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Bioenergetic investigations on tea-tree and related essential oils

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Abstract

Direct and indirect calorimetry were used to determine the metabolic rates of the bacterium *Escherichia coli* and the baker's yeast *Saccharomyces cerevisiae* under the influence of five tea-tree oils and some of their constituents. Bacteriostatic and bactericidal as well as fungicidal effects were observed, dose-effect curves established, and the kinetics of the inactivation evaluated. Experiments showed that subpopulations of the *E. coli* suspensions were less sensitive against these essential oils than the whole population and were able to continue to metabolize and to grow.

Some general information about tea-tree oils, their origin, application and composition is given.

Keywords: Calorimetry; Essential oils; Metabolism; Microorganism; Polarography; Tea-tree

1. Introduction

Modern human health care — confronted with a steadily increasing resistance of pathologic microbes against usual drugs — is forced to search for alternative and possibly also more natural substances in the fight against infections, inflammations and other diseases. Thus, interest goes for plant products used in the ancient and mediaeval folk medicine, but also for medicines well-known in traditional treatments in Non-European, often tropical countries. This paper presents results of direct and indirect calorimetric experiments with essential oils of the tea-trees on two microorganisms, the eukaryotic baker's yeast *Saccharomyces cerevisiae* and the prokaryotic bacterium *Escherichia coli*.

Talking about tea-trees and their essential oils, a group of different, but botanically related species are meant which all served James Cook and his crew as medical teas in case of illness or injury. These trees are: (i) the ''true'' or paper bark tea-tree *Melaleuka alternifolia*, (ii) the cajeput (kajoepuetih) tree *M. cajeputi* (*M. leukadendron*), (iii) the niaouli paper bark tea-tree *M. viridiflora gaertner* (*M. quinquenervia*), (iv) the manuka tree *Leptospermum scoparium* (supposedly the original tea-tree of James Cook), and (v) the kanuka or white-manuka tree *Kunzea ericoides* (*Leptospermum ericoides*). All five belong to the family of Myrtaceae, well-known for some spicy or odorous members like clove, nutmeg, allspice (pimento), myrthe, and of course eucalyptus. Many further members of this family are used in traditional medicine and none of this family is poisonous. Common for all of them is a high concentration of essential oils which can be extracted by steam distillation of their leaves and small twigs.

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The interesting feature of tea-trees are their essential oils with a broad and strongly varying chemical composition. The most important classes of compounds found in all tea-tree oils are: (i) monoterpenoles (especially terpenen-4-ol) with up to 45% (in tea-tree oil), (ii) monoterpenes with up to 65% (in kanuka oil), (iii) oxides (especially 1,8 cineole) up to 60% (in cajeput oil), (iv) sesquiterpenes up to nearly 70% (in manuka oil), (v) sesquiterpenoles in lower concentrations (<15%), and (vi) triketones (<25%), specially in manuka oil. The concentrations of the compounds differ considerably, not only between the five trees, but also with provenience, time of harvesting and with the years. The strongest influence on microorganisms is ascribed to terpinen-4-ol and cineole, the latter with the drawback that it seems to give some irritation of the human skin. Besides this observation and a few allergic reactions, no negative side effects have been described for the application of tea-tree oils.

Tea-tree leaves have been used in folk medicine for thousands of years by the aborigines of Australia and New Zealand against infections, inflammations, colds, diarrhoea, and for wound healing [1]. The positive effects of these plants became well-known in Europe in the 19th century, but fell into oblivion till the first quarter of this century when the Australian chemist Penfold performed spectacular screening tests on the effects on malignant microbes [2]. He determined an antiseptic action of tea-tree oil ten times stronger than that of the that-time common phenol. With the growing influence of synthetic chemistry in pharmacy tea-tree oil was forgotten again, till it experienced a renewed interest in the last two decades due to search for alternative medical treatments. Besides its analgesic effect, anti-allergic, anti-bacterial, anti-microbial, anti-mycotic, anti-rheumatic, and anti-viral activities were found [3]. Moreover, its stimulating effects, psychic stabilization and support of good moods were used in the so-called ''aroma-therapy'' and led to the title ''smallest household pharmacy of the world'' for (the true) tea-tree oil.

Microbiological investigations on various tea-tree oils or their components were performed by various authors with different methods, including the measurement of optical density to monitor growth or cell lysis in the presence of oils, dilution techniques to determine the minimal inhibition concentration (MIC) or the minimal lethal dose (MLD), and agar well diffusion techniques on solid medium. But energetic investigations on aerobic or anaerobic microbial metabolism are rare. Cox and coworkers [4] applied a Clark type oxygen electrode to follow the decrease of respiration in *E. coli* under the influence of tea-tree oils. Until now no direct calorimetry has been performed in this field, neither on the oils themselves nor on their components, and only on one other essential oil, the lemongrass [5]. Thus, the intention of this paper was to introduce this promissing technique into tea-tree oil research and to get — hopefully — a deeper insight into the actions of essential oils on microorganisms.

