Microcalorimetric investigation of the antibacterial activity of curcumin on *Staphylococcus aureus* coupled with multivariate analysis

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Abstract The antibacterial effect of Curcumin on *Staphylococcus aureus* growth was evaluated by microcalorimetry. The heat flow power–time curves and nine quantitative parameters of the *S. aureus* growth were applied to investigate the inhibitory effect with Curcumin. By analyzing these curves and some quantitative parameters using multivariate analytical methods, similarity analysis and principal component analysis, the antibacterial activity of Curcumin on *S. aureus* could be accurately evaluated from the change of the two main parameters, the second exponential growth rate constant k_2 and the maximum heat flow power P_m^2. The main two thermal parameters played more important role in the evaluation: at low concentration (0–10.5 µg mL^{-1}), Curcumin hardly influence the growth of *S. aureus*, while at high concentration (10.5–43.4 µg mL^{-1}) it could notably inhibit the growth. All these illustrated that the antibacterial activity of Curcumin on *S. aureus* was enhanced with the increase of the concentration of this compound. This study might provide an useful method and idea accurately evaluate the antibacterial effects of Curcumin, which provides some useful methods for evaluate the nature antibacterial agents.

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Introduction

Staphylococcus aureus is the most significant human pathogen among the staphylococci, as other species are considered as opportunists [1]. It can be detected in water, dust, air, food, and food processing or storage, which is a frequent cause of diseases in humans and animals, even life-threatening infections, including endocarditis, septic arthritis, osteomyelitis, and alimentary toxinfection. Therefore, it is necessary and urgent of developing an effective food preservative and food additives, which possess antimicrobial activities.

Food safety is of major concern both to consumer and food industries as there are increasing numbers of food-associated infections. Consumers prefer foods of high nutritional quality with little or no chemical preservatives due to their adverse effect [2]. Recent years have seen an increasing demand in developing new food preservation techniques or natural food additives to prevent food contamination. In fact, herbs and spices have been used to prevent spoilage and extend shelf life of foods for centuries [3, 4]. Many researches showed that food additives (i.e., essential oils etc.) with antimicrobial activity might be against food-borne pathogens [5, 6]. Furthermore, researchers have reported that many plants extract possessed antimicrobial activity against *S. aureus* [7, 8]. Curcumin (diferuloylmethane)-(1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural compound isolated from the dietary spice turmeric [9], which is widely applied as food additive. Researches indicated that extracts of turmeric have anti-fungal and antibacterial activities [10–13]. In turmeric, curcuminoids have

been taken as active ingredients, which mainly contain Curcumin and its natural analogs: demethoxycurcumin and bisdemethoxycurcumin [14, 15]. As Curcumin is the major component in turmeric, it is assumed that Curcumin might contribute to the effectiveness of turmeric in food preservation. However, no scientific study has been reported on the activity of Curcumin against food-borne pathogens. In this study, we evaluated the antimicrobial activity of Curcumin against *S. aureus* by microcalorimetry coupled with multivariate analysis.

Microcalorimetry, considered to be an efficient, noninvasive, and versatile method for measuring the heat production in many fields, could be used to monitor the growth of bacterium. Microcalorimetry provides a means of studying the energetics of biomolecular processes directly at the molecular and cellular levels. It can be applied to reflect various reaction processes of physics, chemistry, life science, and environmental science [16, 17]. Besides the good sensitivity, accuracy, and reproducibility, microcalorimetry has some peculiar advantages in the new drug discovery and response for the antibacterial challenge. For example, continuous, real-time, quantitative detecting can be realized to obtain abundant thermokinetic/dynamic information or quantitative structure–activity relationship, even the information about the mechanism of action of the antimicrobial drug [18, 19]. As an established procedure, microcalorimetry has been extensively applied as an useful method for rapid screening antimicrobial drugs on *Escherichia coli*, *Bifidobacterium adolescentis*, *Shigella dysenteriae*, *Candida albicans*, mice *splenic lymphocytes* [20–24], etc. By analyzing the heat flow power–time curves of microbial metabolism, kinetic parameters such as rate constant for bacterial growth, peak power, and the heat flow for microbial activity could be obtained. Therefore, the antibacterial investigation using appropriate approach should be important index for antibacterial drugs.

