

Abstract

Fresh chilled fish and minimally processed fish-based products are prone to microbiological spoilage. Psychrotrophic *Pseudomonas* spp. including *Pseudomonas psychrophila*, whose metabolic activity is dependent on the quorum sensing system, are mainly responsible for spoilage. In quorum sensing system, autoinducers (AI) are recognized by receptor proteins that are transcriptional regulators of a specific group of genes. Interference in the above mechanism with essential oils and their components may affect the synthesis of enzymes and exopolysaccharides (EPS) or the process of lipids degradation by microorganisms. Anti-quorum sensing verification in *in vitro*, *in silico* and *in situ* systems may support the development of alternative/additional solutions in the future, aimed at eliminating or/and reducing the physiological activities of saprotrophic microorganisms in the food matrix.

The major aim of the current study was the assessment of the impact of tarragon (TEO) and black pepper (BPEO) essential oils on quorum sensing system and metabolic activity of *P. psychrophila* KM02 isolated from fish.

In the work, TEO and BPEO were obtained by hydrodistillation, and their chemical compositions were determined with the GC-MS system. Subinhibitory concentrations (suMIC) of essential oils and their major compounds were estimated with the serial dilutions method. GC-FID system was used for the assessment of the fatty acids profile of cellular envelopes. Anti-quorum sensing potential of analyzed factors were characterized by: (i) the estimation of the changes in AI synthesis with the UHPLC-MS/MS system, and (ii) the molecular docking analysis of major compounds of essential oils with receptor proteins.

The whole genome and transcriptome analyses were conducted in order to assess the *P. psychrophila* KM02 potential to grow/metabolic activity in the food matrix. Molecular docking and RT-qPCR analyses were used to evaluate the impact of the analyzed agents on quorum sensing-related efflux and T2SS systems. The degree of inhibition of proteolytic and lipolytic properties, and the reduction of EPS synthesis were verified with spectrophotometric and RT-qPCR analyses. The Koch plate method was used to characterize the growth of *P. psychrophila* KM02 in a model food product containing subMIC of TEO and BPEO.

In the work, the subMICs of TEO (70-75 $\mu\text{L/mL}$) and BPEO (100-135 $\mu\text{L/mL}$) interfered with the quorum sensing system and disturbed the metabolic activity of

examined microorganisms. The exposition of *Pseudomonas* spp. cells on tested agents resulted in the reduction of unsaturated fatty acids and with branched structure in cellular envelopes. *Pseudomonas* spp. synthesized AI belonging to AHL group (i.e. 3-oxo-C12-HSL, 3-oxo-C14-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL, C12-HSL, C4-HSL, C6-HSL) and quinolone compound PQS *in vitro*. SubMICs of TEO, BPEO and their components (i.e. methyl eugenol (ME) 10-12 $\mu\text{L}/\text{mL}$, β -phellandrene (PHE), 8-10 $\mu\text{L}/\text{mL}$, limonene (LIM) 60-65 $\mu\text{L}/\text{mL}$ and β -caryophyllene (CAR) 20-35 $\mu\text{L}/\text{mL}$) inhibited AI synthesis in *Pseudomonas* spp. ME, PHE, LIM and CAR bonded with the receptor proteins LasR, RhIR, TraR and PqsR of quorum sensing system and with proteins MFS, MexB and MuxB of efflux system of *Pseudomonas* spp. The genome and transcriptome of *P. psychrophila* KM02 showed the presence of factors indicating the the ability of the bacteria to decompose of proteins and lipids and secrete the enzymes. SubMIC of TEO, BPEO, ME, PHE, LIM and CAR reduced the proteolytic and lipolytic activity and EPS synthesis by *P. psychrophila* KM02. Tested agents introduced into vinegar-oil marinade inhibited *P. psychrophila* KM02 growth in fish-based product.

The current work showed impact of TEO and BPEO and major components, i.e. ME, PHE, LIM and CAR on quorum sensing system and metabolic activity of *P. psychrophila* KM02. The above relationship may delay the spoilage process of fish stored under refrigeration, in which *P. psychrophila* KM02 is involved. The results obtained in this work introduce new information about the quorum sensing system in saprotrophic bacteria isolated from foods

Key words: quorum sensing, fish spoilage, efflux system, molecular docking, genome, transcriptome

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