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ORIGINAL PAPER

Antibacterial activities of plant-derived compounds and essential oils against *Cronobacter* strains

Anna Berthold-Pluta[1] · Lidia Stasiak-Różańska[2] · Antoni Pluta[1] · Monika Garbowska[3]

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Abstract

Essential oils (EOs) are liquid preparations produced from plant materials. Their use as inhibitors of the growth of spoilage and pathogenic microorganisms is a good alternative to the chemical additives in foods. The disc-diffusion method was used to screen the EOs from thyme, cinnamon, clove, peppermint, marjoram, cumin, rosemary, fennel, basil, lime, bergamot orange, orange, lemon, grapefruit, mandarin, cardamom, anise, and ginger, against 21 strains of *Cronobacter* species, including: *C. sakazakii, C. muytjensii, C. turicensis, C. condimenti*, and *C. malonaticus*. In addition, the minimum inhibitory concentration (MIC) and the maximum tolerable concentration (MTC) of thymol, *trans*-cinnamaldehyde, eugenol, and menthol were determined for five strains of *Cronobacter* spp. The most effective EOs were: thyme > cinnamon > marjoram. In turn, EOs from clove, cumin, and fennel had a moderate inhibiting effect against only some of the analyzed strains. The majority of the tested EOs: peppermint, rosemary, basil, cardamom, anise, ginger, and all EOs from citrus fruits were ineffective against all the studied strains.

Keywords *Cronobacter* spp. · Essential oils · Thymol · Eugenol · Cinnamaldehyde · Menthol

Introduction

The *Cronobacter* genus bacteria are Gram-negative motile bacilli representing the *Enterobacteriaceae* family which includes microorganisms responsible for inducing the highest number of food poisonings in humans worldwide [1]. Currently, the following seven species: *C. sakazakii, C. turicensis, C. malonaticus, C. muytjensii, C. universalis, C. dublinensis*, and *C. condimenti*, have been classified to the *Cronobacter* genus [2, 3]. Among them, *C. turicensis, C. sakazakii, C. malonaticus*, and *C. universalis* are of clinical significance [4]. The most extensively described species from the *Cronobacter* genus is *C. sakazakii*, which is an opportunistic pathogen that may cause bacteremia, sepsis, meningitis, and necrotizing enterocolitis in neonates [5–7]. *Cronobacter* infections also occur in adults (especially immunocompromised, suffering from serious illnesses, and elderly patients), and cause the following symptoms: conjunctivitis, biliary sepsis, urosepsis, appendicitis, wound infections, and pneumonia [4]. It has the ability to adapt to several environmental stresses including drying, heating (though it does not survive pasteurization), chilling, and to osmotic stresses. In addition, it easily colonizes various environments due to the capability for biofilm formation, which facilities its attachment to stainless steel, glass, silicon, latex, polycarbonate, and polyvinyl chloride (PVC) [8, 9]. All these traits allow these microorganisms to survive under conditions of food production processes [10, 11]. Bacteria belonging to the genus *Cronobacter* have been isolated from various food products, including these intended for infants and children, from dairy and meat products, plant-derived foods, water, and others [12–20].

✉ Anna Berthold-Pluta
anna_berthold@sggw.pl

[1] Division of Milk Biotechnology, Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences, SGGW, Nowoursynowska 159C St, 02-787 Warsaw, Poland

[2] Division of Food Biotechnology and Microbiology, Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences, SGGW, Nowoursynowska 159C St, 02-787 Warsaw, Poland

[3] Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, Inter-Department Problem Group for Dairy Industries, Rakowiecka 36 St, 02-532 Warsaw, Poland

Natural antimicrobial agents derived from plant sources (for example, the essential oils—EOs) have been recognized and used for centuries in food preservation as well as for food flavor enhancement. While choosing EO dose added to a food product, attention should be paid not only to the expected technological effect of EO but also to sensory changes its components may evoke in a given food product. As food additives, the EOs may be used as food preserving agent, as they extend storage time of food products or enter into synergistic reactions with the preserving agents present in food to enhance their efficacy [21, 22].

Several plant-derived EOs have been demonstrated to display the antimicrobial properties [23, 24], and a variety of their active components have been identified. EOs contain multiple substances which exhibit antibacterial activity against pathogenic bacteria, *Listeria monocytogenes, Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Bacillus cereus*, and *Staphylococcus aureus* [25–30]. Only a few studies, however, have investigated the inhibitory effects of EOs and carvacrol, thymol, eugenol, and cinnamic acid or *trans*-cinnamaldehyde against *C. sakazakii* [10, 31–33]. The knowledge about the potential susceptibility of other species from the genus *Cronobacter* to EOs and active substances of plant origin is still very scanty.

The first goal of this study was to determine the antimicrobial activity of 18 selected plant EOs against 21 strains representing five species from the genus *Cronobacter*. The second objective was to establish the susceptibility of the five tested species: *Cronobacter sakazakii, C. malonaticus, C. muytjensii, C. turicensis*, and *C. condimenti* to the major active substances of thyme, cinnamon, clove, and peppermint oils, i.e., to thymol, *trans*-cinnamaldehyde, eugenol, and menthol, respectively. Effects of some of these antimicrobials have not been reported previously. The manuscript is also the first to provide data on the susceptibility of *C. muytjensii, C. turicensis*, and *C. condimenti* species to EOs.

