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Microcalorimetry studies on the antimicrobial actions of volatile oil of dry ginger

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Abstract The rate of heat output is one of the suitable measurements of metabolic activity of the organism. In this article, microcalorimetry was first applied to study the effect of volatile oil of dry ginger (ginger oil) on *Escherichia coli* and *Staphylococcus aureus* growth. The power–time curves were plotted with a TAM air isothermal microcalorimeter. The parameters such as the growth rate constant μ, the peak-time T_p, inhibitory ratio I, and half-inhibitory concentration IC_{50} were calculated. From the data, the relationships between μ and the concentration of ginger oil c were established. The results revealed that the μ of *E. coli* and *S. aureus* both gradually declined with the increase of the c, there were linear relationships between μ and c, and ginger oil had stronger inhibitory effect on *S. aureus* than on *E. coli*. Results obtained from our study strongly suggest that microcalorimetry is an ideal method to investigate the effect of drug on microorganism.

Keywords Dry ginger · Volatile oil · *E. coli* · *S. aureus* · Microcalorimetry

Introduction

Ginger is a traditional Chinese medicine, which is belonging to the family of Zingiberaseae. Dry ginger which is widely used in the world as one of the important spice and traditional herbs is the dried root of Zingiber officinal Roscoe. A number of studies illustrated that ginger and its extracts present some pharmacological activities, including antioxidant activity [1], anti-inflammatory [2], and anti-rheumatic for musculoskeletal disorders [3]. Ginger oil is the volatile component extracted from dry ginger, the main components of which are sabinene, terpinen-4-ol, bsesquiphellandrene, triquinacene 1,4-bis (methoxy), triquinacene 1,4,7-tris (methoxy),1-[4-(1-methyl-2-propenyl) phenyl] ethanone, and ferulic acidmethyl ester [4]. The activities of ginger oil reported are as follows: modulator effects of immune response [5], moderate-to-severe knee pain [6], and antidepressant-like synergism [7]. It also has been reported that ginger oil could inhibit the growth of *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* and so on. Although the activity of ginger oil has been widely investigated, techniques used were qualitative and half quantitative with less information [8, 9].

Microcalorimetry, as a high sensitive and accurate technique, appears as a suitable technique to carry out both qualitative and quantitative comparative studies of microbial activity, which is a general analytical technique for the characterization of the microbial growth process. This technique has been widely used in study of the interactions between drugs and microorganism. The power–time curves of microorganism can be plotted by microcalorimeter. Using mathematical growth model, the growth rate constant μ, minimal inhibitory concentration MIC, peak-height P_m, peak-time T_p, total heat Q_{tot}, and a series of kinetic parameters can be calculated [10]. It allows for interaction in a heterogeneous medium; monitoring the process without disturbing the system; measuring the thermal effect of the system; and giving abundant thermodynamic and kinetic information [11, 12]. In recent years, microcalorimetry has been widely applied in the research of ligand binding studies, assessment of physical and chemical stabilities, and investigations of living systems [13–15], which

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has also been used to investigate the interaction between drugs and microbial cells [16–18].

The purpose of this study is to investigate the effect of ginger oil on *E. coli* and *S. aureus* by microcalorimetry.

Experimental

Instrument

A 3114/3236 TAM air microcalorimeter (Thermometric AB, Sweden) was used to determine the thermal effect of *E. coli* and *S. aureus*. It is consisted with a measurement system, a temperature-control system and a data output system. It is equipped with eight twin calorimetric channels with one side for the sample and the other for a static reference. Measurements are carried out in sealed 24 mL glass ampoules. Experiments can be carried out at the temperature range from 5 to 90 °C. The temperature error is within ±0.02 °C. The detection limit is 2 μw and baseline stability is 2×10^{-6} μw over a period of 24 h. PLW32 Recorder is used to monitor and record the heat output in real time [19, 20].

Materials

Bacteria *E. coli* and *S. aureus* were used as the test bacteria. They were routinely cultured in a Luria–Bertani (LB) culture medium composed of 5 g NaCl, 5 g yeast extract, and 10 g peptone per liter (pH 7.0–7.2). The LB culture medium was sterilized by autoclaving at 121 °C for 20 min before the experiment.

Ginger oil was distilled by steam extraction and diluted with DMF.

