

## 체다치즈의 숙성 전과정에 대한 수학적

김 중 균

부산수산대학교 생물공학과

# A Mathematical Model for the Whole Ripening Process of Cheddar Cheese

Joong Kyun Kim

Department of Biotechnology and Bioengineering  
National Fisheries University of Pusan, Pusan 608-737, Korea

### ABSTRACT

A model to explain the observed kinetics in a whole process of Cheddar-cheese ripening has been developed. It includes growth and lysis of cells in the cheese matrix, cell-wall bound proteinases and intracellular dipeptidases that are released into cheese upon cell lysis, and the production of dipeptides and amino acids from casein in cheese. Model simulations have been conducted to figure out the crucial factors in the process of the cheese ripening. The influential factors have been found to be the cell numbers and the dipeptidase activity at the beginning of the cheese ripening, and the cell-lysis rate of cheese starters. The simulation results have also suggested the use of a mixed culture as well as the experimental screening for a more suitable organism as a cheese starter. Hence, the model shows how to accelerate the cheese ripening.

### INTRODUCTION

The production of a Cheddar-cheese starts with the fermentation of milk. Pasteurized milk is fermented during which coagulation occurs to form cheese curd. After the cheese curd is cooked, cheddared, salted, and pressed, it shapes a cheese block. Then, the block is ripened conventionally at 2 to 15°C for several months to acquire the unique cheese flavor, body and texture(1). It is really a time- and energy-consuming process.

In the process of cheese ripening, cheese starter and non-starter bacteria in the cheese block grow

to some extent at the expense of lactose, and cell-lysis also occurs at the same time. After the lactose is exhausted, cells only lyse for several months. During this period., a portion of casein is solubilized and the cheese structure is modified so that the cheese mass becomes more integrated, softer, and smoother (2). Thus, the cheese ripening is the most important process that determines the quality of ripened cheese in the market.

The whole process in cheesemaking must be controlled properly, otherwise off-flavor and bitter taste easily can be obtained(3). Mixed cultures of the cheese starters have been used since

less proteolytic mutant cells produce significantly less bitterness than the parent cells do (4). To reduce the costs of cheese production the ripening process must be shorten while maintaining the quality of cheese. Thus, the characteristic reactions of cheese starter cells during cheese ripening should be studied in order to determine which factors are important in this process. A mathematical model which describes the whole ripening process is necessary and is herein proposed.

### MODEL ASSUMPTIONS

The followings are based in this model (5, 6):

1. The cheese components (cells, proteins, fats and moisture) are uniformly distributed in the cheese block :
2. Only homogeneous enzyme-catalyzed reactions take place.
3. There are no enzyme concentration gradients.
4. There is no mass or heat transfer between the inside and outside of the cheese block.

Thus, in essence, the local enzymatic reactions dominate and the effects of diffusion are neglected in this model.

### MODEL DEVELOPMENT

The ripening process of Cheddar cheese can be divided into two phases. In the first phase, cells grow at the expense of residual unfermented-lactose, which is converted to lactic acid. Some lysis of cells may also occur concomitantly. The rates of cell growth and cell lysis in this phase are closely related to the level of enzymes at the beginning of ripening. After the lactose is fermented, the second phase starts and lasts for several months. In this phase, cells H : only lyse and any lysis of cells will result in the release of dipeptidases into the cheese matrix. Our mathematical model is established for the second phase first, followed by the extension to cover the cell growth in the first phase, and followed by the simulations of the whole ripening period.

The cheese considered here is a quality Cheddar cheese which is made by the normal cheese-making process. The process of cheese ripening is an enzymatic process where several enzymes participate and give the cheese its unique characteristics. Proteinases present in milk, rennet and starter bacteria bread down casein to peptides, and peptidases break down the peptides further to amino acids. The proteinases are present on the surface of starter cells while the dipeptidases are intracellularly located. Thus, dipeptidases act on the peptides mainly when they are present in the cheese matrix after cell lysis. Since proteinases are bound at the cell wall, their concentrations are not affected by the cell lysis. The activities of both these enzymes are affected by their own deactivation in the cheese matrix. The composition of amino acids and peptides in cheese will be used as an index of the effectiveness of the ripening process since cheese flavor has been known to be governed by those composition (7, 8, 9).

#### Kinetic Equations

Table 1 presents six equations which govern the ripening reactions of the second phase where cell lysis is the only cell-related event. These equations are considered only the microbial proteinases and dipeptidases since peptides and amino acids have been known to be formed during cheese ripening mainly as a result of the

**Table 1. Equations which simulate the phase II of cheese ripening**

Cell: $dX/dt = -k_1 \cdot X$ ,	$X(0) = X_0$
Proteinases: $d\hat{e}_1/dt = -[(k_1/k_2) \cdot k_2 - k_1] \cdot \hat{e}_1$ ,	$\hat{e}_1(0) = 1.0$
Dipeptidases: $de_2/dt = k_1 \cdot \alpha_2 + (k_1 - k_2) \cdot e_2$ ,	$e_2(0) = e_{20}$
Casein: $dA/dt = -V_i \cdot e_{10} \cdot [A/(A + K_m)] \cdot X \cdot \hat{e}_1$ ,	$A(0) = A_0$
Dipeptides: $dB/dt = -1.08 \cdot (dA/dt) - (1/1.08) \cdot (dC/dt)$ ,	$B(0) = B_0$
Amino acids: $dC/dt = V_o \cdot [B/(B + K_m)] \cdot X \cdot e_2$	$C(0) = C_0$

1. Initial conditions are designated by the subscript  $_0$
2. Parameter  $k_1$  has been redefined as the product of  $k_1/k_2$  and  $k_2$ .
3. The enzyme activities have been normalized as follows:  $\hat{e}_1 = E_1(X \cdot e_{10})$  and  $e_2 = E_2/X$ .

activities of those enzymes (10, 11). The factors 1.08 in the governing equation for dipeptidases have been introduced to account for addition of one molecule of water every time a dipeptide bond is hydrolyzed. The experimental data to estimate model parameters were obtained from the published papers (7, 8, 12-19). None of these data sets was complete in the different variables considered in this model. Hence, the missing values of variables in the data sets were assumed to be average values for quality Cheddar cheese reported in the literature (20). The solutions of these equations allow us to follow the profiles of cell count, casein, dipeptides, amino acids, and the enzymatic activities in the cheese matrix during the phase II of cheese ripening.

