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A novel approach for calculating shelf life of minimally processed vegetables

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Abstract

Shelf life of minimally processed vegetables is often calculated by using the kinetic parameters of Gompertz equation as modified by Zwietering et al. [Zwietering, M.H., Jongenburger, F.M., Roumbouts, M., van't Riet, K., 1990. Modelling of the bacterial growth curve. Applied and Environmental Microbiology 56, 1875–1881.] taking 5×10^7 CFU/g as the maximum acceptable contamination value consistent with acceptable quality of these products. As this method does not allow estimation of the standard errors of the shelf life, in this paper the modified Gompertz equation was re-parameterized to directly include the shelf life as a fitting parameter among the Gompertz parameters. Being the shelf life a fitting parameter is possible to determine its confidence interval by fitting the proposed equation to the experimental data. The goodness-of-fit of this new equation was tested by using mesophilic bacteria cell loads from different minimally processed vegetables (packaged fresh-cut lettuce, fennel and shredded carrots) that differed for some process operations or for package atmosphere. The new equation was able to describe the data well and to estimate the shelf life. The results obtained emphasize the importance of using the standard errors for the shelf life value to show significant differences among the samples.

Keywords: Gompertz equation; Microbial growth; Mathematical modeling; Shelf life; Fresh-cut vegetables

1. Introduction

Fresh fruits and vegetables have both an important nutrition-health and an economic value. Recently, the market demand for minimally processed fruits and vegetables has undergone an important rise because of busy lifestyles, increasing purchasing power and increasingly health-conscious consumers (Baldwin et al., 1995). Minimally processed vegetables, due to processing operations that alter the physical integrity of these products, are more perishable than the original raw materials. The understanding of the processes that result in quality degradation after processing is essential to develop technologies to extend shelf life and to maintain quality during processing and distribution.

Predictive microbiology is a useful tool to determine shelf life of food products. Several attempts have been made toward predictive modelling of the growth of microorganisms inside, or on the surface of foods as a function of time during refrigerated storage. These models are analytical expressions, such as the Gompertz or the logistic curve (e.g. Zwietering et al., 1991) which exhibit the typical sigmoidal appearance of the bacterial growth curve, or are sets of ordinary differential equations (Baranyi and Roberts, 1995).

The empirical sigmoid-like analytical expressions used in predictive food microbiology are attractive because of their simplicity. For example, researchers have used the Gompertz model to estimate parameters of the model as function of the effects of substrate composition (salt concentration, pH, temperature) for the growth of *Listeria monocytogenes* (Buchanan et al., 1989). The accuracy in predicting growth depends on the number of parameters used in the sigmoidal model. Modified versions of the Gompertz equation can include three or more parameters to describe the behaviour of the bacterial growth curve and a modified version of the Gompertz model to describe the bacterial population growth curve was used by Zwietering et al. (1990).

The kinetic parameters derived by the Gompertz equation have been often used to calculate the shelf life of numerous minimally processed vegetables (Lanciotti et al., 1999; Riva

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et al., 2001; Corbo et al., 2004; Sinigaglia et al., 2003) taking into account that, according the French regulation (Ministere de l'Economie des Finances et du Budget, 1988), 5×10^7 CFU/g is the maximum acceptable contamination value at the end of the microbiological shelf life of these products. However, this method does not allow to estimate the standard error of the shelf life; as a consequence, the aim of this paper was to re-parameterize the Gompertz equation modified by Zwietering to insert, among the Gompertz parameters, that of the shelf life accompanied by standard errors. The model was tested by using it for the mesophilic bacteria cell load from different minimally processed vegetables (packaged fresh-cut lettuce, fennel and shredded carrots).

