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Critical Reactions in Ripening of Cheeses

A Kinetic Analysis

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ABSTRACT

A mathematical model has been developed for the key reactions taking place during cheese ripening. It includes growth and lysis of cells in the cheese matrix, cell-wall bound proteinases and intracellular peptidases that are released into cheese upon cell lysis, and the production of peptides and amino acids from casein in cheese. The model parameters have been estimated using published experimental data for cheddar cheese, and model simulations have been conducted to suggest effective means of reducing ripening times of cheeses. The time required for ripening of cheeses can be significantly reduced by carefully controlling the cell numbers at the beginning of cheese ripening and their proteinase and peptidase activities.

Index Entries: Kinetic model; cheddar cheese; proteases; peptidases; cell lysis.

NOMENCLATURE

A	amount of casein/g of cheese, mg/g
b_{11}	growth associated lactic acid formation, mg lactic acid/cfu
b_{12}	nongrowth-associated lactic acid formation rate, mg lactic acid/(cfu·day)

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B amount of dipeptides/g of casein, mg/g
C amount of amino acids/g cheese, mg/g
e_1 specific proteinase activity relative to that at the beginning of ripening
$e_{1,o}$ units of proteinase/cell at the beginning of ripening, U/cfu
E_1 proteinase activity in the cheese matrix, U/g cheese
E_2 dipeptidase activity in the cheese matrix, U/g cheese
k_1 specific rate of degradation of proteinases, day^{-1}
k_2 specific rate of degradation of extracellular peptidases, day^{-1}
k_l specific rate of lysis of cells in cheese matrix, day^{-1}
K_A Michaelis-Menten constant for proteinase activity, mg/g
K_B Michaelis-Menten constant for dipeptidase activity, mg/g
K_l Monod's constant for cell growth on lactose, mg/g
L amount of lactose in cheese, mg lactose/g cheese
t ripening time, h
U amount of enzyme that gives rise to 1 μmol product/min; also unit function
V_b maximum dipeptidase activity, mg amino acids/(U·day)
V_f maximum proteinase activity, mg casein/(U·day)
X number of cells in cheese, cfu/g cheese
Y_x yield of cells on lactose, cfu/mg lactose
Y_p yield of lactic acid on lactose, mg lactic acid/mg lactose

Greek Letters

α_1 proteinase activity in the cells, U/cfu
α_2 dipeptidase activity/cell, U/cfu
μ_m maximum specific growth rate of cells, day^{-1}

Superscripts

intra intracellular amount/cell
total total amount/g cheese block
crit critical value

INTRODUCTION

Cheese ripening is an enzymatic (chiefly proteolytic) process during which cheese body, texture, and flavor develops, and different cheeses attain their unique characteristics. The quality of ripened cheeses is strongly dependent on this time-consuming process, which traditionally takes place over several months at low temperatures. Although many studies (*1–4*) of enzymatic reactions during the ripening of cheeses have been conducted, no comprehensive mathematical model for the process of cheese ripening has been published so far. Basch et al. (*5*) have

reported a simple model for enzyme-catalyzed, time-dependent changes in protein composition of cheddar cheese during storage. This article represents an effort to mathematically model the reactions that take place during cheese-ripening, with a view to suggest strategies for reducing the ripening period.

Production of cheese starts with fermentation of pasteurized milk in the presence of starter and/or some nonstarter bacteria with rennet. After curd-formation owing to coagulation of proteins is complete, the curd suspension is cut into small pieces, cooked, and whey is removed by cheddaring, pressing, and salting. The cheese block is then set aside for ripening. At the beginning of the ripening process, the cheese block contains curd (protein aggregates), starter and nonstarter bacteria retained in the curd, milk proteinases, and salt. Small amounts of lactose and rennet also remain trapped by the cheese matrix. All these components influence the ripening process and finally the quality of cheese.

The ripening of cheddar cheese has been a subject of several studies (*2,3,16–18*). In the initial period of the ripening process, cells trapped in the curd grow at the expense of residual lactose. The rate of cell growth is closely related to the level of enzymes (proteinases and peptidases) present in the system. Some lysis of the cells also takes place during this time and it results in release of intracellular enzymes into the cheese matrix (*17*). After lactose is exhausted, primarily the lysis of cells with the accompanying release of enzymes in the matrix takes place.

The proteolytic system of starter bacteria is generally classified into two groups: the proteinases and the peptidases (*18,19*). The proteinases are cell-wall bound (*19–23*) and hydrolyze essentially the extracellular proteins, such as casein, into peptides. Peptidases are present in the cell membrane (*24*) and in the cytoplasm (*25*). Lysis of cells releases peptidases into the cheese matrix and these are responsible for the production of amino acids from peptides (*26–30*). The degradation products of casein make the greatest contribution to the intensity of cheese flavor (*31*). Amino acids are known to be among the major flavor compounds in cheddar cheese (*32–37*). Other known flavor-agents are carbonyl compounds, fatty acids, hydrogen sulfide, methanethiol, dimethyl sulfide, and peptides (*15,38–46*). Accumulation of low-mol-wt peptides is related to bitterness in cheddar cheese (*47–50*).