Materials and methods

Essential oils and crude plant-derived substances

The study was conducted with 18 essential oils from the following plants: *Thymus vulgaris* (thyme), *Cinnamomum aromaticum* (cinnamon), *Syzygium aromaticum* (clove), *Mentha piperita* (peppermint), *Origanum marjorana* (marjoram), *Carum carvi* (cumin), *Rosmarinus officinalis* (rosemary), *Foeniculum vulgare* (fennel), *Ocimum basilicum* (basil), *Citrus aurantifolia* (lime), *Citrus aurantium* subsp. *bergamia* (bergamot orange), *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus paradisi* (grapefruit), *Citrus reticulate var. madurensis* (mandarin), *Elettaria cardamomum* (cardamom), *Pimpinella anisum* (anise), and *Zingiber officinale* (ginger). They were obtained from FSZ Pollena—Aroma, Nowy Dwór Mazowiecki, Poland.

Analyses were also conducted for the four following active substances of the essential oils: thymol, *trans*-cinnamaldehyde, eugenol, and menthol; all obtained from Sigma-Aldrich, Poland.

Microbial strains

Strains to be analyzed in this study were isolated from market products of plant origin by Berthold-Pluta et al. [13] (the isolates were subjected to genetic identification and differentiation by 16S rDNA sequencing, PCR-RFLP analysis, and RAPD-PCR). They included: 13 strains of *Cronobacter sakazakii* (s12, s14, s21, s22, s41, s42, s44, s45, s47, s48, lv25, lv27, lv28), four strains of *C. muytjensii* (s16, s34, s50, s51), two strains of *C. turicensis* (lv53, lv54), 1 strain of *C. condimenti* (s37), and one strain of *C. malonaticus* (lv31) (Table 1). The tested strains were stored at − 48 °C. Prior to use, they were grown freshly on tryptone soya broth (TSB, Argenta, Poland).

Table 1 Tested *Cronobacter* strains

Species	strain	Origin (product)	References
C. sakazakii	s12	Alfalfa sprouts	Berthold-Pluta et al. [11]
	s14	Alfalfa sprouts	
	s41	Sunflower sprouts	
	s42	Sunflower sprouts	
	s44	Mix of sprouts	
	s45	Mix of sprouts	
	s21	Leek sprouts	
	s22	Leek sprouts	
	s47	Mix of sprouts	
	s48	Mix of sprouts	
	lv25	Rucola	
	lv27	Endive escarola	
	lv28	Rucola	
C. muytjensii	s16	Lentil sprouts	
	s34	Broccoli sprouts	
	s50	Lentil sprouts	
	s51	Lentil sprouts	
C. turicensis	lv53	Mix of leaf vegetables	
	lv54	Mix of leaf vegetables	
C. condimenti	s37	Small radish sprouts	
C. malonaticus	lv31	Lamb's lettuce	

Screening of antimicrobial activity of plant essential oils

A disc-diffusion assay described by Rusenova and Parvanov [34] with small modifications was used in the study. The bacterial inoculum was prepared from an overnight culture on tryptone soya agar (TSA, Argenta, Poland). Colonies were directly suspended in 0.85% saline to obtain turbidity comparable to that of the 0.5 McFarland standard (approximately 8 log CFU mL^{-1}). Aliquots (0.1 mL) of the inoculum were spread over the surface of pre-dried tryptone soya agar plates with a sterile spreader. Sterile 6 mm filter paper discs (BTL Sp. z o.o., Poland) were placed on the plates and 10 µL portions of the essential oils were added immediately. Sterile paper discs were used as the control. The plates were left for 30 min at room temperature to allow oil diffusion and then were incubated at 35 °C for 24 h. Diameters of inhibition zone were measured in triplicate in millimeters. The scale of measurement was as follows: strong inhibition—zone of inhibition ≥ 20 mm (disc diameter included), moderate inhibition—zone < 20 − 12 mm, and no inhibition—zone < 12 mm. The assay was carried out in triplicate. Values are presented as means ± SD.

Determination of the minimum inhibitory concentration and maximum tolerable concentration of thymol, *trans*-cinnamaldehyde, eugenol, and menthol against *Cronobacter* strains

The susceptibility of the five selected strains of the *Cronobacter* genus bacteria (*C. sakazakii* lv27, *C. malonaticus* lv31, *C. muytjensii* s50, *C. turicensis* lv53, and *C. condimenti* s37) to the active substances of essential oils was evaluated with a slightly modified method of Oussalah et al. [35]. Prior to the experiment, working cultures were prepared by subculturing 100 µL of each culture in 9 mL of TBS, and incubated at 35 °C for 24 h.