2. Materials and methods

2.1. Characteristics of the samples

Data used to test the re-parameterized version of the Gompertz equation were from different minimally processed vegetables:

a) packaged cut lettuce salads and shredded carrots were processed at a local company producing "ready-to-eat salads" according to four different processes (Sinigaglia et al., 1999). The main differences among the four options were:
   1. treatment with a solution containing 150 ppm of free chlorine;
   2. treatment with a solution containing 100 ppm of free chlorine;
   3. treatment with a solution containing 100 ppm of free chlorine and washing after cutting for lettuce or shredding for carrots in order to reduce the residual chlorine concentration;
   4. pause of 12 h at room temperature (15–18 °C) before the treatment with a chlorine solution having 100 ppm of free chlorine and washing in order to eliminate the residue of the chlorine.

   These samples were labeled as: C1 (shredded carrots produced according to process I), C2 (shredded carrots produced according to process II), C3 (shredded carrots produced according to process III), C4 (shredded carrots produced according to process IV), L1 (lettuce salads produced according to process I), L2 (lettuce salads produced according to process II), L3 (lettuce salads produced according to process III), L4 (lettuce salads produced according to process IV).
b) Shredded carrots from two different trademarks (labeled C5 and C6) were purchased in a retail store in Foggia, brought to our laboratory in refrigerated bags, and immediately analysed and for every day of their shelf life.
c) Fresh-cut fennels, washed at room temperature with 100-ppm chlorinated water to avoid risk of microbial development, were packaged in high barrier plastic bags [Nylon/Polyethylene, 102 μm (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] by means of S100-Tecnovac equipment. The bags were 170 mm wide × 250 mm long with properties specified by the manufacturer as follows: CO_2 and O_2 permeability of 3.26×10^{-19} mol m m^{-2} s^{-1} Pa^{-1} and 9.23×10^{-19} mol m m^{-2} s^{-1} Pa^{-1}, respectively, and water vapor transmission rate of 1.62×10^{-10} kg m^{-2} s^{-1}. The samples were packaged in air, under vacuum and in a modified atmosphere (70% N_2, 30% CO_2), stored at 4 °C, and labeled F1, F2 and F3, respectively.

For microbiological analysis, 10 g of each sample were diluted with 90 ml of 0.1% peptone solution in a Stomacher bag (Seward, London, England) and blended for 1 min in a Stomacher Lab Blender 400 (Seward). Serial dilutions of vegetable homogenates were plated on Plate Count Agar (PCA, Oxoid, Milan, Italy) and incubated at 32 °C for 48 h for mesophilic bacteria.

3. Results and discussion

As reported above the Gompertz equation as modified by Zwietering et al. (1990) has been often used to determine the shelf life of minimally processed fruits and vegetables (Lanciotti et al., 1999; Riva et al., 2001; Corbo et al., 2004; Sinigaglia et al., 2003). The method generally adopted (Zwietering et al., 1990) consists in estimating the Gompertz's parameters by fitting the following equation to the experimental data:

$$\log(\mathrm{CFU}) = K + A\cdot\exp\left\{-\exp\left\{\left[(\mu_{\max}\cdot 2.7182)\cdot\frac{\lambda - t}{A}\right] + 1\right\}\right\} \tag{1}$$

where: K is the initial level of bacterial count (log CFU/g), A is the increase in log CFU/g between time = 0 and the maximum population density achieved at the stationary phase, $\mu_{\max}$ is the maximal growth rate (Δlog (CFU/g)/day), λ is the lag time (days) and t is the time (days). Once the modified

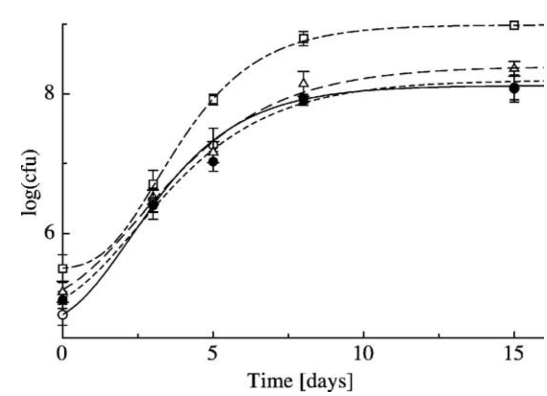

Fig. 1. Evolution of mesophilic bacteria load as a function of storage time for shredded carrots C1, C2, C3 and C4: (○) sample C1, (●) sample C2, (△) sample C3, (□) sample C4; (–) best fit of Eq. (5) to sample C1 data, (- - - -) best fit of Eq. (5) to sample C2 data, (– –) best fit of Eq. (5) to sample C3 data, (- – -) best fit of Eq. (5) to sample C4 data.