In the first phase of cheese ripening, cells grow and lyse simultaneously. It has been assumed that the growth rate of the cheese starter depends on the concentration of limiting lactose which is utilized to produce lactic acid and cell mass. The equation proposed by Luedeking and Piret (21) was chosen for the description of acid production. This equation is known to be valid for a number of strains involved in lactic acid production (22). Dipeptidases are synthesized intracellularly when cells grow in this phase, and some of dipeptidases are released into the cheese matrix since cells lyse at the same time. Hence, only extracellular dipeptidases degrade as cheese age. The synthesis rate of intracellular dipeptidases has been considered as a growth-associated activity. The specific activity of dipeptidases was found to be the highest in the active cell-growth phase (23).

It has been observed in many studies (16, 24, 25) that the formation of amino acids by dipeptidases does not occur in the early phases of cheese ripening. This could be an effect of catabolite repression. In the absence of any further information, the lactose effect on enzyme expression has been written in the form of a threshold concentration ( $L^{crit}$ ) above which no dipeptidase production occurs; at lactose concentrations below  $L^{crit}$ , enzyme production is dependent upon lactose concentration as per the Michaelis-

**Table 2. Equations which simulate the phase I of cheese ripening**

Cell: $dX/dt = \mu_m * [L / (L + K_1)] * X - k_1 * X$
Lactose: $dL/dt = -1/y_p * (b_{11} * \mu_m * (L / (L + K_1)) + b_{12}) * X - 1/y_x * u_m * [L / (L + K_1)] * X$
Total dipeptidases: $dE_2^{tot}/dt = \alpha_2 * \mu_m * [L / (L + K_1)] * X * [1 - u(L - L^{crit})] - k_2 * (E_2^{tot} - E_2^{intra})$
Intracellular dipeptidases: $dE_2^{intra}/dt = \alpha_2 * \mu_m * [L / (L + K_1)] * X * [1 - u(L - L^{crit})] - k_1 * E_2^{intra}$
Initial condition*: $X(0) = X_0$
$L(0) = L_0$
$E_2^{tot}(0) = E_{2,0}^{tot}$
$E_2^{intra}(0) = E_{2,0}^{intra}$

\* Designated by the subscript  $\alpha$ .

Menten kinetics. The kinetic equations for the phase I are tabulated in Table 2. Published data (16, 24, 26) were used to estimate the model parameters.

Unfortunately, these data sets were incomplete in the different variables considered in this mode. The experimental data were collected for *Streptococcus cremoris* NCDO 924. Hence, the parameter,  $k_1$ , was estimated again in order to obtain the cell growth pattern for this particular strain. The degradation rate of dipeptidases was assumed to be the same as that estimated in the phase II since cheese ripening process takes place under fairly constant temperature and pH. The solution of these equations subject to initial conditions allow us to follow the profiles of cell count, lactose, and dipeptidase activity in the cheese matrix.

Based upon the analysis of the two phases of cheese ripening, a comprehensive model for the whole ripening process was developed and the cheese ripening was simulated. It would involve growth of cells, consumption of lactose, production of the proteinases and the dipeptidases, degradation of casein and the peptides, and formation of amino acids. Since dipeptidases are released into the extracellular environment due to cell-lysis, separate equations for the intracellular and total dipeptidases are required. The eight governing equations involved in the model are shown in Table 3.

**Table 3. Equations which simulate the whole process of cheese ripening**

$dX/dt = \mu_m^* [L/(L+K_1)]^* X - k_1^* X$
$dL/dt = -1/y_p^* (b_{11}^* \mu_m^* [L/(L+K_1)] + b_{12}^*) X - 1/y_x^* \mu_m^* [L/(L+K_1)]^* X$
$dE_2^{xs}/dt = \alpha_2^* \mu_m^* [L/(L+K_1)]^* X^* [1-u(L-L^m)] - k_2^* (E_2^{xs} - E_2^{rms})$
$dE_2^{rms}/dt = \alpha_2^* \mu_m^* [L/(L+K_1)] + (k_1 - \mu_m^* [L/(L+K_1)] - k_1)^* \hat{e}_1$
$dA/dt = -V_f^* e_{1,0}^* [A/(A+K_m)]^* X^* \hat{e}_1$
$dB/dt = -(1.08)^* (dA/dt) - (1/1.08)^* (dC/dt)$
$dC/dt = V_b^* [B/(B+K_m)]^* (E_2^{xs} - E_2^{rms})$
Initial conditions*: $X(0) = X_0$
$L(0) = L_0$
$E_2^{xs}(0) = E_{2,0}^{xs}$
$E_2^{rms}(0) = E_{2,0}^{rms}$
$\hat{e}_1(0) = 1.0$
$A(0) = A_0$
$B(0) = B_0$
$C(0) = C_0$

\* Designated by the subscript  $_0$

In our model, only the starter cultures have been considered to contribute to cheese flavor (1, 24, 27). No distinction has been made between different types of the cells since proteinases are bound at the cell wall and there is no difference in dipeptidase components in the parent cell and the mutant cell (28). The effect of phage infection on the bacterial population has not been also considered since its influence on cheese flavor has not been found to be important (18).

## RESULTS AND DISCUSSION

Marquardt's algorithm (29) was used in each estimation of model parameters in order to fit all the available experimental data.