In this study, the thermal activity monitor (TAM III) isothermal microcalorimeter was used to investigate the inhibitory effect of Curcumin on *S. aureus* growth coupled with multivariate analysis. The aim is to provide an useful idea for investigating the effect of drugs on microbes by the combination of microcalorimetry and multivariate analysis.

Experimental

Materials

Curcumin (purity ≥98%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100051, P. R. China. Ethanol (EtOH, analytical purity grade). Curcumin was dissolved in EtOH

with a concentration of 2.65 mg mL^{-1}. Different concentration of Curcumin solution was freshly prepared before use. All the glasswares were soaked in chromic acid for at least 24 h and sterilized by autoclave at 121 °C for 30 min before use. Triply distilled water was used throughout the study.

Bacterial strain and cultural methods

Staphylococcus aureus (*S. aureus* CCTCCAB910393) was provided by China Center for Type Culture Collection, Wuhan University, Wuhan, P. R. China. The bacterial strain was propagated in broth culture medium, which contains peptone (10 g), beef extract (6 g), and NaCl (5 g) per 1000 mL (pH 7.0–7.2). The culture medium was sterilized by autoclave at 121 °C for 30 min. *S. aureus* were inoculated in 25 mL broth culture medium and incubated with shaking (120 rev min^{-1}) for 8 h at 37 °C. Bacteria were prepared for microcalorimetric measurements. The strain was stored at 4 °C before inoculation. Luria–Bertani (LB) culture medium per 1000 mL contains (pH 7.0–7.2) peptone (10 g), yeast extract (5 g), and NaCl (5 g). The culture medium was sterilized autoclave at 121 °C for 30 min and stored in a refrigerator at 4 °C before use.

Instruments

The thermal activity monitor (TAM III, Thermometric, Järfälla, Sweden) isothermal microcalorimeter was used to determine the growth heat of *S. aureus* cells. TAM III thermostats can be set at any temperature within an instrument's performance range (e.g., 15–150 °C) with high accuracy, typically within 0.02 °C. In this instrument, each channel is a twin calorimeter where one unit positioned on top of the other. One of the multi-channel calorimetric units is set as reference. In this study, the microcalorimetric thermostat was set at 37 °C. For details of the instrument, see [25]. The TAM III was operated in static ampoule mode (see [16]).

Microcalorimetry analysis

4 mL ampoules with prepared cultures (see above) were placed into the TAM III. In all experiments, heat flow (Q/t μW) was measured. The experiment procedure was as follows: all 4.0 mL ampoules were cleaned, sterilized and filled with 1.5 mL of LB medium containing *S. aureus* at an initial density of 1×10^6 CFU mL^{-1}. The experimental group was then added at different concentrations of Curcumin at the beginning of the experiment. The culture medium with samples was filled in glass ampoules and sealed. After equilibration for at least 45 min at 37 °C, the ampoules were lowered into the equilibration position,

waiting for recorder returned to the baseline, and then the heat flow power–time curves were began to record. All data were collected continuously until the recorder returned to the baseline using the dedicated software package.

Multivariate analysis

Similarity analysis (SA)

By employing SA, the heat flow power–time curves of *S. aureus* growth affected by different concentrations of Curcumin could be evaluated by their similarities, which come from the calculation on the correlative coefficient of original data. Thus, the correlation coefficient of the *S. aureus* growth curves without Curcumin and the *S. aureus* growth curves affected by different concentrations of Curcumin were calculated with included angle cosine using software of Microsoft Excel 2003 [26].

Principle component analysis (PCA)

The PCA is the simplest of the true eigenvector-based multivariate analysis; it could reveal the internal structure of the multivariate dataset by "shadow" the variables in a lower-dimension picture. It reduces the dimensionality of the original data set by explaining the correlation among a large number of variables in terms of a smaller number of underlying factors (principal components or PCs) without losing much information [27]. In recent years, PCA had been widely used in many fields, especially in life science [28–30]. Here, the quantitative thermokinetic parameters obtained by analyzing the heat flow power–time curves of *S. aureus* growth affected by Curcumin were selected for PCA using SAS statistical software (SAS for Windows 8.0, SAS Inc., USA).