Most of the enzymatic activity leading to formation of peptides and amino acids is owing to the starter bacteria (*2*). In some cheeses, lactobacilli which form the majority of nonstarter bacteria, grow to large numbers within a few weeks of cheese-ripening process. However, their contribution to the flavor developed in cheddar cheese is questionable (*2*). Milk proteinases, rennet, and other nonstarter bacteria also contribute to ripening in various degrees, but are not very important in production of amino acids in cheese (*51*).

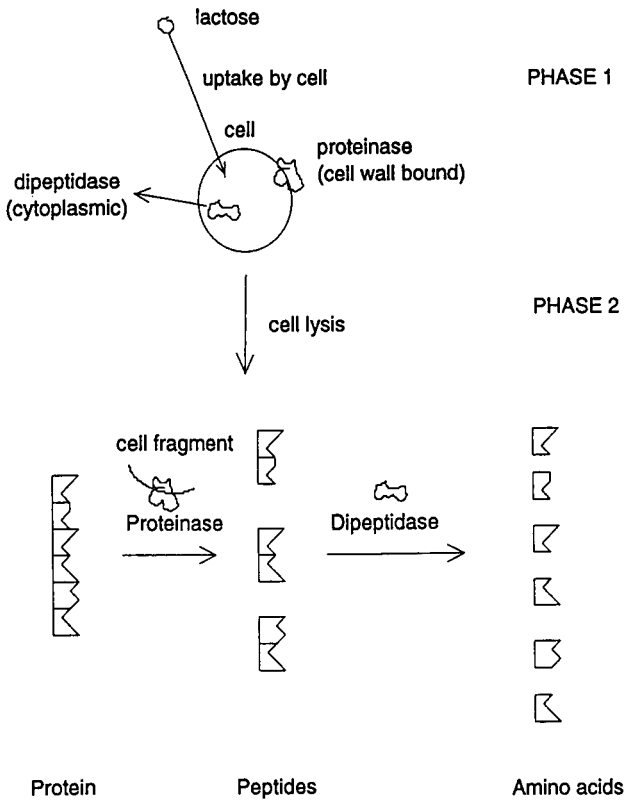

Fig. 1. A schematic representation of interactions taking place during cheese ripening.

ASSUMPTIONS OF THE MODEL

The key interactions of the cheese-ripening process modeled in this work are shown in Fig. 1. These are based on the significant amount of research work involving ripening of cheddar cheese that has recently been published. The following assumptions have been used to simplify the complex process.

1. Only one type of bacterial cells is present in the cheese matrix. These cells grow on lactose present in the cheese block and lyse at a constant specific rate (*52*).
2. Cell growth is associated with production of extracellular lactic acid and of proteinases/peptidases in the cell.
3. Only the microbial proteinases and peptidases participate in the ripening process (*53–55*). Over 90% of the rennet added to milk is lost in the process of whey removal (*56*) and thus rennet does not contribute significantly to proteinase activity. No peptidase activity was detected in cheeses made without

starter cultures (*3*). Even though both the proteinases and peptidases represent a collection of proteolytic and peptidolytic activities, single enzymes have been assumed to represent the activities of each group in this model.

4. All the enzymatic activity of interest takes place in the cheese matrix outside of intact cells only. Since cell lysis releases peptidases into cheese matrix, the total peptidase activity has been divided into intracellular and extracellular activities.
5. Degradation of proteinases and peptidases exposed to the extracellular environment takes place according to a first order process. No degradation of intracellular peptidases takes place.
6. Fats in the cheese matrix are not hydrolyzed (*57,58*).
7. Water activity in cheddar cheese remains high (>0.95) and its effect on enzymatic reactions can be neglected (*59*).
8. pH and temperature remain constant during the ripening period (*26*).
9. The cheese matrix can be treated as a homogeneous phase, with no concentration gradients of cells, proteins, enzymes, fat, moisture, and salt. As a result, only the local reaction kinetics have been considered.
10. Although several different chemicals contribute to cheese flavor, amino acids are the chief flavor-precursors in cheddar cheese (*32–37*). Hence, the levels of PTA-soluble amino acids have been assumed to be reliable indicators of cheese flavor (*8*).

GOVERNING EQUATION IN THE MATHEMATICAL MODEL

Cell mass, lactose, proteinases, intracellular and total peptidases, casein, peptides, and amino acids are the variables in this model. In writing the mathematical expressions for the accumulation of different quantities of products in the cheese matrix, an attempt has been made to utilize the simplest forms that can justify the reported experimental observations. Thus, the cells in cheese have been assumed to follow Monod's kinetics of growth on lactose and a first order lysis reaction. This is consistent with the observations of Kim (*60*). As a result, the governing equation for cell concentration in the cheese matrix becomes:

$$(dX / dt) = \mu_m [L / (K_l + L)] X - k_l X \quad (1)$$

During cell growth, lactose is consumed with a constant yield coefficient. Lactose is also assumed to be consumed for lactic acid production, which occurs according to Leudeking and Piret's model (*61,62*) with a constant

yield coefficient. Hence, the governing equation for lactose concentration becomes:

$$(dL / dt) = -(1 / Y_x)\,\mu_m\,[L / (K_l + L)]\,X - (1 / Y_p)\,\{b_{11}\,\mu_m\,[L / (K_l + L)]\,X + b_{12}\,X\} \quad (2)$$