### Model for the Phase II of Ripening

A total of six model parameters in Table 1 were estimated. The initial values used in the estimation of the parameters were set from the published papers (7, 16, 20);  $X_0 = 1 \times 10^7$  cfu/g,  $e_{2,0} = 2.0 \times 10^{-9}$  units/cfu,  $A_0 = 245$  mg/g,  $B_0 = 25$  mg/g, and  $C_0 = 2.0$  mg/g, respectively.  $V_f^* e_{1,0}$  was estimated as a single parameter and the values of  $K_m$

**Table 4. Estimation of parameters for the phase II of ripening<sup>+</sup>.**

Parameters: $k_1 = 8.56 \times 10^{-3} \pm 1.11 \times 10^{-3}$	[1/day]
$k_2 = 2.35 \times 10^{-2} \pm 2.29 \times 10^{-3}$	[1/day]
$\alpha_2 = 8.72 \times 10^{-9} \pm 4.84 \times 10^{-10}$	[units/cfu]*
$k_1/k_2 = 1.00 \times 10^{-4} \pm 1.19 \times 10^{-4}$	[-]
$V_f^* e_{1,0} = 4.46 \times 10^{-8} \pm 6.81 \times 10^{-9}$	[mg/cfu/day]
$V_b = 3.88 \pm 2.89 \times 10^{-1}$	[mg/units/day]*

+ The estimates are in 95% confidence limits.

\* One unit is  $\mu$ mole of product liberated  $\text{min}^{-1}(\text{ml extract})^{-1}$ .

and  $K_m'$  were fixed at 0.207 and 1.15 mg/g, respectively (30, 31). In any case, the value of  $K_m$  is not crucial since casein is always present in excess in the system. The estimated values for the model parameters are shown in Table 4. It has been assumed that the parameters are the same for all of the strains used as cheese starters. The environmental conditions is cheese matrix during cheese ripening would affect the model parameters since the activities of microbial enzymes vary by the conditions. In a conventional process, however, it has been known to be least affected by the conditions, such as pH (32), the moisture content (33), and the uniformity of salt concentration (34).

The estimation errors were under  $\pm 15\%$  except  $k_1/k_2$ , and these small errors support the initial assumptions that all the strains can be characterized by the same set of parameters. The ratio ( $k_1/k_2$ ) was estimated to be very small. This indicates that the cell-wall bound proteinases are much more stable than dipeptidases, which is also reported by Law *et al.* (7), because proteinases remain attached to the cell wall after cell lysis while dipeptidases are released in the cheese matrix as free enzymes. This is also in agreement with the report that immobilized enzymes (cell-wall proteinases) have longer half life than suspended enzymes (free dipeptidases) (18). The errors in  $k_1/k_2$  could result in a significant miscalculation of the activity of the proteinases in the cheese matrix. This is not the surprising, as no applicable experimental data for the activity of proteinases were available.

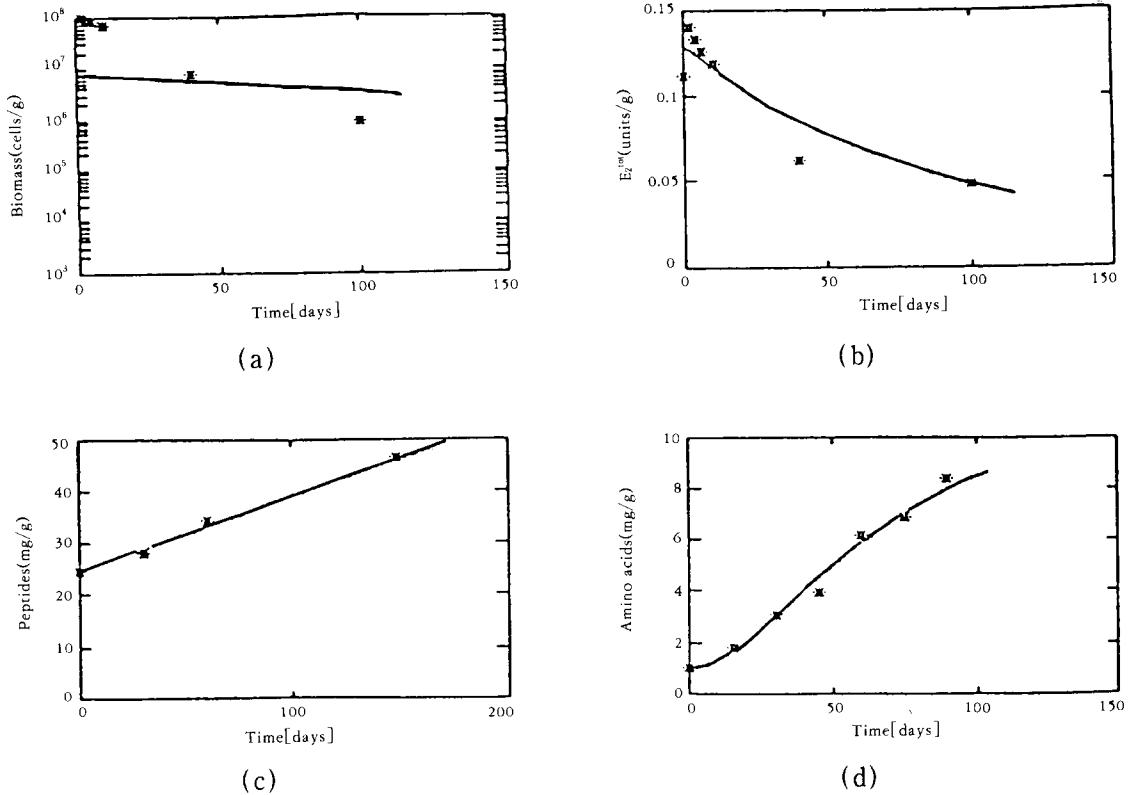


Fig 1. Simulated results for the second phase of ripening (data from the literature [7, 12, 13]. a; Viable cell count [7], b; Total dipeptidase activity [7], c; Peptide concentration [12], d; Amino acid concentration [13].

