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Model for Estimating CO_2 Concentration in Package Headspace of Microbiologically Perishable Food

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Abstract

Levels of carbon dioxide gas, a metabolite of microbial growth, have been reported to parallel the onset of microbial spoilage and may be used as a convenient index for a packaged food's shelf life. This study aimed to establish a kinetic model of CO_2 production from perishable food for the potential use for shelf life control in the food supply chain. Aerobic bacterial count and package CO_2 concentration were measured during the storage of seasoned pork meat at four temperatures (0, 5, 10 and 15°C), and their interrelationship was investigated to establish a mathematical model. The microbial growth at constant temperature was described by using model of Baranyi and Roberts. CO_2 production from the stored food could be explained by taking care of its yield and maintenance factors linked to the microbial growth. By establishing the temperature dependence of the microbial growth and CO_2 yield factor, CO_2 partial pressure or concentration in package headspace could be estimated to a limited extent, which is helpful for controlling the shelf life under constant and dynamic temperature conditions. Application and efficacy of the model needs to be improved with further refinement in the model.

Key words: CO_2 production, package headspace, microbial growth, aerobic bacteria, Baranyi model

INTRODUCTION

Because of the high concerns about microbial spoilage in perishable foods, shelf life control to ensure their microbial quality has been of great interest to the industry. As an endeavour to conveniently control the shelf life on real-time basis, metabolites produced from the microbial growth or spoilage have been investigated as useful indicators for microbial quality. The presence and concentration of volatile metabolites such as trimethylamine and some sulfides in the package headspace can be detected by appropriate sensors to give an indication about the microbial growth without contacting the food (1). Among the volatiles, carbon dioxide gas in the food package has been reported to have high correlation with microbial spoilage and can serve as an indicator for microbial spoilage (2-4).

Sensors monitoring CO_2 gas concentration change in the package can work to infer the microbial food quality, perceive the onset of spoilage and screen out the spoiled foods as safety measures. Understanding the behaviour of the package's CO_2 gas concentration change will also augment the sensor system in terms of shelf life indication. A looped feedback mechanism of predicting and sensing the CO_2 concentration in the package headspace will be able to help control shelf life *in situ*. In this context, prediction of CO_2 concentration as a function of package and storage conditions will serve as a useful auxiliary tool to control and manage the shelf life in food supply chain.

Therefore, this study aims to develop a predictive model for CO_2 package concentration in a format to be applied to controlling the microbial shelf life of perishable food.

MATERIALS AND METHODS

Seasoned pork meat

As a typical seasoned side dish in ready-to-cook form for Korean food meals, seasoned pork was selected for this study and prepared according to a general standard recipe (4), which is described below. One kg of the fresh meat was marinated in 234 g of seasoning (Cheongjeongwon, Cheonan, Korea) and then aged at 6°C for 2 hours. The seasoned meat had a salt content of 0.4%, pH of 5.6 and water activity of 0.95.

One hundred grams of the prepared food were filled into 250 mL cylindrical glass bottles (diameter of 6.0 cm and height of 10.8 cm), which were closed hermetically by gas-tight lug caps. The gas impermeable property of the glass jar does not allow any gas loss out of the container and thus makes it possible to easily

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quantify total CO_2 production from the food. Free volume measured by filling the bottle with water was 150 mL. The bottled food packages were stored under constant or dynamic temperature conditions, with package headspace CO_2 concentration being measured and aerobic bacterial count of the foods being determined. In a package experiment involving dynamic temperature, a one liter jar package containing 300 g food (free volume 702 mL) was used with the attachment of a CO_2 sensor and thermocouple temperature senor.

Package headspace CO_2 concentration and aerobic bacterial count

Package headspace CO_2 concentration was measured for one milliliter of gas samples taken from the bottle by using a gas-tight syringe. A Varian Model 3800 Gas Chromatograph (Varian Inc., Palo Alto, CA, USA) equipped with an Alltech CTR I Column (Alltech Associates Inc., Deerfield, IL, USA) and a thermal conductivity detector was used. This procedure allows non-destructive measurement of the package headspace CO_2 concentration. Ideal gas law was used to convert the CO_2 concentrations to its partial pressure (usually in kPa). For online monitoring of headspace CO_2 concentration, a nondispersive infrared CO_2 sensor (Model K33, SenseAir, Delsbo, Sweden) was placed under the cap of the package and the CO_2 concentration data were transferred to computer.

