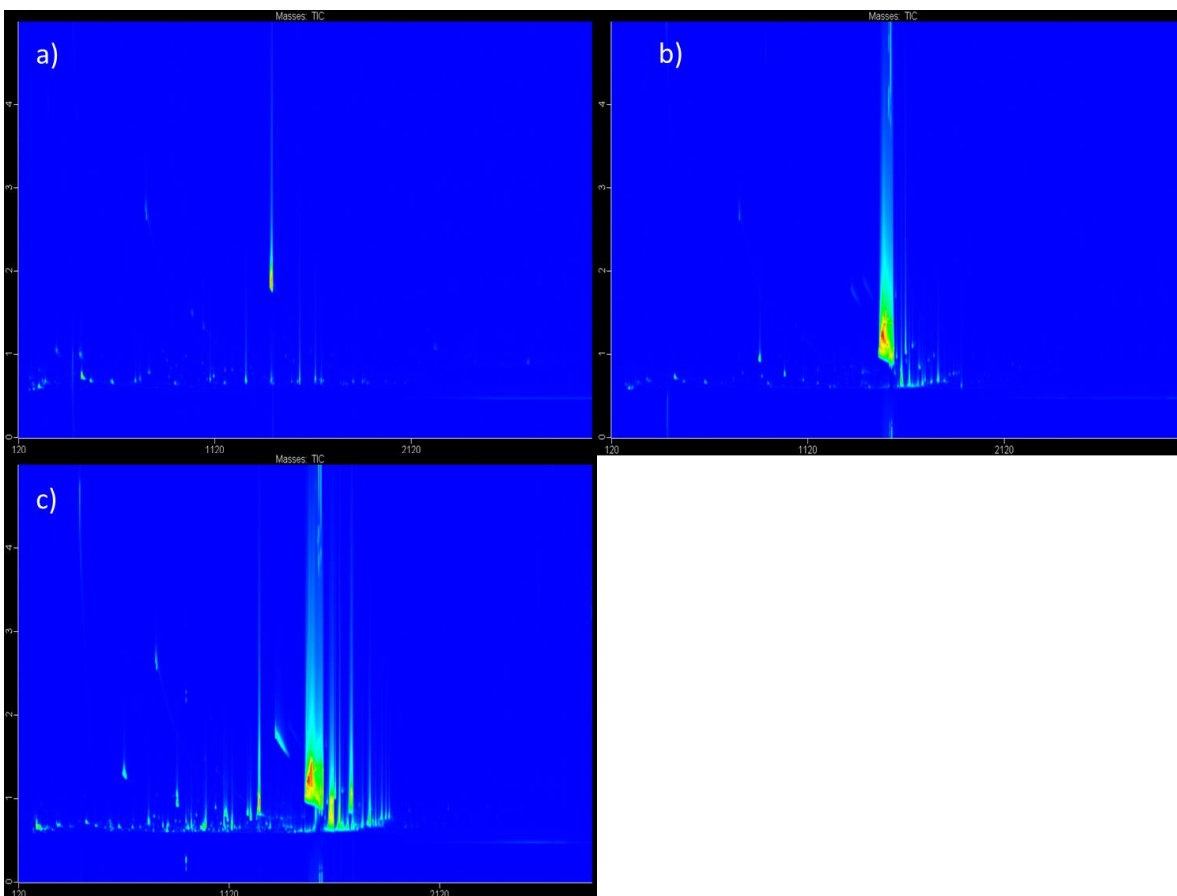


Figure SI.10 GCxGC chromatograms obtained from a) bacteria culture, b) bacteria culture treated with pure eugenol and c) bacteria culture treated with clove oil. Total Ion Current (TIC) is displayed.

