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Predicting the Bacterial Growth in MAP Chilled Beef by Artificial Neural Networks

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An efficient Artificial Neural Networks(ANN) method was developed to predict the microbial growth in beef. The targeted environmental factors were: temperature(-2, 0, 5 and 10°C) and Modified Atmosphere Packaging(MAP) air component(65% O_2, 35%CO_2 and 80% O_2, 20%CO_2). The ANN model used the three-vector model and was further developed into a four-vector model(bacterial species was imported into the model as an extra vector) which can predict all microbial growth in the single model. It turned out that both the ANN models were closely matching to the modeling datasets. And the disparity between two models was also not significant in testing datasets. This indicated that the more variables introduced did not affect the accuracy of ANN model. Using this model, the bacterial counts and remaining shelf-time of beef can be rapidly predicted by filling the air component and temperature into input layer.

Key words: Artificial neural networks, Modified atmosphere packaging, Chilled beef.

Beef products are highly perishable foods. Organisms involved in the beef spoilage usually are *Pseudomonas, Lactobacillus, B. thermosphacta* and Coliform; their contributions to spoilage flora will largely depend on oxygen availability(Borch *et al*.,1996). Chilled beef with MAP packaging is getting increasingly popular, since it is with attractive and fresh appearance, and capable of retarding bacterial spoilage. The MAP packaging and chilled conditions(0-4°C) are proved to be fairly efficient to extend its shelf-life(Pennacchia *et al*., 2011). The actives of major microbes are inhibited under the chilled temperature, and resulted in the slower perishing rate. The effectiveness of MAP in shelf-time extension of meat is based on the presence of CO_2. The presence of CO_2 in the head-space of meat MAP packages can lead to the inhibition of bacterial growth and provoke a shift of the dominant microbes to the bacterial groups with less spoilage potential(Limbo *et al*., 2010).

The MAP chilled beef have been highly purchased in supermarkets nowadays. Therefore, predicting the spoilage bacterial growth in the this kind of food is a meaningful work that is closely related to daily life and has significant value in our food safety. Artificial neural networks(ANN) offer an advanced technique[1] to incorporate multiple parameters into the total evaluation, they can provide accurately modeling for microbial survival and growth and can deal with the high level of variability and uncertainty that is typically associated with microbial responses(Jeyamkondan *et al*., 2001). Therefore, the ANN model can predict the various bacterial growth in beef by importing the "bacterial species" into its input layer. Because of the promising future and performances in several applications(Borchf *et al*.,1996; Chowdhury

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et al., 2007; Panagou *et al*., 2011), ANN is gaining increased popularity in predictive microbiology. For instances, Keeratipibul and Phewpan (Keeratipibul *et al*., 2011;Khuri *et al*., 2010) employed the ANN model to predict the *E. coli* and coliforms in vegetables; Günay and Nikerel(Günay*et al*.,2008) modeled the enzyme production and biomass growth in recombinant *E. coli* using ANN; and Fernández-Navarro and Valero (Fernández *et al*., 2010) developed an ANN model to determine the microbial growth/no growth interface.

The effect of MAP on meat products has been frequently discussed. There are no published work that can predict the spoilage bacterial growth in chilled beef with MAP packing. Therefore, in present study, an ANN model based on back-propagation algorithm was constructed to describe the bacterial growing curves in chilled beef under different storing conditions(temperature:-2, 0, 5 and 10°C and MAP:65%O_2, 35%CO_2 and 80%O_2, 20%CO_2).

MATERIALS AND METHODS

Sample preparation

Fresh fillets were purchased from beef carcass a meat company in Shanghai. Samples were kept in a cool box(1±1°C) and transported to laboratory within 1 h. Each meat was further divided into portions of 50-100g minced beef immediately.

Sample storage

PET bags(Polyethylene terephthalate, low penetrability and resisting to organic solvent) with modified atmosphere(65% O_2, 35%CO_2 and 80% O_2, 20%CO_2) were applied to hold these samples. Then the packaged meat was stored under 4 controlled isothermal conditions(-2,0,5 and 10°C), and analyses were carried out in a given period. Samples stored at -2°C(30 samples) and 0°C(25 samples) were analyzed after 0, 3, 6, 9, 12 , 15, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26 days; those stored under 5°C(20 samples) after 0, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 days and the ones at 10°C(15 samples) after 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 days of storage. Last two temperatures with fewer and closer time points recorded was because the perishing rate of beef samples was greater. Each sample was analyzed twice. Therefore, there were totally eight experimental conditions.