Methods

The microcalorimetric measurement was made with the ampoule method. One ampoule with bacteria suspension of *E. coli* or *S. aureus* was the blank control. Other ampoules added different concentrations of ginger oil. All the ampoules were with the final volume 10 mL. The ampoules were sealed and placed in the microcalorimeter. The temperature was controlled at 37 °C. The nutrient and oxygen were limited, because the growth process was monitored under isothermal and isochoric conditions. The power–time signals were recorded at an interval of 1 min.

Results

Microcalorimeter monitored the growth of bacteria in real time and plotted the power–time curves. On the basis of power–time curves and theoretical model, the growth rate constant μ, peak-time T_p, inhibitory ratio I, and half inhibiting concentration IC_{50} were calculated. The relationships between μ and c were also established.

Power–time curves

The power–time curves of the growth processes of *E. coli* and *S. aureus* without ginger oil are shown in Figs. 1 and 2. The heat production growth curves of *E. coli* and *S. aureus* can be divided into four phases, i.e., lag phase, exponential phase, stationary phase, and decline phase [21].

In the exponential phase, bacteria were in a good environment with sufficient nutrient matter and less metabolic products. It was suit to study the effect of drug on the bacteria. The exponential phases of *E. coli* and *S. aureus* with different concentrations of ginger oil are shown in

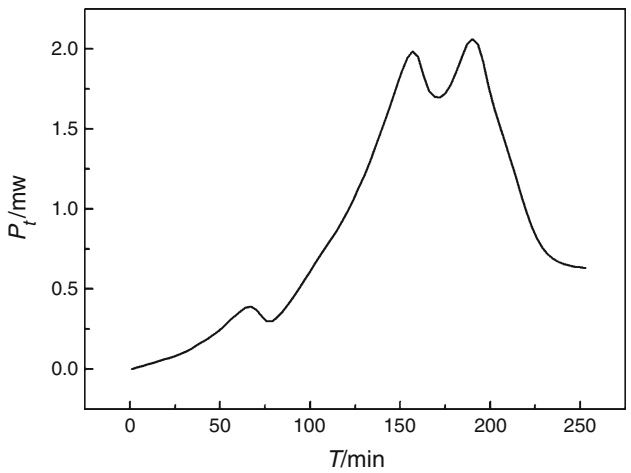

Fig. 1 Power–time curve of the growth processes of *E. coli* without ginger oil

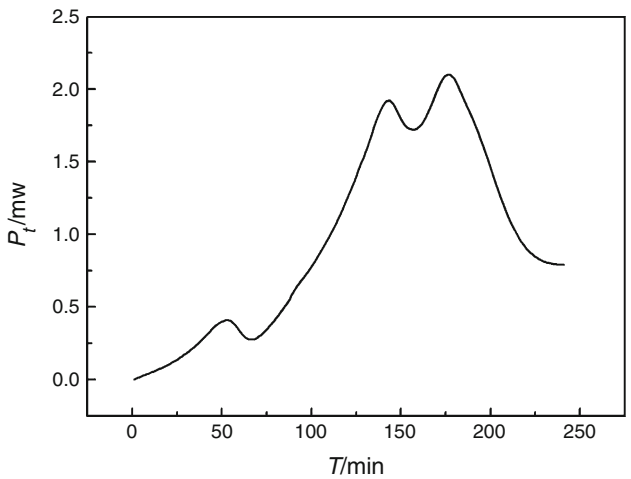

Fig. 2 Power–time curve of the growth processes of *S. aureus* without ginger oil

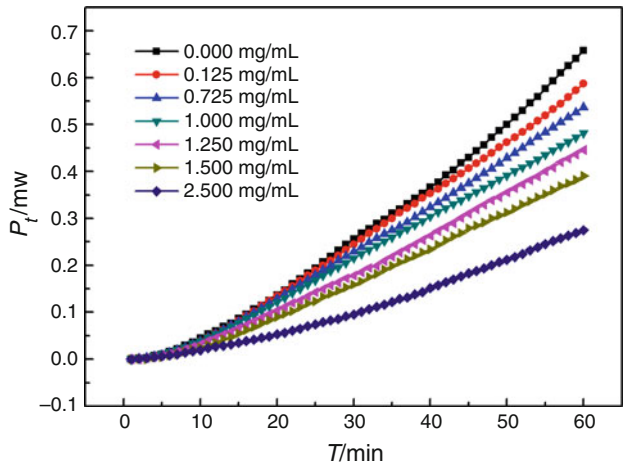

Fig. 3 Exponential phase of *E. coli* with different concentrations of ginger oil