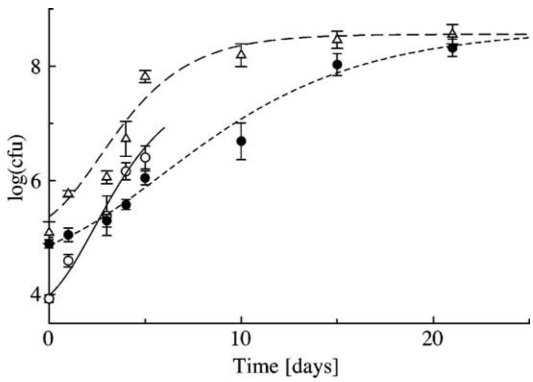

Fig. 2. Evolution of mesophilic bacteria load as a function of storage time for fresh-cut fennels F1, F2 and F3: (○) sample F1, (●) sample F2, (△) sample F3; (–) best fit of Eq. (5) to sample F1 data, (- – -) best fit of Eq. (1) to sample F1 data, (- - - -) best fit of Eq. (5) to sample F2 data, (······) best fit of Eq. (1) to sample F2 data, (– –) best fit of Eq. (5) to sample F3 data, (- - - - - -) best fit of Eq. (1) to sample F3 data.

Gompertz function's parameters are estimated the shelf life (SL) of the produce is calculated through the following expression:

$$\mathrm{SL} = \lambda - \frac{A\cdot\left\{\ln\left[-\ln\left(\frac{\log(5\cdot 10^7)-K}{A}\right)\right]-1\right\}}{\mu_{\max}\cdot 2.7182} \tag{2}$$

where: 5×10^7 is the acceptability limit for the microbial population. It is worth noting that, even when it is possible to use Eq. (1) for estimating the confidence interval of each of the Gompertz's parameters, it is not possible to estimate the confidence interval of the shelf life since it does not appear explicitly in Eq. (1). The difficulty of estimating the shelf life confidence interval is a main drawback of using the above approach to estimate the shelf life of fresh produces.

An alternative way for estimating the shelf life of minimally processed products consists in rearranging Eq. (1) in such a way that the shelf life appears directly as a parameter of the equation relating log (CFU/g) to storage time.

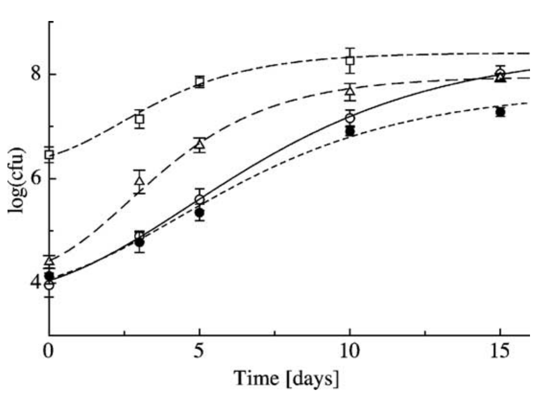

Fig. 3. Evolution of mesophilic bacteria load as a function of storage time for fresh-cut lettuces L1, L2, L3 and L4: (○) sample L1, (●) sample L2, (△) sample L3, (□) sample L4; (–) best fit of Eq. (5) to sample L1 data, (- - - -) best fit of Eq. (5) to sample L2 data, (– –) best fit of Eq. (5) to sample L3 data, (- – -) best fit of Eq. (5) to sample L4 data.