Cell wall-associated proteinases are produced constitutively in the cells. These proteinases are exposed to the extracellular environment and degrade according to a first order kinetics. The governing equation for proteinase concentration in the cheese block is

$$(dE_1 / dt) = \alpha_1\,\mu_m\,[L / (K_l + L)]\,X - k_1\,E_1 \quad (3)$$

Since no experimental measurements of the time variations of proteinase activity in ripening cheeses are available from the literature, the proteinase concentration in the cheese block has been replaced by a relative concentration, e_1, where

$$e_1 = (E_1 / X\,e_{1,o}) \quad (4)$$

Here, $e_{1,o}$ is the initial proteinase activity per cell at the beginning of the ripening process. Hence, Eq. (3) can be rewritten as

$$(de_1 / dt) = (\alpha_1 / e_{1,o})\,\mu_m\,[L\,(K_l + L)] - k_1\,e_1 - (e_1 / X)\,(dX / dt) \quad (5)$$

The peptidase activity has been characterized by depeptidases that are considered to be essential for the formation of amino acids in cheeses. These enzymes are produced by the growing cells, are cytoplasmic, and are released into the cheese matrix upon cell lysis (*60*). The intracellular enzymes have been assumed to be stable, but the extracellular ones are degraded by a first order process. The governing equation for the intracellular peptidases is

$$(dE_2^{intra} / dt) = \alpha_2\,\mu_m\,[L / (K_l + L)]\,X\,[1 - U\{L - L^{crit}\}] - k_l\,E_2^{intra} \quad (6)$$

The accumulation of extracellular amino acids does not occur in the early periods of cheese ripening (*2–4,63*). This could be a result of rapid consumption of amino acids by growing cells in the early phase of ripening or owing to difficulty of measuring low concentrations of amino acids in the cheese block. In the present model, this observation has been reconciled by considering a critical lactose concentration above which dipeptidase activity is repressed. Here U is a unit function that has a value of 0 for $L < L^{crit}$ and 1 for $L \geq L^{crit}$. The second term in the above equation represents loss of intracellular dipeptidases owing to cell lysis. The governing equation for the total dipeptidase activity is

$$(dE_2^{total} / dt) = \alpha_2\,\mu_m\,[L / (K_l + L)]\,X\,[1 - U\{L - L^{crit}\}] - k_2\,[E_2^{total} - E_2^{intra}] \quad (7)$$

Degradation of milk protein, casein, by proteinases has been modeled according to a Michaelis-Menten equation, thus disregarding the presence of heterogeneities in the cheese matrix. The governing equation for casein concentration is

$$(dA / dt) = - (V_f e_{1,o}) e_1 [A / (K_A + A)] X \quad (8)$$

The production of extracellular amino acids in the cheese block is related to the activity of extracellular dipeptidases and the concentration of dipeptides. Using a Michaelis-Menten kinetics for this reaction:

$$(dC / dt) = V_b [E_2^{\text{total}} - E_2^{\text{intra}}] [B / (K_B + B)] \quad (9)$$

The governing equation for dipeptides is obtained from Eqs. (8) and(9) as

$$(dB / dt) = - (1.08) (dA / dt) - [(1 / 1.08) (dC / dt)] \quad (10)$$

The factors 1.08 have been introduced to account for addition of one molecule of water every time a dipeptide bond is hydrolyzed (assuming the mol-wt of a typical amino-acid residue in milk protein to be 225).

PARAMETERS OF THE MATHEMATICAL MODEL

Published literature data (*2,3,7–10,12,13,15,16*) were used by Kim (*60*) to estimate the parameters in the model Eqs. (1), (2), and (5)–(10). Even though a significant amount of published information is available for the kinetic processes taking place during cheddar cheese ripening using *Lactococcus cremoris*, two major problems were encountered in estimation of parameters. First, none of the experimental data-sets were complete in all the different variables used in this model. Hence, experimental data from all the authors were pooled together for the sake of parameter estimation. Second, although several authors used *Lactococcus cremoris*, different strains were used (BK5, EB6, E8, ML1, C13, 266, UC). As a result, an implicit assumption was that the parameters are the same for all strains. The parameter values thus obtained can, therefore, be construed only as estimates of the actual values among different strains.

Marqurdt's method (*64*) was used to estimate parameters from the experimental data. For the sake of ease of parameter estimation, the ripening process was divided into two phases: phase 1 during which cells grow and produce the enzymes, and phase 2 in which primarily the enzymatic reactions take place. Phase 1 typically lasts 2–4 wk.

In phase 2 of the ripening process, Eqs. (1) and (5)–(10) are used. Since lactose is completely exhausted, all of the intracellular dipeptidase will be active and its concentration will be given by

$$E_2^{\text{intra}} = \alpha_2 X \quad (11)$$

Table 1
Initial Conditions for Simulations

Variable	Phase 1 and overall		Ref.	Phase 2		Ref.
X	1×10^9	cfu/g	*67,68*	1×10^7	cfu/g	*2,3*
L	8.5	mg/g	*69,70*	0		
e_1	1.0			1.0		
E_2^{intra}	0.005	U/g	*69,70*	—		
E_2^{extra}	—			0.02	U/g	69,70
E_2^{total}	0.028	U/g	*69,70*	—		
A	258	mg/g	69,70	245	mg/g	69,70
B	13.1	mg/g	*69,70*	25	mg/g	69,70
C	1.33	mg/g	*69,70*	2.0	mg/g	*69,70*

