Research Paper

Effect of *Citrus* essential oil addition upon growth and cellular lipids of *Yarrowia lipolytica* yeast

Seraphim Papanikolaou[1], Olga Gortzi[2], Eleni Margeli[1], Ioanna Chinou[3], Maria Galiotou-Panayotou[1] and Stavros Lalas[2]

[1] Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece
[2] Department of Food Technology, Technological Educational Institute of Larissa (Karditsa Branch), Karditsa, Greece
[3] Department of Pharmacy, Division of Pharmacognosy, Chemistry of Natural Products, University of Athens, Athens, Greece

The chemical composition of the essential oil from fruits of the Greek citrus hybrid *Citrus sinensis* cv *New Hall* – *Citrus aurantium* was investigated by GC and GC-MS. Forty-four compounds were identified, representing 92.83% of the total oil. Limonene, myrcene, β-pinene, α-pinene and α-terpineol constituted the major components of the oil. The effect of the addition of this oil upon the behavior of *Yarrowia lipolytica* yeast was also investigated. The microorganism was aerobically grown on glucose and oil was added to the culture medium in various initial quantities (from 0.0 to 1.5 mL/L). The strain underwent significant inhibition exerted by the added essential oil; a decrease of the highest achieved biomass concentration, even if the oil was added to the culture medium at small concentrations (*e.g.* 0.3 mL/L), was observed. The more oil was added, the more the lag phase of the growth increased, while the biomass yield on glucose consumed decreased with the addition of the oil. When the essential oil was added to the medium, even at small concentrations, a significant increase of cellular medium-chain saturated fatty acids (12:0 and 14:0) resulting in an increase of saturated fatty acid content in the cellular lipids, was observed.

Keywords: Cellular lipids / *Citrus sinensis* / Essential oil / Microbial growth / *Yarrowia lipolytica*

Received: April 1, 2008; accepted: May 26, 2008

DOI 10.1002/ejlt.200800085

1 Introduction

Aromatherapy is the therapeutic use of volatile, aromatic essential oils extracted from plants. Aromatic forms of medicine have been used throughout history for spiritual, medicinal, social and beauty purposes. Aromatic substances were used in the ancient Egypt in the preservation of the body afterlife, in the medicine and the spiritual rituals. In more recent history, herbal and aromatic substances have been used in the Middle Ages against airborne infections, such as the plague [1]. The antiviral and antimicrobial activities of several essential oils have been described in the literature [2, 3]. Besides their antimicrobial activity, several essential oils more recently have been qualified as natural antioxidants and proposed as potential substitutes in specific sectors of food preservation where their use is not in contrast with their aroma. Natural antioxidants are extensively studied also for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer. Essential oils are known to possess potential as natural agents for food preservation; in fact, their effectiveness against a wide range of bacteria including antibiotic-resistant species and fungal species has been repeatedly demonstrated [2, 3].

In recent investigations, the potential of strains belonging to the species *Yarrowia lipolytica* for the production of various microbial metabolites of high added value (*e.g.* microbial lipid, aromatic compounds, microbial biomass, lipases, organic acids, *etc.*) has been extensively studied, with investigations being focused on the levels of biochemistry, fermentation technology, process modeling and genetic engineering [4–17]. Specifically, the strain *Yarrowia lipolytica* ACA-DC 50109 has

Correspondence: Seraphim Papanikolaou, Department of Food Science and Technology, Agricultural University of Athens, 75 Iera Odos., 11855 Athens, Greece.
E-mail: spapanik@aua.gr
Fax: +30-210-5294700

been efficiently cultivated on various agro-industrial residues and produced significant quantities of high-added-value metabolites (microbial lipids presenting composition similarities with cocoa butter, citric acid, lipases, microbial biomass) [18–22].

Essential oils of plants and spices in general are considered as agents that display significant antimicrobial, antiparasitical and antioxidant activities, thus presenting important and useful properties for food technology and cosmetology. The antimicrobial effect of such compounds has been studied by various authors [23–25]. However, so far a scarce number of reports have appeared in the literature that correlate the addition of various natural compounds (*e.g.* aqueous extracts, essential oils, *etc.*) with the fatty acid composition of lipid reserves of various microorganisms [22, 26–30]. The present investigation aims at studying the chemical composition of the essential oil deriving from the *Citrus sinensis* cv *New Hall – Citrus aurantium* plant. Furthermore, the effect of the addition of this oil, at various concentrations, on the biochemical behavior (biomass production, substrate uptake, potential metabolites production, inhibition exerted by the essential oil, composition in fatty acids of cellular lipids) of the strain *Yarrowia lipolytica* ACA-DC 50109 in submerged cultures was investigated. Biochemical interpretations concerning the yeast behavior were considered.

2 Materials and methods

2.1 Plant material

About 370 g of fresh plant material (pericarps) was collected during morning hours from citrus trees of the *Citrus sinensis* cv *New Hall – Citrus aurantium* hybrid (grown in the Poros Horticulture Institute, Poros Island, Greece) in October 2003. The identification of species was carried out according to the protocol of the Institute.

2.2 Extraction of essential oil

The plant material was air-dried in the shade prior to distillation of essential oils. The pericarps were cut into small pieces and subjected to hydrodistillation for 3 h in 1 L water, using a Clevenger-type apparatus, with a water-cooled oil receiver to reduce formation of artifacts due to overheating during hydrodistillation. The essential oils were collected in high-purity *n*-penten and dried with anhydrous sodium sulfate (Panreac Quimica SA, Barcelona, Spain) and stored at 4–6 °C until use.

2.3 GC and GC-MS component analysis of the essential oil

GC analysis was carried out on a Perkin-Elmer Clarus 500 gas chromatograph, with a RTX-5 (30 m × 0.25 mm × 0.25 μm) fused capillary column and equipped with a flame ionization detector. The GC-MS analysis of the essential oil and the isolated fractions was carried out using a Hewlett-Packard 5973-6890 GC-MS operating in EI mode (equipped with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column). Helium (2 mL/min) was used as carrier gas. The injection temperature was set at 290 °C and the detector temperature at 300 °C. Temperature program for GC and GC-MS: the initial temperature of the column was 60 °C (for 5 min), then raised to 280 °C at 3 °C/min, and held there for 30 min. The components of the oils were identified by comparison of their mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley mass spectral database. They have also been confirmed by comparison of their retention indices (RI) [31] and retention times (RT), either with those of authentic compounds or with published data [32].