Results and discussions

The heat flow power–time curves of *S. aureus* growth at 37 °C

The growth thermogenic curve of *S. aureus* growth at 37 °C without any substance is shown in Fig. 1. It was a typical heat flow power–time curve of *S. aureus* and could be divided into two stages (stages I and II) and four phases, i.e., the first exponential phase (A, B), the stationary phase (B, C), the second exponential growth phase (C, D), and a decline phase (D, E) [31].

The corresponding heat flow power–time curves of *S. aureus* growth affected by different concentration of Curcumin are shown in Fig. 2. As could be seen from the
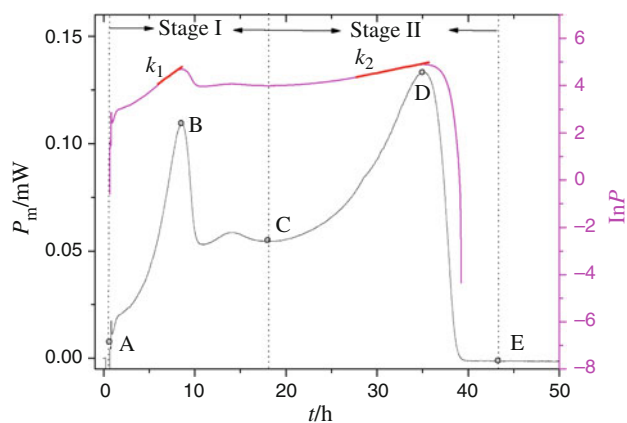

Fig. 1 The power–time curve of *S. aureus* growth at 37 °C without any substance. It was a typical metabolic profile of *S. aureus* culturing in LB culture medium supplemented without any substance monitored by the microcalorimeter at 37 °C and could be divided into two stages (stages I and II) and four phases: the first exponential phase (A, B), the stationary phase (B, C), the second exponential growth phase (C, D) and a decline phase (D, E). The straight line (k_1, k_2) in the upper curve is the fit linear results of the corresponding phases
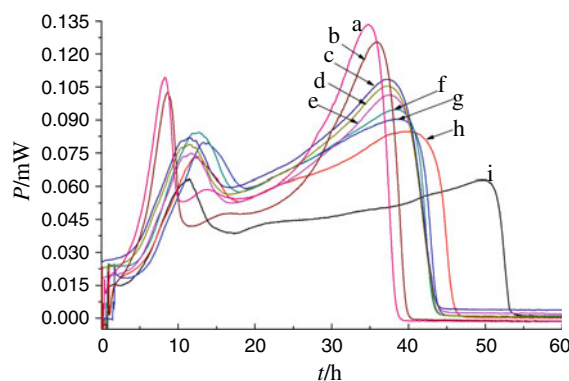

Fig. 2 The heat flow power–time curves o f *S. aureus* growth at 37 °C affected by different concentrations of Curcumin. The concentrations of Curcumin were (*a*) 0, (*b*) 7.2, (*c*) 10.5, (*d*) 14.5, (*e*) 18.1, (*f*) 21.7, (*g*) 31.0, (*h*) 36.2, and (*i*) 43.4 μg mL^{-1}, respectively. These curves changed with the increase of concentration of Curcumin, showing that different concentrations of Curcumin had varied effects on *S. aureus*

profile of those curves, the growth of *S. aureus* was influenced by curcumin apparently.

Mechanisms of *S. aureus* diauxic growth

Microcalorimetry provides a continuous real-time electronic signal proportional to the amount of heat being produced by an ampoule containing microorganisms. It is possible to study energy metabolism directly by energy variations. It is capable of providing a lot of kinetic and thermodynamic information for the study of growth process of living microbial. The combination of the calorimetric investigations with biomass, carbon sources, and

metabolites provides a systematic view of the energy metabolism for the investigated microbial cell. In this study, the TAM III isothermal microcalorimeter was used to investigate the inhibitory effect of Curcumin on *S. aureus* growth by the heat flow power–time curves. However, as heat flow is an excellent proxy for microbial activity, the heat evolved provides valuable information. Heat flow and activity reflect metabolic rates and, on the other hand, heat is an indication of the quantity of substrate consumed or metabolic product released. Moreover, in certain cases, heat is also directly linked to the electron acceptors consumed, and their reduced counterpart produced.