The datasets was divided into modeling datasets and testing datasets. The modeling datasets were consisted of all the five bacterial counts(including TVC) under 7 experimental conditions(excluding 0°C, 65%O_2 35%CO_2). While the testing datasets were obtained from the counts under conditions 0°C, 65%O_2 35%CO_2 and used for validating the models.

Microbiological analysis

25g of minced meat from the surface of sample was steriled in 225 ml sterile saline water and homogenized for 60 s in a stomacher at room temperature. Series of 10-fold dilutions were made in sterile saline water and a 0.1 ml aliquot from the appropriate dilution was plated onto five types of media for enumeration.

(1)Total viable counts(TVC) were count on Plate Count Agar(PCA, Merck), incubated at 37°C for 48 h. (2)*Pseudomonas* were determined by plating on cetrimidefucidincephaloridine agar(CFC, Merck) at 25°C for 48 h; all the colonies on the plate are typical colonies. (3)*Lactobacillus* count was enumerated on deMan, Rogosa, Sharpe(MRS, Merck) agar incubated at 30°C for 72 h; the white colonies with circular shape are the typical colonies. (4)Coliform number was determined using violt red bile agar(VRBA, Merck) and inoculated plates are incubated at 35°C for 24 h; the typical colonies display fuchsia. (5)*B.thermosphacta* was counted on *B.thermosphacta* selected culture medium plates provided by Beijing Luqiao Company, and aerobically incubated at 10°C for 10 days.

Plates were removed from the incubator and typical colonies were enumerated visually from plates with three seriate dilutions. The microbiological data was transformed into logarithms of the number of colony forming units(log CFU/g).

Artificial Neural Network model

The supervised back-propagation(BP) network is based on searching an error surface for points with minimum error. Forward activation to produce an output, then a backward propagation of the computed error to modify the weights according to the discrepancy between the output and the original data(Basheer*et al*., 2000; Lou *et al*.,2001;McMeekin *et al*., 2002;Slongo *et al*., 2009). The ANN model applied in the study was a three-layer BP network. The first layer was input layer. It

consisted of the input neurons which were bacterial species, storing time, storing temperature and MAP component. The second layer was called hidden layer, and used for processing the nonlinearity of the input information. The appropriate number of neurons in hidden layer is found empirically varied from 3 to 12. Since the superfluous neurons will partition the input space into too many small subspaces and can over-fit the training patterns, increasing the number of neurons in hidden layer may not lead to an improved precision of prediction. Thus, the best performance configuration was held, while others discarded. Each ANN network topology was trained at an average of three times due to the possible random influence of the initial weights. The third layer(output layer) was with one output neuron, representing the corresponding microbial counts. When the counts under each storing condition were obtained, the Modified Gompertz(MG) equation(Eq.1) was applied to describe the growing curve:

$$log(N_t) = log(N_0) + log\left(\frac{N_{max}}{N_0}\right) \times exp\left(-exp\left(\frac{e*\mu_{max}}{log\left(\frac{N_{max}}{N_0}\right)} \times (\lambda - t) + 1\right)\right) \quad ...(1)$$

Where λ is the lag phase extension(days); μ_{max} is the maximum exponential microbial growth rate(days^{-1});*t* is the storage time(days); $log(N_0)$ is the initial bacterial density(log cfu/g); $log(Nt)$ is the counts of bacteria in *t* days and $log(N_{max}/N_0)$ is the differential between the maximum counts and the minimum counts of microbes(log cfu/g).

The neural networks can be viewed as a set of neurons connected in forms of a structural layered network. Each neuron is implemented by an activation function. The activation function employed in hidden and output layers are hyperbolic tangent transfer function and linear transfer function(Eq.2 and Eq.3), respectively.

$$f(x) = [exp(x) - exp(-x)]/[exp(x) + exp(-x)] \quad ...(2)$$

$$f(x) = x \quad ...(3)$$

Creating a BP network via the Matlab7.0 subroutine "newff". Then training with "train" until the network error was below 0.001, and the largest training times was 5000. After training, the subroutine "sim" was employed to simulate the testing results.