To measure the aerobic bacterial count of the stored product, 30 g of food samples from each bottle were aseptically transferred to sterile Stomacher bags, and 90 mL of sterile 0.05% peptone water was added. The samples were then homogenized for 4 min at 300 rpm in a Stomacher (400 Circulator, Seward Limited, Worthing, West Sussex, England). Aliquots were plated out directly or as ten-fold dilutions in 0.05% peptone water onto Plate Count Agar (Difco Laboratories, Detroit, MI, USA). Microbial colonies were counted after incubation of 30°C for 72 hr and multiplied to express the microbial count as colony-forming units (CFU) per gram of sample. All of the measurements were conducted for triplicate packages. Standard deviation of microbial count was usually around 0.3 in unit of log (CFU/g).

Modelling of CO_2 production related to microbial growth

CO_2 production was linked mathematically to aerobic bacterial growth. Even though there are different microbial species associated with microbial spoilage of seasoned pork, aerobic bacteria were reported to be highest in the count during the storage, and they represent the specific spoilage organisms (4-7). Thus, for the sake of simplicity, CO_2 production was assumed to result from aerobic bacterial growth, which has been described by the model of Baranyi and Roberts (8):

$$\frac{dN}{dt} = \mu_{max}\left(\frac{q}{1+q}\right)\left(1-\frac{N}{N_{max}}\right)N \quad (1)$$

where N is the microbial count in CFU/g at time t, μ_{max} is the maximum specific growth rate (day^{-1}), N_{max} is the maximum cell density in CFU/g, and q of a conceptual component critically needed for the microbial growth is accumulated by first order kinetics of Equation 2.

$$\frac{dq}{dt} = \mu_{max} q \quad (2)$$

Equations 1 and 2 can also be given in an integrated form with initial conditions of q and N (q_o and N_o):

$$\log N = \log N_o + \frac{\mu_{max}}{\ln(10)}\cdot\left[t + \frac{1}{\mu_{max}}\cdot\ln\left\{\frac{e^{-\mu_{max}t}+q_o}{1+q_o}\right\}\right] - \frac{1}{\ln(10)}\cdot\ln\left(1+\frac{e^{\mu_{max}A}-1}{10^{(\log N_{max}-\log N_o)}}\right) \quad (3)$$

where A isand the parameters, N_o, q_o, N_{max} and μ_{max} can be obtained by using nonlinear regression technique. Because q_o and μ_{max} are related to lag time (t_{lag}, day) as:

$$t_{lag} = \frac{\ln\left(1+\frac{1}{q_o}\right)}{\mu_{max}} \quad (4)$$

the parameter set of Baranyi and Roberts model is often given as N_o, t_{lag}, N_{max} and μ_{max}, and they were obtained in this study by using DMFit (Institute of Food Research, Norwich, UK), which is offered in a website (http://modelling.combase.cc).

CO_2 production of the microbial growth was assumed to result from energy supply for cell biosynthesis and viable cell maintenance, and thus modelled as sum of growth-associated part ($Y_{CO2/N}\frac{dN}{dt}$) and non-growth-associated part (M·N) (9):

$$\frac{dm_{CO2}}{dt} = Y_{CO2/N}\frac{dN}{dt} + M\cdot N \quad (5)$$

where $Y_{CO2/N}$ is yield factor (mg CFU^{-1}) and M is maintenance coefficient (mg CFU^{-1} day^{-1}).