This *S. aureus* growth heat flow power–time curves is obtained in a batch culture, a culture in which the medium is retained in a limited volume and is not supplied with fresh nutrients at any stage of growth. When a bacterium is inoculated into a flask containing fresh culture medium and incubated, this phase represents an active growth during which the bacteria prepare for reproduction, synthesizing DNA etc. The bacteria were transferred to a new medium or fresh nutrients were added to the medium. Therefore, during this phase, there may be increase in size (volume) and metabolic rates speed up, on the other hand, heat is an indication of the quantity of metabolic product released quickly (Fig. 1A–B). Since the bacteria are not transferred to a new medium or no fresh nutrients are added to the medium, it might be possible that the cells may be old and depleted of ATP (B–C). Heat flow degrade the bacteria cells until it divide by subsequent binary fissions and increases its population at a maximal rate and their generation time reaches a minimum and remains constant (C–D). The growth in this phase is quite balanced (i.e., all cellular constituents are synthesized at constant rates relative to each other) hence, the most uniform in terms of chemical and physiological properties, the log phase cultures are usually used in biochemical and physiological studies. This phase is called log phase because the logarithm of the bacterial mass increases linearly with time, and bacterial cells are most active metabolically, and in industrial production, this is the period of peak activity and efficiency.

Since the bacteria are growing in a constant volume of medium of batch culture, and no fresh nutrients are added, the bacterial cells start dying and the number of such cells balances the number of newborn cells, and the bacterial population stabilizes. Eventually, the number of dying cells begins to exceed the number of newborn cells and thus the number of viable bacterial cells present in a batch culture starts declining (D–E). This condition represents the bacterial death or decline phase that continues until the population is diminishing to a tiny fraction of more resistant cells, or it may die out entirely. Like exponential growth, death is also exponential, but inverse, as the number of viable bacterial cells decreases exponentially.

Calculation of the growth rate constant of *S. aureus*

The exponential model of metabolism of *S. aureus*, the heat flow power–time curves obeyed the following equation [32]:

$$P_t P_0 \exp(kt) \text{ or } \ln P_t = \ln P_0 + kt, \tag{1}$$

where P_0 represents the heat flow power at time $t = 0$, and P_t represents the power at time t. Thus, using the data $\ln P_t$ and t taken from the curves to fit a linear equation, the growth rate constants k of the quick growth phase of *S. aureus* at 37 °C without curcumin could be calculated. The relative standard deviation (RSD) for k_1 and k_2 was 1.67 and 1.64%, respectively, showing that good reproducibility was obtained under identical experimental conditions.

Thus, other quantitative thermokinetic parameters, such as the maximum power output P_m^1 in the first exponential phase and P_m^2 in the second exponential phase, the appearance time of the maximum power output t_m^1 in the first exponential phase and t_m^2 in the second exponential growth phase, the heat output Q_{sta}^I of stage I and Q_{sta}^{II} of stage II, and the total heat output Q_t were calculated from the heat flow power–time curve of *S. aureus* growth affected by Curcumin are shown in Table 1.

Relationship between quantitative thermokinetic parameters and concentration of Curcumin

In order to explain the thermal parameters and the concentration of Curcumin, 2D histograms of the relationship between quantitative thermokinetic parameters are shown in Table 1 and concentration of Curcumin is shown in Fig. 3. It could be concluded from these histograms that in the range of 0–43.4 μg mL^{-1}, the values of k_2 and P_m^2 decreased while t_m^2 prolonged with the concentration of curcumin increased. The values of Q_{sta},^{II} and Q_t increased with the increasing of curcumin at a lower concentration (0–10.5 μg mL^{-1}), then decreased with curcumin increasing at a higher concentration (10.5–43.4 μg mL^{-1}). While the values of other quantitative parameters such as k_1, t_m^1, P_m^1, and Q_{sta},^{I} fluctuated irregularly with a concentration range (0–43.4 μg mL^{-1}) of curcumin. This phenomenon made it difficult to evaluate the effect curcumin on *S. aureus* growth objectively and exactly. So it was needed to employ some suitable Chemometrics methods in this evaluation.