Ten final concentrations of the tested substances [0.000%, 0.003%, 0.006%, 0.013%, 0.025%, 0.05%, 0.1%, 0.2%, 0.4%, and 0.8% (vol/vol)] were obtained by adding their various volumes from the 10% (wt/vol) suspension in 96% ethanol to the molten Mueller Hinton Agar (MHA, Argenta, Poland) (15 mL). Immediately after thorough mixing, the MH agar with an appropriate amount of the tested substance was poured onto sterile Petri plates and left to solidify (30 °C, 3 h). Then, 100 µL of each diluted culture was individually spread on the surface of the plates. Dilution of the *Cronobacter* culture was adjusted to ensure the number of colonies grown after plates' incubation in the range from 30 to 150. The suspension of microorganisms was carefully spread onto the plate using a sterile spreader, until its complete absorption. The positive control consisted of MHA without the tested substances, inoculated with the diluted medium culture. Non-inoculated plates containing the tested substances served as the negative control. The test and control plates were then incubated at 35 °C for 48 h. Results obtained allowed determining the minimum inhibitory concentration (MIC; concentration of the tested active substance at which no growth of the test strain was observed) and maximum tolerable concentration (MTC; the maximum concentration of the analyzed active substance at which the number of grown colonies of the test strain did not differ significantly from the number of colonies grown on the plate with the medium not containing the analyzed active substance). Determinations were made in triplicate.

Results and discussion

Antimicrobial activity of plant essential oils against *Cronobacter* strains

The zones of inhibition resulting from the exposure of *Cronobacter* strains to the plant essential oils (10 µL/disc) measured using the disc-diffusion method are shown in Table 2.

In general, the plant extracts showed a high antibacterial activity against Gram(+) bacteria and a lower activity against Gram(−) bacteria. The observed resistance of the Gram(−) bacteria could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism. The hydrophilic cell wall structure of Gram(−) bacteria, constituted essentially by a lipopolysaccharide, blocks the penetration of hydrophobic components of oils [23].

The greatest zones of growth inhibition of bacterial strains from the genus *Cronobacter* were observed in the case of the thyme oil, i.e., from 15.8 to 31.8 mm. It turned out to be strongly inhibitory (growth inhibition zone > 20 mm) against 13 strains. The most susceptible strains to this EO, i.e., the strains with the greatest inhibition zones, included these from the species *C. muytjensii*, and the mean diameter of their growth inhibition zone reached 27.1 mm. More resistant to the effects of thyme oil appeared to be strains of *C. turicensis, C. malonaticus*, and *C. condimenti* species (mean diameter of growth inhibition zone of ca. 17 mm).

The major components of thyme EO include: thymol, *p*-cymene, γ-terpinene, and also carvacrol, linalool, and β-caryophyllene [36, 37]. Among these, the greatest load of data about the antimicrobial activity is available for thymol and carvacrol. The mechanism of their action consists in disrupting the cytoplasmic membrane of a cell, which increases its permeability and depolarizes its potential [38]. Using the disc-diffusion method, Soković et al. [37] demonstrated growth inhibition zones of 22 mm for both *Enterobacter cloacae* and *E. coli* exposed to thyme oil.

Table 2 Antimicrobial activity of plant essential oils (10 µL/disc) against *Cronobacter* strains using disc-diffusion method