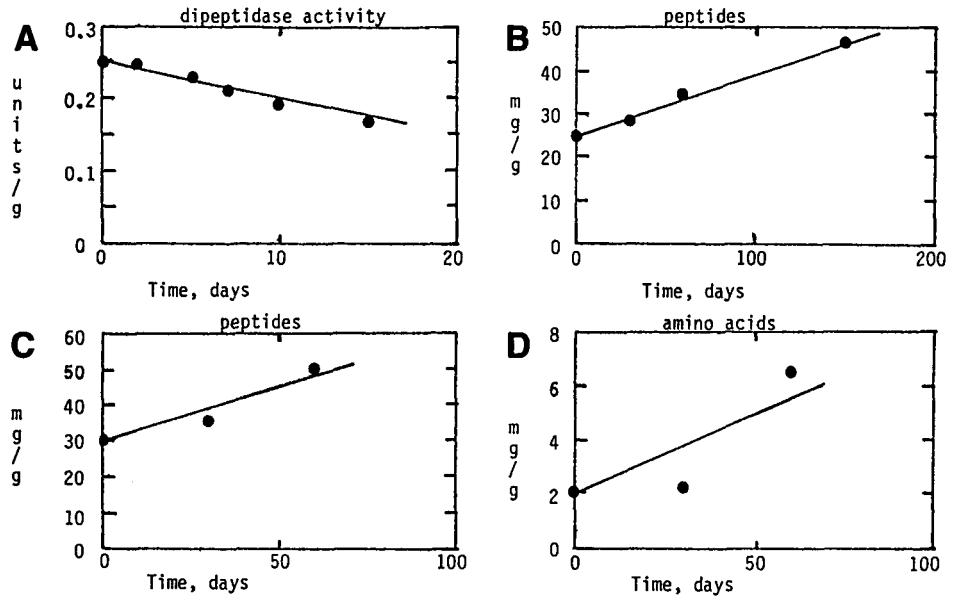

Fig. 2. Experimental data (●) and simulation results (——) for the second phase of ripening. **A.** dipeptidase activity from cell free extract (*2*). **B.** peptide concentration (*13*). **C.** peptide concentration (*15*). **D.** amino acid concentration (*15*).

Experimental data from this phase were used to estimate the parameters k_l, α_2, k_2, k_1/k_2, $V_f \cdot e_{1,o}$, and V_b. The parameters K_A and K_B were available from literature (*65,66*). Initial conditions used in simulations of this phase are listed in Table 1 and comparison between experimental and predicted data are shown in Figs. 2 and 3. Estimated parameters along with their 95% confidence limits are presented in Table 2. For all the variables except k_1/k_2, the maximum estimation errors are $< 15\%$, which sup-

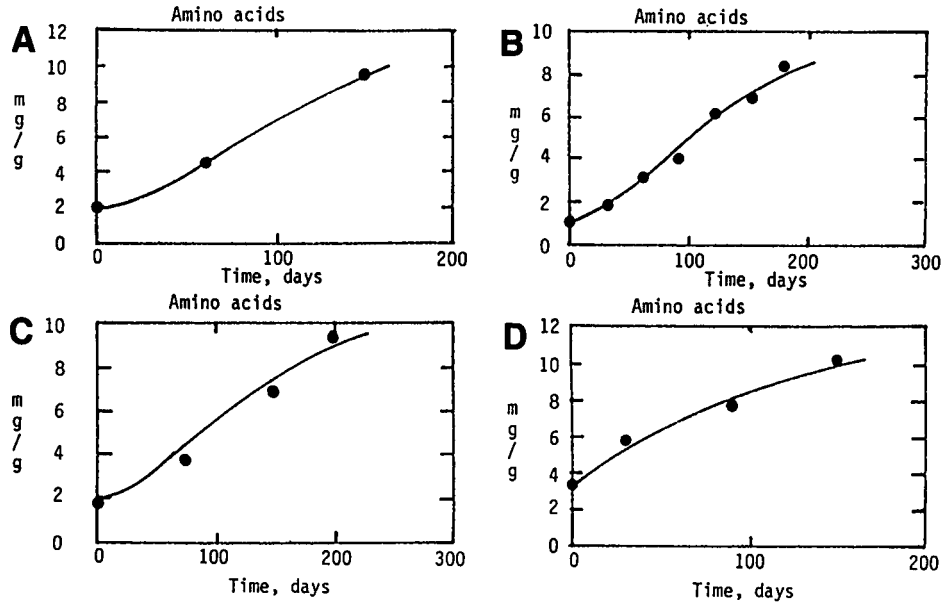

Fig. 3. Experimental values of amino acid concentrations in cheese (●) and simulation results (——) for the second phase of ripening. **A.** (*8*). **B.** (*16*). **C.** (*12*). **D.** (*7*).

ports the initial assumption that the different strains may be characterized by the same set of parameters. The ratio of degradation rate constants for proteinases and dipeptidases (k_1/k_2) is very small, suggesting that the cell-wall bound enzymes are significantly more stable than those in the cheese matrix. This assertion is also supported by the observation of Law et al. (*3*). The errors in estimation of k_1/k_2 are of the order of 100%, which is not surprising since no measurements of proteinase activity were available.

