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Use of a simple catalase assay for assessment of aerobic microbial contamination on vegetables

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Abstract This paper presents a rapid catalase test for monitoring the aerobic microbial contamination associated with vegetables. The microbial loads of celery, bell pepper and ready-to-eat salad were serially tested over a 2-week period under common storage conditions. At each time point, samples were surface-sampled for catalase activity with a Pasteur pipette method in 5 min. Simultaneously, the aerobic viable microbial counts of the samples were determined using PCA, PIA and DRBC plates. It was found that the catalase activity (% gas produced) in the sample is positively correlated to the log concentration of aerobic bacteria and fungi. The catalase activity assay appears to be a fairly effective method for rapid assessment of aerobic microbial contamination on vegetables tested. The model based on the correlation between catalase activity and aerobic microbial loads was established and tested, with results that show accuracy of prediction at higher levels of contamination.

Keywords Catalase test · Vegetables · Spoilage · Prediction · Rapid detection · Spoilage contamination

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Introduction

Total aerobic microbial counts per unit weight of solid food (colony-forming unit, CFU/g) or per unit volume of liquid food (CFU/mL) are commonly used to evaluate microbial contamination of food. Although food spoilage is not precisely defined, in general, greater than 10^7 CFU/g or CFU/mL is regarded as an indicator for food that is spoiled (Ellis and Goodacre 2001). The total viable cell counts of microorganisms in food products may be determined by standard plate culture methods, which are reliable but cumbersome and labor-intensive. Currently, alternative methods based on impedometric techniques, dye reduction assays, or chromogenic/fluorogenic tests for detecting food microbial contamination are available, but they require minimal growth period of usually no less than 8–16 h (Feng 2001; Fung 2002; Kang et al. 2002). Ideas including special developments in early sensing of viable colonies on agar, electronic sensing of viable cells under the microscope, improvements of vital stains to count living cells, and more effective sensing of most probable number (MPN) of samples have been discussed or explored to improve the viable cell count procedure (Fung 2002). The importance of rapid methods for determination of total aerobic microbial counts has been emphasized (Fung 2002; Wu 2008).

Catalase is a constituent enzyme of most aerobic microorganisms (Wang and Fung 1986; Bin Jasass and Fung 1998; Kang et al. 2002). It is one of the mechanisms aerobes use to break down hydrogen peroxide by catalyzing the following reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$ (Gladwin and Trattler 2007). Since catalase is an obligatory enzyme of aerobic bacteria, the concentration (activity) of catalase may be positively correlated to the total aerobic viable cell counts of aerobic bacteria. Based on this hypothesis, catalase activity has been used for determining the total

viable microbial counts on chicken surfaces (Wang and Fung 1986), fish (Bin Jasass and Fung 1998), and milk (Kang et al. 2002). The safety of vegetables is becoming important due to an increased frequency of foodborne outbreaks associated with various vegetable products. According to U.S. Food and Drug Administration, (US FDA 2008) there have been 72 food-borne illness outbreaks associated with fresh produce from 1996 to 2006. The availability of a rapid method for testing these products is of particular importance since these products may be directly consumed without going through a cooking process, which normally kills most of the bacteria and makes the consumption safe. Currently, the feasibility of utilizing catalase activity in determining the total aerobic viable cell counts in vegetables is untested. The development of simple models for predicting aerobic microbial contamination using catalase analysis has not been reported yet. Therefore, the objectives of this study were to (1) establish a simple predictive model for unacceptable vegetables based on the correlation of catalase activity and aerobic viable microbial counts for celery, bell pepper, and ready-to-eat salad (lettuce), and (2) verify the aforementioned model by analyzing random samples.

Materials and methods

Food products and reagents

Celery, bell pepper, and ready-to-eat salad mix (lettuce) were bought fresh from the local grocer's shop and stored at 4°C within 24 h of purchase. Three of each type of sample were selected at random and prepared for the catalase analysis and microbial enumeration at 0, 2, 4, 6, 8, 11, 14, and 16 days of storage.

Sample preparation

For each sampling time, each sample (10 g) was placed into a separate stomacher bag with 9 mL 0.1% sterile peptone water and mixed thoroughly by manual shaking and rubbing. After mixing, the original sample solutions were used for the catalase assays. Appropriate 10-fold serial dilutions were made from the original sample solutions (10 g sample in 9 mL 0.1% sterile peptone water) using 0.1% sterile peptone water for microbial enumeration.