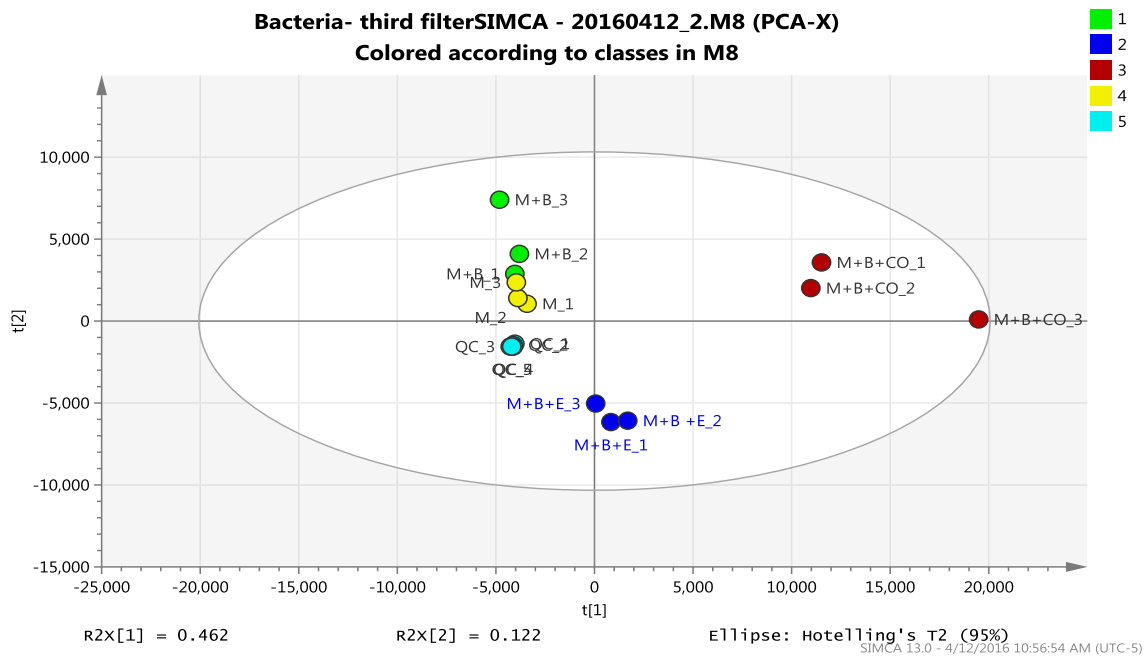


Figure SI.11 Score plot corresponding to PCA analysis carried out on VOC profiles of control samples and samples treated with clove oil and eugenol, sampled by HS-SPME-GC-ToF/MS.

#### References

- 1 S. Risticvic, E. A. Souza-Silva, J. R. Deell, J. Cochran and J. Pawliszyn, *Anal. Chem.*, 2016, **88**, 1266–12274.
- 2 S. Roller, *Natural Antimicrobials for the Minimal Processing of Foods*, Woodhead Publishing Ltd, Cambridge, England, 2003.
- 3 R. Di Pasqua, N. Hoskins, G. Betts and G. Mauriello, *J. Agric. Food Chem.*, 2006, **54**, 2745–9.
- 4 D. R. Morris and R. H. Fillingame, *Annu. Rev. Biochem.*, 1974, **43**, 303–325.
- 5 C. N. Wendakoon and M. Sakaguchi, *Dev. food Sci.*, 1992.
- 6 S. L. Taylor, S. S. Sumner, D. E. Kramer and J. Liston, *Sea Food Quality Determination*, Elsevier Science Publishers, Amsterdam, 1986.
- 7 C. N. Wendakoon and M. Sakaguchi, *Food Prot.*, 1995, **52**, 280–283.
- 8 J. Thoroski, G. Blank and C. Biliaderis, *Food Prot.*, 1989, **52**, 399–403.
- 9 H. Pelicano, D. S. Martin, R.-H. Xu and P. Huang, *Oncogene*, 2006, **25**, 4633–46.
- 10 B. L. Bassler, *Cell*, 2002, **109**, 421–424.
- 11 M. Hentzer and M. Givskov, *J. Clin. Invest.*, 2003, **112**, 1300–1307.
- 12 F. Nazzaro, F. Fratianni and R. Coppola, *Int. J. Mol. Sci.*, 2013, **14**, 12607–19.
- 13 M. S. A. Khan, M. Zahin, S. Hasan, F. M. Husain and I. Ahmad, *Lett. Appl. Microbiol.*, 2009, **49**, 354–60.