Multivariate analysis

SA

The differences of heat flow power–time curves of *S. aureus* growth affected by Curcumin were evaluated by SA

Table 1 Quantitative thermokinetic parameters for *S. aureus* growth at 37 °C affected by different concentrations of Curcumin

$c/\mu g\ mL^{-1}$	k_1/h^{-1}	R_1	t_m^1/h	P_m^1/mW	k_2/h^{-1}	R_2	t_m^2/h	P_m^2/mW	Q_{sta}^I/J	Q_{sta}^{II}/J	Q_t/J
0	0.3258 ± 0.0032	0.9998 ± 0.0002	8.6355 ± 1.0024	0.1094 ± 0.0012	0.1204 ± 0.0018	0.9983 ± 0.0005	34.7427 ± 1.0235	0.1335 ± 0.0018	2.0895 ± 0.0199	7.7689 ± 0.0698	9.8584 ± 0.0898
7.2	0.3228 ± 0.0035	0.9994 ± 0.0003	8.6907 ± 1.1003	0.1024 ± 0.0015	0.1098 ± 0.0024	0.9985 ± 0.0004	35.7757 ± 1.0345	0.1255 ± 0.0021	2.0809 ± 0.0162	7.6433 ± 0.0674	9.7242 ± 0.0821
10.5	0.1523 ± 0.0028	0.9986 ± 0.0003	11.4078 ± 0.9678	0.0820 ± 0.0024	0.0738 ± 0.0025	0.9994 ± 0.0003	37.3641 ± 1.0028	0.1085 ± 0.0024	2.0385 ± 0.0166	8.8875 ± 0.0676	10.9260 ± 0.0822
14.5	0.1452 ± 0.0024	0.9995 ± 0.0002	11.4083 ± 0.7835	0.0789 ± 0.0027	0.0631 ± 0.00188	0.9995 ± 0.0002	37.3648 ± 1.1098	0.1054 ± 0.0023	2.0285 ± 0.0169	8.6544 ± 0.0717	10.6829 ± 0.0819
18.1	0.1596 ± 0.0025	0.9994 ± 0.0004	11.417 ± 0.6758	0.0749 ± 0.0025	0.0533 ± 0.00198	0.9995 ± 0.0006	37.6223 ± 0.9976	0.1014 ± 0.0019	2.0337 ± 0.0189	8.424 ± 0.0658	10.4577 ± 0.0917
21.7	0.1404 ± 0.0023	0.9920 ± 0.0009	12.2465 ± 0.5683	0.0843 ± 0.0028	0.0469 ± 0.00181	0.9979 ± 0.0008	38.6053 ± 0.8943	0.0947 ± 0.0015	2.0497 ± 0.0175	8.2876 ± 0.0654	10.3373 ± 0.0809
31.0	0.1392 ± 0.0026	0.9991 ± 0.0006	12.5103 ± 0.4678	0.0798 ± 0.0031	0.0329 ± 0.00191	0.9931 ± 0.0010	38.2980 ± 1.1065	0.0905 ± 0.0022	2.032 ± 0.0181	7.8470 ± 0.0668	9.8790 ± 0.0829
36.2	0.1488 ± 0.0022	0.9993 ± 0.0002	12.0068 ± 0.9867	0.0731 ± 0.0025	0.0274 ± 0.00197	0.9945 ± 0.0016	40.3513 ± 1.0089	0.0848 ± 0.00185	2.0458 ± 0.0176	7.7481 ± 0.0658	9.7939 ± 0.0814
43.4	0.1458 ± 0.0019	0.9973 ± 0.0008	11.3713 ± 0.1004	0.0629 ± 0.0027	0.0146 ± 0.00192	0.9982 ± 0.0009	49.7940 ± 1.0104	0.0632 ± 0.0019	1.859 ± 0.0185	7.8045 ± 0.0653	9.6635 ± 0.0828