In order to accelerate the network training speed, the input and output data were normalized within the range [-1, 1], using the subroutine "premnmx". Consequently, all variables acquire same significance during the training process. The normalized value(Xt) for each raw input/output dataset(Xi) was calculated as:

$$X_t = -1 + 2 \times [(X_i - X_{min})/(X_{max} - X_{min})] \quad ...(4)$$

whereXminand Xmax are the minimum and maximum values of the raw data.

All the models were written by authors using Matlab 7.0 software.

Criteria for comparison

To evaluate the developed models, three indices are employed, which were defined as root mean square error(RMSE), bias(Bf) factor and accuracy(Af) factor. In the below equations, XpandXdare the predicted and detected value, respectively; n is the number of training or testing data.

TheRMSEwhich measures the average distance between the detected and predicted values:

$$RMSE = \sqrt{\frac{\sum(X_p - X_d)^2}{n}} \quad ...(5)$$

the smaller the value of this index the better prediction of the model.

The Bfindicates whether, on average, the observed counts are above or below the line of equity(y=x), and if so, by how much. It is defined as:

$$B_f = 10^{\frac{\sum log\left(\frac{X_p}{X_d}\right)}{n}} \quad ...(6)$$

A value = 1 indicates a perfect model where the predictions are in full agreetment with detections. TheBf value > 1 indicates that the model over-estimates, while <1 means under-estimation. The accuracy factor(Af):

$$A_f = 10^{\frac{\sum\left|log\left(\frac{X_P}{X_d}\right)\right|}{n}} \quad ...(7)$$

it indicates the deviation between predictive and detective values, and how close they are. The values are ? 1, the smaller the value of Af the better performance of the model.

RESULTS AND DISCUSSION

The experimental data were fitted by MG equation. Then The spoilage bacterial growing parameters were calculated (Table.1). The μ_{max} was

dramatically increased with the raising temperature and O_2 component, while λ decreased remarkably in the total trend.On the other hand, the lgN_0 and $lg(N_{max})$had little changed. This indicated that the bacteria grow quicker when the beef was stored under higher temperature and oxygen environment, but the initial and final counts was little affected.

Two types of ANN model were constructed which were three-vector model and four-vector model. The input layer in the three-vector model was consisted of three neurons: storing time, temperature and air components in the MAP. The output layer had one neuron, representing the counts at its corresponding time points. Since five species of major spoilage bacteria were discussed, five three-vector models were demanded to simulate the growth of related organisms. The optimal hidden layer configuration were obtained when the model had lowest mean square error(Fig.1, the model for TVC was given as

Table 1. The growth parameters of spoilage bacteria in chilled beef under targeted storing conditions (TVC given as example)

MAP	T'	Parameters				
		$logN_0$	$log(N_{max}/N_0)$	μ_{max}	λ	R^2
65%O2 35%CO2	-2	5.0176	1.2245	0.0620	5.4141	0.9798
	0	4.7573	1.5635	0.1220	4.1697	0.9937
	5	5.1365	2.3497	0.2662	5.1676	0.9960
	10	5.5833	2.3511	0.4013	0.9752	0.9947
80%O2 20%CO2	-2	5.0475	1.2097	0.1061	7.5402	0.9966
	0	4.7426	1.6519	0.1068	3.5671	0.9963
	5	5.1131	3.0019	0.3351	4.1238	0.9960
	10	5.5266	3.0671	0.4046	0.7930	0.9928

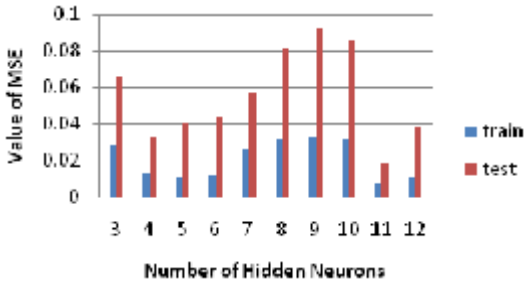

Fig. 1. Comparison of the mean square error(*MSE*) of three-vector models for TVC with different configurations