Unfortunately, this error will be propagated in the form of errors in predictions of the amounts of casein that is degraded by proteinases. As a result, the parameter values obtained here can be constructed only as estimates of the actual values. The proposed use of these model equations and the parameters is expected to be an investigation of the most sensitive variables the control of which will result in the maximum return, in terms of reduction in the ripening times while maintaining cheese quality.

Comparisons of the simulation results with published results can be used to look into the validity of the model expressions. Several calculated profiles and experimental data have been shown in figures 1(a-d). Solid lines in these figures are

results of simulations corresponding to initial conditions of each investigation. Experimental data, where available are shown as stars (7, 12, 13). Some of other figures show somewhat more scatter but indicate similar trends. The estimated curves for the viable cell counts do not fit the experimental data very well as seen in figure 1A. This would be caused by the errors in plate-counting or by the different cell-lysis rate of distinct starter cultures. The characteristics of starter cells such as salt tolerance and thermal tolerance, in the cheesemaking process would influence the cell-lysis rate. Another possibility would be that the starter cells may lyse more rapidly when in competition with contaminants usually present in the cheese block than in their absence

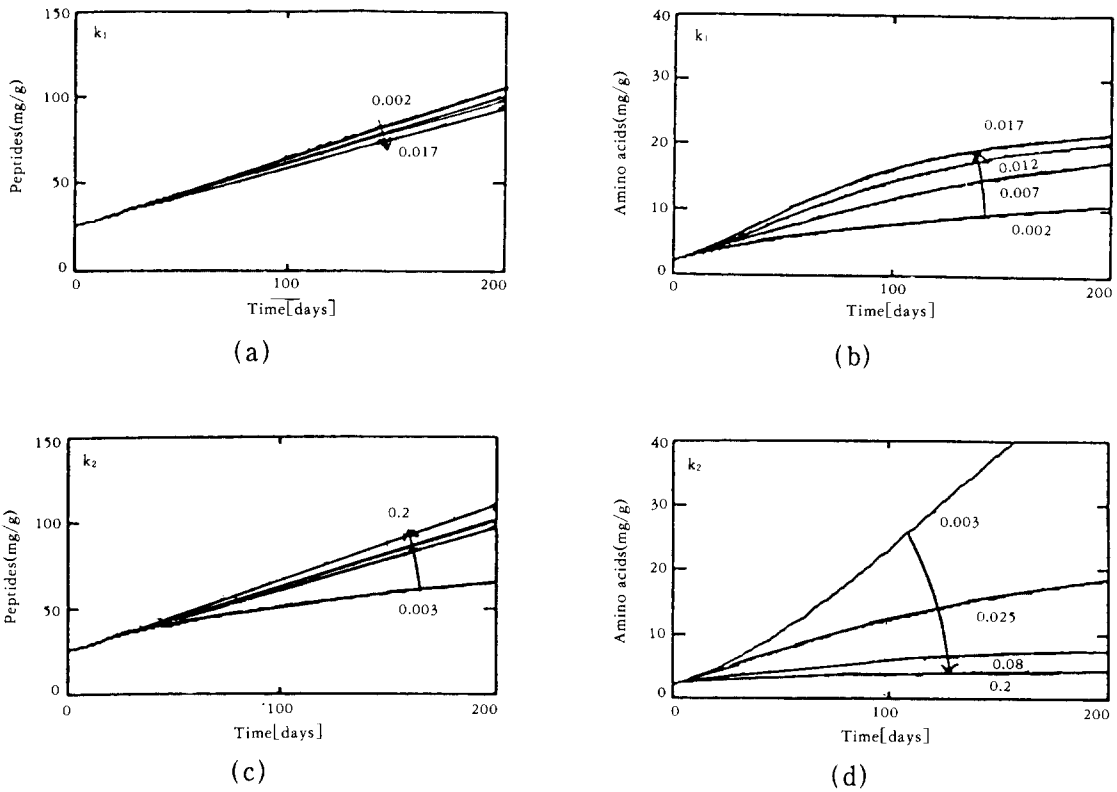


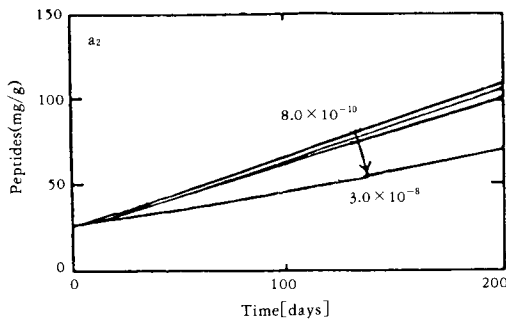
Fig 2. Sensitivity analysis for  $k_1$  and  $k_2$  in the second phase of ripening a & c; Peptide concentration, b & d; Amino acid concentration.

(19). The viable cell counts have been found to be different even in two lots of the same cheese (35).

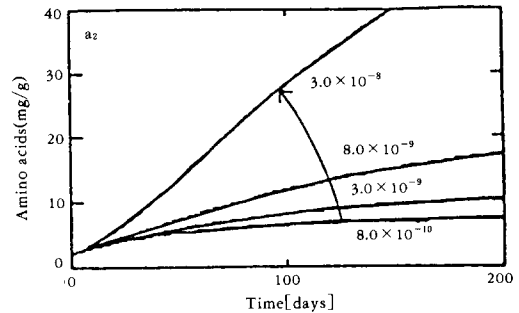
From the results, the model equations describe the degradation of casein and dipeptides and do the formation of amino acids very well. In particular the estimated profile of total dipeptidases fitted very well. The activity of dipeptidases in the cheese matrix clearly is shown to be affected by cell lysis. This is responsible for cheese flavor. This fact can be seen in the study of Law *et al.* (16). They demonstrated by using lysozyme-treated cells that the free amino acid concentration at the beginning of cheese ripening increased about three times compared with the control cheese.