2.4 Microorganism and culture conditions

The *Yarrowia lipolytica* strain ACA-DC 50109 [first registration as LGAM S(7)1] was used. Maintenance of the strain has been presented previously [20, 21]. The composition of salts in the growth medium was (in g/L): KH_2PO_4 7.0, Na_2HPO_4 2.5, $MgSO_4 \times 7H_2O$ 1.5, $CaCl_2 \times 6H_2O$ 0.15, $FeCl_3 \times 6H_2O$ 0.15, $ZnSO_4 \times 7H_2O$ 0.02, $MnSO_4 \times H_2O$ 0.06; $(NH_4)_2SO_4$ 5.0; yeast extract 1.0. The initial pH after autoclaving in all trials was 6.0 ± 0.1. The carbon source used was commercial-industrial glucose (provided by the "Hellenic Industry of Sugar"), a low-added-value material utilized in various confectionary industries [purity 95%, impurities composed of maltose (2 wt-%), maltodextrines (0.5 wt-%), water (1.5 wt-%) and salts (0.5 wt-%)]. The initial concentration of glucose (Glc_0) used was 25.0 g/L and all cultures were carried out in carbon-limited conditions (initial molar C/N ratio around 10). Trials, carried out in batch mode, were performed in 250-mL conical flasks containing 50 ± 1 mL growth medium. All components (including glucose and yeast extract) were added to the flasks, and thermal sterilization (115 °C/20 min) was performed. No glucose concentration decrease was observed due to the thermal treatment. The pH of the medium after sterilization was 6.0 ± 0.2 in all trials. Flasks were then inoculated with 1 mL of a 24-h exponential pre-culture (around 1×10^8–3×10^8 cells, initial cell concentration 0.12 ± 0.02 g/L). The pre-culture was carried out in the above-mentioned synthetic medium, the same as for the principal culture. All experiments were performed in an orbital incubator (Gallenkamp, Leicestershire, UK) at temperature T = 28 °C and agitation rate 185 rpm. The pH in the culture medium throughout culture was maintained within the range of 5.0–6.0 by adding (periodically and aseptically) small quantities (*e.g.* 500–600 μL) of 5 M KOH to the flasks (for more details see Papanikolaou et al. [22]).

2.5 Determinations and analyses

Cells were harvested by centrifugation (Heraeus Sepatech Suprafuge-22 apparatus) at 7000 × g/20 min, washed once with distilled water and re-centrifuged under the same conditions. Cell concentration (X, g/L) was determined from dry weight (90 ± 5 °C/24 h). The dissolved oxygen (DO) concentration in the growth medium was determined with the aid of a selective electrode (oxi200 Sensodirect, Lovibond). Before harvesting, the shaker was stopped and the probe was placed in the flask. Then, the shaker was switched on and the measurement was taken after DO equilibration (usually within 10 min). All trials were conducted under full aerobic conditions [DO >40% (vol/vol)]. pH measurements were conducted in a Jenway 3020 pH-meter. Furthermore, besides dry weight, cell viability assays of the cultures were performed at the end of the fermentations carried out. This process was performed with the aid of total plate counting in PDA-based petri plates. This viability was expressed as CFU per mL of culture medium. Determination of the glucose concentration (Glc) in the medium was conducted according to the DNS method [33]. The concentration of organic acids in the culture medium was determined with the aid of HPLC as previously indicated [22]. Total microbial oil was extracted from the dry biomass with a mixture of chloroform/methanol 2 : 1 (vol/vol) and was determined gravimetrically. Lipids were converted to methyl esters in a two-step reaction with methanolic sodium and hydrochloric methanol and were analyzed by GLC as previously described [20–22].

In order to ascertain the repeatability of the experimental results related with yeast growth, all of the fermentations were carried out in (at least) triplicate experiments, in which different inocula were employed. Moreover, each experimental point of the kinetics presented in the tables and figures related with the cultures of *Y. lipolytica* is the mean value of three determinations. In all cases, standard error was calculated and it was found to be less than 15%.

3 Results

3.1 Chemical composition of essential oil

The hydrodistillation of the pericarps of *Citrus sinensis* cv *New Hall – Citrus aurantium* gave a white-yellow oil in good yield (0.54%, vol/wt), with distinct citrus odor. Forty-eight different compounds were identified in the essential oil. The composition, as obtained by GC-MS analysis, is illustrated in Table 1. Limonene proved to be the major component (67.96%) followed by myrcene, β-pinene, α-pinene, and α-terpineol, sabinene, valencene, linalool, terpinen-4-ol, and decanal were also present in high amounts (4.79, 4.01, 2.88, 2.13, 1.19, 0.91, 0.90, 0.79, and 0.60%, respectively). The essential oil did not contain any small-, medium- or long-chain fatty acids.

Table 1. GC-MS analysis of essential oil from *Citrus sinensis* cv *New Hall – Citrus aurantium*.

No.	Compound	[%]
1	α-Pinene	2.88
2	Sabinene	1.19
3	Myrcene	4.79
4	β-Pinene	4.01
5	Limonene	67.96
6	γ-Terpinene	0.35
7	Octanol (*n*-)	0.47
8	Terpinolene	0.50
9	Linalool	0.90
11	Menthadien-1-ol (2,8-*trans*-para-)	0.34
12	Limonene oxide (*cis*-)	0.13
13	Limonene oxide (*trans*-)	0.16
14	Citronellal	0.10
15	Terpinen-4-ol	0.79
16	α-Terpineol	2.13
17	Decanal	0.60
18	Carveol (*trans*-)	0.41
19	Citronellol	0.54
20	Neral	0.21
21	Carvone	0.19
22	Geraniol	0.26
23	Geranial	0.19
24	Perillaldehyde	0.26
25	Limonen-10-ol	0.15
29	Neryl acetate	0.12
30	α-Copaene	0.29
31	Geranyl acetate	0.10
32	β-Cubebene	0.28
33	Elemene (beta-)	0.15
34	Caryophyllene (*trans*-beta-)	0.26
35	β-Copaene	0.19
36	α-Humulene	0.11
37	Germacrene D	0.28
38	Valencene	0.91
39	α-Farnesene	0.10
41	δ-Cadinene	0.43
44	Caryophyllene oxide	0.10
	Total	92.83

3.2 Effect of essential oil addition on growth of *Yarrowia lipolytica* yeast

Y. lipolytica was cultivated in carbon-limited medium with industrial glucose used as the sole substrate at an initial concentration (Glc_0) of 25 g/L and three initial concentrations of essential oil (namely 0.3, 0.5 and 1.5 mL/L) added to the culture medium, in order to investigate the effect of the addition of the essential oil on the physiological behavior of the strain. The culture without essential oil addition was used as the control experiment. All trials were carried out under full aerobic conditions (DO >40%, vol/vol, for all culture steps).