Catalase assay

Pasteur Pipettes (Fisherbrand 9" Borosilicate Glass; Fisher Scientific, Pittsburgh, PA, USA) were used for catalase analysis. The tips of Pasteur pipettes were heat-sealed. For each sample, 100 μL of the original mixed/stomached sample was transferred to a pipette. The liquid was forced to the narrow portion of the pipette by using centrifugal force in a motion similar to the one used when shaking mercury into the tip of a thermometer. Subsequently, a total of 100 μL of 3% hydrogen peroxide were added to the same pipette, and forced to the narrow portion in a similar fashion. The length of the liquid was measured from the sealed tip using a ruler. The pipette was inverted upside down for 5 min at room temperature (25°C) (Wang and Fung 1986; Bin Jasass and Fung 1998; Kang et al. 2002). During this period, the air bubbles generated, representing oxygen produced from the reaction, raised. The total length of the air column was recorded at the end of 5 min. If there were multiple air columns separated by the liquid, the combined length of the air columns was recorded. The catalase activity was expressed as:

$$\%\,\text{Catalase} = \frac{\text{Length of gas production in mm}}{\text{Length of the sample liquid plus reagent column in mm}} \times 100$$

Microbial enumeration

Serial 10-fold diluted sample solutions were spread-plated onto Plate Count Agar (PCA; Oxoid, UK), *Pseudomonas* Isolation Agar (PIA; Acumedia, Neogen, Lansing, MI, USA), and Dichloran Rose Bengal Chloramphenicol (DRBC; Acumedia) used to enumerate total aerobic microorganisms, *Pseudomonas* spp., and fungi, respectively. PCA and PIA plates were incubated at 37°C for 2 days whereas DRBC at 25°C for 4 days. The counts were done in duplicate.

Application of the established predictive models using random samples

The predictive models of aerobic microbial concentrations were established based on the correlation between catalase activity and aerobic microbial loads using linear regression trend lines and viable cell count (VCC) grouping. Samples from an actual environment were taken and the catalase assay, as described previously, was performed. In order to obtain actual VCC, the samples were serially diluted and examined using the procedures for microbial enumeration as described previously. Once the actual VCC had been obtained, the number was converted into $\log_{10}$ CFU/g. The percent catalase production was also recorded, and applied to the equation for the best-fit line of the standard curve as the y-value, and then solved for the x-value, or the $\log_{10}$ CFU/g predicted. The established predictive models were used to assess the aerobic microbial concentrations, and the predicted results were compared to actual results in order to ascertain the functionality of the model.

Data analysis

Experiments were conducted in triplicate. Bacterial numbers were converted to $\log_{10}$ CFU/g. Standard curves were established for each food and each type of culture plates. Linear regressions and correlation coefficients were performed between the catalase activity and the log concentration of total microorganisms using Microsoft Excel 2007 to establish predictive models for assessment of microbial concentrations. The data were further analyzed by grouping according to the concentration of total aerobic microorganisms, and the catalase activity within each group was averaged. The results were expressed as bar graphs as an alternative predictive model. For the application of established predictive model using additional random samples, the standard curves obtained from the time point study were used to estimate the concentration of total aerobic microorganisms.

Results

Relationships between catalase activity and total aerobic viable cell counts (VCC)