2. Methods and materials

2.1. Calorimetry

A flow-calorimeter (type 10700, LKB, Bromma/Sweden) with a flow-through cell of 0.587 ml was used at 37°C. The sensitivity amounted to 65.5 mV/W, the time constant to 65 s. The intensively stirred microbial culture of 25 ml was kept in a thermostatted cylinder at the same temperature and pumped (Pharmacia LKB pump P-1, silicon tubing 1 mm × 1 mm) at a constant rate of 0.56 ml/min (33.6 ml/h) to the calorimeter and back to the cylinder. At these settings it took 5.5 min for the sample to reach the flow-through cell. The thermal signal was monitored by a recorder (Micrograph BD5, Kipp and Zonen, Delft/The Netherlands) at a sensitivity of 50 μV full scale and a paper speed of 0.5 mm/min.

The power–time curves of the calorimeter were digitized with an electronic digitizer (Digikon, Kontron, Munich/Germany), smoothed and stored for further evaluations. Typical fingerprint-like structures of the calorimetric curves gave hints to normal, undisturbed microbial growth and metabolism or to changes due to the application of tea-tree oils. Peak height, time of peak appearance, reduction of heat flow amplitude after oil addition and the time till a new exponential growth (lag phase) served as measure for the observed effects.

Dose–effect curves (as usual in microbiological investigations of irradiation hazards) of the action of tea-tree oils on bacteria or yeast were constructed

by plotting the rate reductions in a logarithmic scale as function of the final concentrations of oils. The concentration that rendered a 50% decrease was taken as LD_{50} (50% lethal dose) irrespectively of the question whether such a reduction was due to a bactericidal or bacteriostatic effect. These LD_{50} values served to compare the effectivity of different tea-tree oils or their constituents.

2.2. Photometry

Microbial growth and possible lysis after oil application were monitored by a spectrophotometer (UV-120–01, Shimadzu, Kyoto/Japan) at a wavelength of 650 nm. Absorption spectra of the cells and of the different oil solutions or emulsions were obtained by means of a UV-VIS Scanning Spectrophotometer (UV 2102 PC, Shimadzu, Kyoto/Japan) connected to a PC with corresponding software.

2.3. Polarography

The aerobic metabolism of maintenance was monitored with an oxygen electrode of the Clark type (Bachofer, Reutlingen/Germany). For all experiments an active, intensively stirred volume of 1 ml was used, the temperature set to 30°C for yeasts and to 37°C for *E. coli*. Investigations were run in a 0.1% glucose buffer of the correct pH, fully aerated before and — if necessary — during a break of the measurement. The electrode signal, proportional to the oxygen concentration in the suspension, was recorded and later evaluated for the rate of oxygen consumption. Usually, the results are given in percent of the metabolism without tea-tree oil. For this end, 1 ml of the pure suspension was analyzed first and then treated with the wanted amount of oil and analyzed again. For the evaluation of the reaction kinetics the same sample was left in the electrode chamber for a chosen time, aerated again and measured.

2.4. Tea-tree oils

The following essential oils were purchased from Taoasis, Lemgo/Germany: (i) ''True'' tea-tree oil (*M. alternifolia*) with a cineole concentration 2.5% and a terpinen-4-ol concentration 39.2%, thus easily satisfying the Australian Standard AS2782 of less than 15 and more than 30%, resp., (ii) cajeput oil (*M. cajeputi*) with 51.8 and 0.69%, resp., (iii) niaouli oil (*M. viridiflora gaertner*) with 59.5 and <1%, resp., (iv) manuka oil (*L. scoparium*) with 1 and <1%, resp., (v) kanuka oil (*K. ericoides*) with 4.5 and <1%, resp. Some special compounds of these oils like cineole, terpinen-4-ol, α-terpineol, α-and γ-terpinene (Aldrich/Sigma, Steinheim/Germany) as well as the antibiotic kanamycin solution (Sigma, Steinheirn/Germany) served to further evaluate the antimicrobial actions of tea-tree oils or as an inactivation standard. Dissolution of the oils was difficult as already discussed in the literature. The oils were dissolved in 0.1% Tween80 (Aldrich, Steinheim/Germany), in 90% ethanol or in a 0.2% agar solution.

2.5. Microorganisms

The experiments were mainly aimed at the gram-negative bacterium *E. coli*, strain K12 C600 (from the stock collection of the institute). It was grown at 37°C in a YT medium (0.5% yeast extract, 1% tryptone, 1% sodium chloride, pH 7.5) as an overnight preculture, harvested, washed three times and set to the wanted cell concentration. Calorimetric growth experiments were performed with the same medium, agar diffusion tests run with this medium, but solidified by addition of 2.5% agar. For calorimetric experiments on the metabolism of maintenance and for the determination of oxygen consumption rates (indirect calorimetry) a potassium hydrogen phosphate buffer (0.05 mol/l, pH 7.5) was applied. All investigations were performed at 37°C.

Orientational experiments with an eukaryotic microorganism were done with a commercial baker's yeast (*S. cerevisiae*, Uniferm, Werne/Germany), mainly in connection with the polarographic determination of oxygen consumption rates. Again, a potassium hydrogen phosphate buffer, but this time of pH 5.5, was used at 30°C.