In the kinetics carried out, the point at which maximum concentration of biomass (X_{max}, g/L) and maximum yield of biomass produced per glucose consumed ($Y_{X/Glc}$, g/g) was achieved is illustrated in Table 2. It can be understood that, given that kinetic studies were done, the X_{max} value for the four cultures carried out was not achieved at the same fermentation time (see Table 2). Additionally, the kinetics of biomass (X, g/L) production and substrate (glucose – Glc) consumption in all trials is shown in Fig. 1. From Table 2 and Fig. 1, it can be deduced that, although biomass production and sugar consumption were observed in all cultures carried out, the addition of the essential oil, even at small concentrations, negatively affected the growth of the employed strain; the maximum biomass concentration achieved (X_{max}) was significantly reduced. It is surprising that, although the addition of the essential oil even at small concentrations drastically reduced the X_{max} value, the carbon substrate (glucose) was completely consumed by the microorganism. Therefore, besides the X_{max} value, the addition of essential oil to the culture medium substantially affected (reduced) the biomass yield on glucose consumed ($Y_{X/Glc}$) (see Table 2).

Addition of the oil even in minimal quantities (*e.g.* 0.3 mL/L) resulted in a considerable decrease (of around 35–40%) of both X_{max} and $Y_{X/Glc}$ values compared with the trial in which no essential oil was added to the medium (see Table 2). Furthermore, the addition of the oil in higher quantities (*e.g.* 0.5 and 1.5 mL/L) induced a slight further decrease of the X_{max} and $Y_{X/Glc}$ values, compared with the trial in which oil was added in the quantity of 0.3 mL/L. Likewise, the addition of the highest quantity of essential oil tested (1.5 mL/L) resulted in a prolonged lag phase (of around 20 h) compared with all other trials (see Fig. 1a). Despite the fact that the addition of the essential oil in various quantities decreased the X_{max} con-

Table 2. Quantitative data of *Yarrowia lipolytica* originating from kinetics in glucose-based carbon-limited media with various initial *Citrus sinensis* cv *New Hall – Citrus aurantium* essential oil additions to the medium. Representation of maximum biomass concentration (X_{max}, g/L), remaining glucose quantity (Glc_r, g/L), and maximum conversion yield of biomass produced per glucose consumed ($Y_{X/Glc}$, g/g). The fermentation time is different between the cultures, since maxiumum biomass concentration (X_{max}) was not achieved simultaneously for the trials carried out. Glc_r values and fermentation time are presented when the maximum concentration of biomass were achieved. Culture conditions: growth in flasks at 185 rpm, initial pH = 6.0 ± 0.1, pH ranging between 5.0 and 6.0, DO >40% (vol/vol), incubation temperature T = 28 °C. Three (at least) lots of independent cultures were conducted by using different inocula. In all of the determinations, the calculated standard error was less that 15%.

Essential oil [mL/L]	Fermentation time [h]	Glc_r [g/L]	X_{max} [g/L]	$Y_{X/Glc}$ [g/g]
0.0[§]	72	0.0	14.5	0.58
0.3	120	0.0	7.3	0.29
0.5	65	0.1	6.2	0.25
1.5	114	0.0	6.0	0.24

[§] Control experiment, no addition of essential oil.

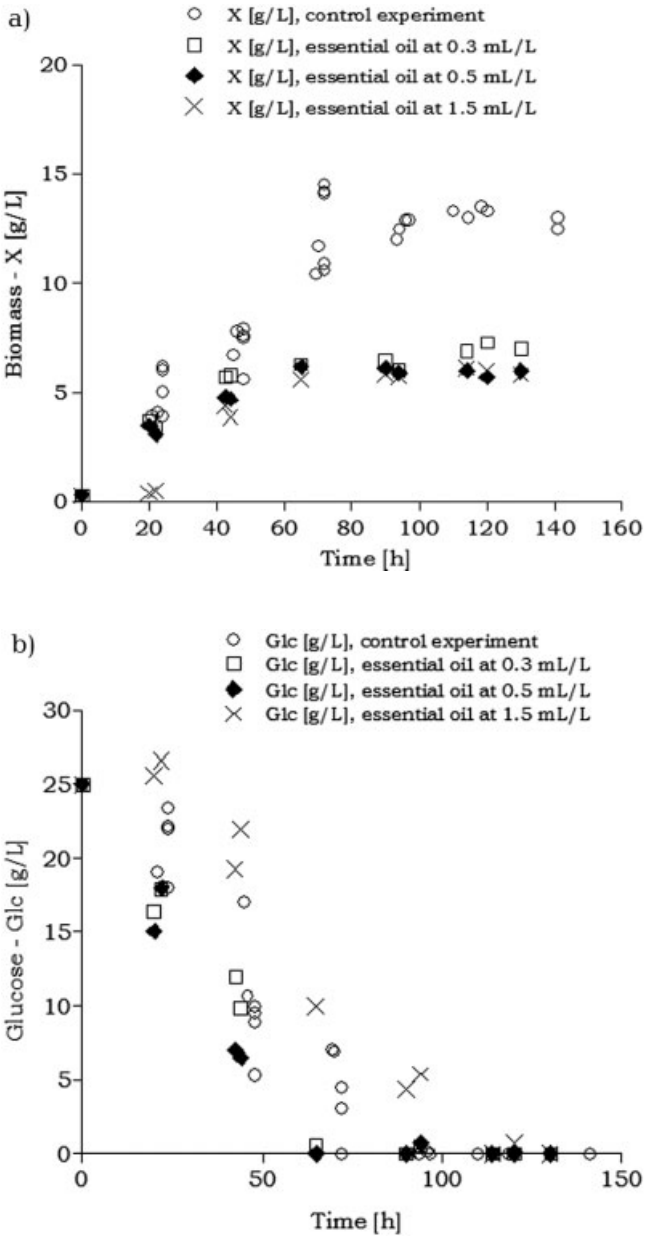

Figure 1. Changes of biomass (X, g/L) (a) and glucose (Glc, g/L) (b) during growth of *Yarrowia lipolytica* in carbon-limited flask experiments, with glucose used as the sole substrate and essential oil of the *Citrus sinensis* cv *New Hall – Citrus aurantium* plant added at various concentrations. Culture conditions: Glc_0 = 25 g/L; pH ranging from 5.0 to 6.0; incubation temperature T = 28 °C; DO >40% (vol/vol) for all growth phases. Three lots (at least) of independent cultures were conducted by using different inocula. In all of the determinations, the calculated standard error was less than 15%.

centration, the maximum specific growth rate (μ_{max}), estimated in the early exponential growth phase, was almost unaffected by the presence of the oil in the culture medium (see slopes of biomass curves in Fig. 1a; $\mu_{max} = 0.22 \pm 0.02\ h^{-1}$ for all trials). It is also noted that biomass production reached its plateau values at the same time, regardless of the addition of essential oil to the medium (around 70 ± 10 h after inoculation). Furthermore, the addition of essential oil influenced the viability of the cells. Cell viability was measured in the late stationary phase (114 h after inoculation) for all trials; in the control experiment, around 7.9×10^8 CFU/mL of culture medium were observed. Addition of essential oil at 0.3 mL/L resulted in a drastic decrease of the above value (around 4.1×10^8 CFU). When essential oil was added at 0.5 mL/L, around 3.8×10^8 CFU/mL of culture medium was measured; almost equal cell concentration (3.7×10^8 CFU/mL) was observed when 1.5 mL/L of essential oil was added.

