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Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity

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Abstract

Isolation of carrot fruit (*Daucus carrota* L., cultivar "Chanteney") essential oil by supercritical carbon dioxide was investigated from the pretreatment of herbaceous matrix and extraction conditions to the chemical composition of obtained extract and its antimicrobial activity. The qualitative and quantitative analyses of the supercritical extract, as well as of the essential oil obtained by hydrodistillation, were done by GC/FID and GC/MS methods. Antimicrobial properties of both samples were investigated against ten species of microorganisms. Experimental results showed that the particle size had no influence on the extraction process. The highest yield was obtained at 40 °C and 10 MPa. The main component of the supercritical extract, as well as of the essential oil was carotol. The supercritical extract was characterized by the presence of heavier molecular weight compounds, while some lighter compounds, e.g. pinenes, were not detected. The supercritical extract and the essential oil were the most effective against Gram-positive bacteria.
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Keywords: Carrot fruit; Carotol; Supercritical fluid extraction; Essential oil; Antimicrobial activity

1. Introduction

Essential oils may have great potential use as food flavours and preservatives. The antimicrobial activity of essential oils and their components has long been recognized. Spices, which are added as flavouring agents to foods, are present in insufficient quantities for their antimicrobial properties to be significant. Moreover, spices are often contaminated with bacterial and fungal spores, due to their volatile oil content being enclosed within oil glands with antimicrobial activity (Dorman & Deans, 2000). Therefore, essential oils, which often contain the main aromatic and flavouring components of herbs, when added as flavours to food, would also retard microbial contamination. Carrot fruit essential oil is widely used as a flavour ingredient in most major food categories, and as a fragrance component in perfumes, cosmetics, and soaps (Lawless, 2002). It is the source of sesquiterpenic alcohols, carotol and daucol, and the sesquiterpene β-caryophyllene. The conventional method for carrot essential oil isolation is steam-distillation of dried fruits. Claimed properties of the oil include antibacterial (Giraud-Robert, 2005; Kilibarda, Nanusevic, Dogivic, Ivanic, & Savin, 1996; Staniszewska, Kula, Wieczorkiewicz, & Kusewicz, 2005), fungicidal (Batt, Solberg, & Ceponis, 1983; Dwivedi, Dwivedi, Pandey, & Dubey, 1991; Giraud-Robert, 2005; Staniszewska et al., 2005), hepatocellular regenerator, general tonic and stimulant, lowering of high cholesterol and cicatrisant (Giraud-Robert, 2005). Traditional uses are for hepatic and renal insufficiency and skin disorders, e.g. burns and furuncules (Bergonzelli, Donnicola, Porta, &

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Corthesy-Theulaz, 2003; Giraud-Robert, 2005). Antimicrobial activity of carrot fruit essential oils has recently been investigated by Staniszewska et al. (2005). Essential oils obtained by hydrodistillation of cultivars "Koral" and "Perfekcija", as well as Polish wild grown carrot were tested against two species of Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two species of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two species of fungi (*Candida albicans* and *Peicillium expansum*). The oils obtained from cultivated carrot fruits were the most effective against all the organisms tested. Minimal inhibitory concentrations (MICs) obtained by the agar dilution method were in range 2–8 μl/ml. The lowest values of MICs were obtained in the case of gram-positive bacteria. Chemical composition (Benecke, Reichold, Kessel, & Schmidt, 1987; Gonny, Bradesi, & Casanova, 2004; Jian-Qin, Perineau, Delmas, & Gaset, 1989; Lawrence, 1999; Mazzoni, Tomi, & Casanova, 1999; Perineau, Ganou, & Gaset, 1991; Pinilla, Perez-Alonso, & Valeasco-Negueruela, 1995; Sead, El-Sharkawy, & Halim, 1995) as well as antimicrobial and fungicidal activities (Batt et al., 1983; Dwivedi et al., 1991; Giraud-Robert, 2005; Kilibarda et al., 1996; Staniszewska et al., 2005) of carrot fruit essential oils obtained by hydrodistillation have been the subject of frequent researches. Yet, to the best of our knowledge, there is no information available in the literature on chemical composition or antimicrobial activity of carrot fruit essential oil extracts obtained by supercritical fluid extraction.

