The Conformability of Two Equations for Bacterial Growth in Pork

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Abstract

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Pork is now distributed as cut meat, which increases the chance of contamination with bacteria. The rate of bacterial growth can be expressed by an exponential function. In order to find how the number of contaminating bacteria increases, we compared two functional equations for a growth curve. They are logistic: $Y_t = K (1 + m\, e^{-at})$ (1) and Gompertz: $\log Y_t = \log K + (\log a) b^t$ (2) equations (where Y_t = the number of bacteria at the time t in min, m and a = coefficient, e = natural logarithm, K maximum number of bacteria). 90 ml of physiological salt solution was added to 10 g of pork. It was homogenized for 3 min, then incubated at 35°C for 13 hrs. The number of bacteria was counted every hour. We found from these data that the above two equations can be expressed as follows: $Y_t = 23\,535 (1 + 16269\, e^{-1.1608t})$ and $\log Y_t = 8.9940 + (-3.1124) \times 0.7839^t$. The theoretical and actual values matched well in equation (1), and the number of bacteria can be predicted accurately using this equation at a given time after incubation. The theoretical and actual values did not match well in equation (2) and its accuracy to predict the number of bacteria was very low except the initial number of bacteria was high.

Keywords: logistic equation; mathematical model; micro-organismus; Gompertz

There are models for the mathematical estimation of bacterial growth (Olley & Ratkowsky 1973). The concept of Hazard Analysis Critical Control Point (HACCP) is generally accepted in food hygiene. The ability to estimate bacterial growth in food is important in studying the occurrence of food poisoning as well as in general practice in food hygiene.

It is well known that bacteria grow exponentially with time under ideal conditions. Their growth rate increases proportionally to the concentration of nutrients (Olley & Ratkowsky 1973; Genigeorgis *et al*. 1971). These mathematical models are concerned only with a certain phase of bacterial growth. We tried to determine in this report how well the two theoretical equations, Verhulst's logistic curve (McMeekin *et al*. 1992) and Gompertz curve (Viniegra-Gonzalez *et al*. 1993; Whiting & Buchanan 1994), fit bacterial growth.

MATERIAL AND METHODS

Material: Pork was obtained at a store in Tokyo. Ten grams of meat were prepared and put in a sterilised homogenizer cup with 90 ml of 0.85% physiological salt solution. The meat was homogenised at 8 000–10 000 rpm for 2 min. The samples were then kept in an incubator at 35°C.

Methods: The number of bacteria was counted by a conventional method immediately after the meat was homogenized. The homogenized samples were then incubated in the homogeniser-cup. Bacteria were counted once every hour for 13 hours.

The meat was well mixed before a sample was taken for bacterial counts. The sample was diluted to adjust the number of colonies on the plate to 30–300. They were then incubated for 48 ± 3 hrs at 35°C in agar culture by the diluted plate culture method. The number of colonies after incubation is the number of bacteria.

RESULTS AND DISCUSSION

Growth of Bacteria

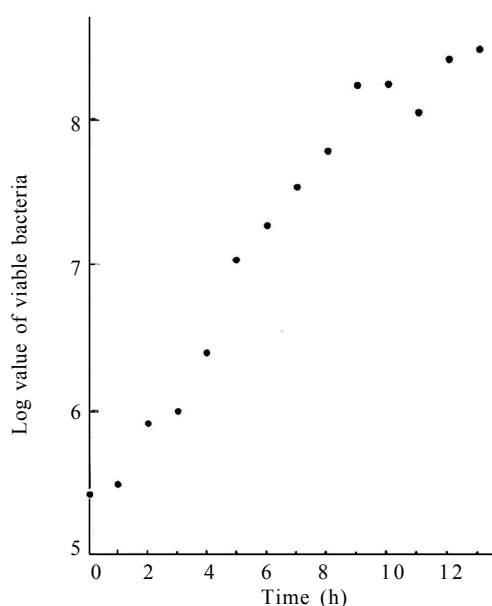As shown in Fig. 1, the growth pattern showed three phases of growth, the induction, exponential and maxi-

Fig. 1. The growth of bacteria (total count) in pork meat homogenate – measured data

mum stationary phases, which is the same pattern as in the growth of a colony with one species of bacteria. In general the number of bacteria after t minutes in the exponential phase can be derived by Kimata's equation:

$$b = B2^n \ldots \qquad (1)$$

It can be transformed into the following two equations:

$$n = (\log b - \log B)/\log 2 \qquad (2)$$

$$g = t \log 2/(\log b - \log B) \qquad (3)$$

where: B – number of bacteria at the beginning
　　　b – number of bacteria after t minutes
　　　t – time in minutes
　　　n – number of divisions in t minutes
　　　g – time in minutes needed for one division

Using the numbers of bacteria Fig. 1, the number of divisions per unit period and the time (t) needed for a division can be derived from equations (2) and (3). The maximum numbers of cell divisions were 1–2 in 3–9 hrs after the start of incubation. The fastest division time was 23–54 min. The rate increased rapidly in 3–9 hrs after the start of incubation. The meat contained various species of bacteria, so the division time was unstable. It appears, however, that the induction phase lasted 4 hrs, followed by 5 hrs of exponential phase. The rest of the period was the maximum stationary phase.