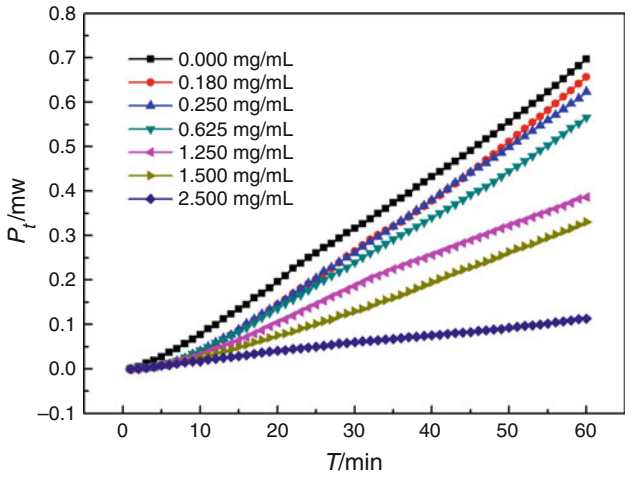

Fig. 4 Exponential phase of *S. aureus* with different concentrations of ginger oil

Figs. 3 and 4, respectively. As they are shown, with the increasing concentration of ginger oil, the heat production raised slowly.

Thermokinetics

In the exponential phase of growth, the power–time curves of *E. coli* and *S. aureus* obey the exponential equation [22]. If the bacteria number is n_0 at time 0, and n_t at time t, then:

$$n_t = n_0 \exp(\mu t), \tag{1}$$

where μ is the growth rate constant. If the power output of each bacteria is w, then:

$$n_t w = n_0 w \exp(\mu t). \tag{2}$$

If the heat output power is P_0 at time 0 and P_t at time t, then:

$$P_0 = n_0 w \text{ and } P_t = n_t w \text{ giving}$$
$$P_t = P_0 \exp(\mu t) \text{ or } \ln P_t = \ln P_0 + \mu t. \tag{3}$$

According to Eq. 3, the rate constant μ can be calculated according to the data P_t and t obtained from the power–time curves.

The inhibitory ratio I is a good index to indicate the inhibition of drug on metabolism of bacteria and it can be defined as:

$$I = [(\mu_0 - \mu_c)/\mu_0] \times 100\%. \tag{4}$$

where μ_0 and μ_c are the growth rate constant of bacteria without and with ginger oil, respectively. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called half-inhibitory concentration (IC_{50}).

The corresponding values of μ, T_p, I, and IC_{50} are shown in Tables 1 and 2, where r is correlation coefficient, T_p is the value of the first peak-time, and IC_{50} is the half inhibiting concentration.

Relationship between μ and c

From Tables 1 and 2, it could be seen that the μ of *E. coli* and *S. aureus* gradually declined with the increase of the c, which showed that ginger oil had inhibitory effect on the growth of *E. coli* and *S. aureus*. When the range of con-

Table 1 Parameters of *E. coli* growth at different concentrations of ginger oil

c/mg mL^{-1}	$\mu/10^{-3}$ min^{-1}	r	T_p/min	I/%	IC_{50}/mg mL^{-1}
0	8.716	0.9989	80	–	
0.125	8.450	0.9994	81	3.05	
0.750	7.540	0.9995	89	13.39	
1.000	6.934	0.9994	98	20.45	2.439
1.250	6.482	0.9988	100	25.63	
1.500	5.879	0.9987	111	33.55	
2.500	4.250	0.9990	128	51.24	

Table 2 Parameters of *S. aureus* growth at different concentrations of ginger oil

c/mg mL^{-1}	$\mu/10^{-3}$ min^{-1}	r	T_p/min	I/%	IC_{50}/mg mL^{-1}
0	8.956	0.9996	70	–	
0.180	8.823	0.9991	71	1.49	
0.250	8.604	0.9996	80	3.99	
0.625	7.815	0.9987	83	13.26	1.663
1.250	5.855	0.9999	89	39.68	
1.500	5.017	0.9987	98	43.98	
2.500	1.769	0.9999	106	80.25	

centration of ginger oil was 0–2.5 mg mL^{-1}, there was a linear relationship between μ and c, which could be established as follows:

$$\text{For } E.\,coli: \mu = -0.0018c + 0.0087\ (r = 0.9954);$$
$$\text{For } S.\,aureus: \mu = -0.0029c + 0.0093\ (r = 0.9904).$$

According to Eq. 4, the inhibitory ratios I were calculated, which are shown in Tables 1 and 2. The inhibitory ratios I become bigger with the increasing of c. And the IC_{50} of ginger oil on *E. coli* and *S. aureus* were 2.439 and 1.663 mg mL^{-1}, respectively. From the data, we could get a conclusion that ginger oil had a stronger inhibitory effect on *E. coli* than that on *S. aureus* growth.