As previously stated, in all fermentations, sugar was completely exhausted from the fermentation medium at the end of fermentation (see Fig. 1b), while surprisingly the substrate consumption rate r_{Glc}, expressed as $r_{Glc} = -\Delta Glc/\Delta t$ and estimated during the exponential growth phase, was somehow lower in the control fermentation compared with the cultures in which essential oil was added at 0.3 and 0.5 mL/L (around 0.35 g/L.h in the trials with the oil added against 0.30 g/L.h in the control experiment). It is noted that in the trial in which essential oil was added at the highest concentration (1.5 mL/L), r_{Glc} presented a value almost equivalent to the one obtained in the control experiment (0.29 against 0.30 g/L.h). Therefore, it can be assumed that the addition of the essential oil did not negatively affect the sugar uptake rate by the microorganism tested. Finally, in all experiments and regardless of the quantity of the essential oil added to the medium, the pH presented a drop, specifically in the early and late-exponential growth phase (up to 70 ± 10 h after inoculation). Acetic acid was the principal organic acid produced in all trials (in concentrations of 3.0 ± 0.5 g/L). No quantities at all of citric acids (citric + iso-citric acid) were detected. In the stationary growth phase, the acetic acid concentration slightly declined. The fact that addition of the essential oil (even in small quantities) significantly reduced the X_{max} value while, in contrast, glucose consumption and metabolite accumulation in the medium were not influenced, as compared with the control experiment, indicates that carbon recovery in the cultures with added essential oil was drastically decreased in comparison with the culture with no oil added. This fact suggests that addition of oil induced increased energy of maintenance requirements in comparison with the control culture.

3.3 Cellular lipid analysis

The total cellular lipids of *Y. lipolytica* were extracted (in a mixture of chloroform/methanol 2 : 1, vol/vol), converted to their methyl esters and analyzed by GLC, in order to investigate whether the addition of the essential oil influenced both the quantity and the composition of the cellular lipids of the strain. As it was expected, the culture conditions (low initial C/N ratio) did not favor accumulation of significant quantities of lipid inside the cell structures [28], while, as it has been previously reported, the current microbial strain even under conditions favoring the accumulation of storage lipid from sugar-based substrates (flask experiments in high initial C/N ratio media) does not accumulate remarkable fat quantities [20, 22]. In the present investigation, the total microbial lipid produced corresponded to a quantity of 3.8–7.9 wt-% in dry cell mass for all culture phases and all trials carried out. Addition of the essential oil induced significant differences concerning the composition of total cellular lipid (Table 3). The obtained results concerning the fatty acid composition of *Y. lipolytica* lipid showed that in the control experiment small differences in the fatty acid profile were observed throughout culture; the principal cellular fatty acids observed were mainly oleic (Δ9–18:1), linoleic (Δ9,12–18:2) and palmitic acid (16:0). Stearic acid (18:0) and medium-chain fatty acids (*e.g.* laurate 12:0 and myristate 14:0) were observed in minimal quantities. In the control experiment, a slight decrease of Δ9–18:1, Δ9,12–18:2 and 18:0 and a small increase of 12:0 and 14:0 concentrations as function of the fermentation time were observed. Regardless of the concentration of the essential oil added to the culture medium, the principal cellular fatty acids, as in the case of the control experiment, remained the Δ9–18:1, Δ9,12–18:2 and 16:0 fatty acids. However, compared with the control experiment at the early growth step (exponential phase), the concentration of 18:0 was somehow higher, but it considerably decreased afterwards. Additionally, relatively high concentrations of medium-chain fatty acids (12:0 and 14:0) were detected in the cellular lipids of the strain that was cultivated in the presence of the essential oil, while the more the concentration of the added essential oil increased, the more the concentration (wt-%) of cellular 12:0 and 14:0 fatty acids was elevated (see Table 3). Likewise, the concentration of the cellular Δ9–18:1 fatty acid was inversely correlated with the addition of essential oil to the culture medium, while a noteworthy result of the trials carried out was that the content of saturated cellular fatty acids presented the tendency to increase with the addition of essential oil to the growth medium. This observation was obvious principally for the trial with added essential oil at 1.5 mL/L; in the latter case the concentration of cellular saturated fatty acids was 41–43 wt-% of total microbial lipid, while in the control experiment the respective value was 24–25 wt-% (see Table 3).

4 Discussion

Essential oils are vegetable products that are constituted basically by complex mixtures of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, alcohols and esters [34]. Citrus oil generally contains over 90% of monoterpenes, about 5% of oxygenated compounds and less than 1% of non-

Table 3. Fatty acid composition (wt-%) of cellular lipids produced by *Yarrowia lipolytica* when this microorganism was grown on glucose-based media with essential oil of the *Citrus sinensis* cv *New Hall – Citrus aurantium* plant, added at various concentrations. Culture conditions: Glc_0 = 25 g/L; pH ranging from 5.0 to 6.0; incubation temperature T = 28 °C; DO >40% (vol/vol) for all growth phases. Three (at least) lots of independent cultures were conducted by using different inocula. In all of the determinations, the calculated standard error was less that 15%.

	Lipid [wt-%]	12:0	14:0	16:0	18:0	Δ9–18:1	Δ9,12–18:2	SFA[§]
Control experiment (no essential oil added)								
Exponential phase (20–30 h)	7.9	0.3	0.2	19.0	4.2	51.5	19.9	23.7
Mid stationary phase (60–70 h)	5.6	0.5	1.5	18.0	3.2	49.7	19.5	23.2
Late stationary phase (100–120 h)	4.9	1.5	2.0	17.8	3.5	47.0	19.2	24.8
Essential oil added at 0.3 mL/L								
Exponential phase (20–30 h)	5.6	1.5	2.7	15.8	10.5	49.2	15.5	30.5
Mid stationary phase (60–70 h)	4.5	7.0	7.3	16.8	4.0	41.0	14.9	35.1
Late stationary phase (100–120 h)	3.2	6.0	7.5	17.3	3.2	42.1	14.5	34.0
Essential oil added at 0.5 mL/L								
Exponential phase (20–30 h)	4.5	4.3	2.5	12.0	8.3	47.0	21.5	27.1
Mid stationary phase (60–70 h)	4.6	4.0	4.0	19.5	7.0	45.1	18.0	34.5
Late stationary phase (100–120 h)	4.0	5.7	7.5	16.2	2.9	45.2	16.8	32.3
Essential oil added at 1.5 mL/L								
Exponential phase (20–30 h)	4.6	12.5	2.9	18.9	7.2	39.9	14.9	41.5
Mid stationary phase (60–70 h)	4.2	9.0	10.3	20.1	2.0	39.4	16.0	41.4
Late stationary phase (100–120 h)	3.8	12.2	9.9	19.0	2.0	36.1	15.0	43.1

[§] SFA, Saturated fatty acids.

volatiles such as waxes and pigments. Limonene is a principal compound (about 70%) of monoterpenes. This compound does not contribute much to the flavor or fragrance of the oil but acts as a good solvent for polystyrol in the chemical recycling process [35, 36]. Limonene can also be used in therapeutic trans-dermal delivery systems as a penetration enhancer and is found in chemicals used for histologic and cytologic specimen preparation [2, 3, 34].