3. Results

3.1. Microcalorimetry (direct calorimetry)

Fig. 1 shows a typical power–time curve (solid line) of growth of *E. coli* in the complex medium. After a

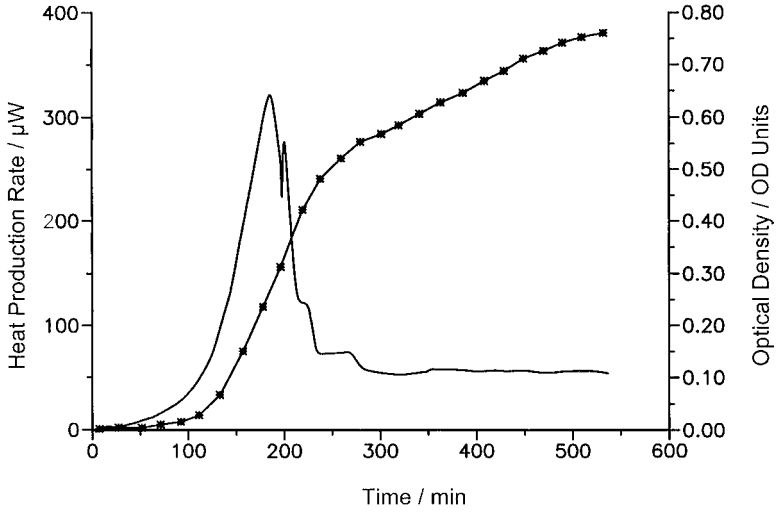

Fig. 1. Calorimetric (solid line) and optical growth curve (line with asterisks) of an *E. coli* culture at 37°C in YT medium, monitored by a flow calorimeter and a separate spectrophotometer. The heat production rate is given for the flow-through cell of 0.587 ml. An optical density of 1.0 corresponds to a bacterium count of 4.2×10^9 ml. The microbial culture was kept in a stirred Erlenmeyer flask outside the calorimeter.

short lag time of about 20 min the exponential growth phase starts and continues for about 3 h until a significant peak after which the heat production rate drops in several steps to a level remaining constant for a long time. The maximum of the curve is due to the consumption of an easily degradable component of the medium and the subsequent slower use of other components or metabolic products. The trace of the optical density (line with asterisks) indicates that the exponential phase endures about 1 h longer than the increasing heat production rate and that growth continues at a much smaller rate after the drop of the calorimetric signal. As cells exhibit a pronounced sensitivity towards chemical and physical noxes in the exponential phase (see e.g. [4,6]), experiments were mainly performed in this part of growth curve.

This in mind, calorimetric runs were done as depicted in Fig. 2. After establishment of the baseline cells were inocculated into the medium (20 min) leading to a significant increase in heat output due to their metabolism of maintenance and after a very brief lag phase to their exponential growth. Short time before the main peak, tea-tree oil was added (arrow) producing an abrupt drop of the heat production rate after a few minutes. Most cells became inactive after this treatment and only a small subpopulation survived. It grew till its number was large enough to render a detectable calorimetric signal. To determine the length of the lag phase (in a rather arbitrary, but consistent manner for all runs) a tangent under 60° was constructed at the slope. The period between the oil addition and this point was defined as the lag phase.

Another information can be obtained from this graph (Fig. 2): the drop of the calorimetric signal from about 415 µW to around 15 µW, rendering a reduction of the heat flow to 3.6% in this case. By varying the amount of tea-tree oil added to the culture all degrees of reduction could be obtained. This is seen in Fig. 3 for the influence of manuka oil. Increasing concentrations reduced the heat output rate down to 14% of the control. The corresponding dose-effect curve renders a straight line (exponential inactivation) with a LD_{50} (lethal dose for 50% inactivation) of 0.59 ml oil or a final concentration of 4% oil in the suspension. It shows that manuka oil is less effective against *E. coli* than the "true" tea-tree oil in the present experiments.

When tea-tree oil was given already at the beginning of the run before growth started, it led to a prolongation of the lag phase from about half an hour to several hours or even to a complete block of growth (2% final concentration) for a preselected period of 24 h.

Although it is known that growing cells are more sensitive towards chemical noxes than stationary or

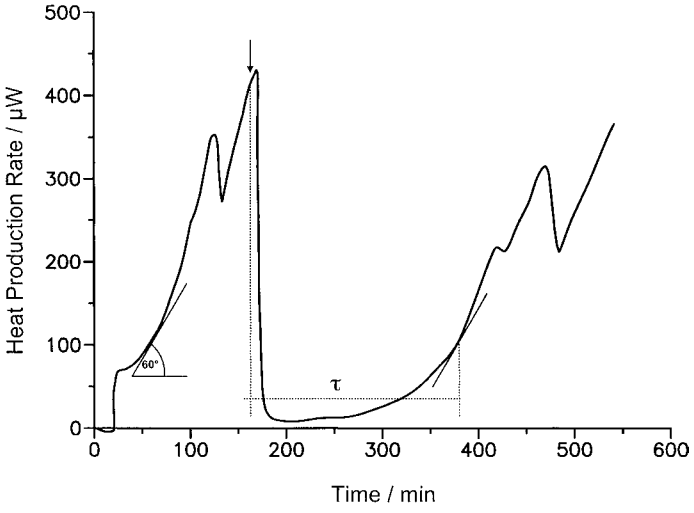

Fig. 2. Power–time curve of growth of an *E. coli* culture at 37°C before and after addition (arrow) of 0.1 ml tea-tree oil (dissolved in 0.1% Tween80, final oil concentration 0.2% v/v). After the essential drop of the heat production rate to nearly zero, a subpopulation starts to grow again and to produce a similar growth curve from which the length τ of the lag phase is taken.