Supercritical fluid extraction (SFE) of active compounds from plant material is a promising field for the industrial application of SFE (Reverchon, 1997) since it has certain advantages over steam-distillation and solvent extraction. Steam-distillation can lead to thermal degradation and partial hydrolysis of some essential oil compounds, while SFE can be performed at lower temperatures, thereby preserving the original extract composition and properties. Carbon dioxide is the most used supercritical solvent in extractions of fragrances and other active compounds when they are used in food, pharmaceutical and cosmetic industries. Carbon dioxide is non toxic and allows SFE at temperatures near room temperature and relatively low pressures (8–10 MPa). To suppress co-extraction of higher molecular weight compounds, Reverchon (1997) suggested performing SFE of essential oils at temperatures of 40–50 °C and pressures below 10 MPa.

In plants, essential oils are stored within secretory structures which can be found either on the surface of plants or within the plant tissue. The type of secretory structure is a characteristic of each plant family. Carrot, as a member of the Apiaceae family, is characterized by secretory ducts as sites of essential oil production and accumulation (Gersbach & Reddy, 2002; Sarafis, Rumpel, Pope, & Kuhn, 1990; Svoboda, Svoboda, & Syred, 2000). In Apiaceae fruits, secretory ducts are called vittae. Recently, techniques involving nuclear magnetic resonance and magnetic resonance imaging have been increasingly used to study plant physiology and metabolism, including localization of triglyceride and essential oil accumulation in Apiaceae family fruits (Gersbach & Reddy, 2002; Sarafis et al., 1990). Results of these investigations showed that the fruit was shizocarp with two single-seeded mericarps, which had six oil canals (vittae). Reserve oil (triglycerides) was located in the seeds. Therefore, it was observed that the essential oil and triglycerides were located in separate, well-defined compartments, mericarp channels and seed endosperm. In Fig. 1, a SEM micrograph (scanning electron microscopy) of carrot fruit is presented. The oil channels (vittae) are clearly visible. According to a previous investigation (Zizovic, Stamenić, Orlović, & Skala, 2007), which showed that, in the case of SFE of essential oils from plants with secretory ducts, particle size had no effect on evolution of extraction yield, it can be expected that particle size will have no influence on the carrot fruit essential oil SFE process. The aim of this study was to investigate SFE of carrot fruit essential oil from the pretreatment of herbaceous matrix to the chemical composition of obtained extract and its antimicrobial activity. Antimicrobial activity of carrot fruit SFE extract was investigated using ten species of microorganisms and compared with the antimicrobial activity of essential oil obtained by hydrodistillation. The MICs were determined by the agar dilution method and broth dilution method. Chemical compositions of the SFE extract and essential oil obtained by hydrodistillation were analyzed by GC/FID and GC/MS methods.

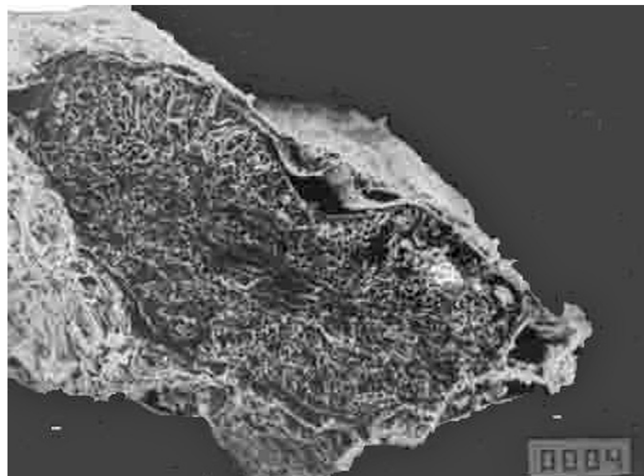

Fig. 1. Cross section of carrot fruit (bar = 700 μm).

2. Materials and methods

2.1. Plant material

Fruits of *Daucus Carota* L. (cultivar "Chanteney"), produced in northern Serbia and examined by the Agricultural Station Novi Sad, Serbia (320-6-00015-89/2002–04), were used in experimental studies. The plant material moisture content was 9.1 wt%.