Using the model Eqs. (1) and (7)–(11), the phase 2 experimental data for total dipeptidases, peptides, and amino acids, can be fitted well by the model. But the predicted profiles of viable cells does not agree well with the experimental data. This is probably a reflection of the difficulties in measurements of viable cell count, which varied significantly between two lots of the same cheese (*51*).

In phase 1 of the ripening process, mainly cell growth and enzyme production occurs; some hydrolysis of casein also takes place here. Hence. Eqs. (1), (2), and (5)–(10) were used to estimate μ_m, k_l, b_{11}, b_{12}, $\alpha_1/e_{1,o}$, and $V_f \cdot e_{1,o}$ with the initial conditions presented in Table 1. The parameter values are listed in Table 2. The computed and experimental results are shown in Figs. 4 and 5. The parameter $V_f \cdot e_{1,o}$ needed to be estimated again, since the value of $e_{1,o}$ at the beginning of phase 1 may be different from that at the beginning of phase 2. As a matter of fact, a comparison of $V_f \cdot e_{1,0}$ from the two phases suggested that significant amounts of proteases are produced during the first phase of cheese ripening.

Table 2
Estimated Parameters and Their 95% Confidence Limits[a]

Parameter		Estimated from phase 1	Estimated from phase 2	Obtained from literature	Parameters used in simulations
μ_m	(day^{-1})	0.149 ± 0.0131			0.149
K_l	(mg/g)			0.296 (*71*)	0.296
k_l	(day^{-1})	0.255 ± 0.003	0.00856 ± 0.00111		0.00856
Y_x	(cfu/mg)			$1.04\ 10^9$ (*61*)	$1.04\ 10^9$
Y_p	(mg/mg)			0.8 (*72*)	0.8
b_{11}	(mg/cfu)	$1.89\ 10^{-9} \pm 1.95\ 10^{-9}$			$1.89\ 10^{-9}$
b_{12}	(mg/cfu/day)	$6.59\ 10^{-11} \pm 1.32\ 10^{-10}$			$6.59\ 10^{-11}$
$\alpha_1/e_{1,o}$			0.792 ± 0.467		0.792
k_1/k_2			$1\ 10^{-4} \pm 1.19\ 10^{-4}$		$1\ 10^{-4}$
α_2	(U/cfu)	$8.72\ 10^{-9} \pm 4.8\ 10^{-10}$			$8.72\ 10^{-9}$
L^{crit}	(mg/g)			1.5	1.5
k_2	(day^{-1})		0.0235 ± 0.00229		0.0235
$V_f\ e_{1,o}$	(mg/cfu/day)	$8.53\ 10^{-11} \pm 3.0\ 10^{-11}$	$4.46\ 10^{-8} \pm 6.81\ 10^{-9}$		$8.53\ 10^{-11}$
K_A	(mg/g)			0.207 (*65,66*)	0.207
V_b	(mg/cfu/day)		3.88 ± 0.289		3.88
K_B	(mg/g)			1.15 (*65,66*)	1.15

[a]Unless otherwise stated in the text.

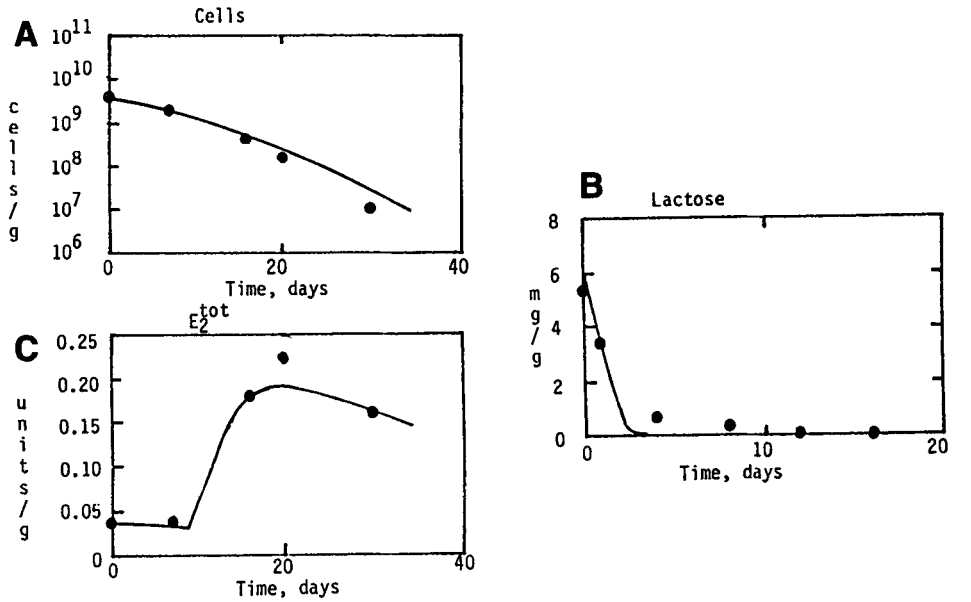

Fig. 4. Experimental data (●) and simulation results (——) for the first phase of ripening. **A.** Viable cell count (2). **B.** Lactose concentration (73). **C.** Total dipeptidase activity (2).