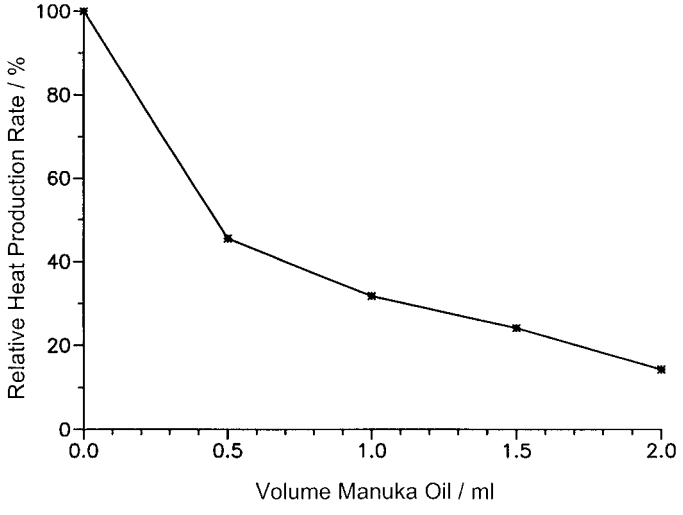

Fig. 3. Effect of the essential oil of the manuka tree (*L. scoparium*) (dissolved in 0.1% Tween80) on *E. coli* cells. 2.0 ml oil render a final concentration of 4% v/v. The graph corresponds to an exponential inactivation with an $LD_{50} = 0.59$ ml.

resting cells, it is worthwile to investigate also the metabolism of maintenance and to compare direct and indirect calorimetry. Fig. 4 presents such a power–time curve of *E. coli* cells in a buffer supplemented with glucose as an energy source, but without nitrogen. After establishment of the baseline the suspension was pumped through the calorimeter leading at first to a rapid increase in the heat production rate and a subsequent steady decrease with time. This decline was enhanced by two successive additions of tea-tree oil (arrows) with a dramatic drop after an incubation period of about half an hour. Apparingly, a more resistant subpopulation remained metabolizing at a nearly constant rate. An injection of a larger amount of oil influenced also this population. In the end, plain buffer was used to return to the baseline.

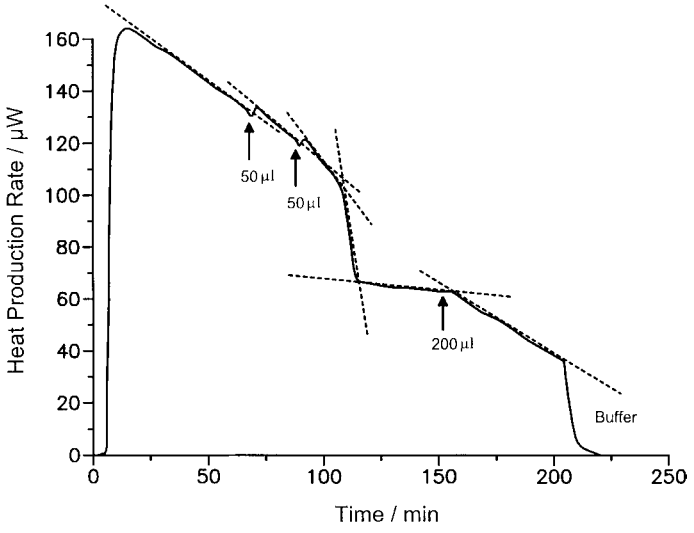

Fig. 4. Power–time curve of the maintenance metabolism of an *E. coli* suspension at 37°C under the influence of tea-tree oil. At the indicated points (arrows) two times 50 µl and one time 200 µl oil are added. The dashed lines are used to determine the reduction degree of the metabolic rate after poisoning.

3.2. Polarography (indirect calorimetry)

The oxygen consumption rate of resting yeast cells was determined with a Clark type electrode connected to a recorder. Kinetic experiments with yeast suspensions showed that the electrode time constant of about 50 s did not distort the polarographic signal so that all information was taken directly from the original graphs. The rate of a strongly aerated cell suspension without treatment with tea-tree oil served as a control (left part of Fig. 5). When all oxygen was consumed, a chosen amount of oil was injected into the same sample. After an incubation time of 10 min inside the electrode chamber the suspension was aerated again and the new rate measured (right part in Fig. 5). The ratio of the two angles α_1 and α_2 gave the relative decrease of respiration expressed as percentage of the original consumption. In a separate experiment the time course of inactivation with tea-tree oil was determined, showing a biphasic behaviour with a very steep drop in the first five minutes and a constant level or very slow decrease afterwards (Fig. 6). Since the small increase around 20 min was not significant, 10 min incubation time with the oils appeared sufficient for the present investigations. Fig. 7 shows the dependence of the reduction as function of the added amount of tea-tree oil. Already a volume of 30 µl oil soluted 1 : 1 in 0.1%Tween80 (corresponding to an absolute concentration of 1.5% v/v) produced the final reduction to about 8% which remained constant with increasing oil concentrations (Fig. 7). A volume of 7.5 µl (0.4% final concentration) of tea-tree oil reduced the undisturbed respiration rate to 50% of the initial value (LD_{50}).