Conformity with Logistic Curve

The logistic curve is based on an assumption that a certain long-term variation goes through the growth pattern that consists of initial, developmental, saturation and stable phases. It can be shown in the following equation.

$$Y_t = K/(1 + me^{-at}) \qquad (4)$$

where: Y_t　– number of bacteria after t minutes
　　　K　– maximum number of bacteria
　　　T　– time in minutes
　　　m, a – coefficients

The coefficients m and a, and the maximum bacteria counts (K) in equation (4) can be derived by the least squares method.

(i) Calculation of logarithm of the initial number of bacteria $P = a$ and q.

$$\Sigma R = nP + q\Sigma Y$$

$$\Sigma \Delta Y = p\Sigma Y + q\Sigma Y^2 \qquad (5)$$

Using the simultaneous equations in (5) we calculated the values of p and q as 1.2818 and –0.000056, respectively, as shown in Table 1.

(ii) Calculation of K, the maximum number of bacteria $K = -q/P$, so $K = 22\ 879$ using the values in Table 1.

Table 1. The coefficients of logistic curve equality

No.	$P = a$	q	K	m	t
1	0.7164	–0.000025	28 725	308	8
2	1.2818	–0.000056	22 897	28 407	8
3	0.5282	–0.000036	14 680	68	8
4	1.9638	–0.000089	22 053	18 380	5
5	2.278	–0.000099	23 088	88 434	5
6	1.0875	–0.000045	24 183	6 143	8
7	1.0443	–0.000036	29 253	4 249	8
8	0.8449	–0.000042	20 135	159	6
9	0.702	–0.000026	26 806	275	8
Mean	1.608	–0.00005	23 535	16 269	7.1

(iii) Calculation of the point of inflection (t_r).

The time needed to reach 1/2 of K or $Y_t = 0.5K$ is expressed as t_r. Using the values on average we get $t_r = 7.1$.

(iv) Calculation of m, which the point of inflection over time in the growth curves of bacteria

$$t_r = 1/a \log e \times m \qquad (6)$$

Equation (6) gives $m = 28\ 407$. Therefore, the logistic curve is

$$Y_t = 22\ 897/(1 + 2\ 840e^{1.2818t})$$

Fig. 2 shows its actual curve. Table 1 shows the values for logistic curves that were derived from the averages of actual values in the experiments.

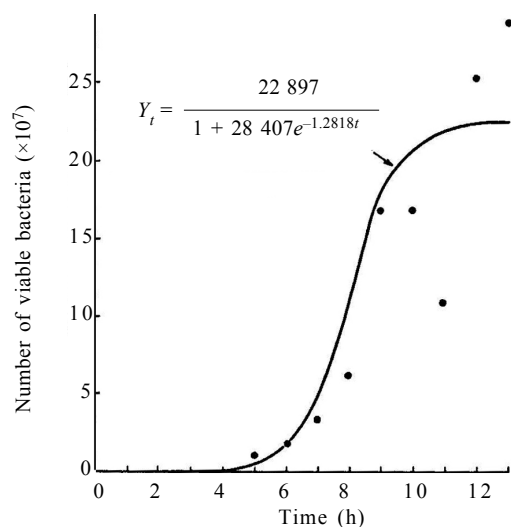Based on the average of 10 values, we obtained the following equation.

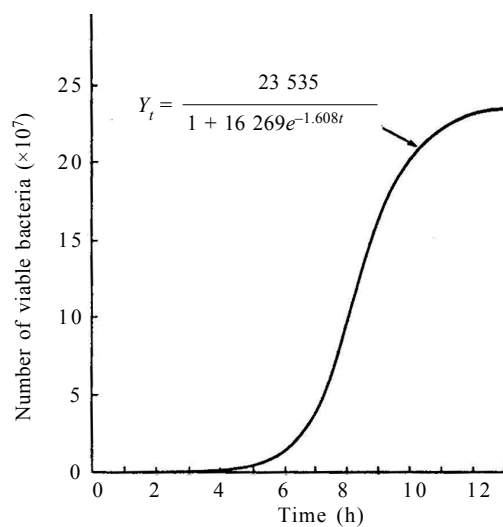Fig. 2. The logistic curve that was defined from the averages of actual values in the experiment

Fig. 3. The logistic curve that was defined from actual theoretical values

$$Y_t = 23\ 535/(1 + 16\ 269e^{-1.1608t})$$

Table 2 shows the process to obtain theoretical values. The actual theoretical values are shown in Fig. 3.