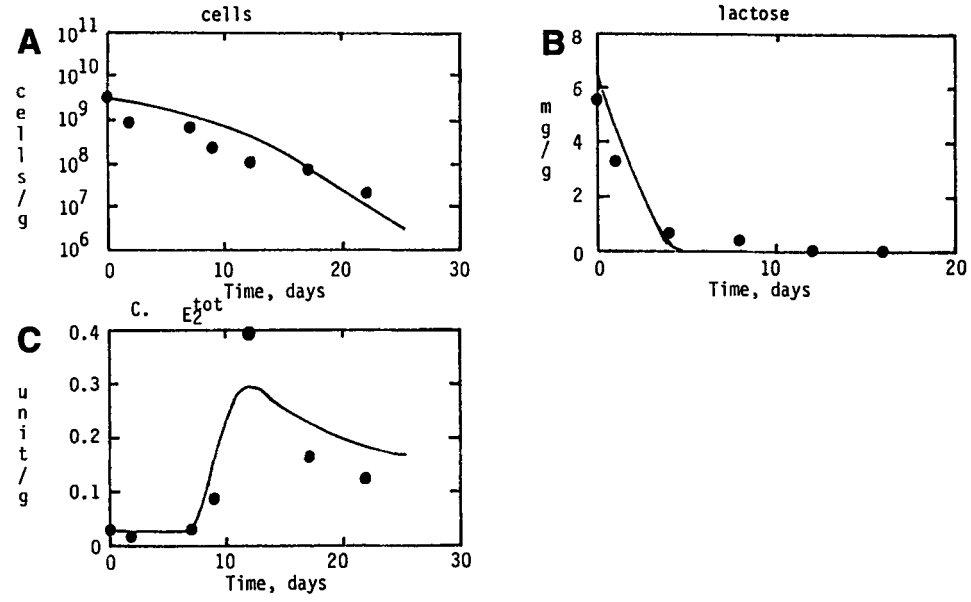

Fig. 5. Experimental data (●) and simulation results (——) for the first phase of ripening. **A.** Viable cell count (3). **B.** Lactose concentration (73). **C.** Total dipeptidase activity (3).

Another major difference between phase 1 and phase 2 is seen in the value of parameters k_l, the specific rate of lysis of cells, which is estimated to be approx 30 times more during phase 1 than that from phase 2 data. The reasons why this should be so are not clear, even though it has been reported in literature that presence of bacterophages can significantly enhance the lysis of cells. This aspect, however, was not pursued any further.

SIMULATION OF CHEESE RIPENING

As suggested earlier, the concentrations of peptides and amino acids are two key measures of the rate of ripening of cheeses. The strategies for reducing the ripening times center around achieving similar concentrations of peptides and amino acids in shorter time. These strategies range from increasing the concentration of cells in the cheese block to modifying the contents of proteinases and peptidases within the cells. Hence simulations were conducted to investigate the effects of different variables and parameters on the evolution of peptides and amino acids. The model equations (1), (2), and (5)–(10) were solved with initial conditions and average parameter values listed in Tables 1 and 2 using a standard differential equation solver that utilizes a step-size control and is capable of handling stiff equations.

The effect of initial cell concentrations is presented in Fig. 6. Increasing the initial cell concentration fivefold from 1.0×10^9 increases the rate of amino acid production without significantly influencing the peptide concentrations. A further doubling of cell concentration causes a transient build-up of peptides that eventually reduce to small values by the 60th day. But another doubling of cell concentration to 2.0×10^{10} is predicted to result in a large increase in the concentration of peptides, which will increase the probability of bitterness in cheese.

The parameter α_2 represents the amount of peptidases per cell, and manipulations of starter strains will result in a change of this parameter. The simulation results, with a 10-fold change in the value of this parameter (Fig. 7), show only a slight change in the concentration-profiles of peptides and amino acids. Similarly, reducing the parameter $\alpha_1/e_{1,0}$ by an order of magnitude also did not change the amino acid and peptide concentration-profiles much. Hence, manipulations of these parameters by selection of different starter strains, may not be very effective in changing the ripening times.

A change in the temperature during ripening will also affect several parameters in the kinetic model. The effect of changing the value of V_f (the specific rate constant for proteinases) has been shown in Fig. 8. As the value of this parameter is increased 2.5-fold from its base value (the estimated value), the production of peptides and amino acids increases enough to reduce the ripening time (time required to achieve a given