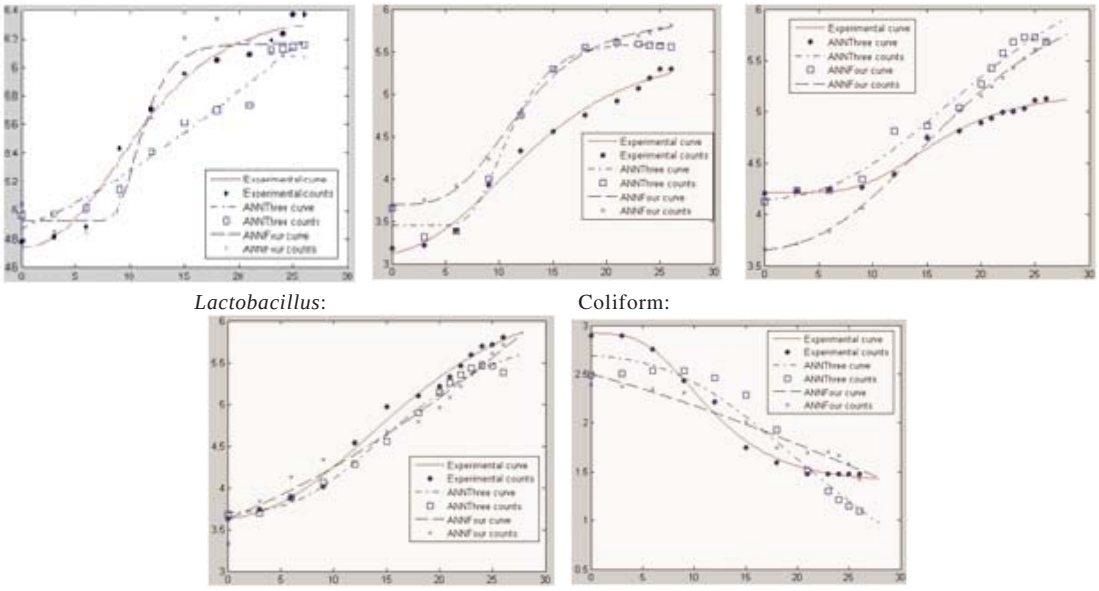

Fig. 2. Comparison of growing curves predicted by ANN models(Testing data)

an example). The configurations of each three-vector model were given in Tab.2. Then the counts at each time point predicted by the models were fitted by the MG equation to describe the curves(Fig.2).

Compared to three-vector model, the four-vector ANN model had an extra neuron in the input layer representing the bacterial species(Fig.3). Thus the single four-vector model was able to predict all the bacterial growth in beef. Best performance was obtained while the hidden neuron number was 11. The predicted counts of each species were also fitted by the MG equation(Fig.2).

In the modeling datasets(Tab.2), both models had fairly good fits(R^2>0.95). The indexes RMSE, $A_f$and B_f were all within the satisfactory range, indicating they were capable of generating precise prediction.

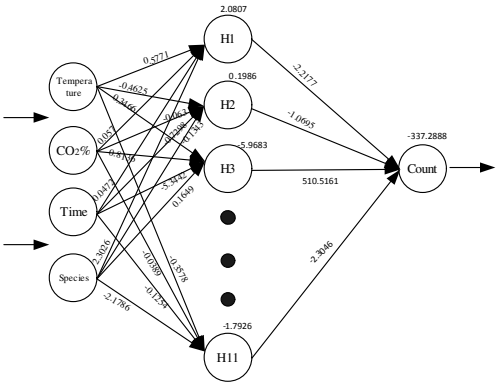

Fig. 3. The configuration of four-vector ANN model for predicting the five bacterial species in beef

Table 2. Comparison of the performance of models(Modeling data)

Model	Bacteria	Configuration	RMSE	A_f	B_f	R^2
Three-vectormodel	TVC	3-11-1	0.1018	1.0273	1.0002	0.992
	Pseudomonas	3-10-1	0.1576	1.0537	1.0007	0.987
	B.thermosphacta	3-11-1	0.0959	1.0311	1.0004	0.990
	Lactobacillus	3-10-1	0.1306	1.0458	1.0008	0.989
	Coliform	3-12-1	0.0799	1.1082	1.0044	0.984
	Total[a]		0.1221	1.0511	1.0017	0.988
Four-vectormodel	TVC	4-11-1	0.1743	1.0539	1.0051	0.966
	Pseudomonas	4-11-1	0.1811	1.0647	1.0037	0.979
	B.thermosphacta	4-11-1	0.2711	1.0902	0.9897	0.905
	Lactobacillus	4-11-1	0.2317	1.0908	0.9952	0.962
	Coliform	4-11-1	0.2186	1.1734	1.0020	0.955
	Total[b]		0.2152	1.0946	0.9992	0.953

Total[a] and total[b] are used to evaluate the performance of the hole modeling data.