The characteristic flavor of citrus oil is provided by the oxygenated terpenes that mainly consist of alcohols, aldehydes and esters such as linalool, citral (neral and geranial) and linalyl acetate. The results are difficult to be compared with those of other researchers because, as indicated by Ojeda de Rodriguez *et al.* [34], the differences in composition of the diverse citrus essential oils are rather of quantitative than qualitative order. However, some of them have certain distinctive compounds. In fact, the multiple components of the oils from each species depend on its own genetic program, though the composition can be influenced by several environmental and physiological factors (maturity, climatic season, soil type, storage conditions and extraction method) [34].

Y. lipolytica presented efficient cell growth when cultures were carried out in carbon-limited glucose-based medium (X_{max} = 14.5 g/L, μ_{max} = 0.22 h^{-1}, biomass yield $Y_{X/Glc}$ = 0.58 g/g). The relatively rapid uptake of the sugar substrate and the increased conversion yield of biomass produced per sugar consumed ($Y_{X/Glc}$ = 0.58 g/g) suggest the potential of utilization of the present *Y. lipolytica* strain for single-cell protein production during growth in various glucose-based residues. Furthermore, in all trials, in spite of the fact that cultures were carried out in carbon-limited conditions (low initial C/N ratio), a drop in the medium pH value was observed, indicating accumulation of some quantities of organic acids in the medium The principal organic acid produced was acetate (in quantities of 3.0 ± 0.5 g/L), while surprisingly citric acid was not at all detected in the culture medium. Acetic acid in small concentrations was produced by *Y. lipolytica* strains when cultures were carried out in ethanol utilized as substrate [10], while in general *Y. lipolytica* strains are considered as promising producers of citric acid [4, 5, 10–12, 15–17] and to a lesser extent pyruvic acid [13] and iso-citric acid [12], when growth is effectuated in nitrogen-limited conditions and various fermentation configurations. The microorganism used in the current investigation (strain ACA-DC 50109) has been extensively and successfully used in previous investigations for the valorization of various hydrophobic or hydrophilic residues used as substrates in submerged fermentations (*e.g.* tallow derivatives, raw glycerol, olive mill wastewaters, *etc.*) and various metabolites of industrial significance have been produced in noticeable quantities (*e.g.* microbial lipid resembling cocoa butter, lipases, citric acid, *etc.*) [18–22].

Addition of the essential oil deriving from *Citrus sinensis* cv *New Hall – Citrus aurantium* fruits to the flask cultures of *Y. lipolytica* yeast induced a non-negligible decrease of the X_{max} and $Y_{X/Glc}$ values (reduction of around 45–50% compared with the medium in which no addition of essential oil

was carried out), even if the quantity of oil added to the medium was small (*e.g.* 0.3 mL/L). When the concentration of essential oil added increased, a slight further decrease of the X_{max} and $Y_{X/Glc}$ values was observed. Surprisingly, the μ_{max} and r_{Glc} values were not affected by the addition of the oil. It may hence be assumed that the essential oil added presented a non-negligible inhibitory effect upon the production of biomass by the present *Y. lipolytica* strain. Given that the addition of the essential oil did not affect the uptake rate of the carbon substrate, the concentration of which at the end of fermentation presented negligible values, it may be suggested that this addition shifted the microbial metabolism, since significant carbon quantities were presumably absorbed for energy of maintenance requirements rather than anabolic activities (*e.g.* production of biomass). The reduced values of X and $Y_{X/Glc}$ in the cultures with the added essential oil provide strong evidence for the above assumption.

The antimicrobial effect of essential oils, spices and similar types of compounds has been studied by several authors in the previous years [23–25]. Specifically, this effect upon growth of (principally) food spoilage yeasts has been quantified in solid or liquid media or even in various foodstuffs [37–41]. For instance, essential oils of allspice, cinnamon, clove, garlic, onion, oregano, savory and thyme were revealed to be extremely inhibitory against yeasts of the genera *Candida*, *Hansenula* and *Lodderomyces* [37]. Specifically, garlic oil was a potent inhibitor of yeast growth at concentrations found in the magnitude of 25 ppm (concentrations significantly higher than the ones of the present investigation). Furthermore, addition of oils of onion, oregano and thyme at the above levels (around 25 ppm) were equally strongly inhibitory [37]. Essential oil addition also induced morphological changes in the tested yeasts, since their addition delayed pseudomycelium formation by *Hansenula anomala*, whereas some of the oils tested stimulated pseudomycelium production by *Lodderomyces elongisporus.* Cinnamon and clove oils were clearly stimulatory for pseudomycelium production by *Saccharomyces cerevisiae.* In contrast, the essential oils had no effect on pseudomycelium production by *Candida* (*Yarrowia*) *lipolytica* [37]. Likewise, addition of the essential oil of *Origanum vulgare* L. at concentrations of 5–20 mL/L (concentrations somewhat higher than in the current investigation) inhibited growth of the yeasts *Candida krusei*, *Candida tropicalis*, *Candida albicans*, *Rhodotorula rubra* and *Saccharomyces cerevisiae*, with growth inhibition critically influenced by the amount of the essential oil added [40]. Moreover, the combined effects of a mild heat treatment and the presence of aroma compounds [*e.g.* citron essential oil, citral, and (E)-2-hexenal] on the spoilage of non-carbonated beverages inoculated with different amounts of a *Saccharomyces cerevisiae* strain was evaluated [41]. It was found that essential oil concentrations within 100–500 ppm of the above-mentioned compounds accompanied by a small thermal treatment were revealed capable of preventing yeast growth, even if the bottles were inoculated with high yeast populations (around 10^5 CFU per bottle) [41]. Additionally, the combined preservative effect of oregano essential oil and modified atmospheres was studied in minced meat, and when essential oil was added in somewhat higher quantities compared to the present investigation (*e.g.* from 0.05 to 1% vol/wt), the microbial population (including yeasts) was drastically decreased [39]. From all the above stated, it can be easily assumed that there is a remarkable inhibitory effect of *Citrus sinensis* cv *New Hall – Citrus aurantium* essential oil against *Y. lipolytica* yeast (a species that is frequently met in various foodstuffs), even if this oil is added in small amounts to the growth medium. Therefore, this essential oil can be considered as a promising agent for the preservation of various edible products, and its addition in minimal quantities can potentially satisfactorily prevent microbial alteration.