R correlation coefficient

The data were expressed as means ± SD of triplicate parallel experiments

and expressed by correlation coefficient. The correlation coefficient of *S. aureus* growth curves without curcumin and those of *S. aureus* growth curves affected by Curcumin was calculated and listed as 0.9992, 0.9610, 0.9453, 0.8906, 0.8562, 0.8214, 0.8178, and 0.7086. These results showed that the influence of curcumin on the *S. aureus* growth curves was changeable. The diminishing of correlation coefficient also showed that the change was enhanced with the concentration of Curcumin increase. All these illustrated that Curcumin at different concentrations had varied effects on *S. aureus* growth.
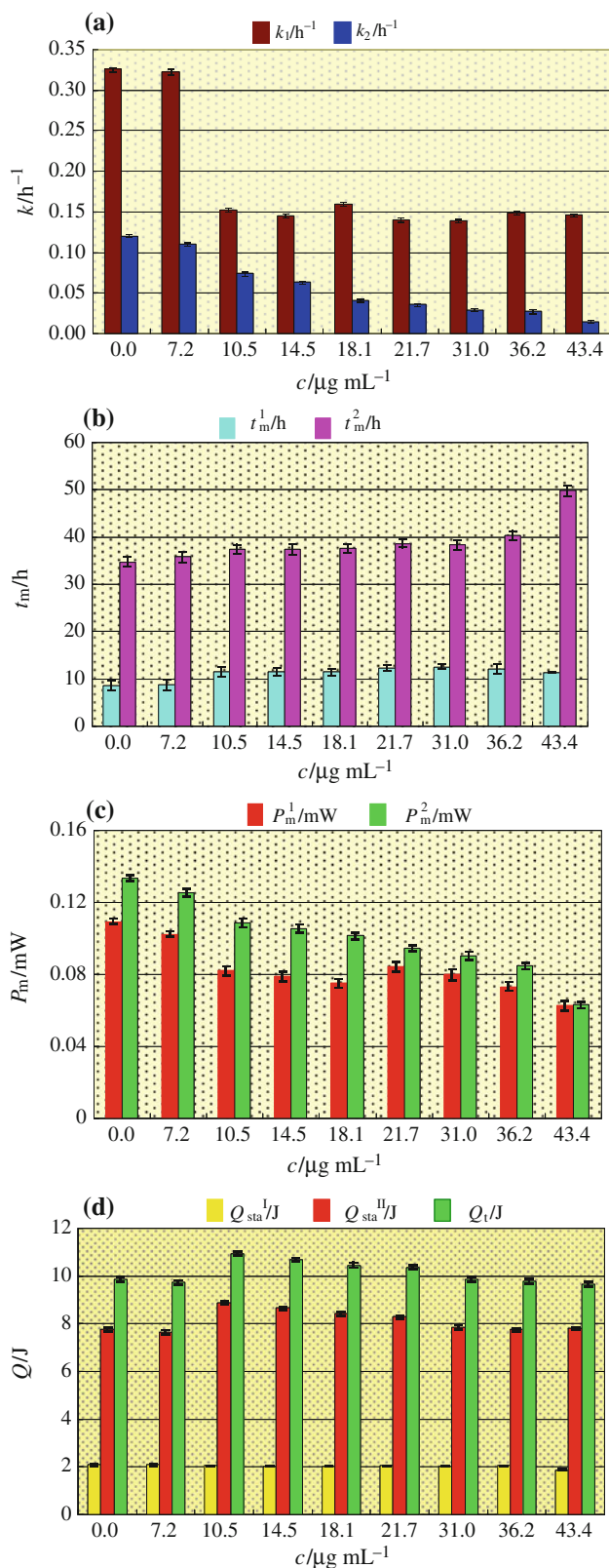
PCA

The PCA is a popular method in applied statistical work and data analysis, and it has a good ability to summarize multivariate variation. It allows visualizing the information of the data set in a few principal components retaining the maximum possible variability within that set. PCA was employed to reduce the computation burden. Therefore, the nine quantitative thermokinetic parameters (k_1, t_m^1, P_m^1, k_2, t_m^2, P_m^2, Q_{sta}^I, Q_{sta}^{II}, and Q_t in Table 1) obtained by analyzing the heat flow power–time curves of *S. aureus* growth affected by Curcumin were performed for PCA using statistics software of Windows SAS 8.0 (SAS for Windows 8.0, SAS Inc., USA). The result of the PCA showed that the first two principal components (Z_1 and Z_2) contained 90.598% of the information of the original seven indexes from the results of "Eigenvalue of the Correlation Matrix", the equations of which were:

$$Z_1 = 0.900k_1 - 0.8477t_m^1 + 0.933P_m^1 + 0.958k_2 - 0.803t_m^1 + 0.968P_m^1 + 0.783Q_{sta}^I - 0.179Q_{sta}^{II} - 0.063Q_t$$

$$Z_2 = -0.401k_1 + 0.352t_m^1 - 0.061P_m^1 + 0.540k_2 - 0.485t_m^2 + 0.640P_m^2 + 0.367Q_{sta}^I + 0.434Q_{sta}^{II} + 0.470Q_t.$$