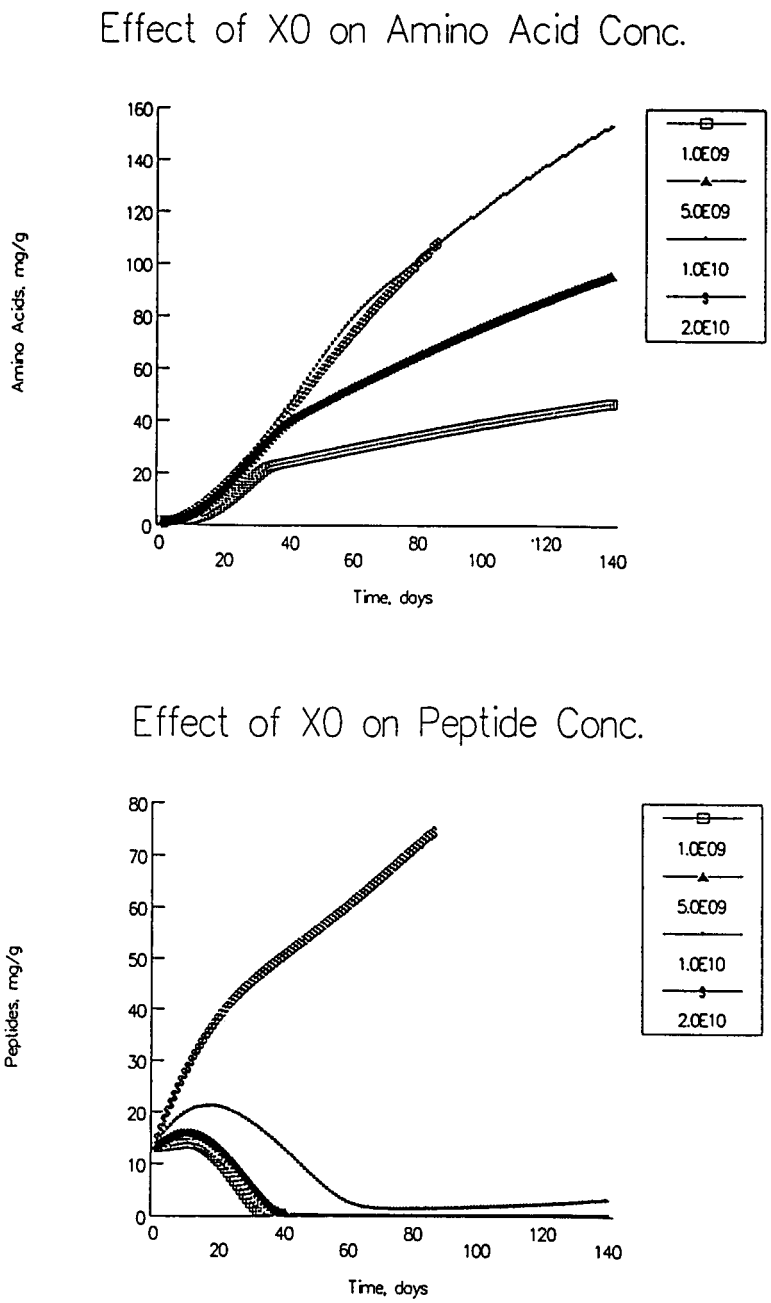

Fig. 6. Effect of initial cell concentration (Xo, cfu/g cheese) on the concentrations of amino acids and peptides during cheese ripening. Simulation results. —□— : 1.0E09; —▲— : 5.0E09; —●— ; 1.0E10; —S— : 2.0E10.

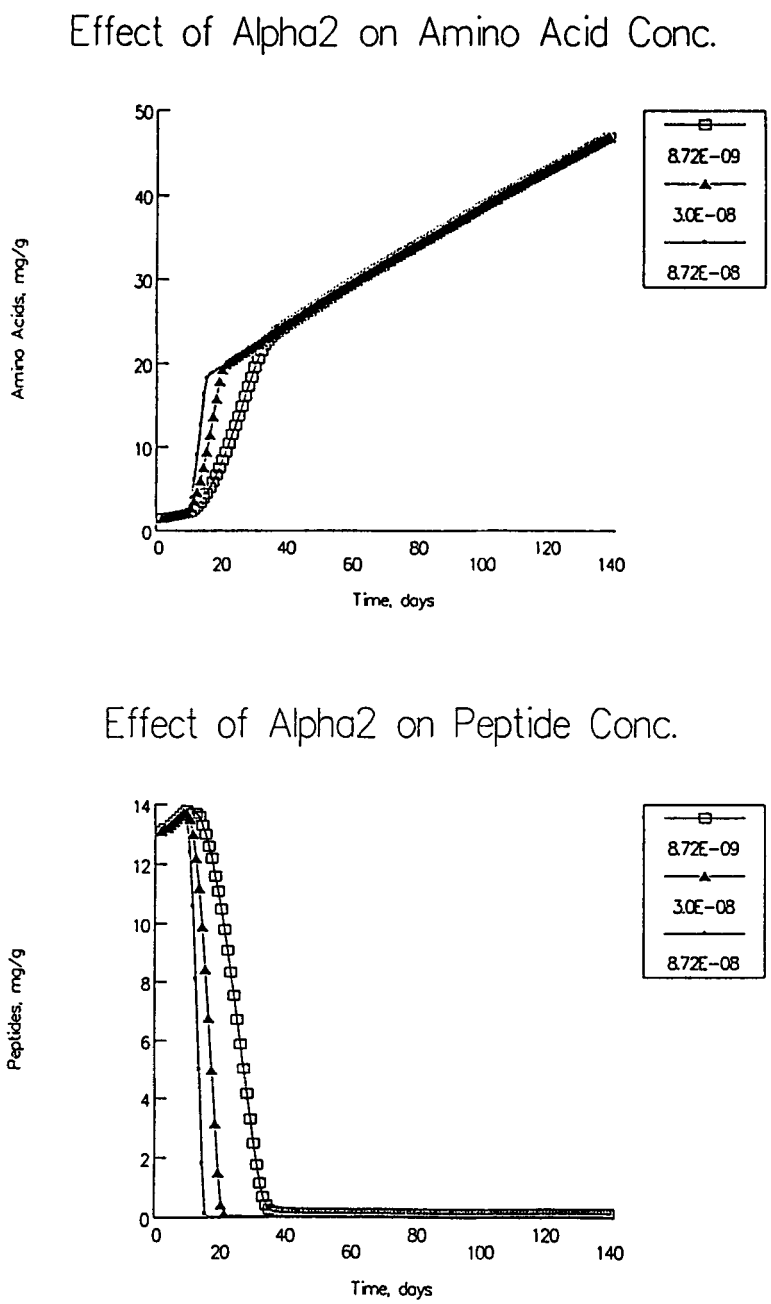

Fig. 7. Effect of dipeptidase concentration per cell (α_2, U/cell) on the concentrations of amino acids and peptides during cheese ripening. Simulation results. —□— : 8.72E-09 —▲— : 3.0E-08; —●— ; 1.72E-08.