The effect of the addition of different types of plant products (*e.g.* aqueous extracts, essential oils, *etc.*) on growth of various microorganisms of biotechnological significance has attracted and attracts much interest, since addition of these types of products can enhance, alter or modify the production of various metabolites [28–30, 42]. For instance, addition to the growth medium of aqueous extracts of plants belonging to the Lamiaceae family (*Sideritis montana*, *Origanum dictamus*, *Mentha piperita*, *Rosmarinus officinalis* and *Origanum marjorana*) considerably increased the lag time of the strain used in the present investigation (ACA-DC 50109) in flask glucose-based media [30]. Addition of all aqueous extracts significantly increased the yield $Y_{X/Glc}$ (from 25 to 75%) when growth was carried out in nitrogen-limited conditions, while a slight decrease (from 10 to 19%) was observed in carbon-limited media [30]. Furthermore, it was found that addition of an aqueous extract of the *Teucrium polium* L. plant slightly inhibited microbial growth of *Y. lipolytica* and *Saccharomyces cerevisiae* strains cultivated in flask glucose-based carbon-limited experiments, while a Monod-type model quantitatively describing the inhibitory effect of the aqueous extract upon microbial growth (in terms of μ_{max} and K_i) was developed [28]. Also addition of the same aqueous extract in nitrogen-limited glucose-based chemostats significantly enhanced accumulation of storage lipid and assimilation of sugar in the currently studied *Y. lipolytica* strain [29].

The addition of the essential oil of *C. sinensis* cv *New Hall – C. aurantium* to the culture medium of *Y. lipolytica* induced a change in the composition of total cellular fatty acids; an increment of the essential oil concentration significantly increased the cellular concentration of the fatty acids 12:0 and 14:0, decreasing simultaneously the concentration of Δ9–18:1. The total cellular saturated fatty acid concentration (12:0, 14:0, 16:0, 18:0) noticeably increased with the essential oil amount increase in the culture medium. The literature suggests that significant modification of the fatty acid composition in the cellular lipids of various microorganisms (principally, studies have been elaborated with the current *Y. lipolytica* strain) can be achieved during growth of the studied strains on media containing fatty acids, since fatty acids can be incorporated inside the cell structures with dif-

ferent assimilation rates [18–21]. Therefore, the fatty acid composition of microbial lipid can be remarkably modified, with this modification being related to the composition of fatty acids found in the culture medium [18, 19]. However, the current investigation, in which addition of the essential oil induced significant modifications in the cellular fatty acids of *Y. lipolytica*, was not due to the above phenomenon; as it was previously stated (see Section 3.1), the essential oil was completely devoid of fatty acids. On the contrary, it seems that the presence of the essential oil induced noticeable biochemical changes in the levels of the intracellular biosynthesis of microbial fatty acids. Precisely, it appears that the addition of *Citrus* essential oil to the medium provokes an inhibition of the level of (principally) cellular acyl-SCoA elongases and (to a lesser extent) cellular desaturases (mainly Δ9 desaturase). It is known that the synthesis of cellular lipids (in both oleaginous and non-oleaginous microbial strains) is carried out by condensation reactions of acetyl-SCoA molecules, by virtue of inversion of the β-oxydation process, reactions catalyzed by acyl-SCoA elongases [42]. In the current investigation, it appears that the addition of the essential oil, even at small concentrations, induced an inhibition of the level of acyl-SCoA elongases catalyzing the elongation reactions of 12:0 → 14:0 and 14:0 → 16:0 fatty acids, thus resulting in increased concentrations of the fatty acids 12:0 and 14:0 in the cellular lipids. Slight inhibition of the Δ9 desaturase activity (catalyzing the reaction 18:0 → Δ9–18:1) also seemed to occur, since at the early fermentation steps somewhat increased cellular 18:0 fatty acid concentrations were observed (see Table 3). The above facts (principally inhibition of fatty acyl elongation reactions) could also explain the global inhibition exerted by the essential oil on biomass production by *Y. lipolytica*. The appearance of medium-chain fatty acids (12:0 and 14:0) in the microbial lipids of *Y. lipolytica* at appreciable concentrations when growth was carried out in the presence of essential oil in the medium was also a noteworthy result, since the above fatty acids can potentially present a slight toxic effect on various microbial strains. It is known that growth of various yeast strains (including *Y. lipolytica*) in the presence of small- or medium-chain fatty acids (*e.g.* acetic acid, butyric acid, oleic acid, *etc.*) in the growth environment can be somehow restricted, since the aforementioned compounds present some inhibiting effect towards yeasts [43]. In general, the more the length of the fatty acid chain increases, the more the toxic effect decreases [43], but in some cases inhibitory effects can be observed even by C_{18} fatty acids (*e.g.* discrimination of *Apiotrichin curvatum* strains against stearic acid (18:0); see Lee *et al.* [44]).

As a general remark, it must be stressed that yeast lipid (*e.g.* genera *Candida*, *Rhodosporidium*, *Yarrowia*, *etc.*), when growth is carried out on sugars or similarly metabolized compounds, is principally composed of oligo- and poly-unsaturated fatty acids [20, 26–29, 45–48] while, in accordance with the results of the present investigation, addition of various natural oils (*e.g.* cyclopropenic fatty acids) to the culture medium induced an increase of the cellular saturated fatty acid concentration in various (principally oleaginous) yeasts [26, 42]. This is of significant interest given that, with the above strategy, oleaginous yeasts were revealed capable of producing microbial equivalents of cocoa butter [26, 42]. It is known that cocoa butter is mainly composed of 16:0, 18:0 and Δ9–18:1 fatty acids, with saturated fatty acids being on the level of 60–70 wt-% of total lipids [19, 42]. Sterculic and malvanic acid, which are cyclopropenic fatty acids found in the seed oil of different plants of the families Malvaceae and Sterculicaceae (*e.g.* sterculia oil), have been added to the growth medium of oleaginous yeasts, since it is known that these plant compounds reduce the activity of cellular Δ9 desaturases [26, 42]. Numerous yeast strains had been tested (*e.g. Candida* sp. 107, *Trichosporon cutaneum*, *Rhodosporidium toruloides*, *etc.*) under conditions that favor lipid biosynthesis (growth on glucose-based nitrogen-limited media), and although relatively significant quantities of inhibitor were added to the medium (up to 2 mL/L of sterculia oil), cell growth was not altered (compared with trials in the absence of inhibitors) while significant modification of the reserve lipids was observed; decreased amounts of cellular Δ9–18:1 fatty acid were detected (around 10–20 wt-% of total lipids), whilst a significant rise of the 18:0 fatty acid amount was observed (around 33–45 wt-% of cellular lipid) [26]. From all the above analyses, it may hence be assumed that it may be of interest to investigate the effect of the addition of the essential oil deriving from *C. sinensis* cv *New Hall – C. aurantium* on the growth of yeasts that can accumulate huge quantities of fat during growth on glucose-based substrates (*e.g.* in the case of *Rhodosporidium toruloides* or *Lipomyces starkeyi* strains [45–48]) in order to potentially alter their metabolism and produce cocoa butter substitutes. For instance, a newly isolated *R. toruloides* strain has been efficiently cultivated in batch [45] and fed-batch [46] fermentation systems and has produced significantly high amounts of microbial lipids (concentrations of around 73 g/L; see Li *et al.* [46]), and various *L. starkeyi* strains can efficiently convert wastes or renewable resources (*e.g.* sewage sludge, xylose, *etc.*) into microbial oil [47, 48], while in contrast with the utilization of various cyclopropenic fatty acids, essential oils present therapeutic and anti-cancer effects [1–3]; therefore they can be used in the fermentation medium with no caution or danger.