2.2. Apparatus and methods

Extractions with supercritical carbon dioxide (SC CO_2) were carried out in the Autoclave Engineers Screening System shown in Fig. 2. The supercritical extraction screening system is designed for small batch research runs, using CO_2 as the supercritical medium. Liquid CO_2 is supplied from a CO_2 cylinder by a siphon tube. The CO_2 is pumped into the system by the liquid metering pump until the required pressure is obtained. Back pressure regulators are used to set the system pressure (in extractor and separator). The extractor vessel (150 ml) is filled with the plant material from which a substance is to be extracted. Heaters are supplied on the extractor vessel for temperature elevation. The SC CO_2 flows through the extractor and enters the separator vessel. Samples of the extracted substance can be taken by opening the ball valve located at the bottom of the vessel. A flowmeter is provided to indicate the flow rate of CO_2 being passed through the system and the flow can be adjusted by a micrometering valve. The CO_2 continues to flow out of the separator through the flowmeter/totalizer and out to the atmosphere. Carrot fruit was fine milled and sieved to the particle diameter of 0.75 mm or coarsely ground. Mass of the plant sample was 47 g and the solvent flow rate was 0.3 kg/h in all experiments. Extractions were carried out at temperatures of 40 °C and 50 °C and pressures of 9 and 10 MPa. To investigate the influence of particle size on SFE yield, extractions of fine milled, as well as coarsely ground plant material were carried out at 40 °C and 10 MPa. Commercial carbon dioxide (99% purity, Thenho-gas, Belgrade, Serbia and Montenegro) was used for SFE.

Carrot fruit essential oil was isolated by hydrodistillation in a Clevenger-type apparatus for 4 h, up to the point at which the oil contained in the herbaceous matrix was exhausted. The obtained essential oil was dried over anhydrous sodium sulphate.

2.3. Analytical procedures

Qualitative analyses of the samples were carried out using a Hewlett-Packard GC (FID) analytical system, Model HP-5890 Series II, equipped with a split–splitless injector, HP-5 capillary column (25 m × 0.32 mm, film thickness 0.52 μm) and a flame ionization detector (FID), was employed. Hydrogen was used as carrier gas (1 ml/min, measured at 210 °C). Sample solutions in ethanol (1 μl) were injected in split mode (1:30). The injector was heated at 255 °C, the detector at 300 °C, while the column temperature was linearly programmed from 50–285 °C (4.3 °C/min).

Quantitative analyses were carried out under the same analytical conditions. An HP 5890 Series II gas chromatograph was used with HP G 1800 C GCD Series II (GC-FID) detector, equipped with a split/splitless injector

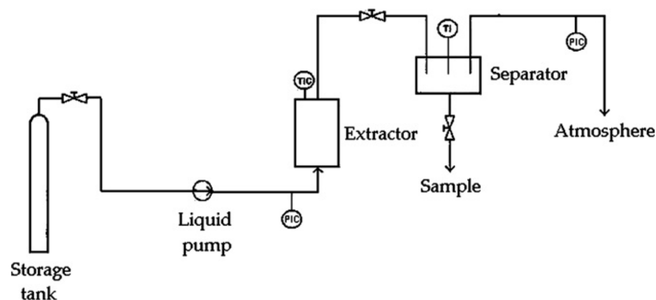

Fig. 2. Schematic presentation of The Autoclave Engineers Screening System.

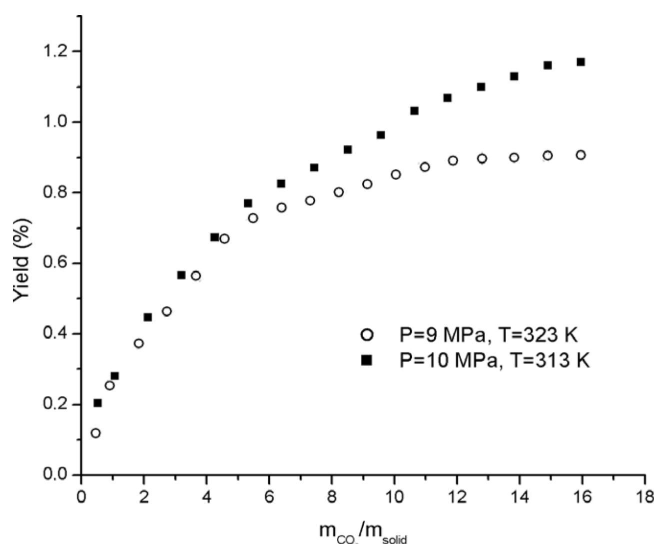

Fig. 3. Yield of total extract as a function of the specific amount of solvent, m_{CO_2}/m_{solid} (kg CO_2/kg herbaceous material) for SFE from carrot fruit.