Relationship between T_p and c

As seen from Tables 1 and 2, the first peak-time T_p with ginger oil was longer than that of the blank control. An increase in the values of T_p was observed when c was increasing. There was similar characteristic on *E. coli* and *S. aureus*. The results indicated that ginger oil had dose-dependent inhibitory effect on the bacteria.

Discussion

The μ of *E. coli* and *S. aureus* gradually declined with the increase of the concentration of ginger oil. And there were linear relationships between μ and c. They were mainly because some bacteria were inhibited or killed, and some metabolized continuously which maintained at a lower heat production rate. This rate directly depended on the concentration of ginger oil [23]. Compared to the blank, the concentration of ginger oil increased the first peak-time T_p prolonged. This was mainly because after the treatment of ginger oil, the growth and metabolism of bacteria became slow. Overall, the information indicated that ginger oil had inhibitory effect on the growth of *E. coli* and *S. aureus*. It also could be inferred that they had similar characteristics according to the relationship between μ and c. The effect of other volatile oils on *E. coli* by microcalorimetry had been reported, such as Radix et Rhizoma Rhei Palmati, Herba Artemisiae Capillaris, and Herba Ephedrae Sinicae [24], which were similar with ginger oil, the volatile oils had inhibitory effect on *E. coli* with linear relationships between μ and c. The IC_{50} of ginger oil on *E. coli* and *S. aureus* were 2.439 and 1.663 mg mL^{-1}, respectively. It could be indicated that ginger oil had a greater inhibitory effect on *S. aureus* than on *E. coli*. The microcalorimetric method was successfully used to analyze the inhibitory effect of different concentrations of ginger oil. Heating output was an effective method to evaluate the metabolic activity of bacteria and investigate the effect of drugs and bacteria. While, conventional bacteriological technique, for example, agar diluted method [8, 9], according to the size of inhibition zone on agar plate, we can get the information IC_{50} and the MIC. However, it has such disadvantages as strenuousness, long laboratory cycle, and a visual representation of information. Our study proved that the microcalorimetry was a quantitative, inexpensive, versatile, and convenient method. It provided kinetic and thermodynamic information which could not be obtained with conventional bacteriological technique. And all this information was interesting for studying the effect of drugs on microbes. Microcalorimetry was an useful technique that could monitor the metabolic activity of microbes.

Conclusions

In this research, microcalorimetry was used to study the inhibitory effect of ginger oil on the growth of *E. coli* and *S. aureus*. We got series of kinetic parameters, such as rate constant μ, the peak-time T_p, inhibitory ratio I, half-inhibitory concentration IC_{50}, and the relationship of μ and c. The μ of the *E. coli* growth gradually declined with the increase of the concentration of ginger oil. And *S. aureus* had similar characteristics. According to the inhibitory ratio I of different concentration of ginger oil on the bacteria, we could draw the conclusion that ginger oil showed stronger inhibitory effect on *S. aureus* than on *E. coli*. The study also proved that microcalorimetry is an useful technique that can be applied to study microbial growth and estimate the efficiency of drugs.

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References

1. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (Zingiber officinale). Food Chem. 2007;102:764–70. doi:10.1016/j.foodchem/2006/06/023.
2. Altman RD, Marcussen KC. Effects of a ginger extract on knee pain in patients with osteoarthritis. Arthr Rheum. 2001;44:2531–8.
3. Srivastava KC, Mustafa T. Ginger (*Zingiber officinale*) in rheumatism and musculoskeletal disorders. Med Hypotheses. 1992;39:342–8.
4. Yang ZN, Luo SQ, Peng QC, Zhao C, Yu ZW. GC–MS analysis of the essential oil of coral ginger (Zingiber corallinum Hance) rhizome obtained by supercritical fluid extraction and steam distillation extraction. Chromatographia. 2009;69:785–90. doi:10.1365/s10337-009-0971-9.