In contrast to the results obtained in the current study, addition of *Teucrium polium* L. aqueous extract to the culture medium in carbon-limited cultures led by *S. cerevisiae* and *Y. lipolytica* significantly increased the unsaturation index of lipid produced by both microorganisms [28]. Likewise, in carbon-limited cultures of *Y. lipolytica*, the addition of aqueous extracts of plants belonging to the Lamiaceae family increased both the oleic and linoleic acid concentration in the cellular microbial lipid, while in nitrogen-limited media the above plant extracts had an adverse effect upon these fatty acids [30]. Furthermore, cultivation of the present *Y. lipolytica* strain (ACA-DC 50109) in olive mill wastewater-based media

containing significant quantities of phenol compounds (around 2.1 g/L) resulted in almost negligible inhibition exerted by the inhibitor (phenol compounds) added to the growth medium, while a significant rise in the concentration of cellular unsaturated fatty acids (principally the Δ9–18:1 and Δ9–16:1 fatty acids) compared with the blank experiment (no addition of olive mill wastewater in the medium) was observed [22].

As a conclusion, limonene was proved to be the main constituent of the essential oil derived from the fruits of *C. sinensis* cv *New Hall – C. aurantium*. The effect of the addition of this essential oil on the biochemical behavior of *Y. lipolytica* ACA-DC 50109 was investigated. The microorganism underwent inhibition exerted by the essential oil added to the medium. Finally it was found that, when the essential oil was added to the medium, an increase of the cellular saturated fatty acids in both microorganisms was observed.

Acknowledgments

This study has been co-funded by 75% from the EU and 25% from the Greek Government under the framework of the Education and Initial Vocational Training Program – Archimedes II.

Conflict of interest statement

The authors have declared no conflict of interest.

References

[1] C. J. Stevensen: Aromatherapy in dermatology. *Clin Dermatol.* 1998, **16**, 689–694.

[2] G. Ruberto, M. T. Baratta: Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 2000, **69**, 167–174.

[3] A. Prashar, P. Hilib, R. G. Venessa, C. S. Evans: Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. *Phytochemistry* 2003, **63**, 569–575.

[4] L. Moeller, B. Strehlitz, A. Aurich, A. Zehnsdorf, T. Bley: Optimization of citric acid production from glucose by *Yarrowia lipolytica*. *Eng Life Sci.* 2007, 7, 504–511.

[5] S. B. Imandi, V. R. Bandaru, S. R. Somalanka, H. R. Garapati: Optimization of medium constituents for the production of citric acid from byproduct glycerol using Doehlert experimental design. *Enzyme Microb Technol.* 2007, **40**, 1367–1372.

[6] Y. Pagot, A. Endrizzi, J. M. Nicaud, J. M. Belin: Utilization of an auxotrophic strain of the yeast *Yarrowia lipolytica* to improve γ-decalactone production yields. *Lett Appl Microbiol.* 1997, **25**, 113–116.

[7] K. Mličková, Y. Luo, S. d'Andrea, P. Peč, T. Chardot, J. M. Nicaud: Acyl-CoA oxidase, a key step for lipid accumulation in the yeast *Yarrowia lipolytica*. *J Mol Catal B Enzym*. 2004, **28**, 81–85.

[8] P. Fickers, F. Fudalej, J. M. Nicaud, J. Destain, P. Thonart: Selection of new over-producing derivatives for the improvement of extracellular lipase production by the non-conventional yeast *Yarrowia lipolytica*. *J Biotechnol.* 2005, **115**, 379–386.

[9] P. Fickers, J. M. Nicaud, C. Gaillardin, J. Destain, P. Thonard: Carbon and nitrogen sources modulate lipase production in the yeast *Yarrowia lipolytica*. *J Appl Microbiol.* 2004, **96**, 742–749.

[10] T. Finogenova, S. Kamzolova, E. Dedyukhina, N. Shishkanova, A. Il'chenko, I. Morgunov, O. Chernyavskaya, A. Sokolov: Biosynthesis of citric and isocitric acids from ethanol by mutant *Yarrowia lipolytica* N1 under continuous cultivation. *Appl Microbiol Biotechnol.* 2002, **59**, 493–500.

[11] S. Kamzolova, N. Shishkanova, I. Morgunov, T. Finogenova: Oxygen requirements for growth and citric acid production of *Yarrowia lipolytica*. *FEMS Yeast Res.* 2003, **3**, 217–222.

[12] S. Kamzolova, T. Finogenova, Y. Luniva, S. Perevoznikova, L. Minachova, I. Morgunov: Characteristics of the growth on rapeseed oil and synthesis of citric and isocitric acids by *Yarrowia lipolytica* yeasts. *Microbiology (Moscow)* 2007, **76**, 20–24.

[13] I. Morgunov, S. Kamzolova, O. Perevoznikova, N. Shishkanova, T. Finogenova: Pyruvic acid production by a thiamine auxotroph of *Yarrowia lipolytica*. *Proc Biochem.* 2004, **39**, 1469–1474.

[14] I. Musiał, W. Rymowicz, E. Cibis: Optimization of single-cell-biomass production by *Yarrowia lipolytica* using response surface methodology and pulse method. *Electron J Pol Agric Univ.* 2004, **7**, #2.

[15] W. Rymowicz, A. Rywińska, B. Żarowska, P. Juszczyk: Citric acid production from raw glycerol by acetate mutants of *Yarrowia lipolytica*. *Chem Pap.* 2006, **60**, 391–394.

[16] W. Rymowicz, E. Cibis: Optimization of citric acid production from glucose syrup by *Yarrowia lipolytica* using response surface methodology. *Electron J Pol Agric Univ.* 2006, **9**, #20.

[17] W. Rymowicz, A. Rywinska, B. Żarowska: Biosynthesis of citric acid from crude glycerol by *Yarrowia lipolytica* in repeated-batch cultivations. *J Biotechnol.* 2007, **131**, S149–S150.