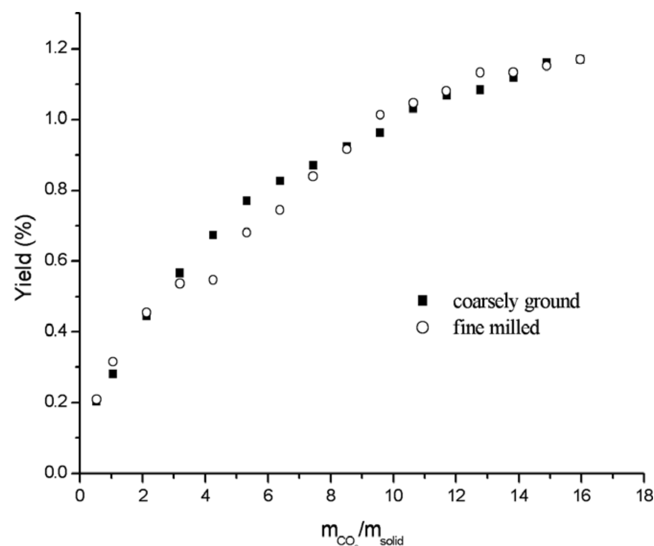

Fig. 4. Yield of total extract as a function of the specific amount of solvent, m_{CO_2}/m_{solid} (kg CO_2/kg herbaceous material) for SFE from fine milled and coarsely ground carrot fruit at 10 MPa and 313 K.

Table 1
Chemical composition of essential oil and supercritical extract of Carrot fruit

Peak no.	Compound	RI	Essential oil (%)	Supercritical extract (%)	SD	Mode of identification
1	α-Thujene	935	0.32	–	0.01	GC, MS
2	α-Pinene	939	7.95	–	0.01	GC, MS
3	Camphene	953	0.71	–	0.03	GC, MS
4	Thuja-2,4(10)diene	957	0.46	–	0.02	GC, MS
5	Sabinene	976	18.7	1.07	0.01	GC, MS
6	β-Pinene	980	*t*	–	0.01	GC, MS
7	β-Myrcene	991	1.35	–	0.03	GC, MS
8	E-3-Caren-2-ol	995	0.46		0.01	GC, MS
9	Car-2-ene	1001	0.55	–	0.02	GC, MS
10	*o*-Cymene	1021	1.38	–	0.21	GC, MS
11	Limonene	1027	1.68	–	0.02	GC, MS
12	γ-Terpinene	1062	0.58	–	0.26	GC, MS
13	Terpinolene	1080	0.37	–	0.04	GC, MS
14	Linalool	1098	0.40	–	0.01	GC, MS
15	3(10)-Caren-2-ol	1111	0.31	0.67	0.01	GC, MS
16	Born-5-en-2-ol	1125	1.14	*t*	0.01	GC, MS
17	Pinocarveol	1139	1.37	*t*	0.02	GC, MS
18	*cis*-Verbenol	1144	2.76	1.13	0.02	GC, MS
19	Sabina ketone	1158	1.30	0.96	0.04	GC, MS
20	Pinocarvone	1162	0.45	–	0.08	GC, MS
21	4-Terpineol	1176	0.47	–	0.05	GC, MS
23	Myrtenol	1193	1.15	0.51	0.05	GC, MS
24	Verbenone	1204	1.08	0.87	0.01	GC, MS
25	*trans*-Carveol	1217	0.33	*t*	0.02	GC, MS
28	Geraniol	1254	0.82	–	0.02	GC, MS
29	Bornyl acetate	1285	0.56	0.65	0.02	GC, MS
30	Cuminalcohol	1287	0.86	*t*	0.01	GC, MS
31	γ-Terpinene-7-ol	1325	0.35	1.27	0.05	GC, MS
32	α-Terpineol acetate	1350	0.36	0.89	0.05	GC, MS
33	Isoledene	1373	1.1	–	0.01	GC, MS
34	Geranyl acetate	1383	4.40	7.22	0.05	GC, MS
35	β-Caryophyllene	1418	5.04	6.47	0.02	GC, MS
36	(Z)-β-Farnesene	1443	0.63	0.96	0.01	GC, MS
37	α-Humulene	1448	0.43	0.54	0.01	GC, MS
38	(E)-β-Farnesene	1458	0.51	0.79	0.06	GC, MS
39	γ-Muurolene	1470	1.29	2.16	0.02	GC, MS
40	Curcumene	1483	0.79	0.78	0.01	GC, MS
41	β-Selinene	1485	4.18	6.66	0.04	GC, MS
42	α-Selinene	1494	1.08	1.93	0.02	GC, MS
43	β-Bisabolene	1508	1.11	2.85	0.01	GC, MS
44	β-Sesquiphellandrene	1524	0.86	0.65	0.07	GC, MS
45	Aromadendrene epoxide	1565	0.38	0.68	0.11	GC, MS
46	Caryophyllene oxide	1584	4.42	7.38	0.31	GC, MS
47	Carotol	1594	20.3	30.3	0.08	GC, MS
48	Humulene epoxide	1606	*t*	0.72	0.03	GC, MS
49	Daucol	1638	0.97	2.46	0.02	GC, MS
50	Longifollenaldehyde	1678	0.70	1.07	0.02	GC, MS
51	Juniper camphor	1691	0.72	–	0.01	GC, MS
52	Aristolone	1756	*t*	1.49	0.01	GC, MS
53	Nonadecene	1900	–	0.46	0.02	GC, MS
54	Methyl palmitate	1927	–	0.83	0.02	GC, MS
55	Palmitic acid	1968	–	2.77	0.03	GC, MS
56	Methyl oleate	2128	–	0.61	0.01	GC, MS
57	Oleic acid	2132	–	3.17	0.03	GC, MS
58	Stearic acid	2137	–	1.60	0.03	GC, MS
59	Tricosane	2300	–	0.49	0.03	GC, MS
60	Methyl arachate	2322	–	0.55	0.02	GC, MS
61	Bis (2-ethylhexyl) phtalate	2540	–	0.66	0.03	GC, MS
62	Heptacosane	2700	–	*t*	0.01	GC, MS
63	Nonacosane	2900	–	0.60	0.01	GC, MS
64	Stigmasterol	3332	–	*t*	0.01	GC, MS
65	Sitosterol	3408	–	*t*	0.01	GC, MS
Total identified		97.13		94.9		