The parameters in Equation 5 ($Y_{CO2/N}$ and M) were estimated according to the algorithm of Fig. 1 from experimental CO_2 production and microbial count data for temperatures of 0, 5, 10 and 15°C. CO_2 produced from the stored food (m_{CO2} in mg) was obtained as sum of that in package headspace ($V_h C_{CO2}$) and that dissolved in food ($V_f D_{CO2}$):

$$m_{CO2} = V_h C_{CO2} + V_f D_{CO2} \quad (6)$$

where V_h is volume of package headspace (mL) and C_{CO2} is headspace CO_2 concentration (mg mL^{-1}). Because the aqueous and fatty phases of food are mainly responsible for dissolving CO_2 (10-12), CO_2 dissolved in food was

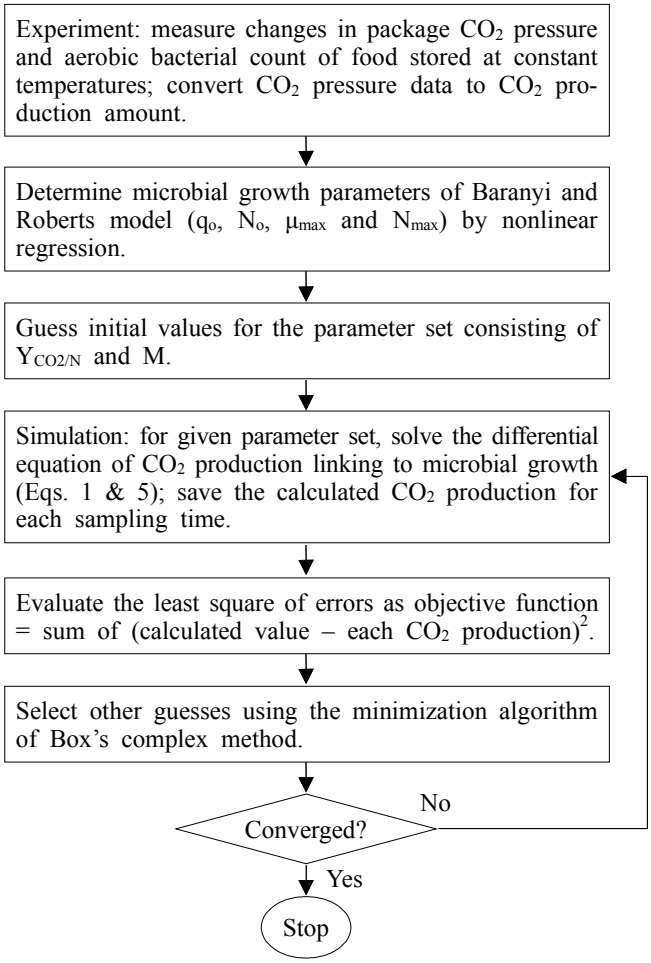

Fig. 1. Algorithm to determine CO_2 production model parameters.

taken into account by water and fatty fractions (V_f in mL), which were estimated from the literature as 74.9 and 6.9%, respectively (13).

The D_{CO2}, CO_2 concentration dissolved in the aqueous and fatty phases, was estimated using Henry's Law,

$$D_{CO2} = k_{CO2}\ P_{CO2} \quad (7)$$

where P_{CO2} is the partial pressure of CO_2 in the headspace (bar) calculated from C_{CO2} by using the Ideal Gas Law, and k_{CO2} is Henry's Law constant (mg mL^{-1} bar^{-1}). The k_{CO2} was obtained from Rammert and Paderson (14) as function of temperature and CO_2 partial pressure under the assumption of zero dissolved oxygen.

$$k_{CO2} = 3.43764 - 0.014P_{CO2} - 0.12723T + 2.8256 \times 10^{-3}\ T^2 - 3.3597 \times 10^{-5}\ T^3 + 1.5933 \times 10^{-7}\ T^4 \quad (8)$$

where T is temperature (^oC).

The algorithm in Fig. 1 determines the CO_2 production parameters ($Y_{CO2/N}$ and M) by an iteration scheme of Box's complex method, which fits the model to CO_2 production data based on the microbial growth provided by Equation 1. Due to the uncertainties in initial content of CO_2 present in the product, the correction factor for the initial amount contained in the product was applied in the search for the parameter.

With the model parameters obtained, Equations 1 and 5 can estimate the microbial growth and CO_2 production under constant and dynamic temperature conditions.