Cronobacter strain	Thyme	Cinnamon	Clove	Peppermint	Marjoram	Cumin	Rosemary	Fennel	Basil	Lime	Bergamot orange	Orange	Lemon	Grapefruit	Mandarin	Cardamom	Anise	Ginger
	Inhibition zone diameter, mean ± SD (mm)																	
C. sakazakii s12	21.9 ± 2.1	17.8 ± 0.9	12.4 ± 0.8	11.4 ± 0.7	12.5 ± 0.9	10.2 ± 1.2	0.0	10.3 ± 0.8	8.8 ± 0.5	0.0	10.3 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s14	21.5 ± 2.0	17.6 ± 1.2	11.6 ± 0.9	11.0 ± 0.9	13.0 ± 0.7	10.8 ± 0.5	0.0	14.3 ± 2.0	9.1 ± 0.5	9.4 ± 0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s41	24.5 ± 0.7	20.5 ± 0.5	11.5 ± 0.4	10.8 ± 1.4	16.8 ± 1.0	11.2 ± 0.6	10.0 ± 0.6	10.0 ± 0.2	10.1 ± 0.3	9.2 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s42	24.7 ± 1.9	18.3 ± 1.3	11.9 ± 0.5	9.2 ± 0.4	12.9 ± 0.5	10.1 ± 0.6	0.0	11.3 ± 1.0	9.0 ± 0.4	9.8 ± 0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s44	23.8 ± 0.8	16.6 ± 0.4	12.4 ± 0.6	11.4 ± 1.5	12.8 ± 0.3	12.7 ± 2.8	9.3 ± 0.5	14.2 ± 0.8	0.0	9.3 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s45	22.3 ± 2.0	18.5 ± 08	12.1 ± 0.8	10.3 ± 2.3	13.7 ± 0.4	10.8 ± 0.6	8.1 ± 0.6	12.7 ± 0.5	8.7 ± 0.2	10.3 ± 0.6	8.3 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s21	18.1 ± 0.9	14.5 ± 0.9	11.1 ± 0.2	8.6 ± 0.3	13.0 ± 0.5	0.0	8.9 ± 0.5	11.0 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s22	20.4 ± 0.9	17.0 ± 0.5	11.9 ± 0.6	10.9 ± 0.8	15.5 ± 0.6	12.8 ± 0.9	8.7 ± 0.9	11.9 ± 1.0	9.9 ± 0.5	9.5 ± 0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s47	23.9 ± 1.1	15.7 ± 0.6	11.9 ± 0.3	10.6 ± 2.3	15.1 ± 1.0	10.5 ± 0.4	8.6 ± 0.3	12.0 ± 0.6	9.2 ± 0.5	9.4 ± 0.6	8.4 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii s48	23.8 ± 0.7	17.5 ± 1.0	10.8 ± 0.6	10.6 ± 1.2	17.9 ± 2.4	0.0	9.8 ± 1.1	12.5 ± 0.5	8.1 ± 0.3	9.9 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii lv25	20.3 ± 1.1	16.4 ± 1.1	11.1 ± 0.3	9.5 ± 0.7	13.3 ± 0.5	0.0	0.0	11.6 ± 0.7	9.6 ± 0.6	0.0	9.3 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii lv27	18.2 ± 0.9	19.6 ± 0.6	12.9 ± 0.7	10.8 ± 1.7	14.0 ± 1.4	15.9 ± 0.9	9.0 ± 0.9	10.9 ± 0.7	9.5 ± 0.6	9.8 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sakazakii lv28	19.4 ± 2.0	14.1 ± 0.5	12.4 ± 0.5	10.3 ± 0.4	13.0 ± 0.6	0.0	9.2 ± 0.4	9.1 ± 0.5	11.2 ± 0.2	8.5 ± 0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. muytjensii s16	21.6 ± 1.0	13.0 ± 0.6	10.3 ± 0.4	9.8 ± 0.4	14.2 ± 1.6	0.0	9.3 ± 0.3	10.3 ± 0.5	8.8 ± 0.3	9.9 ± 0.4	10.0 ± 1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. muytjensii s34	26.6 ± 1.2	17.0 ± 0.5	12.0 ± 0.4	9.0 ± 0.5	13.7 ± 1.1	10.1 ± 0.4	8.7 ± 0.5	14.1 ± 0.5	8.4 ± 0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. muytjensii s50	31.8 ± 1.5	19.6 ± 0.7	13.3 ± 0.8	11.8 ± 0.6	13.6 ± 1.2	0.0	8.8 ± 0.4	9.0 ± 0.5	0.0	9.4 ± 0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. muytjensii s51	28.3 ± 2,7	20.7 ± 0.7	12.3 ± 0.7	11.9 ± 1.1	14.9 ± 1.4	0.0	0.0	11.1 ± 0.8	0.0	9.7 ± 0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. turicensis lv53	18.1 ± 1.0	17.8 ± 0.6	15.7 ± 1.3	9.1 ± 0.2	18.3 ± 3.1	11.1 ± 0.7	8.7 ± 0.7	13.1 ± 0.4	10.1 ± 0.4	8.6 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. turicensis lv54	15.8 ± 1.0	20.5 ± 0.8	16.8 ± 2.9	10.8 ± 1.4	21.5 ± 1.8	9.5 ± 0.5	11.6 ± 1.1	11.9 ± 1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. condimenti s37	17.9 ± 0.7	19.4 ± 0.3	12.5 ± 0.4	11.0 ± 0.3	15.6 ± 0.5	0.0	8.8 ± 0.3	12.2 ± 1.1	10.9 ± 0.3	10.0 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2 (continued)

Cronobacter strain	Thyme	Cinnamon	Clove	Peppermint	Marjoram	Cumin	Rosemary	Fennel	Basil	Lime	Bergamot orange	Orange	Lemon	Grapefruit	Mandarin	Cardamom	Anise	Ginger
	Inhibition zone diameter, mean ± SD (mm)																	
C. malonaticus lv31	17.4 ± 1.6	15.3 ± 0.5	12.4 ± 1.3	8.0 ± 0.4	14.1 ± 1.1	15.0 ± 1.2	9.3 ± 1.5	14.8 ± 0.5	9.6 ± 0.3	10.0 ± 0.7	10.2 ± 0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cinnamon oil contains many substances, such as cinnamaldehyde (and *trans*-cinnamaldehyde), cinnamate, cinnamic acid, cinnamyl acetate, eugenol, L-borneol, camphor, caryophyllene oxide, β-caryophyllene, α-terpineol, and many others, which protect plants against competitors and pathogens [39]. Both oil and pure cinnamaldehyde were equally effective in inhibiting the growth of various bacteria including the Gram-positive (*S. aureus*) and the Gram-negative ones (*E. coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae*, and *S*. Typhimurium), and fungi including yeasts (*Candida* spp.) and molds (*Aspergillus* spp. and *Fusarium* spp.). The MICs of both oil and cinnamaldehyde ranged from 0.0075 to 0.06% for bacteria, from 0.01 to 0.045% for yeasts, and from 0.0075 to 0.015% for molds [40].