It was shown by other authors (see discussion) that the action of tea-tree oil has a double kinetics of a strong initial influence and a much weaker one in a later phase, both in a temporal (Fig. 6) as well as a concentration sense (Fig. 7). The dose–effect-curve presented in Fig. 8 underlines this observation with a steep exponential decline of the respiration rate at low oil concentrations and a very flat dependence above 30 µl oil. Such dose–effect curves are typical for irradiation induced inactivation of microbial growth when two differently sensitive subpopulations are present in the suspension.

Orientational polarographic investigations were performed also on manuka honey, the only tea-tree honey which was commercially available in Germany. The honey was diluted in the usual yeast buffer as stock solution and mixed with the yeast suspension. A final concentration of 6% v/v manuka honey reduced the respiration rate to 50% (LD_{50}).

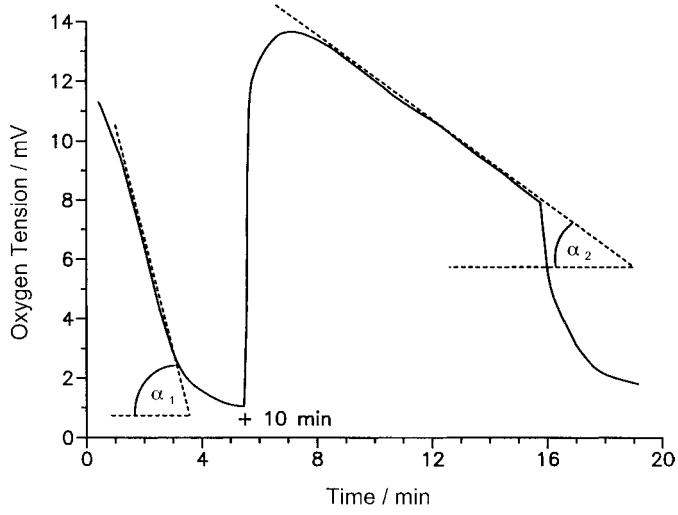

Fig. 5. Polarographic determination of the oxygen tension in a yeast suspension as a function of time. A voltage of about 14 mV corresponds to an air saturation of 0.24 mmol/l oxygen at 30°C. The left part shows the oxygen consumption rate before the addition of tea-tree oil (control), the right part that after the addition of 20 µl oil (final concentration 1% v/v) and 10 min incubation time. The angles α_1 and α_2 serve for the evaluation of the reduction ratio. At the end of the experiment, sodiumdithionit is added to consume all oxygen and to determine the zero line.

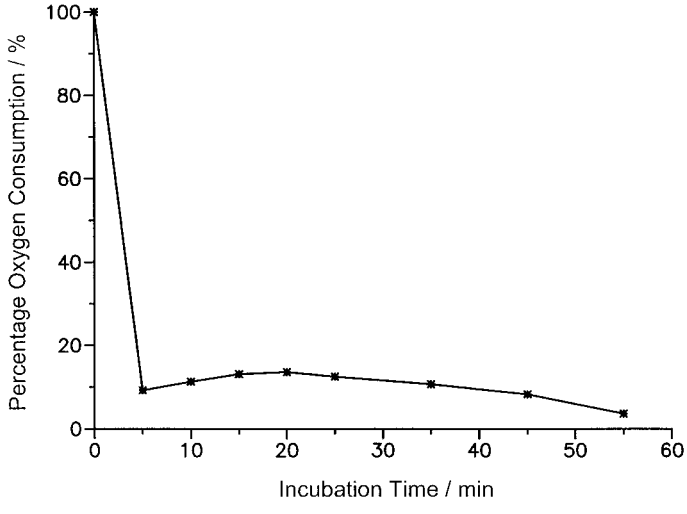

Fig. 6. Relative oxygen consumption rate of a yeast suspension after the addition of 30 µl tea-tree oil dissolved 1 : 1 in 0.1%Tween80 (final concentration 1.5% v/v).

4. Discussion

In the last decades one could observe a back orientation to traditional — or folk — medicine, forming an alternative to the steadily increasing application of synthetic chemical drugs and the growing resistance of microbes against them. Folk medicine is often connected with medicaments derived from officinal plants or parts of them. Many essential oils of botanical origin are among them, not only used in the modern Western "aroma therapy", but for thousands of years in different cultures around the Earth [1,3].