Sensitivity tests of the model for each parame-

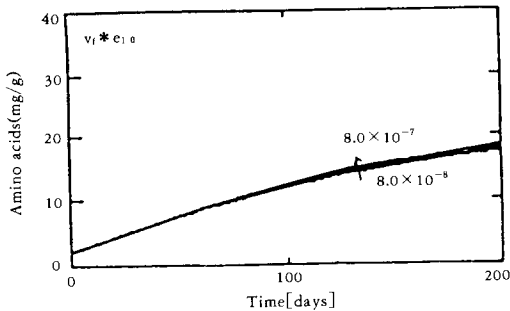
ter and their initial values were also made. Parameters  $k_1$ ,  $k_2$ , and  $\alpha_2$  and initial values of  $X(0)$  and  $e_2(0)$  were found to be significantly affect the results (see Figures 2-4). On the other hand, the effect of  $V_0$  and  $V_1^*e_{1,0}$  on cheese flavor were not large and that of the ratio ( $k_1/k_2$ ) was almost none. The parameters  $\alpha_2$ ,  $X(0)$  and  $e_2(0)$  can be manipulated by use of different amounts of starter cultures. If a higher concentration of starter cultures,  $X(0)$ , is used, more amino acids are produced. This is deemed to be good because the amino acids are the precursor of good cheese flavor (36). An imbalance of proteinases and peptidases can however result in formation of too much bitter peptides. This results in a degraded cheese flavor. The use of mixed cultures of the parent cells and less proteolytic mutants has been



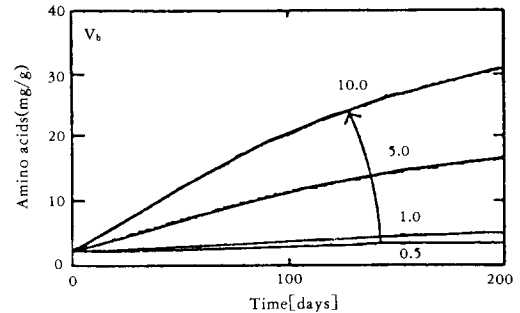
(a)



(b)



(c)



(d)

Fig 3. Sensitivity analysis for  $\alpha_2$ ,  $V_f * e_{1,0}$  and  $V_b$  in the second phase of ripening a; Peptide concentration, b, c, d; Amino acid concentration.

suggested as a means to correct this problem (4). Manipulations of the mixed cultures in the phase preceding the phase II of ripening influence the relative proportions of proteinases and peptidases. Hence this factor must be considered in the phase I when  $E_1$  is synthesized since the control of bitterness is essentially the control of total proteinase activity in the cheese matrix.

The effects of  $k_2$  and  $\alpha_2$  on the model variables are shown in Figure 3. Both the parameters significantly affected the activity of dipeptidases and amino acid formation, whereas these parameters did not affect cell counts. Thus, even under a constant cell population cheese ripening can be accelerated by selecting appropriate strains of cheese starters which have lower  $k_2$  or/and a higher  $\alpha_2$ . The experimental work of Langsrud *et*

*al.* (37), who investigated the autolytic properties of some starter streptococci, supports this conclusion.

#### Model for the Phase I of Ripening

The estimated parameters for the phase I of ripening are shown in Table 5. The initial values used in the estimation of the parameters were fixed from the values reported in literature (16, 20, 24, 38):  $L_0=8.5\text{mg/g}$ ,  $E_{2,0}^{\text{tot}}=0.028\text{units/g}$ , and  $E_{2,0}^{\text{intra}}=0.005\text{units/g}$ , respectively. The value of constant  $K_1$  was  $0.296\text{ mg/g}$  obtained from the published data (39). For the yield coefficients  $y_p$  and  $y_x$ ,  $0.80\text{g/g}$  (40) and  $1.04 \times 10^9\text{ cfu/mg}$  (41) were used, respectively. From a comparison of the profiles of dipeptidases and of lactose, the critical lactose concentration ( $L^{\text{crit}}$ ) was set to