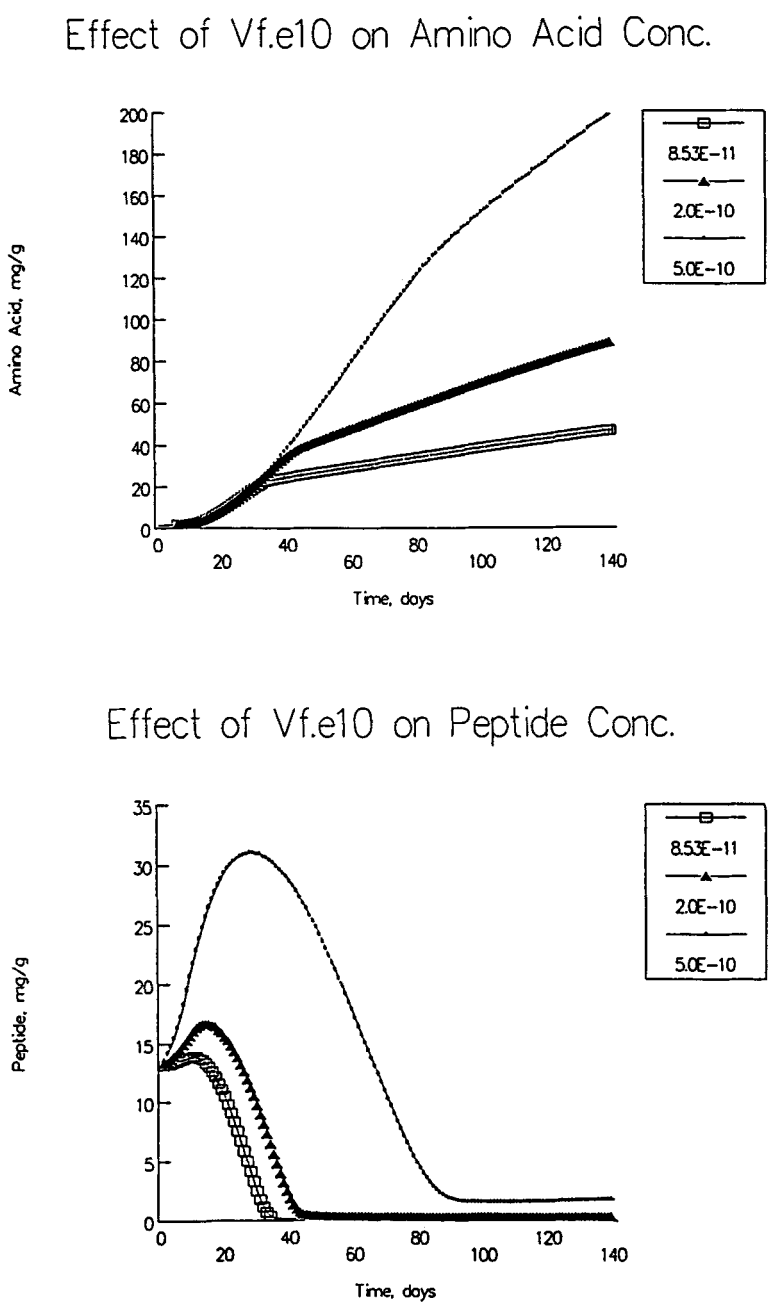

Fig. 8. Effect of proteinase activity (Vfe_{10}, mg casein/cfu·day) on the concentrations of amino acids and peptides during cheese ripening. Simulation results. —⊟— : 8.53E-11; —▲— : 2.0E-10; —●— ; 5.0E-10.

amount of amino acids) by half. By the end of this time, the peptide concentration will be reduced to small values again. However, another 2.5-fold increase in the value of V_f will cause a large undesirable increase in the peptide concentration. Simulation involving the effect of V_b, the specific rate constant for dipeptidase activity, suggested an effect similar to that of V_f. As a result, it may be concluded that small changes in temperature of ripening process may be helpful in reducing ripening time. Again, a sharp increase in temperature will be detrimental owing to the excessive production of bitter peptides in cheese.

CONCLUSIONS

Growth and enzymatic processes taking place during the ripening of cheddar cheese have been investigated with the help of a mathematical model. As a result of simulations, it was determined that the number of cells at the beginning of ripening has the most profound effect on formation of peptides and amino acids. The specific activities of proteinases and peptidases also influence the profiles of peptides and amino acids in the cheese matrix. The most surprising conclusion was that variations in the amount of proteinases and peptidases per cell had no significant influence on the peptides and amino acids.

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