In order to validate the models, the datasets of condition 0°C, 65%O_2 35%CO_2 were experimentally gathered and tested with these models. In the testing datasets(Tab.3), the disparity between two models were also not significant. Compared to the three-vector model, the four-vector model got the relativelylowererror(RMSE: 0.4548 for three-vector model and 0.3572 for four-vector model), while the A_f and B_f were littleinferior to the three-vector model. This was caused by the different mathematical algorithm between these three indexes. The B_f for ANN models(1.021 for three-vector model and 1.0493 for four-vector model) was close to 1 indicating no significant bias and a slight overestimation for the five species. This can be further demonstrated by the predicted curves(Fig.2). The ANN predicted curves and counts were relatively higher than the experimental curves.

Moreover, based on the calculated indexes for the modeling datasets and testing datasets, it can be concluded that both models yielded good prediction. And the accuracy were not observably declined when the additional vector(bacterial species) was added to the four-vector ANN model(A_f: 1.1878 for three-vector model and 1.1886 for four-vector model in the testing datasets). This indicated that the more complicated four-vector ANN model can get reasonable good results for testing after appropriate training.

Table 3. Comparison of the performance of models(Testing data).

Model	Bacteria	Configuration	RMSE	A_f	B_f	R^2
Three-vectormodel	TVC	3-11-1	0.2452	1.0964	0.9461	0.914
	Pseudomonas	3-10-1	0.4695	1.2097	1.2078	0.938
	B.thermosphacta	3-11-1	0.4321	1.1706	1.1628	0.951
	Lactobacillus	3-10-1	0.2120	1.0793	1.9344	0.973
	Coliform	3-12-1	0.3459	1.4368	0.8873	0.743
	Total[a]		0.4548	1.1878	1.0210	0.942
Four-vectormodel	TVC	4-11-1	0.2131	1.0755	0.9903	0.869
	Pseudomonas	4-11-1	0.5670	1.3171	1.3171	0.979
	B.thermosphacta	4-11-1	0.3856	1.1828	1.0302	0.981
	Lactobacillus	4-11-1	0.2308	1.1095	0.9512	0.938
	Coliform	4-11-1	0.2819	1.2923	1.0147	0.892
	Total[b]		0.3572	1.1886	1.0493	0.940

Total[a] and total[b] are used to evaluate the performance of the hole testing data

In this study, there were four major spoilage bacteria(*Pseudomonas*, *B.thermosphacta*, *Lactobacillus* and coliform) in the chilled beef. The four-vector model we constructed was able to show a full-scale prediction by adding an extra neuron(bacterial species) into the input layer. All the bacterial counts and growth can be simulated in the model. Two factors (storing temperature and MAP air component) were taken into account in this experiment. And these two factors are the main environmental storing conditions in the super-markets. Using this model, the bacterial counts and remaining shelf-time can be rapidly predicted by filling the air component and Temperature into input layer.

In fact, the bacterial counts and shelf-time were also influenced by the characters of beef like pH and a_w. In further research, more environmental factors would be considered to improve this model. And the ANN is capable of performing massively parallel computations for data processing. It is reliable in predicting the complicated conditions such as nonlinear and time-variant biological process(Jeyamkondan *et al*., 2001; Geeraerd *et al*., 1998; Liu *et al*.,2008; Gracia-Gimeno, *et al*., 2005;Ross,1996; Ross,1999). After a proper training process, the ANN model can reach the desired precision as people set.

In conclusion, the bacterial growth in our daily consumed MAP chilled beef was discussed and predicted. The spoilage bacteria growing quicker with the increased temperature and oxygen content. As a result, the shorter shelf-time was obtained. The artificial neural networks is

an efficient method for predicting the bacterial growth as a food quality and safety assessment. It is a much simpler approach without setting multiple polynomial equations compared to high dimensional regression method. In this study, the two ANN models were demonstrated to got the satisfied prediction in both modeling and testing datasets. And the four-vector ANN model which adding an extra neuron into the input layer was proved to be able to present all the five bacteria(including TVC) growth in a single model. The precision of ANN model did not decline when the vector changed from three to four. Whilemore factors(like characters of beef)are directly added into the input layer to modified this model, the networks can be easily trained and improved its accuracy.

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