The relationships between catalase activity expressed as percentage and log CFU/g for celery, bell pepper, and ready-to-eat salad (lettuce) are shown in Fig. 1. The results include viable cell counts per gram of food, cultured on PCA, PIA, and DRBC plates. The scatter plots from model 1, which analyzes data points with all viable cell counts, show that catalase activities correlate reasonably well with the overall aerobic VCC. For celery, r^2 ranged from 0.58 to 0.74, and for bell pepper, r^2 was from 0.87 to 0.94, whereas r^2 ranged from 0.77 to 0.83 for ready-to-eat salad (lettuce). There was a stronger correlation between catalase activity and aerobic VCC when microbial loads were higher than 4 log CFU/g (analyzed by model 2, data not shown). An improvement in r^2 was shown with ranges from 0.89 to 0.98 for celery and 0.88 to 0.94 for bell pepper. For ready-to-eat salad (lettuce), models 1 and 2 showed the same r^2 ranges, since all the data points had viable cell counts greater than 4 log CFU/g. The data were also grouped according to the range of VCC and the corresponding average catalase activities were calculated (from 1–2 to 8–9 log ranges, model 3, data not shown). For celery, the VCC groups 2–3, 3–4, 4–5, and 5–6 (log CFU/g) were related respectively to the average catalase activities in each VCC group as 1.9, 2.4, 9.3, and 13.74 for PCA plates; groups 1–2, 2–3, 3–4, 4–5 and 5–6 (log CFU/g) as 1.91, 1.89, 5.98, 7.01, and 18.48 for PIA; and groups 3–4, 4–5, 5–6 and 6–7 (log CFU/g) as 1.72, 5.99, 11.30, and 18.48 for DRBC. For bell pepper, the VCC groups 3–4, 4–5, 5–6, 6–7, 7–8, and 8–9 (log CFU/g) were related respectively to the average catalase activities in each VCC group as 1.1, 1.21, 6.54, 46.44, 86.02, and 98.28 for PCA plates; as 1.1, 19.77, 29.05, 63.83, 75.31, and 98.28 for PIA; and as 1.1, 1.21, 29.05, 46.44, 75.31, and 98.28 for DRBC. For ready-to-eat salad (lettuce), the VCC groups 4–5, 5–6, 6–7, and 7–8 (log CFU/g) were related respectively to the average catalase activities in each VCC group as 21.44, 31.99, 43.44, and 47.13 for both PCA and PIA plates; and groups 5–6, 6–7, 7–8 and 8–9 (log CFU/g) as 21.44, 31.99, 43.44, and 47.13 for DRBC. The results were expressed in bar graphs, which likewise show reasonably good correlations.

The use of established models to assess total microbial counts on random samples

After the relationships of the catalase activity and the aerobic viable cell counts obtained on PCA, PIA, and DRBC plates were established (Fig. 1), random samples of celery, bell pepper, and ready-to-eat salad (lettuce) were tested for catalase activity to evaluate the established predictive models. The catalase activities for the random samples are in turn used to estimate the aerobic viable cell counts by all three type of models, i.e., linear equation established by including all the data points, linear equation established only including data points containing greater than 4 log CFU/g of viable cell counts, and bar graph established by grouping viable cell counts. The estimates of aerobic viable cell counts for the random samples of celery, bell pepper and ready-to-eat salad (lettuce) are shown in Table 1. All three models give more or less similar estimates. Viable cell counts enumerated on PCA, PIA, and DRBC plates were also done. The cultured results and the estimated results seem to agree reasonably well. Compared to the other models, model 2 provided better estimation of aerobic viable cell counts in celery, especially when cell numbers were greater than 4 log CFU/g. This could be seen from celery samples 1–8 under PCA counts, sample 2 under PIA counts, and samples 1, 2 and 4–8 under DRBC counts (Table 1). Both models 1 and 2 had similar estimation for bell pepper samples. Models 1 and 2 estimated the same results for ready-to-eat salad (lettuce), becuase all the data points used for establishing the model contain more than 4 logs. Model 3 showed the positive relationship between aerobic microbial counts and the average of catalase activities in all foods. It offers a quick estimation of aerobic microbial count ranges without need of calculation.

Discussion

The harmful effects of high concentrations of microorganisms in foods include food spoilage and the possibility of