The absolute value of the coefficient before the nine quantitative thermokinetic parameters was the coefficient between the principal component and the parameter. The bigger the coefficient of the parameter was the better correlation the principal component had with the parameter. The above equations showed that the values of Z_1 and Z_2 (the first and second principal components) were mainly decided by k_2 and P_m^2. The results showing that parameters of the second exponential growth rate constant k_2 and the maximum heat flow power for second exponential phase P_m^2 were main two parameters which played more important role in evaluating the effect of Curcumin. By which, we could quickly and clearly evaluate the effect and the potency of this compound on *S. aureus* growth.

(a)

(b)

(c)

(d)

Fig. 3 Relationships between quantitative thermokinetic parameters and concentration of Curcumin. The column maps of the nine quantitative thermokinetic parameters **a** the growth rate constants k_1 and k_2, **b** the maximum power output P_m^1 and P_m^2, **c** the appearance times of the maximum power output t_m^1 and t_m^2, and **d** the heat output Q_{sta}^I, Q_{sta}^{II}, and Q_t

main parameter of the second exponential growth rate constant k_2. Inhibitory ratio I was defined as [33] $I\% = [(k_0 - k_c)/k_0] \times 100\%$, where k_0 was growth rate constant of the control, k_c was rate constant in the exponential phase of bacterial growth inhibited by inhibitor concentration c. In addition, we can obtain the $I–c$ curve (Fig. 4a). As shown in Fig. 4, the relationship between I and c (Curcumin) is not linear. With the increase in concentrations of Curcumin, the value of inhibitory ratio I increased. When the range of concentration of Curcumin c is 10.5–43.4 μg mL^{-1}, analyses of the inhibitory ratio I and the corresponding Curcumin concentration c, the I versus c was established: $I = 0.0142c + 0.2746$ ($r = 0.9907$, linear range: 10.5–43.4 μg mL^{-1}), from which the value of IC$_{50}$ (the half-inhibitory concentration) of 15.87 μg mL^{-1} for Curcumin can be obtained.
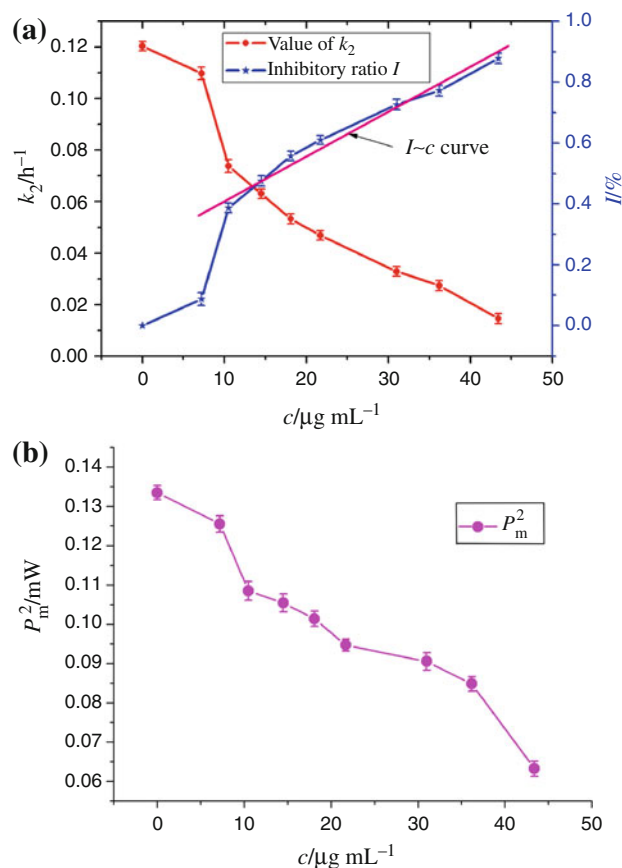