Significant susceptibility to cinnamon oil (growth inhibition zone > 20 mm) was demonstrated for the three following strains: *C. muytjensii* s51, *C. sakazakii* s41, and *C. turicensis* lv54, while the growth of the other strains was moderately inhibited by this EO. An inhibiting effect of cinnamon oil on *C. sakazakii* was also shown by Al-Nabulsi et al. [31] (diameters of growth inhibition zones ranged from 32 to 40 mm). Literature data on the susceptibility of other bacterial species, e.g., *L. monocytogenes*, to this essential oil also confirm its strong antimicrobial properties [34, 35].

The clove oil turned out a poor growth inhibitor (growth inhibition zone < 12 mm) for 9 out of the 21 tested strains. Growth inhibition diameters measured for the other analyzed strains ranged from 12.1 to 16.8 mm, and their average values reached ca. 12 mm for strains of *C. sakazakii, C. condimenti, C. muytjensii*, and *C. malonaticus* species, as well as 16 mm for strains of *C. turicensis* species. Data concerning the antimicrobial properties of clove oil against *Cronobacter* are very scanty. Only Fraňková et al. [33] reported the MIC of clove oil at 0.05% for *C. sakazakii* and *C. malonaticus*.

Among the volatile components of clove oil, eugenol and (−)-α-selinene occur in the highest concentrations, but also *cis*-α-bisabolene, ocimene, santolinatriene, and humulene are its major volatiles, though they are present in lower concentrations [41]. Biofilm inhibition experiments showed that a clove extract inhibited biofilm formation by *C. sakazakii* isolates [42]. Biofilm formation plays a significant part in the pathogenesis of *C. sakazakii*, and development of these biofilms is based on the signal-mediated quorum sensing (QS) system. Therefore, an interference with QS may prevent the development of bacterial biofilms and further infections [42].

None of the tested strains was susceptible to the peppermint oil; diameters of their growth inhibition zones ranged from 8.0 to 11.9 mm. Their resistance to this EO was similar (mean growth inhibition zone diameter reached ca. 10 mm for strains of the same species), except for the strain *C. malonaticus* lv31 which turned out to be little more

resistant (growth inhibition zone diameter of 8.0 mm). The bactericidal properties of the peppermint oil were confirmed by Rusenova and Parvanov [34]; diameters of growth inhibition zones measured for *L. monocytogenes* and *Bacillus licheniformis* reached 18 and 27.4 mm, respectively. This EO evoked the inhibiting effect also on other bacteria, i.e., *S. aureus, E. coli*, and *P. aeruginosa* [37, 43, 44]. The antimicrobial potential of the *Mentha piperita* essential oil was also observed by Tyagi and Malik [45] against the strains of *E. coli* (growth inhibition zone of 10 mm) and *Pseudomonas* spp. (11–14 mm).

The vast majority of the tested strains exhibited moderate susceptibility to the marjoram oil; mean growth inhibition diameters ranged from 12.5 to 17.9 mm. Only both strains of *C. turicensis*—lv53 and lv54 appeared to be more susceptible (growth inhibition zones of 18.3 and 21.5 mm, respectively). The antimicrobial activity of the marjoram oil against *E. coli* (growth inhibition zone of 14 mm) and *B. cereus* (16 mm) was reported by Tserennadmid et al. [46]. In turn, Rusenova and Parvanov [34] demonstrated its inhibiting effect on such species as. *S. aureus, L. monocytogenes, B. lichenformis, E. faecalis*, and *E. aerogenes*, whose growth inhibition diameters reached 17.7, 14.3, 19.3, 13.7, and 13.0 mm, respectively. The major components of the marjoram essential oil include: γ-terpinene, sabinene, linalool, and linalyl-acetate [46]. Literature data on its antibacterial properties are scanty. Soković et al. [37] used the disc-diffusion method to analyze the antibacterial activity of, i.a., linalyl-acetate against *E. coli* and *E. cloacae* and noted 8-mm zones of their growth inhibition.

In our study, only four strains (*C. sakazakii* s44, *C. sakazakii* s22, *C. sakazakii* lv27, and *C. malonaticus* lv31) turned out to be moderately resistant to the cumin essential oil (diameters of growth inhibition zones ranging from 12.7 to 15.9 mm), whereas eight strains remained unaffected by this EO (lack of growth inhibition zones). The antibacterial activity of *Carum carvi* essential oil could be attributed to its polyphenolic compounds. In turn, the antibacterial activity of polyphenols has been ascribed to the inhibition of synthesis of DNA, RNA and other related macromolecules [30]. Sixteen compounds were identified in *C. carvi* EO, out of which cuminaldehyde (22%) was the principal component followed by γ-terpinene (18%), γ-terpinene-7-al (15%), and *p*-cymene (8%) [47]. Fraňková et al. [33] demonstrated a moderate antibacterial activity of its components, i.e. γ-terpinene and *p*-cymene, against *C. sakazakii* and *C. malonaticus*.