As can be seen in Figs. 2 and 3, the maximum bacterial number was reached in 9–10 hrs in the culture, and the average theoretical value was 23×10^7 derived from equation (5). Their actual number ranged between 20×10^7 and 28×10^7, and it conforms to the theoretical value. The theoretical time for the other growth periods also matches those of actual time. Therefore, we can use the logistic curve for estimating the number of bacteria at a certain

time. This logistic curve is strongly influenced by the time to reach the point of inflection. This causes the fluctuation as affected by the storage conditions.

Gompertz Curve

The values of coefficients K, a and b in the equation $\log Y = \log K + (\log a)b^t$ were calculated using the following equations in which the entire growth period was equally divided into three and the sum of the actual bacterial counts in each period, 1, 2 and 3, is expressed as $S_1 \log Y$, $S_2 \log Y$ and $S_3 \log Y$, respectively.

Table 2. The progress derived logistic curve equality from the experimental values by Expt. Mean

Time (h)	$\log e^{at}$	e^{at}	me^{-at}	$1 + me^{-at}$	Y_t
0	0	1	16 269	16 270	1
1	0.5041	5.3192	3 058.6	3 059.6	8
2	1.0082	10.191	1 596.4	1 597.4	15
3	1.5123	32.534	500.07	501.07	47
4	2.0164	103.86	156.65	157.65	149
5	2.5206	331.55	49.07	50.07	470
6	3.0247	1 058.4	15.372	16.372	1 438
7	3.5288	3 378.9	4.815	5.815	4 047
8	4.0329	10 787	1.5082	2.5082	9 333
9	4.5370	34 435	0.4725	1.4725	15 983
10	5.0411	109 930	0.148	1.148	20 501
11	5.5452	350 930	0.0464	1.0464	22 493
12	6.0493	1 120 300	0.0145	1.0145	23 199
13	6.5534	3 576 400	0.0045	1.0045	23 429

$\log Y = 9.2801 - 4.0702 \times 0.8664^t$

$$b_n = (\Sigma_3 \log Y - \Sigma_2 \log Y)/(\Sigma_2 \log Y - \Sigma_1 \log Y) \quad (8)$$

$$\log a = (\Sigma_2 \log Y - \Sigma_1 \log Y) \times (b-1)/(b_n-1)^2 \quad (9)$$

$$\log K = (\Sigma_1 \log Y - ((b_n-1)/(b-1)) \log a)/n \quad (10)$$

Table 3 shows the calculation procedures for the coefficients K, a and b using the values in Tables 1 and 4 and

Fig. 4 show the results of calculations using equations (8), (9) and (10). The equation used for Table 3 is

$$\log Y_t = 9.2801 - 4.0702 \times 0.7839^t$$

Fig. 5 shows Gompertz curve derived by the following equation using the values in Table 4.

$$\log Y = 8.9940 - 3.1124 \times 0.7839^t \quad (11)$$

Table 3. The theoretical values and the progress derived the Gompertz curve equality from the experimental values Expt. No. 3

| Time (h)	$Y (\times 10^5)$	$\log Y$	$\Delta \log Y$	b^t	$(\log a) b^t$	$\log Y$	Y
1	3.175	5.5017	0.4173	0.8664	−3.5262	5.7539	6
2	8.3	5.9191	0.4972	0.7506	−3.0549	6.2252	17
3	9.975	5.9989	0.4039	0.6503	−2.6467	6.6335	43
4	25.28	6.4028	0.6319	0.5633	−2.2929	6.9872	97
$\Sigma_1 \log Y$		23.823				25.6	
5	108.3	7.0348	0.233	0.4881	−1.9865	7.2937	197
6	185.3	7.2678	0.2641	0.4228	−1.721	7.5592	362
7	340.3	7.5318	0.263	0.3663	−1.491	7.7892	615
8	623.5	7.7948	0.4331	0.3174	−1.2917	7.9884	974
$\Sigma_2 \log Y$		26.629				30.63	
9	1690	8.2279	−0.0013	0.2749	−1.1191	8.1611	1449
10	1685	8.2266	−0.1852	0.2382	−0.9695	8.3106	2045
11	1100	8.0414	0.363	0.2064	−0.8399	8.4402	2756
12	2538	8.4044		0.1788	−0.7277	8.5525	3568
$\Sigma_3 \log Y$		32.9				33.464	