RESULTS AND DISCUSSION

Fig. 2 shows the bacterial count and CO_2 production of the seasoned pork meat packaged in glass jars and stored at different temperatures. By fitting the model of Baranyi and Roberts (Equations 3 and 4) to the microbial count data, the parameters of N_o, N_{max}, t_{lag} and μ_{max} were obtained (Table 1) and given as function of temperature in Fig. 3. As shown in Fig. 2, the Baranyi and Roberts model with those parameters can describe the microbial growth well. N_o of asymptotic initial load does not depend on temperature and may be reasoned to be dictated, not by storage temperature, but by processing conditions. N_{max} of maximum cell density at the stationary phase increases with increased storage temperature (Equation 9), which is the usually observed phenomenon for spoilage of many foods (15-17).

$$\log\ N_{max} = 0.1549\ T + 9.3505 \quad (9)$$

Lag time (t_{lag}) and maximum specific growth rate (μ_{max}) are strong functions of temperature: high temperature decreases lag time and increases the growth rate. A square root model can be conveniently used for describing their temperature dependence (18,19):

$$\sqrt{1/t_{lag}} = 0.0489\,T + 0.5951 \quad (10)$$

$$\sqrt{\mu_{max}} = 0.1103\,T + 1.1414 \quad (11)$$

Based on the algorithm of Fig. 1, CO_2 production mod-

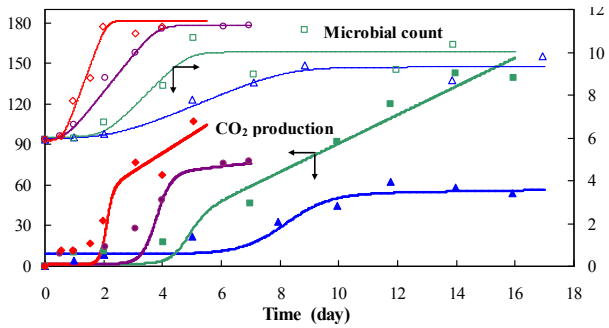

Fig. 2. Aerobic bacterial count and CO_2 production of seasoned pork stored at different temperatures. 100 g of pork product was in a 250 mL glass jar. Triangle, 0^oC; square, 5^oC; circle, 10^oC; diamond, 15^oC. Open and filled symbols are experimental data for microbial count and CO_2 production, respectively. Thick lines are estimated values for CO_2 production, while thin ones are for bacterial count.

Table 1. Parameters for aerobic bacterial growth and CO_2 production

Temperature (°C)	Bacteria growth				CO_2 production	
	log N_o	log N_{max}	t_{lag} (day)	μ_{max} (day^{-1})	$Y_{CO2/N}$ (mg CFU^{-1})	M (mg CFU^{-1} day^{-1})
0	5.95	9.31	2.40	1.33	2.20×10^{-10}	1.54×10^{-12}
5	6.04	10.01	2.02	3.24	3.41×10^{-11}	1.04×10^{-11}
10	5.90	11.26	0.69	3.97	3.69×10^{-12}	1.25×10^{-13}
15	5.88	11.47	0.60	8.58	1.89×10^{-12}	4.62×10^{-13}