Table 1
Values of parameters obtained by fitting Eq. (5) to samples C5 and C6 data

Parameters[a]	Samples[b] C5	C6
A	4.53 [3.99, 5.19]	3.01 [2.51, 11.6]
SL	6.87 [6.04, 7.96]	1.77 [0.347, 2.43]
$\mu_{\max}$	0.421 [0.317, 0.550]	0.339 [0.255, 0.498]
λ	2.90×10^{-13} [2.17×10^{-13}, 4.06×10^{-13}]	8.65×10^{-13} [9.53×10^{-14}, 2.18×10^{-12}]
$\bar{E}$%	1.67	0.600

The 95% confidence intervals of each parameter are shown in the square brackets. They were calculated on the base of 200 converging interactions.

[a] Re-parameterized Gompertz equation parameters: A, maximum cell increase attained at the stationary phase (log CFU/g); SL, predicted shelf life as the time (days) necessary to attain 5×10^7 CFU/g level; $\mu_{\max}$, maximal growth rate; λ, lag phase (days); $\bar{E}$%, relative percent difference between experimental and predicted values.

[b] Samples: C5 and C6, shredded carrots from two different local retails.

According to Eq. (1) one can write:

$$\log(5\cdot10^7) = K + A\cdot\exp\left\{-\exp\left\{\left[(\mu_{\max}\cdot 2.7182)\cdot\frac{\lambda-\mathrm{SL}}{A}\right]+1\right\}\right\} \tag{3}$$

The above equation can be rearranged as follows:

$$K = \log(5\cdot10^7) - A\cdot\exp\left\{-\exp\left\{\left[(\mu_{\max}\cdot 2.7182)\cdot\frac{\lambda-\mathrm{SL}}{A}\right]+1\right\}\right\} \tag{4}$$

By substituting the above equation in Eq. (1) the following expression is obtained:

$$\begin{aligned}\log(\mathrm{CFU}) = \log(5\cdot10^7) &- A\cdot\exp\left\{-\exp\left\{\left[(\mu_{\max}\cdot 2.7182)\cdot\frac{\lambda-\mathrm{SL}}{A}\right]+1\right\}\right\}\\ &+ A\cdot\exp\left\{-\exp\left\{\left[(\mu_{\max}\cdot 2.7182)\cdot\frac{\lambda-t}{A}\right]+1\right\}\right\}\end{aligned} \tag{5}$$

Table 2
Values of Gompertz's parameters obtained by fitting Eq. (1) to samples C5 and C6 data

Parameters[a]	Samples[b] C5	C6
K	4.90 [4.46, 5.29]	7.08 [6.20, 7.47]
A	4.53 [4.02, 5.19]	3.01 [2.51, 17.65]
$\mu_{\max}$	0.420 [0.316, 0.549]	0.338 [0.252, 0.484]
λ	2.98×10^{-13} [2.22×10^{-13}, 3.83×10^{-13}]	1.61×10^{-13} [3.51×10^{-16}, 2.44×10^{-13}]
$\bar{E}$%	1.69	0.600

The 95% confidence intervals of each parameter are shown in the square brackets. They were calculated on the base of 200 converging interactions.

[a] Gompertz equation parameters: K, initial load (log CFU/g); A, maximum cell increase attained at the stationary phase (log CFU/g); $\mu_{\max}$, maximal growth rate; λ, lag phase (days); $\bar{E}$%, relative percent difference between experimental and predicted values.

[b] Samples: C5 and C6, shredded carrots from two different local retails.

Table 3
Comparison of shelf life values obtained according the two approaches presented

Produce[a]		Shelf life parameter	Shelf life calculated by using Gompertz's parameters
Shredded carrots	C1	6.42 [4.65, 7.15]	6.41
	C2	6.92 [6.34, 7.69]	6.88
	C3	6.30 [5.65, 7.00]	6.26
	C4	4.56 [4.27, 4.86]	4.44
Shredded carrots	C5	6.87 [6.04, 7.96]	6.84
	C6	1.77 [0.347, 2.43]	1.74
Fennels	F1	nd[b]	nd
	F2	13.6 [12.3, 15.1]	13.5
	F3	5.84 [5.59, 6.12]	5.80
Fresh-cut lettuces	L1	12.63 [11.5, 14.7]	12.57
	L2	–[c]	–
	L3	9.69 [8.47, 11.7]	9.58
	L4	4.51 [3.62, 5.41]	4.47

The 95% confidence intervals of the shelf life values calculated according to the proposed approach are shown in the square brackets. They were calculated on the base of 200 converging interactions.