Although there have been published some broad screenings of essential oils against a larger variety of

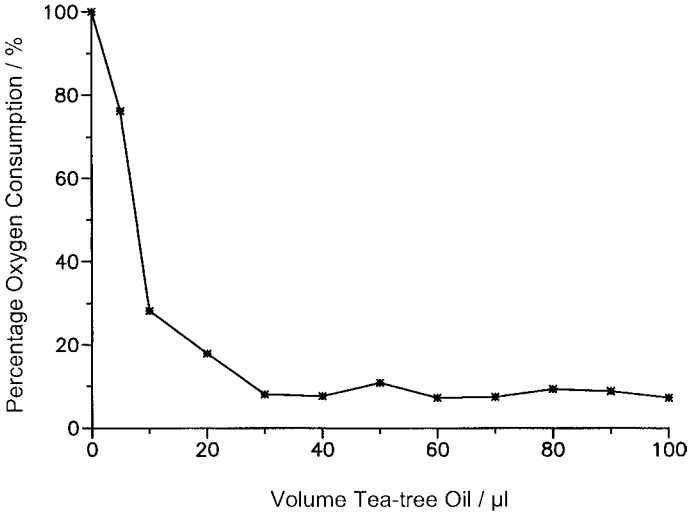

Fig. 7. Relative oxygen consumption rate of yeast suspensions at 30°C after addition of increasing volumes of tea-tree oil (dissolved 1 : 1 in 0.1%Tween80). A volume of 100 µl corresponds to a final oil concentration of 5% v/v.

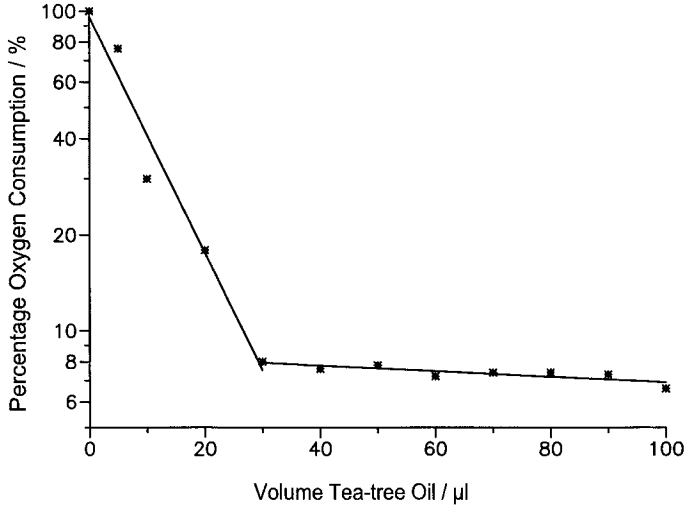

Fig. 8. Dose–effect curve of increasing amounts of tea-tree oil on a yeast suspension (for further details see Fig. 5). The relative oxygen consumption rate is given in a logarithmic scale. A double kinetic of an initial steep decrease and a subsequent flat decline is clearly visible.

microorganisms in recent times (for a survey see [7]), the main microbes included in the usual tests are the prokaryotes: *E. coli* (colonizing the lower intestinal tract of vertebrates and responsible for ureter diseases), *Staphylococcus aureus* (important in postoperative infections, abscesses, pneumonia and sepsis), *Heliobacter pylori* (responsible for dyspepsia and gastritis), and *Pseudomonas aeroginosa* (influence on pus production, wound healing, gall and ureter infection, dysentery). The eukaryotes are: the inoffensive baker's yeast *S. cerevisiae* and the pathogene yeast *Candida albicans* (responsible for severe mykoses). Both groups form a representative profile of microbes envolved in human diseases. Two of them, *E. coli* and *S. cerevisiae* were chosen as examples in the present research.

Tea-tree oils as lipophilic substances are insoluble in water, so that emulsifiers or solvents have to be applied in the test media [8,9]. Tween80 is often used to this end, although it does not guarantee a completely homogeneous solution. Moreover, Altman [10] showed a reduction of the bioactivity of tea-tree oil in the presence of Tween80. Nevertheless, as the solubility of these oils is strongly increased by Tween80, and other authors stated that it has no own antimicrobial effects in the chosen concentration range [8], it can be recommended for such investigations. Other solvents given in the literature are ethanol and DMSO. A low agar concentration (0.15%) was proposed as a stabilizer of an oil/water mixture which remained stable for many hours [9,11]. On this background, mainly Tween80 and ethanol were used with good results by us.

The present direct and indirect calorimetric experiments show that the various tea-tree oils are well suited to influence both, resting and growing cells, even at low concentrations. The effects are seen after a few minutes (Figs. 2, 4 and 6) so that an incubation time of 5 min was already sufficient for yeast cells. The following half hour brought no further reduction of the respiration rate. These results are in accordance with data from literature about the action of other essential oils (e.g. palmarosa, peppermint, eucalyptus, lemongrass [12] and Kiso-Hinoki oil [13]) on bacteria which rendered an instantaneous and irreversible damage of the cells.

Calorimetric and polarographic curves show that the metabolic rates of yeasts and bacteria drop to very low values after the application of oils in the chosen concentration range, but never completely to zero (Figs. 2 and 7). This indicates that a small subpopulation remained active which was less or not at all influenced by the drugs. Even in those cases where the calorimetric signal stayed near the baseline for 24 h, it might well be that after an even longer time growth started again when the few resistant microbes have developed to a detectable population. Such subpopulations, well-known from microbial cultures and often discussed in the literature, are responsible for plateaus as seen in Fig. 4 and for the double kinetics of inactivation (Fig. 8; see e.g. [6]).