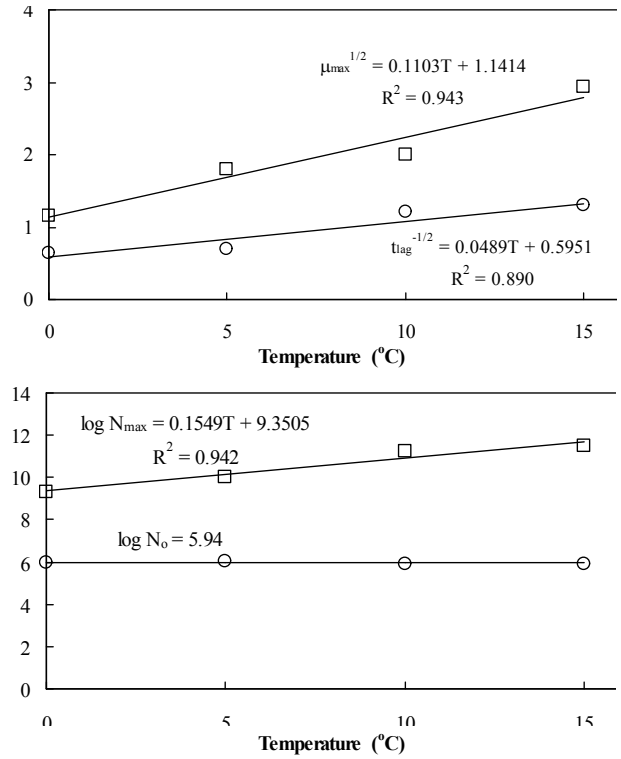

Fig. 3. Dependence of microbial growth model parameters on temperature.

el parameters ($Y_{CO2/N}$ and M) were determined to fit with CO_2 production data in Fig. 2 (Table 1). While the maintenance coefficient M is indifferent with temperature, being log M of -12.009±0.815, the yield factor, $Y_{CO2/N}$ decreases with temperature and its logarithm can be described as a linear function of temperature (Fig. 4):

$$\log Y_{CO2/N} = -0.1432\ T - 9.7464 \quad (12)$$

Now from the models of microbial growth and CO_2 production with temperature dependence of their model parameters, CO_2 production or package CO_2 concentration can be estimated by solution of differential Equations 1, 2 and 5. Lag time of microbial growth with assumed microbial count of N_o was estimated as the time for summed lag phase contribution ($\int F_{lag}\ dt$) to reach 1, where F_{lag} is the inverse of lag time calculated according to Equation 10 for the temperature at each time increment (20,21):

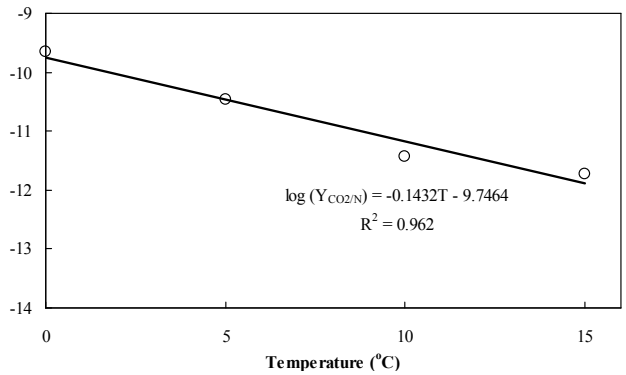

Fig. 4. Dependence of yield factor on temperature.

$$\int_0^{t_{lag}} F_{lag}\ dt = 1.0 \quad (13)$$

The initial physiological state of the cell population at the start of lag phase was assumed to be an averaged value of q_o for the microbial lag time as given by (15):

$$\bar{q}_o = \frac{\int_0^{t_{lag}} q_o\ dt}{t_{lag}} \quad (14)$$

where q_o at each time increment can be obtained from the relationship of Equation 4 given above.

Package CO_2 partial pressure can be calculated numerically from the estimated CO_2 production by the relationship of Equation 6 incorporating Ideal Gas Law and Henry's Law of Equation:

$$m_{CO2} = V_h \frac{10^5 P_{CO2} M_{CO2}}{RT_a} + V_f k_{CO2} P_{CO2} \quad (15)$$

where M_{CO2} is the molecular weight of CO_2 (0.044 kg/mol), R is the gas constant (8.314 J K^{-1} mol^{-1}) and T_a is absolute temperature (K).