Trace, $t < 0.01$; RI: retention indices (Kovats index) on HP-5 column; GC: identification by comparison of retention indices; MS: identification on the basis of the mass spectra NIST AMDIS; SD: standard deviation.

(250 °C) and a HP-5MS column (30 m × 0.25 mm × 0.25 μm film thickness). Carrier gas (He) flow rate was 1 ml/min while column temperature was linearly programmed in a range of 40–240 °C at a rate of 4 °C/min. The transfer line was heated at 260 °C. Electron impact mass spectra (70 eV) were acquired in the m/z range 45–450. Identifications of the compounds were achieved by comparing their retention indices and mass spectra with those found in the literature (Adams, 2001) and supplemented by the NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software version 2.4., GC–MS Libraries. The relative proportions of the essential oil constituents were expressed as precentages obtained by peak area normalization, all relative response factors being take as one.

2.4. Antimicrobial activity

The investigation of the antimicrobial effects was done on referential strains of *Staphylococcus aureus* ATCC 6538 P, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633 BB, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19115, *Rhodococcus equi* ATCC 6939, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 (Becton Dickinson). Mueller Hinton agar (BioLife), Mueller Hinton broth (Becton Dickinson), Brain Heart Infusion agar (Merck), Sabouraud dextrose agar (BioLife) and Sabouraud dextrose broth (BioLife) were used for the investigation. Active substance of gentamicin sulphate (purity 685 μg/mg) by Sigma was used for comparative investigations of referential strain sensitivity. 2,3,5-Triphenyltetrazoliumchloride (Merck, final concentration at 50 mg/l,) was added to Mueller Hinton broth to obtain bacterial growth visibility. Antimicrobial effects of carrot fruit oils obtained by SFE and hydrodistillation were investigated by the agar dilution method and the broth dilution method. The preparation of the investigated bacterial suspension was performed according to prescribed references by NCCLS for bacterial sensitivity to antibiotics investigation (The National Committee for Clinical Laboratory Standards, 2003). Each assay in this experiment was performed in triplicate.

3. Results and discussion

3.1. Essential oil isolation

The content of essential oil in carrot fruit obtained by hydrodistillation was 0.69%. This value is in accordance with literature data on carrot fruit essential oil yield (Guenther, 1965) which varies from 0.4% to 0.8%. In the case of SFE from fine milled plant material, the highest extraction yield of 1.17% was obtained at 40 °C and 10 MPa, and the extract obtained under these conditions has been chosen for further antimicrobial studies. Experimental data of SFE from carrot fruit in the two outermost cases (the highest yield at 313 K and 10 MPa and the lowest yield at 50 °C and 9 MPa) are presented in Fig. 3. Experimental results of the SFE from fine milled and coarsely ground plant material at 40 °C and 10 MPa are shown in Fig. 4. As expected, no significant change in evolution of the extraction yield can be observed besides the reasonable scatter of experimental data. Therefore, particle size had no influence on the extraction process.