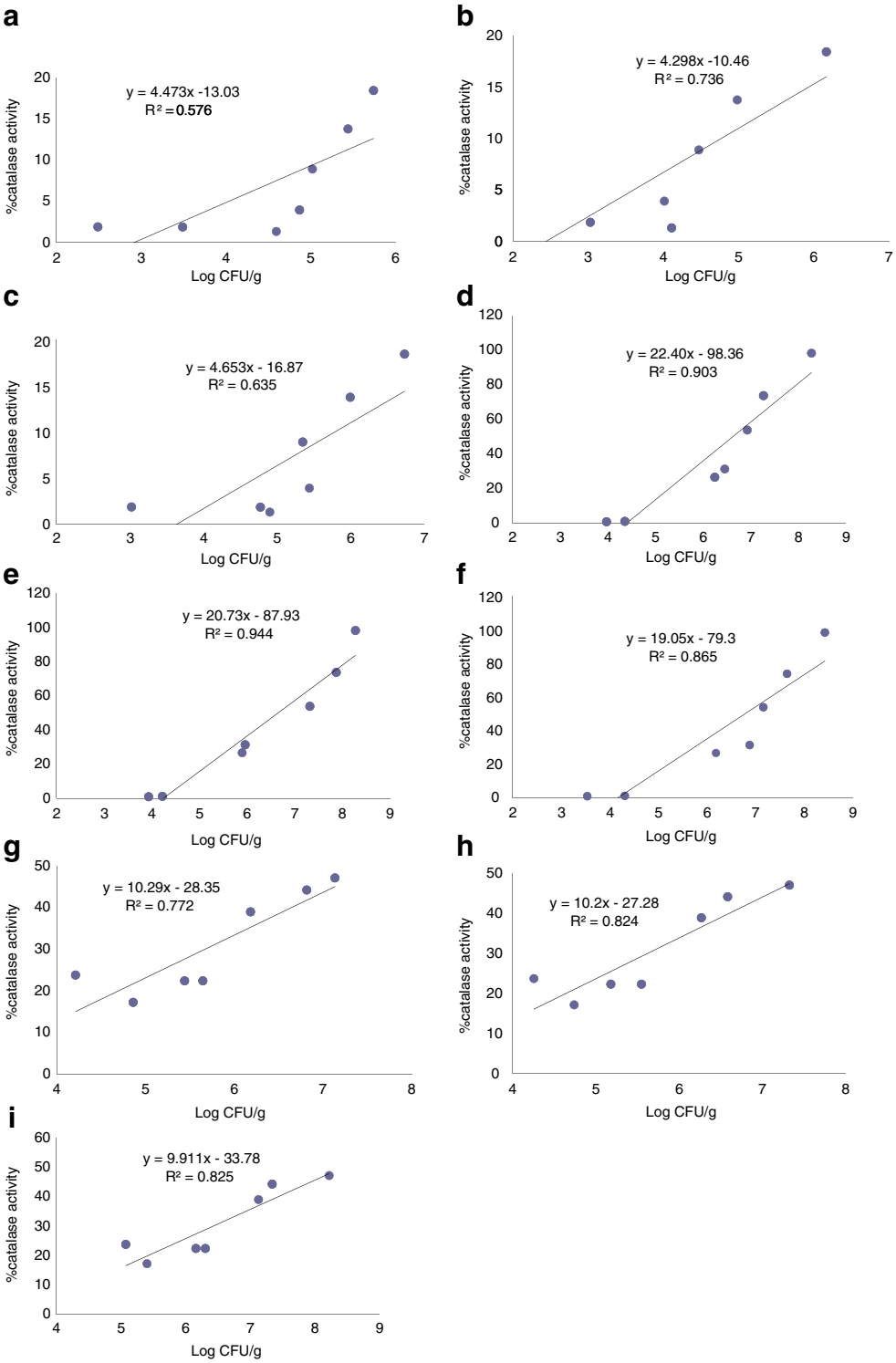

Fig. 1 Relationships of percentage catalase activity and aerobic viable cell counts: in celery cultured on **a** PCA plates, **b** PIA plates and **c** DRBC plates; in bell pepper cultured on **d** PCA plates, **e** PIA plates and **f** DRBC plates;and in ready-to-eat salad (lettuce) cultured on **g** PCA plates, **h** PIA plates and **i** DRBC plates. The results shown as scatter plots are obtained from model 1, which analyzes data points with all viable cell counts. Model 2 analyzes data points with viable cell counts greater than 4 log CFU/g with r^2 equal to 0.981, 0.885, and 0.912 for PCA, PIA, and DRBC plates, respectively (data not shown) for celery, with r^2 equals to 0.908, 0.944, and 0.884 for PCA, PIA, and DRBC plates, respectively (data not shown) for bell pepper. For ready-to-eat salad (lettuce), models 1 and 2 are under the same scatter plots since all the data points have viable cell counts greater than 4 log CFU/g

containing sufficient amounts of pathogenic microorganisms to directly cause diseases in humans. Thus, the concentration of total aerobic microorganisms in foods appears to be a good indicator for detecting the foods that are no longer suitable for consumption. The availability of a rapid detection method is most relevant for those foods that do not require additional cooking. Conventional methods need at least 48 h to enumerate the total microbial counts in vegetables or ready-to-eat foods. A rapid method for monitoring total microbial loads may provide the processors the level of hygiene of their processes and may also enable processors to predict shelf-life of vegetables or