The prediction model described above was applied to the package submitted to the conditions of temperature change (Fig. 5 and 6). The estimation described the CO_2 partial pressure change being in parallel with experimental data but with slight discrepancy. There has been delay of CO_2 pressure increase in the prediction during the initial period of time. This discrepancy may have come from the variation in food samples and the simplified assumption of linking CO_2 production to aerobic bacterial growth. Microbial groups other than aerobic bacteria

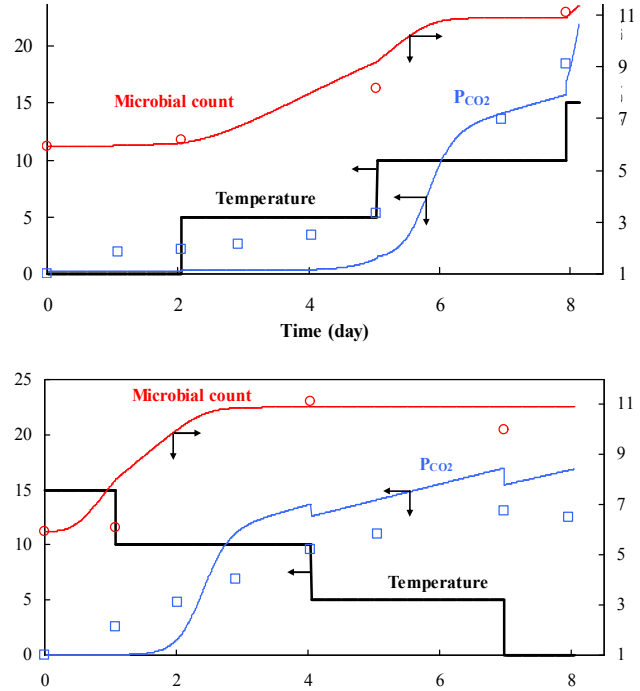

Fig. 5. Two sets of comparison between estimated and experimental CO_2 partial pressures of seasoned pork package exposed to stepwise temperature conditions. Bold line is temperature while thin lines are estimations for microbial growth and CO_2 partial pressure. Upper and lower panels are for different temperature change regimes. ○, experimental microbial count; □, experimental CO_2 partial pressure.

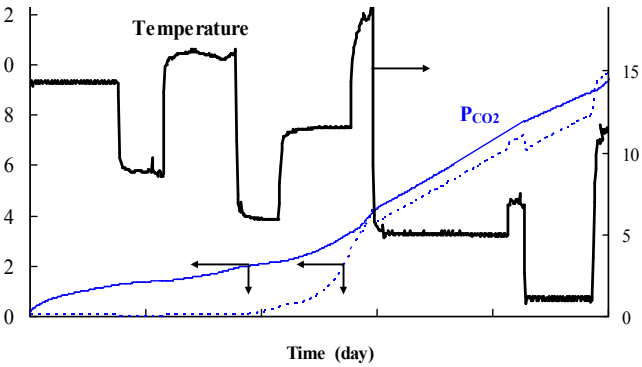

Fig. 6. Comparison between estimated and experimental CO_2 partial pressures of seasoned pork package experiencing dynamic temperature change. Thin solid and dotted lines are measured and estimated CO_2 partial pressures, respectively, while bold solid line is product temperature measured.

would have been involved in CO_2 production, but were not fully covered in the modelling. There would also have been limitation in the simplification of temperature dependence of lag time and rate of microbial growth and functional interrelationship among microbial growth, CO_2 production and further dissolution in the food. For example, microbial growth can be affected by the produced CO_2, which has been shown to have an antimicrobial effect (18). However, predicting CO_2 concentration behaviour, even to a limited extent, can help to control shelf life of microbiologically perishable food as mentioned before. This research needs to be understood as a first step for applying the CO_2 pressure prediction in shelf life management as a subsidiary tool. Further research may widen the application and efficacy of the modelling.

The approach and findings of the present study would be understood to propose and show the way of expressing kinetics of CO_2 production and its contribution to package atmospheric changes of a perishable food. Because CO_2 production behavior varies with types of foods, as reported by Kim et al. (4), kinetic parameters of CO_2 production for specific food types needs to be determined individually to apply the proposed method to other foods. Accumulation of the kinetic parameters may help the potential application of the proposed modelling approach by identifying the significant variables and categorizing the food type in terms of CO_2 production.

CONCLUSION

By modelling the CO_2 production of a perishable food related to aerobic bacterial growth, CO_2 partial pressure or concentration in the package headspace could be estimated to a limited extent, which is helpful for controlling the shelf life under both constant and dynamic temperature conditions. Application and efficacy of the model need to be improved with further refinement in the model.

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