It is stated in the literature (e.g. [4]) and confirmed by our experiments that resting cells are more resistant against tea-tree oils than exponentially growing ones. This is due to the fact that major modifications in the fatty-acid composition of the inner membrane appear at the transition from the exponential to the stationary phase of a culture [4,14] and that the higher amounts of lipopolysaccharides in the outer membrane change the surface charge of the cells [4,15].

Fig. 4 depicts a calorimetric experiment of the action of tea-tree oil on a suspension of resting *E. coli* cells. The first impression is that of a strong reduction of heat production as discussed above. But moreover, a further effect becomes obvious at the first two additions which is often seen at lower oil concentrations: a short-time dip in the curve followed by a stimulation of the heat production rate and a shift to a higher metabolic level from where the decline starts. These phenomena are no calorimetric artifacts like heats of mixing, dilution or neutralization because the addition happens in the fermentor outside the calorimeter and about 5 min earlier than the sample reaches the calorimetric cell. Further experiments are necessary to elucidate reasons for this observation which might be connected with a kind of hormesis.

The experiments described here are in a line of assays to apply calorimetry in monitoring drugs in their action on microbial populations, quite in the sense of an analytical, not quantitative calorimetry. In this field it is important whether a reaction occurs and not to what degree. A summary of the earlier literature for the combination of drug analysis and calorimetry was presented by Beezer and Chowdhry [16,17], stating that such calorimetric tests are rapid, sensitive and reproducible and well suited to support classical assays. More recently, combinations of calorimetry and photometry were applied to monitor the action of chloramphenicol on the bacterium *S. aureus* [18,19]. The authors observed a retarded growth, an increase in the amplitude of the first peak in the power–time curve and a changed period for the return to the baseline, similar to the results of the present investigation. The latter two effects were due to an early loss of anabolic facilities of the cells, followed by a catabolic breakdown later on. Such effects became visible in the calorimetric curves long before the MIC of the classical assays was reached.

The only previous calorimetric investigation on an essential oil was performed with lemongrass oil (*Cymbopogon densiflorus*), which showed fungistatic properties in food storage superior to those of synthetic

commercial fungicides [20] and which is frequently used in traditional medicine, in the culinary domain and for perfumes [5]. Already small concentrations of 0.025–0.05% rapidly stopped the growth of *S. aureus* in the exponential phase. Earlier experiments of the same authors had shown that bacteriostatic drugs influence the ribosomal protein synthesis and the anabolic efficiency of the cells so that more heat could be dissipated by the culture [19]. Power–time curves of *S. aureus* under the influence of lemongrass oil correspond to those of inhibitors of protein synthesis [5]. On the other hand, Ogunlana and coworkers explained the morphological changes in *E. coli* cells after the treatment with lemongrass oil as cell lysis, leakage of potassium and proteins from the cell and formation of abnormal shapes as well as an interaction of the oil with penicillin-binding proteins [21,22]. Still it can be concluded that also structures are involved that support cytoplasmatic and cell wall metabolism. The observations correspond to those of other authors who found a stimulated leakage of intracellular potassium ions and compared it with the effect of membrane active disinfectants in denaturing proteins, disrupting membrane structures and inducing cytoplasmatic leakage [23]. Besides that, electron microscopic pictures showed that a loss of electron dense material occurs, a coagulation of cell cytoplasma and a formation of extracellular blebs [6].

The other tea-tree oils manuka, kanuka, niaouli, and catjeput were also tested polarographically. Preliminary results show that manuka and kanuka are significantly and niaouli just a bit less effective than the true tea-tree oil. However, further investigations, especially calorimetric ones, are necessary to determine exact ratios and kinetics. The most interesting components of tea-tree oil, cineole and terpinen-4-ol, both solved in 90% ethanol, severely influenced the respiration rate of resting yeast cells, at least as strongly as tea-tree oil itself. These results which are in agreement with data from the literature [24–26] will be published elsewhere.

As the essential oils of tea-trees should partly reflect the biochemical composition and the concentration of special compounds in that plant, honey and the bee-glue propolis of identical origin should show a similar action spectrum. Both, honey and propolis in general, are well-known in the traditional medicine for a long time and also introduced into western welfare. Unfortunately, no tea-tree propolis and just manuka honey was commercially available in Germany. The lack of propolis from Australia may be due to the fact that Australia was originally free of honeybees and that propolis is harvested nowadays apparently only from grass trees (genus *Xanthorrhoea*) and eucalyptus [27]. Thus, just a few orientating experiments on respiration rates and growth of yeasts were performed with manuka honey. A 10 min incubation of yeasts in a glucose buffer with a final honey concentration of 6% reduced the metabolic rate by about 40%. A prolonged incubation brought no further inactivation. These values are small compared with data from the literature [28], but this may be due to the low quality of the commercial — and perhaps processed-honey in our experiments in contrast to a ''fresh'' honey directly obtained from the beekeeper (Molan, Hamilton/New Zealand, private communication). Further investigations in this direction including calorimetry are planned for the future.