Table 1 Aerobic viable cell count (log CFU/g) in random samples of celery, bell pepper, and ready-to-eat salad (lettuce) estimated by the established predictive models using catalase activity[a]

Sample	C %	PCA 1/2	PCA 3	VCC^{PCA}	PIA 1/2	PIA 3	VCC^{PIA}	DRBC 1/2	DRBC 3	VCC^{DRBC}
1 celery	4.16	3.8/4.8	3~4	4.5±0.2	3.4/4.1	4~5	3.5±0.2	4.5/5.1	3~4	4.9±0.3
2 celery	5	4/4.8	4~5	4.7±0.1	3.6/4.2	4~5	4±0.1	4.7/5.2	4~5	5.2±0.3
3 celery	2.7	3.5/4.7	3~4	4.5±0.2	3.1/3.9	3~4	3.5±0.3	4.2/4.9	4~5	4.0±0.2
4 celery	2.6	3.5/4.7	3~4	4.7±0.2	3.1/3.9	3~4	3.4±0.2	4.2/4.9	4~5	4.7±0.1
5 celery	2.5	3.5/4.7	3~4	4.3±0.3	3/3.8	3~4	3.3±0.3	4.2/4.9	4~5	4.5±0.3
6 celery	0.8	3.1/4.5	2~3	4.5±0.3	2.6/3.6	2~3	4.6±0.1	3.8/4.7	2~3	4.4±0.1
7 celery	6.66	4.4/5.0	4~5	5.5±0.3	4.0/4.4	4~5	5.3±0.3	5.1/5.4	5~6	5.7±0.2
8 celery	3.5	3.7/4.8	4~5	5.2±0.2	3.2/4.0	4~5	5.0±0.1	4.4/5.0	4~5	4.8±0.2
1 bell pepper	8.0	4.7/5.0	5~6	4.7±0.3	4.6	4~5	4.7±0.4	4.6/4.9	5~6	4.4±0.2
2 bell pepper	4.5	4.6/4.9	5~6	4.6±0.2	4.5	4~5	4.6±0.2	4.4/4.9	5~6	4.2±0.3
3 bell pepper	1.7	4.5/4.8	3~4	3.7±0.2	4.3	3~4	3.7±0.2	4.3/4.8	4~5	4.7±0.3
4 bell pepper	9.91	4.8/5.1	5~6	4.7±0.3	4.7	3~4	4.8±0.2	4.7/5.1	4~5	4.7±0.1
5 bell pepper	2.52	4.5/4.8	4~5	3.7±0.3	4.4	3~4	3.7±0.3	4.3/4.8	4~5	4.7±0.3
6 bell pepper	40	6.2/6.3	6~7	6.3±0.3	6.2	5~6	5.5±0.1	6.3/6.4	6~7	7.0±0.3
7 bell pepper	80	8.0/7.9	7~8	7.0±0.3	8.1	7~8	6.7±0.2	8.4/8.2	7~8	8.0±0.2
8 bell pepper	71.6	7.6/7.5	6~7	6.7±0.2	7.7	6~7	6.0±0.1	7.9/7.8	7~8	7.0±0.2
9 bell pepper	31.5	5.8/6.0	6-7	6.6±0.3	5.8	6-7	5.5±0.2	5.8/5.0	6-7	5.4±0.5
10 bell pepper	28	5.6/5.8	5~6	6.0±0.3	5.6	5~6	5.5±0.3	5.6/5.9	5~6	6.5±0.5
1 lettuce	42.2	6.9	6-7	7.8±0.2	6.8	6-7	7.8±0.2	7.7	7-8	6.4±0.1
2 lettuce	30.08	5.7	5~6	5.1±0.1	5.6	5~6	4.7±0.6	6.4	6~7	5.4±0.3
4 lettuce	41.02	6.7	6~7	6.0±0.1	6.7	6~7	5.9±0.1	7.5	7~8	6.2±0.4
4 lettuce	11.3	3.9	3~4	4.9±0.3	3.8	3~4	4.5±0.2	4.5	4~5^	4.8±0.1
5 lettuce	36.0	6.3	6	5.8±0.2	6.2	6	5.6±0.2	7.0	7	5.6±0.2
6 lettuce	15.8	4.3	4-5	5.2±0.1	4.2	4-5	4.9±0.1	5.0	4-5^	4.5±0.3
7 lettuce	34.9	6.1	6	6±0.1	6.1	6	5.9±0.3	6.9	7	5.5±0.4
8 lettuce	5.12	3.3	3~4	3.8±0.2	3.2	3~4	3.1±0.1	3.9	4~5^	4.6±0.3