The rosemary essential oil had no inhibitory effect on the tested strains regardless of the species. Growth inhibition zones with diameters ranging from 8.1 to 11.6 mm were demonstrated for 16 strains. No growth inhibition zones were observed in the case of the five other strains, including four from the species *C. sakazakii* (s12, s14, s42, and lv25) and one from the species *C. muytjensii* (s51). Our results confirmed earlier findings reported by Al-Nabulsi et al. [31], who demonstrated a negligible antibacterial activity of the rosemary essential oil against the *Cronobacter* genus bacteria (growth inhibition zones up to 8 mm).

Considering the Gram(+) bacteria, the MIC of the rosemary EO was 0.5% for *L. monocytogenes* and *S. aureus*, and 0.06% for *B. cereus*. Up to 1% of this EO had no effect on the Gram(-) bacteria like *E. coli*, or *Salmonella* Enteritidis [48]. The major constituents of rosemary EO were reported to be 1,8-cineole, α-pinene, myrcene, camphor, and camphene [28, 49]. Jiang et al. [49] also reported that the MIC values of rosemary EO, 1,8-cineole and α-pinene for *E. coli* accounted for 0.3%, > 4.0%, and 1.0%, respectively. The effectiveness of 1,8-cineole and α-pinene against *E. coli* was also confirmed by Ojeda-Sana et al. [28] who additionally demonstrated a lack of their antibacterial activity against Gram(+) bacteria like *S. aureus* and *E. faecalis*.

The major components of fennel EO have been reported to be *trans*-anethole, fenchone, estragole (methyl chavicol), and α-phellandrene [50, 51]. The fennel EO has been shown to exhibit a moderately antibacterial effect against foodborne pathogens such as *E. coli, S. aureus*, and *L. monocytogenes* [50, 52, 53]. In our study, this EO also exhibited a moderate antibacterial activity against nine strains (growth inhibition zones from 12.0 to 14.8 mm). In the case of the other *Cronobacter* spp. strains, diameters of growth inhibition zones ranged from 9.0 to 11.9 mm. These results confirm earlier findings reported by Al-Nabulsi et al. [31], who demonstrated very weak inhibiting properties of the fennel EO against *C. sakazakii* (width of growth inhibition zones was less than 1 mm). In turn, Elgayyar et al. [54] showed a strongly inhibiting effect of this EO on *S. aureus* and *Yersinia enterocolitica*; diameters of their growth inhibition zones reached 38 and 57 mm, respectively. The fennel EO was also reported to elicit an inhibiting effect on *B. cereus* [29].

Zones of growth inhibition induced by the basil essential oil were observed for 16 out of the 21 tested strains; their diameters ranged from 8.1 to 11.2 mm. In contrast, this EO had no inhibiting effect on *C. sakazakii* s44 and s21, *C. muytjensii* s50 and s51, and *C. turicensis* lv54 strains. Literature data differ slightly from results obtained in our study and show the antimicrobial activity of basil EO against *E. coli* and *E. cloacae*—diameters of their growth inhibition zones reached 14 and 12 mm, respectively [37]. In turn, Elgayyar et al. [54] demonstrated the antimicrobial activity of basil EO against *S. aureus* and *Y. enterocolitica*, whereas Tajkarimi et al. [29] against *B. cereus*.

As reported by Soković et al. [37], the major components of the EO from *Ocimum basilicum* include linalool (ca. 69%), α-cadinol, γ-cadinene, methyl chavicol, *trans*-β-guaiene, and eugenol. Zones of *E. coli* and *E. cloacae*

growth inhibition by linalool were 12 mm in diameter, and zone of *Bacillus subtilis* growth inhibition was 20 mm in diameter [37]. In turn, eugenol and linalool inhibited the growth of *C. sakazakii* and *C. malonaticus* [33].

Among the six analyzed EOs from citrus fruits, i.e., from: *Citrus aurantifolia* (lime), *Citrus aurantium* subsp. *bergamia* (bergamot orange), *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus paradisi* (grapefruit), and *Citrus reticulate var. madurensis* (mandarin), as many as 4—from orange, lemon, grapefruit, and mandarin—had no effect on the growth of *Cronobacter* (no growth inhibition zones). Diameters of growth inhibition zones of 16 strains of *Cronobacter* induced by the lime EO ranged from 8.5 to 10.3 mm, whereas no growth inhibition zones were observed for five tested strains (*C. sakazakii* s12, *C. sakazakii* s21, *C. sakazakii* lv25, *C. muytjensii* s34, and *C. turicensis* lv54). In turn, the bergamot essential oil inhibited the growth of four strains from the species *C. sakazakii* (s12, s45, s47 and lv25), *C. muytjensii* s16, and *C. malonaticus* lv31 (with diameters of growth inhibition zones ranging from 8.3 to 12.0 mm), whereas it had no effect on the growth of the remaining 15 strains of *Cronobacter*.

Essential oils from citrus plants contain certain components that are typical of all plants from this group, such as: limonene and pinene (α- and β-), but also other compounds present in smaller amounts depending on species, such as: sabinene, myrcene, and telinene in lime EO [47], γ-terpinene [37], and linalool [25] in lemon EO, and citral in orange EOs [25].