(a)

(b)

Fig. 4 Relationship between main quantitative parameters and the concentration of Curcumin demonstrated by $k–c$, $P–c$ curves. **a** the growth rate constants k_2 and **b** the maximum heat flow power P_m^2

Then, to describe the extent of the inhibition of Curcumin against *S. aureus* growth, the inhibitory ratio I and the half-inhibitory concentration (IC$_{50}$) determination by the

In order to show further the results in a quantitative way, we calculated the maximum thermal power (P_m^2) from power–time curves of *S. aureus* growth in the presence of Curcumin of different concentrations. These results are shown in Fig. 4b. From Fig. 4b, it is clear that the heat flow power (P_m^2) evolved in the second exponential growth phase decreases while as the concentrations of Curcumin increase from 0 to 43.4 μg mL^{-1}, which are the same trends as the growth rate constant k_2. The values of P_m^2 decreased linear with the increasing concentrations of Curcumin, so the growth of *S. aureus* were inhibited, further showing the strong antibacterial activity of Curcumin on *S. aureus* growth, and the dose–response relations were determined, it is summarized in Fig. 4b.

Effect of Curcumin treatment on bacterial culture properties

After treatment with different concentrations of Curcumin, the differences of heat flow power–time curves of *S. aureus* growth were obtained by the microcalorimeter. The quantitative parameters have different change trends (increasing or decreasing) that showed that the change enhanced with the concentration of Curcumin increase. By the analysis, the two main quantitative parameters values of k_2 and P_m^2, the treatment of bacteria with the different concentration of Curcumin in the calorimetric second exponential phase resulted in the growth rate constant k_2 and the heat flow power P_m^2 decreased with the increasing concentration of Curcumin. Further study on the actual action mechanism about the inhibitory effects of the Curcumin on microbes is in progress in our research.

Conclusions

Because Curcumin is not hydrosoluble, it was dissolved in EtOH in this study. In the preliminary experiment, the effect of EtOH on *S. aureus* growth was investigated to eliminate the influence of this solvent. The repeated experiments showed that there was no visible antibacterial activity when 25 μL of EtOH was added into the ampoule with the cell suspension of *S. aureus*. So the volume of EtOH was controlled less than 25 μL in all the experiments.

The effect of Curcumin on *S. aureus* growth had been evaluated by microcalorimetry. The thermokinetic parameters obtained from the heat flow power–time curves could be used quantitatively to indicate the effect of Curcumin on *S. aureus*. Curcumin at low concentration had poor inhibitory effect, at high concentration notably inhibited the growth of this tested bacteria. By analyzing these curves and some quantitative parameters using SA and PCA, we could find that the anti-*S. aureus* activity of Curcumin was

strengthened with the increase of concentration of this compound.

The microcalorimetric method is a non-destructive, dynamic, and more sensitive technique to investigate the anti-bacterial activity on microorganism. Microcalorimetry is an established procedure that offers quantitative measurements which has some distinct advantages over traditional antimicrobial test methodologies in that calorimetric measurement can make continuously measurements over real-time, thus the metabolic process of microbes under antimicrobial agents is observed in situ. In this article, the microcalorimetric method was successfully applied to evaluate the inhibitory effect of Curcumin on *S. aureus* growth. The parameters of the second exponential growth rate constant k_2 and the maximum heat flow powers for the second exponential phase P_m^2 were main two parameters and played more important role in evaluating the effect of Curcumin, we could quickly and clearly evaluate the effect and the action potency of this compound on *S. aureus* growth.

In summary, this study provided an important idea of the combination of microcalorimetry and multivariate analysis for studying the activity of other compounds or drugs on organisms. Moreover, this study showed that Curcumin possessed significant in vitro antibacterial properties against a common food-borne pathogenic (*S. aureus*). Curcumin, which is major antibacterial component, has great potential for application as natural food additive for food industry and preservation. However, Curcumin is available in pure form, non-toxic, highly promising natural antibacterial compound with a wide spectrum of biological functions. It is expected that curcumin might be applied as a novel food additives in the near future, particularly on the generalization in food industry.

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