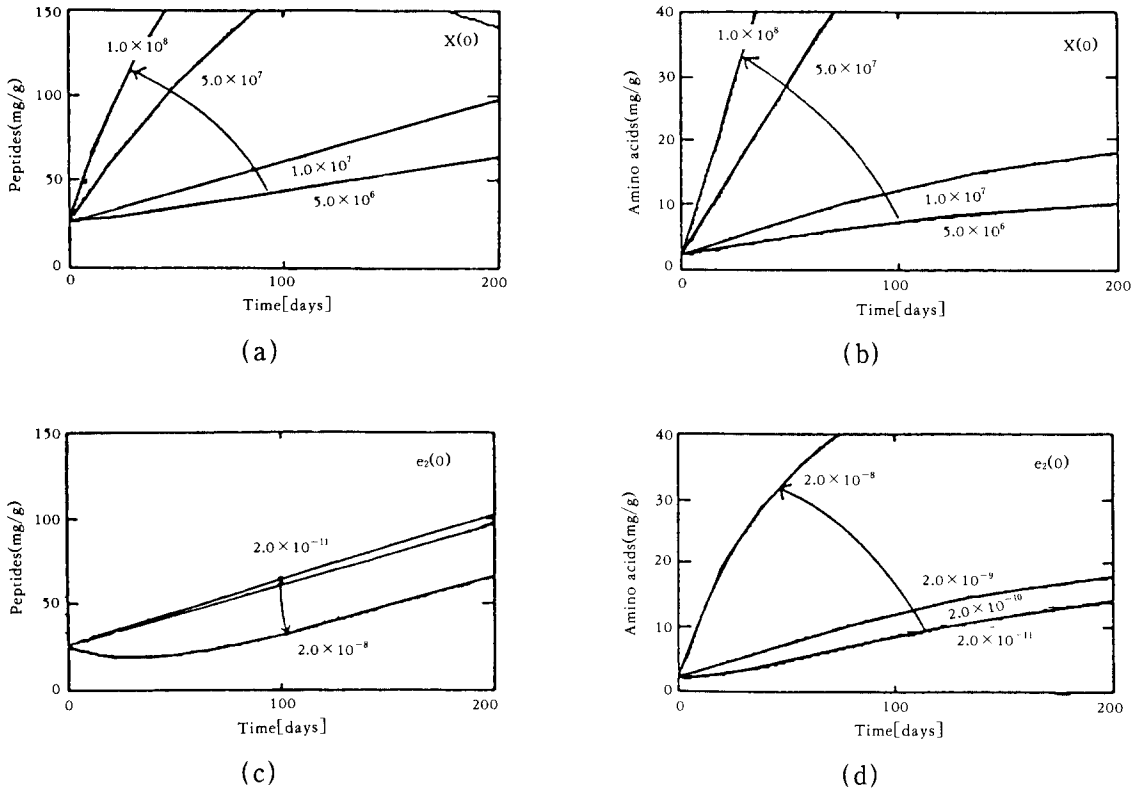


Fig. 4. Sensitivity analysis for  $X(0)$  and  $e_2(0)$  in the second phase of ripening a & c; Peptide concentration, b & d; Amino acid concentration.

Table 5. Estimation of parameters for the phase I of ripening<sup>+</sup>.

Parameters: $K_1 = 2.55 \times 10^{-1} \pm 3.00 \times 10^{-2}$	[1/day]
$\mu_m = 1.49 \times 10^{-1} \pm 1.31 \times 10^{-2}$	[1/day]
$b_{11} = 1.88 \times 10^{-9} \pm 1.95 \times 10^{-9}$	[mg/cfu]
$b_{12} = 6.59 \times 10^{-11} \pm 1.32 \times 10^{-10}$	[mg/cfu/day]
$\alpha_2 = 4.90 \times 10^{-10} \pm 3.73 \times 10^{-10}$	[units/cfu]*

+ The estimates are in 95% confidence limits.

\* One unit is  $\mu\text{mole of product liberated min}^{-1}(\text{ml extract})^{-1}$ .

be 1.5mg/g.

The estimates of few parameters are almost insignificant, as suggested by the large values of the confidence intervals (Table 5). This is a reflection of the small number and the poor quality of data available. The values of  $\alpha_2$  which is relat-

ed to the yield of dipeptidase in growing cells. No measurements were available for the intracellular concentration of dipeptidases. On the other hand, the estimation errors are under 12% for the parameters  $k_1$  and  $\mu_m$ . The estimated value of  $k_1$  for *Streptococcus cremoris* NCDO924 is two orders of magnitude higher than the value of  $k_1$  obtained in the modeling of the phase II for other *S. cremoris* strains. This is likely to be due to variations in the strains (19, 42), although the conditions of ripening may also have played a role. Thus, the strain of cheese starters could significantly affect the cheese flavor. The values of  $\mu_m$  was two orders of magnitude less than that, reported in literature (23), in the initial stages of cheesemaking where milk is fermented to form curd. This is not surprising, since the conditions during ripening



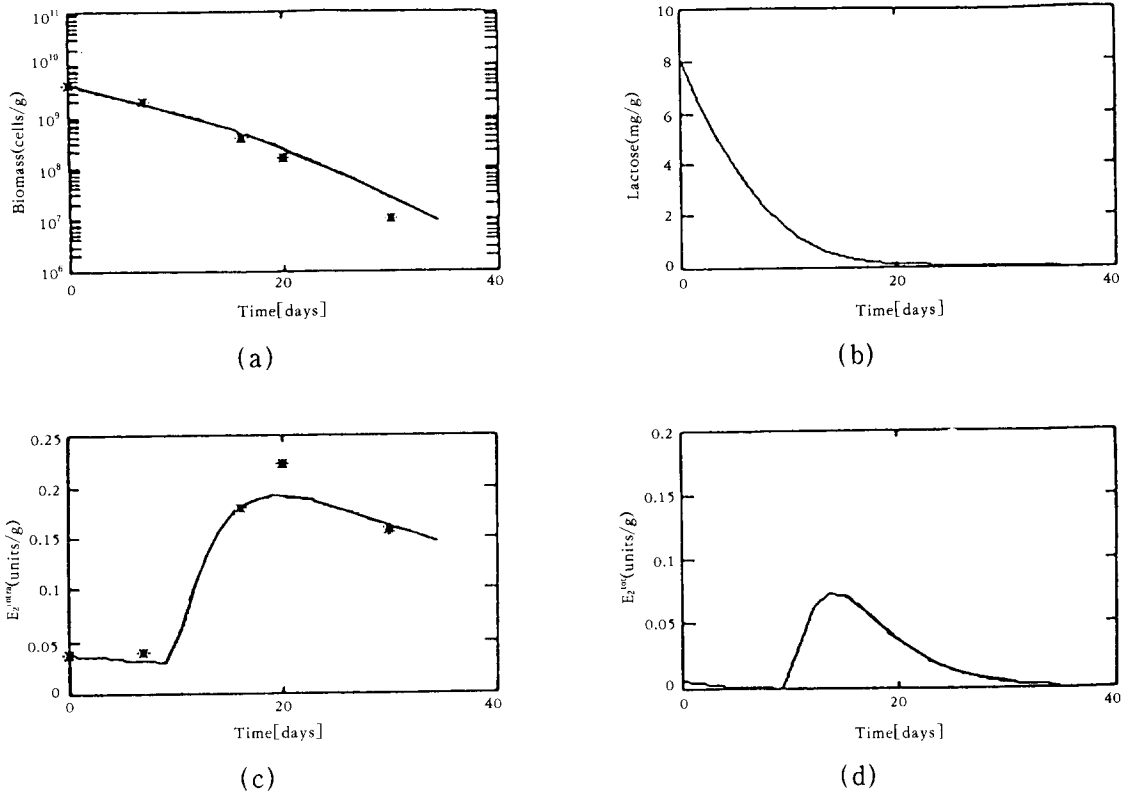


Fig 5. Simulated results for the first phase of ripening (data from the literature [16]). a; Viable cell count, b; Lactose concentration, c; Total dipeptidase activity, d; Intracellular dipeptidase activity.

are not very conducive to growth, due to the lower temperature and high salt concentration. Yet, this analysis still gives some valuable information concerning the behavior of the system.