Lemon and orange essential oils exhibited weak antibacterial properties against *E. coli* and *E. cloacae* [37]. Fisher and Phillips [25] demonstrated that lemon and orange oils had week inhibitory effects on *E. coli* and *Campylobacter jejuni* when assessed with the disc-diffusion method and also, likewise in our study, that bergamot oil was the most effective of the tested citrus essential oils. Fraňková et al. [33] showed an inhibiting effect of limonene (MIC = 0.3%) and weaker effects of α- and β-pinene (MIC > 0.5%) on *C. sakazakii* and *C. malonaticus*, whereas Shi et al. [55] reported the growth inhibition of *C. sakazakii* by citral.

All analyzed strains from the genus *Cronobacter* were completely resistant to the cardamom, anise, and ginger essential oils (lack of growth inhibition zones). Only sparse reports are available in the literature on the antimicrobial activity of these EOs. Al-Nabulsi et al. [31] showed no inhibiting effect of anise EO on strains of the *C. sakazakii* species, whereas Inouye et al. [56] demonstrated a significantly weaker effect of the major component of cardamom oil—camphor—on growth inhibition of Gram(−) bacteria compared to cinnamaldehyde and thymol. As reported by Bellik [57], compounds of the ginger essential oil which exhibited the antibacterial activity were more effective against *E. coli, B. subtilis*, and *S. aureus*, than against *B. cereus*. In turn, Konakchiev et al. [58] demonstrated a weak effect of the ginger EO on growth inhibition of both Gram(+) and Gram(−) bacteria.

MIC and MTC of thymol, *trans*-cinnamaldehyde, eugenol, and menthol against *Cronobacter* strains

Table 3 presents determined values of the maximum tolerable concentration (MTC) and minimum inhibitory concentration (MIC) of EO components for five strains of *Cronobacter* genus bacteria.

The analyzed strains were tolerant to thymol—being the major component of thyme EO, in its concentration ranges from 0.013 to 0.025% (MTC). In turn, the MIC of thymol reached 0.05% for all tested strains. Lee and Jin [10] demonstrated an inhibiting effect of thymol on two strains of *Cronobacter* (MIC 0.019%). In addition, Fraňková et al. [33] noted a similar inhibiting activity of thymol against *C. sakazakii* and *C. malonaticus* (MIC 0.02%). Literature data confirm also the inhibiting effect of thymol on other bacterial species, e.g., thymol was reported to inhibit the growth of *S*. Typhimurium and *E. coli* (MIC 0.02% and 0.04%, respectively) [59, 60].

Equally strong antimicrobial activity was found in our study for *trans*-cinnamaldehyde. Its MTC and MIC values ranged from 0.025 and 0.05% (*C. sakazakii* lv27, *C. malonaticus* lv31, and *C. muytjensii* s50) to 0.05% and 0.1%, respectively (*C. turicensis* lv53 and *C. condimenti* s37). These values are slightly higher from *trans*-cinnamaldehyde concentrations inducing growth inhibition of *C. malonaticus* and *C. sakazakii* bacteria, i.e., MIC from 0.025 to 0.03%,

Table 3 MIC (%) and MTC (%) values of the analyzed components of essential oils determined for strains of the *Cronobacter* genus bacteria

Strain	Thymol		*Trans*-cinnamaldehyde		Eugenol		Menthol	
	MTC	MIC	MTC	MIC	MTC	MIC	MTC	MIC
C. sakazakii lv27	0.013	0.05	0.025	0.05	0.05	0.2	0.4	0.8
C. malonaticus lv31	0.025	0.05	0.025	0.05	0.05	0.1	0.2	0.8
C. muytjensii s50	0.025	0.05	0.025	0.05	0.05	0.1	0.2	0.8
C. turicensis lv53	0.013	0.05	0.05	0.1	0.05	0.2	0.2	0.4
C. condimenti s37	0.013	0.05	0.05	0.1	0.1	0.2	0.4	0.8

respectively [33]. The MIC of *trans*-cinnamaldehyde was also determined in reconstituted milk for infants and ranged from 0.3 to 0.5% depending on treatment temperature and time [32]. In turn, cinnamic acid was found to inhibit the growth of the *Cronobacter* genus bacteria at the concentration of 0.07% [10].

Trans-cinnamaldehyde is an unsaturated aldehyde. It has an acrolein group (α, β-unsaturated carbonyl moiety) in its molecule which is responsible for its antibacterial activity [39]. The mechanism of its antibacterial effect in, e.g., *C. sakazakii*, involves the downregulation of F_1F_0-ATPase which leads to the inhibition of ATP synthesis. The F_1F_0-ATPase complex is a reversible proton-translocating pump that may extrude protons from the cytoplasm and assists in the regulation of cytoplasmic pH [61]. Other mechanisms of *trans*-cinnamaldehyde action include perturbing the cell membrane and altering the lipid profile of the membrane [39]. *Trans*-cinnamaldehyde is able to inhibit guanosine triphosphate (GTP)-dependent FtsZ polymerization, which is the key process during cell division [62]. In addition, it has the ability to suppress gene expression of membrane porins (OmpA, OmpC, and OmpR), and amino acid transporters, which impairs the active transport through the bacterial cell membrane [61, 63]. Amalaradjou and Venkitanarayanan [61] demonstrated that *trans*-cinnamaldehyde can reduce the resistance to osmotic stress and desiccation in *C. sakazakii*. Its sub-inhibitory concentrations caused a significant reduction in both motility of *C. sakazakii* cells and their biofilm formation capability, which was attributable to the downregulation of the genes associated with the flagellar apparatus (*fliD, flgJ, motA*, and *motB*) [63].