3.2. Chemical composition

Chemical compositions of the carrot fruit essential oil and SFE extract are presented in Table 1. As can be seen, carotol was the main component of the SFE extract (30.3%), as well as the essential oil (20.3%). Besides carotol, geranyl acetate, β-caryophyllene and daucol were present at high concentrations in both samples. SFE extract was characterized by higher contents of carotol and heavier molecular weight compounds than the essential oil. As can be seen from the Table 1, lighter components, e.g. α-thujene, α-pinene, β-myrcene, γ-terpinene, *o*-cymene

Table 2
Values of MICs (μg/ml) for the carrot fruit SFE extract, essential oil and gentamicin (average deviation ± 4 μg/ml for the oil samples and ± 0.02 μg/ml for gentamicin)

Source	Strain	SFE extract MIC	Essential oil MIC	Gentamicin MIC
Gram-positive bacteria				
ATCC 6538 P	*Staphylococcus aureus*	640	640	⩽4
ATCC 6939	*Rhodococcus equi*	160	320	⩽4
ATCC 11778	*Listeria monocytogenes*	640	640	⩽4
ATCC 6633 BB	*Bacillus subtilis*	160	80	⩽4
ATCC 11778	*Bacillus cereus*	80	640	⩽4
ATCC 29212	*Enterococcus faecalis*	>1280	>1280	⩽4
Gram-negative bacteria				
ATCC 25922	*Escherichia coli*	>1280	>1280	⩽4
ATCC 13076	*Salmonella enteritidis*	>1280	>1280	⩽4
ATCC 27853	*Pseudomonas aeruginosa*	>1280	>1280	⩽4
Yeast				
ATCC 10231	*Candida albicans*	640	640	–

and linalool, present in essential oil, were not detected in the SFE extract.

3.3. Antimicrobial activity

Results of the antimicrobial activity obtained by the agar dilution method and broth dilution method were identical and they are presented in Table 2. The SFE extract and essential oil obtained by hydrodistillation were the most effective against Gram-positive bacteria. The SFE extract was much more effective against *Bacillus cereus* and slightly more effective against *Rhodococcus equi* than was the essential oil. Against *Bacillus subtilis*, the essential oil showed slightly stronger antimicrobial activity. Against all the other examined strains, both oil samples showed similar antimicrobial activities. The investigated essential oil and SFE extract, at the applied concentrations, failed to show antimicrobial effects on Gram-negative bacterial strains included in this investigation, or on *Enterococcus faecalis* with MIC values less than 1280 μg/ml.

4. Conclusions

In the SFE process of carrot fruit essential oil, particle size had no influence on the extraction rate in the two outermost cases: fine milled material and coarsely ground plant material, which is important on the industrial scale. The highest extraction yield in the SFE process (1.17%) was obtained at 40 °C and 10 MPa. This value was higher than the yield obtained by hydrodistillation, as analytical analyses showed, due to co-extraction of heavier weight compounds during the SFE process.

The SFE extract, as well as the essential oil obtained by hydrodistillation, were the most effective against Gram-positive bacteria, which was in accordance with results of (Staniszewska et al., 2005) who showed that carrot fruit essential oil was more effective against Gram-positive than against Gram-negative bacteria and fungi investigated. Any microbiological activity depends on the chemical composition of oil and the investigated strain sensitivity. Therefore, it can be concluded that the similar antimicrobial activities of essential oil and SFE extract are probably due to carotol, which is the main component of both samples. Carotol itself was previously proved to have fungicidal activity against phytotoxic fungi *Alternaria alternata* isolated from the surface of carrot seed cultivar "Perfekcija" (Jasica-Misiak et al., 2004). Stronger antimicrobial activity of the essential oil could be due to the investigated strain sensitivity to α-pinene, sabinene and the other lighter components present in essential oil only. In the case of *Bacillus subtilis*, essential oil had a stronger antimicrobial effect. This is a reasonable result because the content of sabinene in eseential oil was much higher than in SFE extract, and sabinene was previously proved to inhibit the growth of *Bacillus subtilis* (Dorman & Deans, 2000). Stronger antimicrobial effects of SFE extracts might be due to greater sensitivity of strains to carotol (which has a higher content in SFE extract) or to the synergistic activity of carotol and other heavier molecular weight compounds present in the SFE extract.

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