$\log Y = 9.2801 - 4.0702 \times 0.8664^t$
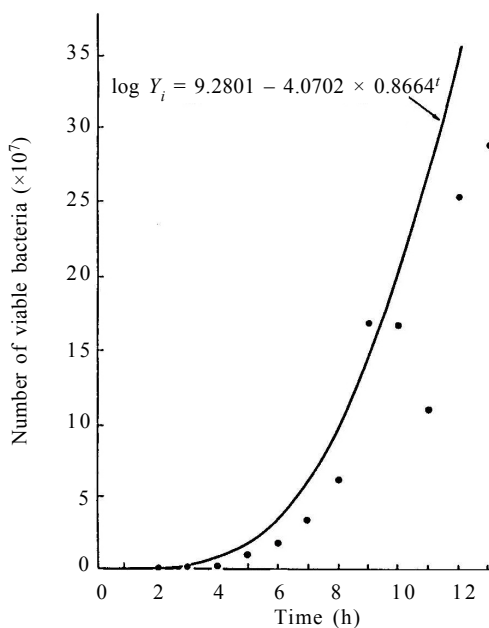
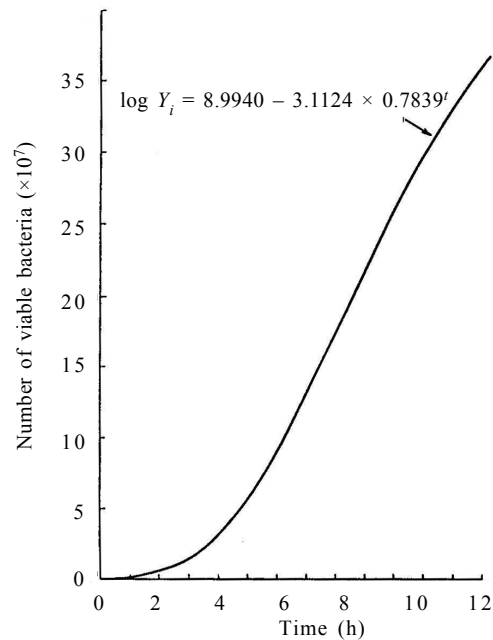Fig. 4. The result of calculations using the equations (8), (9) nad (10) from the experimental values

Fig. 5. The Gompertz curve derived using the values in Table 4 by the equation (11)

Table 4. The coefficients of Gompertz curve

Experiment No.	b	$\log a$	$\log K$
1	0.7975	−1.7702	8.5712
2	0.8664	−4.0702	9.2801
3	0.9138	−5.6086	10.8428
4	0.5921	−2.759	8.3473
5	0.5889	−2.8516	8.4141
6	0.8763	−3.7166	9.3976
7	0.8366	−3.0508	9.0061
8	0.7825	−2.1774	8.5264
9	0.8008	−2.0073	8.5608
Mean	0.7839	−3.1124	8.994

$\log Y = 8.9940 - 3.1124 \times 0.7839^{t}$

Theoretical values obtained from Figs. 4 and 5 indicated a rapid bacterial growth in the period when the actual bacterial growth had already reached the maximum stationary phase. This shows that this equation is not very reliable except for the case when the initial bacterial counts are very high. Therefore we concluded that it is difficult to estimate the number of bacteria using Gompertz curve.

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Abstrakt

MIYAHARA M., MATSUMOTO T., SAKURAI H., PIPEK P. (2002): **Shoda dvou rovnic popisujících bakteriální růst ve vepřovém mase**. Czech J. Food Sci., **20**: 69–73.

Distribuované mělněné maso může být značně kontaminováno bakteriemi. Rychlost bakteriálního růstu lze vyjádřit jako exponenciální funkci. Při sledování tohoto růstu byly srovnány dvě funkční rovnice popisující bakteriální růst. Byl to logistický model $Y_t = K(1 + me^{-at})$ a Gompertzova rovnice $\log Y_t = \log K + (\log a)b^t$. Celkové počty mikroorganismů byly měřeny v hodinových intervalech. Z naměřených dat byly vypočteny koeficienty obou rovnic $Y_t = 23\,535\,(1 + 16\,269e^{-1.1608t})$ a $\log Y_t = 8.9940 + (-3.1124) \times 0.7839^t$. Naměřené hodnoty se dobře shodovaly s hodnotami vypočtenými podle první rovnice a četnost bakterií může být přesně předpovězena s použitím této rovnice v daném čase inkubace. Aktuální hodnoty se již dobře neshodovaly s hodnotami vypočtenými podle druhé rovnice. Přesnost předpovědi bakteriálních četností zde byla velmi nízká s výjimkou případu, kdy výchozí koncentrace mikroorganismů byla vysoká.

Klíčová slova: logistická rovnice; matematický model; mikroorganismy; Gompertzova rovnice

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