Trans-cinnamaldehyde (or cinnamaldehyde) has often been addressed in scientific research considering its ability to inhibit the growth of microorganisms. Hill et al. [64] reported this component of the cinnamon EO to inhibit the growth of *S. Typhimurium* when used in the concentration of 0.04%. The antimicrobial effects of cinnamaldehyde were also studied against bacteria of *E. coli* and *S. aureus* species [34], and the determined MIC values reached 0.015% and 0.03%, respectively. The susceptibility of these bacteria to cinnamaldehyde was also reported by Ye et al. [65] (MIC 0.03%).

Eugenol (4-allyl-2-methoxyphenol) is a phenol naturally occurring in an EO extracted from cloves. The basic mechanism of its antimicrobial effect involves disruption of the cytoplasmic membrane, which increases its non-specific permeability (referred to as the phenomenon of hyperpermeability). It leads to the release of ions and loss of other cellular contents, including the intracellular proteins, which ultimately ends with cell death [66]. The antimicrobial activity of eugenol against pathogenic microorganisms has been widely discussed in the literature. In studies with *S. Typhimurium*, the MIC of eugenol ranged from 0.05% [59] to 0.1% [64]. In turn, Filgueiras and Vanetti [67] analyzed its effects on *L. monocytogenes* and determined its MIC at 0.1%. Eugenol has been reported to exhibit the antimicrobial activity also against *E. coli*, and its MIC value determined for these bacteria ranged from 0.04 to 0.16% [59, 60].

Tolerance of the analyzed strains to eugenol fluctuated between 0.05 and 0.1% (MTC). The least susceptible to this major compound of clove EO in the medium was the *C. condimenti* s37 strain which tolerated the addition of eugenol in the concentration of 0.1%. The MIC values of eugenol ranged from 0.1 to 0.2%. Fraňková et al. [33] reported its similar MIC value (0.1%) for strains of *C. malonaticus* and C. *sakazakii* bacteria. The antibacterial properties of eugenol against bacteria from the genus *Cronobacter* were also investigated by Lee and Jin [10], who determined its MIC at 0.082%.

Menthol turned out to be an EO component with the weakest antibacterial activity. Development of the tested strains was inhibited at menthol concentrations ranging from 0.4 to 0.8% (MIC), but still its MIC value determined for four of the tested strains was as high as 0.8%, and only the *C. turicensis* lv53 exhibited greater susceptibility. In turn, the value of the maximum concentration of menthol tolerated by the analyzed strains (MTC) ranged from 0.2 to 0.4%.

Next to iso-menthone, limonene, β-pinene, menthyl acetate, iso-menthanol, and menthofuran, menthol is the main component of an EO from *Mentha piperita* [37, 45]. No data were found in the literature on the antimicrobial activity of menthol and peppermint EO against *Cronobacter*, whereas some studies are available on the effect of peppermint EO on the development of other microorganisms and respective results have already been published [34, 37, 43, 44, 68]. Slightly lower MIC values of menthol than these determined in our study were reported by Trombetta et al. [69] for *E. coli* (MIC = 0.25%) and by Tyagi and Malik [45] for *E. coli* (MIC ≈ 0.1%) and for *Pseudomonas* spp. (MIC = 0.2%) but in the case of peppermint EO. Significant morphological alterations (shrunk, deshaped, deformed, and ruptured cells) due to the effect of peppermint oil on *B. subtilis* have also been observed using a scanning electron microscope [45].

Conclusions

In summary, this study confirms the antibacterial activity of selected essential oils against *Cronobacter*. According to analyses conducted with the disc-diffusion method, the most effective EOs were thyme > cinnamon > marjoram. The clove, cumin, and fennel oils had moderately inhibiting effects on only some of the tested strains. The majority of the tested EOs: peppermint, rosemary, basil, cardamom, anise, and ginger as well as all EOs from citrus fruits were ineffective against all the analyzed strains.

It is difficult to ascribe the antimicrobial effect of an essential oil to one or a few of its active substances, because extracts often contain a mixture of various chemical compounds. Apart from major constituents, also these present in lower or sometimes even trace amounts may significantly contribute to the antimicrobial properties of EO. Nevertheless, in our study, the order of inhibition (based on MIC values) attributed to the tested natural organic compounds was as follows: thymol > *trans*-cinnamaldehyde > eugenol > menthol, which was also reflected in results obtained for the EOs with the disc-diffusion method (thyme oil > cinnamon oil > peppermint oil).

The use of EOs or natural plant-derived compounds as food additives could be one of the possible ways to control *Cronobacter* in various types of food products. The use of natural EOs inscribes well into the "clean label" trend and allows reducing amounts of chemical preserving agents. However, further studies addressing their antibacterial activities in food matrices and their effects on the organoleptic properties of food products are required prior to their commercial application.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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