Some results of simulation for the phase I of ripening process are shown in Figure 5(a-d). The data points are also shown in the figures as stars. The concentration of  $E_2^{tot}$  are seen to peak at the end of the first phase of ripening. The maximum amount of dipeptidases in the cheese matrix is dependent upon the amount of residual lactose in the cheese. This lactose concentration is decided by the fermenting ability of the starter bacteria and the conditions of the cheese manufacture. This finally affects the formation of amino acids.

#### Model for Whole Ripening Process

The simulation of the whole ripening process were carried out with the parameter values obtained from the modeling of the phase I and II of ripening. Since production of proteinases takes place in the first phase, the values of  $V_f^*e_{1,0}$  and  $c_{11}/e_{1,0}$  were estimated by fitting the results of the simulation of equations in Table 3 into experimental measurements of peptides and amino acids (7, 8, 12-17, 18, 19). The parameter  $c_{11}/e_{1,0}$  was estimated as  $8.53 \times 10^{-11} \pm 3.00 \times 10^{-11}$  mg/cfu/day. The estimated value of the parameter  $V_f^*e_{1,0}$  was found to be  $0.792 \pm 0.467$ , and as expected this value is smaller than that in Table 4.

The initial values of the different values used in the simulations are;  $X_0 = 1 \times 10^9$  cfu/g,  $L_0 = 8.5$  mg/g,  $E_{2,0}^{tot} = 0.028$  units/g,  $E_{2,0}^{intra} = 0.005$  units/g

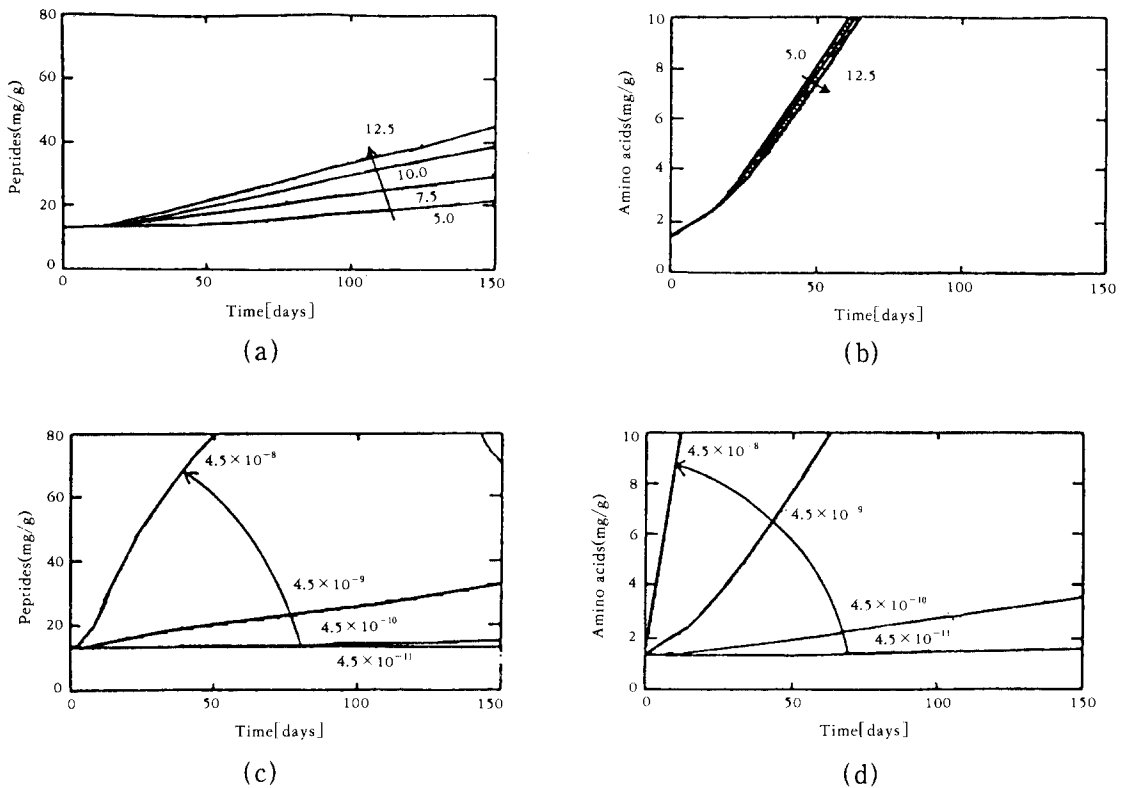


Fig 6. Simulated results for the whole ripening process. a & b; The effect of  $L_0$  on the peptide concentration (a) and on amino acid concentration (b), c & d; The effect of  $V_t^*e_{i,0}$  on peptide concentration (c) and on amino acid concentration (d) at a constant ratio of  $V_b/V_t^*e_{i,0}$ .

$g, e_{i,0}=1.0, A_0=258\text{mg/g}, B_0=13.1\text{mg/g},$  and  $C_0=1.33\text{mg/g}$ , respectively. The values of constants in the equations were the same as the average values in Table 4 and 5. Two different values of the cell-lysis rate constant,  $k_1$  were used in the simulations since the two groups of *Streptococcus cremoris* strains were found to have different  $k_1$ .

An increase in the initial value of lactose caused a delay in the dipeptidase formation (Figure 6a and 6b). This resulted in a decrease of amino acid formation and a rise in the amounts of bitter peptides as well. This trend varies by the fermenting ability of starter cells, and the residual lactose concentration in the cheese block is decided by this factor (32). The salt concentration in the moisture of the cheese block and uni-

form salting is considered to be crucial in developing quality cheese flavors (26).