[a] C % Catalase activity %; *PCA 1&2*, *PIA 1&2, DRBC 1&2* estimated log CFU/g by the scatter plot (model 1 and 2); *PCA 3*, *PIA 3*, *DRBC 3* estimates obtained from bar graphs (model 3), where ^ indicates estimates were obtained from one range below the lowest range available in model 3; the numbers under VCC are the mean values of the actual viable cell counts ± SD obtained from plating (PCA, PIA, or DRBC).

ready-to-eat products. In this project, the feasibility of using catalase assay as a rapid method for a surrogate marker for the concentration of total aerobic microorganisms was tested.

Modifications of microbial AP bioluminescence procedures which require from 5 to 15 min and estimate total microbial contamination in foods have been reported (Griffiths 1991, 1993; Bautista et al. 1995; Siragusa et al. 1995; Samkutty et al. 2001). However, the values of ATP are variable in foods and a specific instrument is needed for the ATP analysis (Siragusa et al. 1995). Chromogenic *Limulus* amoebocyte lysate (LAL) test was reported for estimating aerobic plate counts and coliform counts; however, the test only monitors lipopolysaccharide in Gram-negative bacteria (Siragusa et al. 2000).

The catalase assay used in this project quantifies the amount of molecular oxygen (O_2) released in a 5-min time period in reference to the initial liquid volume after mixing equal volume of 3% hydrogen peroxide and the liquid sample to be tested. The amount of oxygen released is positively related to the total amount of catalase, which, in turn, is positively related to the aerobic microorganisms that are present in the sample. The assay is easy to perform, without the need for expensive equipment or reagents, and the results are available within a few minutes; therefore, it is a very practical method for the implementation of HACCP as well as for catering, for a school cafeteria setting and for all places where ready-to-eat foods are consumed.

It was noted that when aerobic viable cell counts is below 10,000 CFU/g (4 log CFU/g), there is generally insignificant catalase activity. This is best shown in celery samples: when the data points from low viable cell counts (lower than 4 log CFU/g) are excluded, the linear fit of the

equation significantly improves. Bar graph of grouped data also appears to be a good way of denoting the relationship between catalase activity and aerobic viable cell counts, and it does not have the requirement of linearity of the data. In addition, using bar graphs to estimate aerobic viable cell counts has the advantage of simplicity because it does not require calculation. As such, using model 2 or 3 should give estimates that are reasonably in agreement with each other.

From the data presented in Table 1, all three models appear to give estimates that are in agreement with each other; however, models 2 and 3 should work better. In addition, the estimates and culture results appear to be in reasonable agreement.

Following validation experiments, it was concluded that catalase can be used as a rapid method for estimating aerobic microorganisms for unacceptable vegetables between the actual and predicted results. However, it should also be noted that lower concentrations of aerobic bacteria—which yield lower percent catalase activity—produced less accurate results, though the food at the time of testing appeared physically unappealing. This agrees with previous studies. Kang et al. (2002) reported that the detection limit of catalase activities was about 5.0 log CFU/mL for monitoring total mesophilic microbial load in milk, because milk samples with less than 5.0 log CFU/mL of viable cell counts maintained low catalase activities. However, there was a positive relation between catalase activities and aerobic plate counts (>5.0 log CFU/mL) during refrigeration at 4°C. Bin Jasass and Fung (1998) indicated that catalase activities in raw fish were strongly correlated with high numbers of total bacteria counts (>4.0 log CFU/mL or g).

In conclusion, the catalase assay is a simple, reliable and rapid way of detecting concentration of total aerobic microorganisms in contaminated vegetables tested. Catalase activity had better noticeable amounts when aerobic VCC was greater than 4 log CFU/g, which is an approximate non-acceptable level of VCC generally used in the food industry for ready-to-eat food products. It may have potential utility in any other place in which ready-to-eat foods are consumed to estimate the shelf-life or to predict the microbial counts for unacceptable vegetables. The models established will be subject to more random sample testing. We are currently studying the use of the catalase assay to establish Modified Gompertz and Modified Logistic models to predict the growth of *Pseudomonas* spp.

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