5. Zhou HL, Deng YM, Xie QM. The modulatory effects of the volatile oil of ginger on the cellular immune response in vitro and in vivo in mice. J Ethnopharmacol. 2006;105:301–5. doi:10.1016/j.jep/2005/10/022.
6. Yip YB, Tamb ACY. An experimental study on the effectiveness of massage with aromatic ginger and orange essential oil for moderate-to-severe knee pain among the elderly in Hong Kong. Complement Ther Med. 2008;16:131–8. doi:10.1016/j.ctim/2007/12/003.
7. Yi LT, Xu Q, Li YC, Yang L, Kong LD. Antidepressant-like synergism of extracts from magnolia bark and ginger rhizome alone and in combination in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:616–24. doi:10.1016/j.pnpbp/2009/03/001.
8. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 2004;94:223–53. doi:10.1016/j.ijfoodmicro/2004/03/022.
9. Liu Y, Zhang WM, Shan CY, Liu Y, Jiang HF. Study on the antimicrobial activity of ginger essential oil. Sci Technol Food Ind. 2008;29:88–90 (in Chinese).
10. Yang LN, Xu F, Sun LX, Zhao ZB, Song CG. Microcalorimetric studies on the antimicrobial actions of different cephalosporins. J Therm Anal Calorim. 2008;93:417–21.
11. Critter SAM, Freitas SS, Airoldi C. Calorimetry versus respirometry for the monitoring of microbial activity in a tropical soil. Appl Soil Ecol. 2001;18:217–27.
12. Yan D, Jin C, Xiao XH, Dong XP. Investigation of the effect of berberines alkaloids in Coptis chinensis Franch on *Bacillus shigae* growth by microcalorimetry. Sci China Ser B. 2007;50:638–42.
13. Wadsö I. Isothermal microcalorimetry near ambient temperature: an overview and discussion 1. Thermochim Acta. 1997;294:1–11.
14. Wadsö I. Isothermal microcalorimetry: current problems and prospects. J Therm Anal Calorim. 2001;64:75–84.
15. Wadsö I. Microcalorimetric techniques for characterization of living cellular systems. Will there be any important practical applications? Thermochim Acta. 1995;269/270:337–50.
16. Wu YW, Gao WY, Xiao XH, Liu Y. Calorimetric investigation of the effect of hydroxyanthraquinones in Rheum officinale Baill on *Staphylococcus aureus* growth. Thermochim Acta. 2005;429:167–70.
17. Zhang HL, Yu XF, Li XX, Pan XR. A study of promotive and fungistatic actions of steroidal saponin by microcalorimetric method. Thermochim Acta. 2004;416:71–4.
18. Li X, Liu Y, Wu J, Qu SS. The effect of the selenomorpholine derivatives on the growth of *Staphylococcus aureus* studied by microcalorimetry. Thermochim Acta. 2001;375:109–13.
19. Wadsö I. Isothermal microcalorimetry in applied biology. Thermochim Acta. 2002;394:305–11.
20. Kong WJ, Zhao YL, Shan LM, Xiao XH, Guo WJ. Microcalorimetric studies of the action on four organic acids in *Radix isatidis* on the growth of microorganisms. Sheng Wu Gong Cheng Xue Bao. 2008;24:646–50 (in Chinese).
21. Xie CL, Tang HK, Song ZH, Qu SS, Liao YT, Liu HS. Determination of thermograms of bacterial growth. Wei Sheng Wu Xue Bao. 1989;29:149–51 (in Chinese).
22. Xu XJ, Xue Z, Qi ZD, Hou AX, Li CH, Liu Y. Antibacterial activities of manganese(II) ebselen-porphyrin conjugate and its free components on *Staphylococcus aureus* investigated by microcalorimetry. Thermochim Acta. 2008;476:33–8. doi:10.1016/j.tca/2008/07/007.
23. Li X, Liu Y, Wu J, Liang HG, Qu SS. Microcalorimetric study of *Staphylococcus aureus* growth affected by selenium compounds. Thermochim Acta. 2002;387:57–61.
24. Yu Y, Yu M, Zhang HL. Microcalorimetric study of volatile oil on *Escherichia coli*. J Qufu Norm Univ. 2008;34:85–7 (in Chinese).