[a] C1, C2, C3, C4: shredded carrots produced according to processing line I, II, III and IV, respectively; C5 and C6: shredded carrots purchased from two different retails; F1, F2 and F3: fresh-cut fennels packaged in air, under vacuum and in modified atmosphere (70% N_2, 30% CO_2); L1, L2, L3 and L4: fresh-cut lettuces produced according to processing line I, II, III and IV.

[b] Not determined: these samples were not considered for calculating shelf life because of visible growth of molds after 5 days of storage.

[c] Mesophilic bacteria did not attain 5×10^7 CFU/g.

By fitting Eq. (5) to the experimental data it is possible to estimate the equation's parameters and their confidence interval. Therefore, Eq. (3) can be used in place of Eqs. (1) and (2) to determine both the shelf life of the product and its confidence interval.

Figs. 1–3 show the evolution during storage of the microbial population of packed products. The curves shown in each figure are the best fit of Eq. (5) to the experimental data. As an example in Fig. 2 the best fit of Eq. (1) to the experimental data are also shown. As expected, since the equations are only re-parameterized, the fits are exactly equal. The goodness-of-fit was evaluated by means of the relative percent difference, $\bar{E}\%$, or mean relative deviation modulus (Boquet et al., 1978), which is defined by the following expression: $\bar{E}\% = \frac{100}{N} \cdot \sum_{i=1}^{i=N} \frac{|M_i - M_p|}{M_i}$, where: M_i is the experimental value, M_p is the predicted value, N is the number of observations.

As an example, the results of fitting of Eqs. (1) and (5) to the experimental data of the samples C5 and C6 are listed in Tables 1 and 2.

The shelf life values obtained according to the above approaches are listed in Table 3. As one would expect the two shelf life values are practically coincident. However, as pointed out above, using Eq. (5) it is possible to estimate the shelf life confidence interval, which in turn allows to establish if there is a significant difference in the shelf life among the examined produces.

It is worth noting that the method presented measures the noise due to sampling error when constructing the population growth curve from which the shelf life is estimated.

In the case of shredded carrots purchased from two retails (C5 and C6) and of fresh-cut fennels (F1, F2 and F3) a significant difference in the shelf life of the packed produces can be observed; in particular, such significant difference for carrots can be probably attributed to a difference in quality of raw material or to a different processing, while for fennels an expected extension of shelf life was observed in samples packaged under vacuum. It was not possible to use the experimental data from samples packaged in air because after only 5 days the product was unacceptable due to visible growth of molds.

On the contrary, in the case of shredded carrots (C1, C2, C3 and C4) and fresh-cut lettuce (L1, L2, L3 and L4), there is a superposition of the shelf life confidence intervals indicating that there is no significant difference in the calculated values of shelf life. The above results point out that for fresh-cut fennels the treatments are successfully in prolonging the shelf life of the investigated produce, whereas in the last two cases there is no significant influence of the treatment on the shelf life of the packed produces, differing from that reported in a previous work in which the shelf life calculated from the Gompertz's parameters differed because of the treatments in process operations (Sinigaglia et al., 1999).

The examples given above highlight the advantages related to the use of Eq. (5) for calculating the shelf life of packed produces. The proposed approach could be advantageously used to establish the influence of process variables on the quality decay kinetics of many packed foodstuffs whose quality is strictly related to the growth of spoilage microorganisms.

On the contrary, as the initial count is excluded from the Eq. (5), to estimate the effects of the initial process operations (for example, washing or decontamination) seems to be more advantageous to fit with the normal Gompertz equation.

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