[18] S. Papanikolaou, G. Aggelis: Selective uptake of fatty acids by the yeast *Yarrowia lipolytica*. *Eur J Lipid Sci Technol.* 2003, **105**, 651–655.

[19] S. Papanikolaou, L. Muniglia, I. Chevalot, G. Aggelis, I. Marc: Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues. *Curr Microbiol.* 2003, **46**, 124–130.

[20] S. Papanikolaou, M. Galiotou-Panayotou, I. Chevalot, M. Komaitis, I. Marc, G. Aggelis: Influence of glucose and saturated free-fatty acid mixtures on citric acid and lipid production by *Yarrowia lipolytica*. *Curr Microbiol.* 2006, **52**, 134–142.

[21] S. Papanikolaou, I. Chevalot, M. Galiotou-Panayotou, M. Komaitis, I. Marc, G. Aggelis: Industrial derivative of tallow: A promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*. *Electron J Biotechnol.* 2007, **10**, 425–435.

[22] S. Papanikolaou, M. Galiotou-Panayotou, S. Fakas, M. Komaitis, G. Aggelis: Citric acid production by *Yarrowia lipolytica* cultivated on olive-mill wastewater-based media. *Bioresour Technol.* 2008, **99**, 2419–2428.

[23] M. A. Ghannoum: Studies on the anticandidal mode of action of *Allium sativum* (garlic). *J Gen Microbiol.* 1988, **134**, 2917–2924.

[24] H. S. Tranter, C. Tassou, G. J. Nychas: The effect of the olive phenolic compound, oleuropein, on growth and enterotixin B production by *Staphylococcus aureus*. *J Appl Bacteriol.* 1993, **74**, 253–259.

[25] P. Cerrutti, S. M. Alzamora: Inhibitory effects of vanillin on some food spoilage yeasts in laboratory media and fruit purees. *Int J Food Microbiol.* 1996, **29**, 379–386.

[26] R. S. Moreton: Modification of fatty acid composition of lipid accumulating yeasts with cyclopropene fatty acid desaturase inhibitors. *Appl Microbiol Biotechnol.* 1985, **22**, 41–45.

[27] K. Man You, C. L. Rosenfield, D. C. Knipple: Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. *Appl Environ Microbiol.* 2003, **69**, 1499–1503.

[28] G. Aggelis, N. Athanassopoulos, A. Paliogianni, M. Komaitis: Effect of *Teucrium polium* L. extract on the growth and the fatty acid composition of *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. *Antonie van Leeuwenhoek* 1998, **73**, 195–198.

[29] G. Aggelis, M. Komaitis: Enhancement of single-cell oil production by *Yarrowia lipolytica* growing in the presence of *Teuchrium polium* L. aqueous extract. *Biotechnol Lett.* 1999, **21**, 747–749.

[30] M. S. Karanika, M. Komaitis, G. Aggelis: Effect of aqueous extracts of some plants of Lamiaceae family on the growth of *Yarrowia lipolytica*. *Int J Food Microbiol.* 2001, **64**, 175–181.

[31] H. Van den Dool, P. D. Kratz: A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr.* 1963, **11**, 463–471.

[32] R. P. Adams: *Identification of essential oil components by gas chromatography/mass spectroscopy.* Carol Stream, Allured Publishing Corp., IL (USA) 2001.

[33] G. L. Miller: Use of dinitrosalicylic reagent for determination of reducing sugar. *Anal Chem.* 1959, **31**, 426–428.

[34] G. Ojeda de Rodriguez, V. Morales de Godoy, N. Gonzalez de Colmenares, L. Cabrera Salas, B. Sulbaran de Ferrer: Composition of Venezuelan lemon essential oil *Citrus limon* (L.) *Burm. f. Rev Fac Agron (LUZ)* 1998, **15**, 343–349.

[35] C. B. Tirado, E. E. Stashenko, M. Y. Combariza, J. R. Martinez: Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography-mass spectrometry. *J Chromatogr A* 1995, **697**, 501–513.

[36] M. Kondo, M. Goto, A. Kodama, T. Hirose: Fractional extraction by supercritical carbon dioxide for the deterpenation of bergamot oil. *Ind Eng Chem Res.* 2000, **39**, 4745–4748.

[37] D. E. Conner, L. R. Beuchat: Effects of essential oils from plants on growth of food spoilage yeasts. *J Food Sci.* 1984, **49**, 429–434.

[38] R. J. W. Lambert, P. N. Skandamis, P. J. Coote, G. J. E. Nychas: A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol.* 2001, **91**, 453–462.

[39] P. N. Skandamis, G. J. E. Nychas: Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *J Appl Microbiol.* 2001, **91**, 1011–1022.

[40] E. L. Souza, T. L. M. Stamford, E. O. Lima, V. N. Trajano: Effectiveness of *Origanum vulrage* L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control* 2007, **18**, 409–413.

[41] N. Belletti, S. S. Kamdem, F. Patrignani, R. Lanciotti, A. Covelli, F. Gardini: Antimicrobial activity of aroma compounds against *Saccharomyces cerevisiae* and improvement of microbiological stability of soft drinks as assessed by logistic regression. *Appl Environ Microbiol.* 2007, **73**, 5580–5586.

[42] J. Davies, J. Holdsworth: Synthesis of lipids in yeasts: Biochemistry, physiology and production. *Adv Appl Lipid Res.* 1992, **1**, 119–159.

[43] G. Rodrigues, C. Pais: The influence of acetic and other weak carboxylic acids on growth and cellular death of the yeast *Yarrowia lipolytica*. *Food Technol Biotechnol.* 2000, **38**, 27–32.

[44] I. M. Lee, E. G. Hammond, J. L. Cornette, B. A. Glatz: Triacylglycerol assembly from binary mixtures of fatty acids by *Apiotrichum curvatum*. *Lipids* 1993, **28**, 1055–1061.

[45] Y. H. Li, B. Liu, Z. B. Zhao, F. W. Bai: Optimized culture medium and fermentation conditions for lipid production by *Rhodosporidium toruloides*. *Chin J Biotechnol.* 2006, **22**, 650–656.

[46] Y. H. Li, Z. B. Zhao, F. W. Bai: High density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. *Enzyme Microb Technol.* 2007, **41**, 312–317.

[47] C. Angerbauer, M. Siebenhofer, M. Mittelbach, G. M. Guebitz: Conversion of sewage sludge into lipids by *Lipomyces starkeyi* for biodiesel production. *Bioresour Technol.* 2008, **99**, 3051–3056.

[48] X. Zhao, X. Kong, Y. Hua, B. Feng, Z. B. Zhao: Medium optimization for lipid production through co-fermentation of glucose and xylose by the oleaginous yeast *Lipomyces starkeyi*. *Eur J Lipid Sci Technol.* 2008, **110**, 405–412.