The changes in the value of  $c_{11}/e_{i,0}$  did affect only in amounts of peptides. Hence,  $c_{11}/e_{i,0}$  did affect only in amounts of peptides. Hence,  $c_{11}/e_{i,0}$  should be held at a lower value in order not to produce degraded cheeses. The parameter  $V_t^*e_{i,0}$  appears to be not crucial in the development of desirable cheese flavor. It does, however affect bitterness in cheese and should be held at a lower value. Figure 6c and 6d show the effect of changing the parameter  $V_t^*e_{i,0}$  in the cheese block while keeping the ratio of  $V_b/V_t^*e_{i,0}$  constant. At this condition, the activity of the proteinases as well as the peptidases increase at the same time. The figures show that the higher amounts of

amino acid and peptides were produced at a higher value of the ratio ( $V_0/V_1^*e_{1,0}$ ). To reduce the bitterness in cheese, this ratio should be lowered. However, the cheese ripening is retarded due to the lower activity of the peptidases. Hence, a mixed culture with less proteolytic cells is desirable.

So far, no one has reported the proteinase activity and lactose concentration during the cheese ripening with mixed starter cultures. The mixture of different types of starter cells will influence the amounts of proteinases and peptidases in the cells, which are parameters in the proposed model. Hence, this mathematical model can be also applied for mixed stater cultures without any loss of information. Since an imbalance of proteinases and peptidases can result in formation of too much bitter peptides, the appropriate composition of mutant cells in the mixed culture should be decided at an early stage of cheese-making.

## CONCLUSIONS

A model has been developed for the process of cheese ripening. The ripening process can be accelerated by increasing  $X(0)$ ,  $e_2(0)$ , and  $k_1$ . Also selecting appropriate strains which have a lower  $k_2$  and higher  $\alpha_2$  is crucial. However, lower amounts of surface-bound proteinases are required not to produce a degraded cheese flavor. These enzymatic reactions are well described in the model proposed. Thus, the model should prove to useful to cheese makers to control the cheese ripening process and to produce the cheaper quality cheese ultimately. It is believed that this model is the first to be able to make such predictions for the whole cheese ripening process.

## 요 약

치즈의 숙성 전과정을 잘 설명하여 줄 수 있는 수학적식을 만들었다.  $X(0)$ 와  $e_2(0)$  및  $k_1$ 값들을 증가시키므로써 숙성과정을 촉진시킬 수 있었고 낮은  $k_2$ 값과 높은  $\alpha_2$ 값을 가지는 신 균주의 탐색도

필요하다는 것을 알았다. 그러나, 치즈 숙성과정 중 나쁜 치즈맛의 생산을 피하기 위하여서는 낮은 단백질 분해효소 활성도를 갖는 균주가 절대적으로 필요하다. 따라서 이 제안된 수학적 모델 식은 치즈덩어리 내에서 일어나는 효소반응들을 잘 묘사하고 있으며, 궁극적으로는 값싸고 질 좋은 치즈를 생산하는데 유용할 것이다.

## NOMENCLATURE

- A : Casein concentration[ mg/g]
- $A_0$  : Initial casein concentration in the cheese matrix[ mg/g]
- $\alpha_2$  : Fraction of intracellular dipeptidases that are released after cell lysis[ units/cfu]
- $\alpha_2'$  : The amount of intracellular dipeptidases that are formed upon the growth of each cell[ units/cfu]
- B : Dipeptide concentration[ mg/g]
- $B_0$  : Initial dipeptide concentration in the cheese matrix[ mg/g]
- $b_{1,1}$  : Constant for growth-associated product formation[ mg/cfu]
- $b_{1,2}$  : Constant for non-growth associated product formation[ mg/cfu/day]
- C : Amino-acid concentration[ mg/g]
- $C_0$  : Initial amino-acid concentration in the cheese matrix[ mg/g]
- $c_{11}$  : Constant of proteinase formation[ units/cfu]
- $E_1$  : Activity of proteinases[ units/g]
- $E_{1,0}$  : Initial activity of proteinases[ units/g]
- $e_{1,0}$  : Initial specific activity of proteinases in cheese block[ units/cfu]
- $\hat{e}_1$  : Dimensionless activity of proteinases[ -]
- $E_2^{intra}$  : Activity of the intracellular dipeptidases [ units/g]
- $E_2^{tot}$  : Activity of the total dipeptidases[ units/g]
- $E_2$  : Activity of dipeptidases[ units/g]
- $e_2$  : Specific activity of dipeptidases[ units/cfu]
- $e_{2,0}$  : Initial specific activity of dipeptidases [ units/cfu]
- $k_1$  : Rate constant of proteinase degradation [ 1/day]

- $k_2$ : Rate constant of dipeptidase degradation [ 1 /day]
- $k_1/k_2$ : Degradation ratio of the proteinases to the dipeptidases[ -]
- $k_1$ : Rate constant of cell lysis[ 1 /day]
- $k_1$ : Constant of lactose limitation for cell growth[ mg/g]
- $K_m$ : Michaelis constant of casein degradation [ mg/g]
- $K_m'$ : Michaelis constant of amino-acid formation[ mg/g]
- $L$ : Lactose concentration in the cheese matrix[ mg/g cheese]
- $L_0$ : Initial lactose concentration in the cheese matrix[ mg/g]
- $L^{crit}$ : Critical lactose concentration where enzyme induction and repression occurs[ mg/g]
- $\mu_m$ : Maximum cell growth rate[ 1 /day]
- $u$ : Unit step function
- $V_b$ : Maximum velocity of amino-acid formation by dipeptidases[ mg/units/day]
- $V_f$ : Maximum velocity of casein degradation by proteinases[ mg/units/day]
- $X$ : Viable cell count[ cfu/g]
- $X_0$ : Initial number of intact cells per unit weight of cheese[ cfu/g]
- $y_p$ : Yield coefficient of lactic acids produced from lactose[ g/g]
- $y_x$ : Yield coefficient of cells produced from lactose[ cfu/mg]

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