Molan et al. [28–30] made a broad screening research for several hundred New Zealand honey samples to evaluate their effectivity against numerous microorganisms and compared them with phenol as a standard. Among them were *H. pylori*, *S. aureus* and *E. coli*. All sorts of honey are known to act by their high osmolarity and their production of hydrogen peroxide with effectivities up to 58% of that of phenol [28], but some of them also by compounds from floral sources. When the first two reasons for activity were excluded by dilution and addition of katalase (which splits hydrogen peroxide), only manuka honey remained effective, even in concentrations as low as 1.8% v/v [30]. The authors stated as resume that ''It is to be noted that *S. aureus*, a species that has developed resistance to many antibiotics and has become the predominant agent of wound sepsis in hospitals(. . .), is very susceptible to the antibacterial activity of honey, particularly to the non-peroxide activity of manuka honey.'' Here opens a new field for calorimetry and for comparison with the essential oils of tea-trees, specially that of the manuka tree.

References

[1] L. Balchin, S.L. Hart, J. Pharm. Pharmacol. 50(7) (1998) 809.
[2] A.R. Penfold, R. Grant, J. Proc. Royal Soc. New South Wales 59 (1925) 346.

[3] C.F. Carson, T.V. Riley, Lett. Appl. Microbiol. 16 (1993) 49.
[4] S.D. Cox, J.E. Gustafson, C.M. Mann, J.L. Markham, Y.C. Liew, R.P. Hartland, H.C. Bell, J.R. Warmington, S.G. Wyllie, Lett. Appl. Microbiol. 26 (1998) 355.
[5] N.B. Takaisi-Kikuni, D. Krüger, W. Gnann, J. Wecke, Microbios 88 (1996) 55.
[6] J.E. Gustafson, Y.C. Liew, S. Chew, J. Markham, H.C. Bell, S.G. Wyllie, J.R. Warmington, Lett. Appl. Microbiol. 26 (1998) 194.
[7] A.M. Janssen, J.J.C. Scheffer, A. Baerheim Svendsen, Planta Medica 53 (1987) 395.
[8] C.F. Carson, K.A. Hammer, T.V. Riley, Microbios 82 (1995) 181.
[9] C.M. Mann, J.L. Markham, J. Appl. Microbiol. 84 (1998) 538.
[10] P. Altmann, Cosm. Toil. Manufact. 12 (1991) 23.
[11] A. Remmal, T. Bouchikhi, A. Tantaoui-Elaraki, M. Ettayebi, J. Pharm. Belg. 48 (1993) 352.
[12] S. Pattnaik, V.R. Subramanyam, C.C. Rath, Microbios 84 (1995) 195.
[13] S. Koyama, Y. Yamaguchi, S. Tanaka, J. Motoyoshiya, Gen. Pharmac. 28 (1997) 797.
[14] G.W. Huisman, D.A. Siegele, M.M. Zambrano, R. Kolter, in: F.C. Neidhardt (Ed.), *Escherichia coli* and Salmonella, Cellular and Molecular Biology, 2nd ed., ASM Press, Washington, DC, 1996, p. 1672.
[15] A.I. Ivanov, V.M. Fomchenkov, Microbiologiia 58 (1989) 969.
[16] A.E. Beezer, B.Z. Chowdhry, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, p. 195.
[17] A.E. Beezer, B.Z. Chowdhry, Experientia 37 (1981) 828.
[18] D. Krüger, P. Giesbrecht, in: The Target of Penicillin, de Gruyter, Berlin, 1983, p. 273.
[19] D. Krüger, P. Giesbrecht, Experientia 45 (1989) 322.
[20] A.K. Mishra, N.K. Dubey, Appl. Environm. Microbiol. 60 (1994) 1101.
[21] E.O. Ogunlana, S. Höglund, G. Onawunmi, S. Sköld, Microbios 50 (1987) 43.
[22] G.O. Onawunmi, E.O. Ogunlana, Microbios Lett. 28 (1985) 63.
[23] J.F. Gardner, M.M. Peel, in: J.F. Gardner, M.M. Peel (Eds.), Introduction to Sterilization, Disinfection and Infection Control, 2nd ed., Churchill Livingston, London, 1991, p. 170.
[24] G.J. Rhee, K.S. Chung, E.H. Kim, H.J. Suh, N.D. Hong, Yakhak Hoeji 41(1) (1997) 132.
[25] A. Raman, U. Weir, S.F. Bloomfield, Lett. Appl. Microbiol. 21 (1995) 242.
[26] L.R. Williams, V.N. Home, Search 19 (1988) 294.
[27] B. König, Allg. Dtsch. Imker Ztg. (ADIZ) 19 (1985) 310.
[28] K.L. Allen, P.C. Molan, G.M. Reid, J. Pharm. Pharmacol. 43 (1991) 817.
[29] N. Al Somal, K.E. Coley, P.C. Molan, B.M. Hancock, J. Royal Soc. Med. 87 (1994) 9.
[30] D.J. Willix, P.C. Molan, C.G. Harfoot, J. Appl. Bacteriol. 73 (1992) 388.