Checking of population of Listeria monocytogenes in feta cheese during its ripening by cellular automata

Maryam, shirafkan, M.Sc. in computer, Trending software, m.shirafkan14@gmail.com, 09187120514

Saeed, setayeshi, Associate Professor of Department of Nuclear Engineering, Faculty of Nuclear Engineering and Physics, Amirkabir University, setayesh@aut.ac.ir, 02164545252

Mohammad ali, atar, M.Sc. Student in food science and Technology, Ferdowsi University of Mashhad, atar6676@gmail.com

***Abstract*—In this paper, we use cellular automata to provide a model for checking of population of Listeria monocytogenes in feta cheese during its ripening. For this purpose, after determining the characteristics and rules of Listeria monocytogenes, we simulated system in an artificial environment by using software Netlogo. Then, to evaluate the model, we compare the results of the model with real world behavior (laboratory results), in the form of diagram.**

Results of the model such as performed laboratory results witness against is that with reduction in cheese pH, Listeria population reduce.

***Keywords-* cellular automata, feta cheese, Listeria monocytogenes, modeling.**

Introduction

paying of the high cost to cater materials and laboratory appliances, dissipation too much time on the microbial experiments, and in particular, the complexity of understand of the biological systems, has led to the use of computer simulation.

In fact simulation allows in an artificial society composed of agents, different experiments be performed under laboratory conditions. Finally, the results can be observed and measured.

Given the importance of simulation in understanding, optimization and prediction of the important matters in food science, in this study we use simulator software Netlogo, for assessment of population of Listeria monocytogenes in feta cheese during its ripening.

Listeria monocytogenes is one of the known species of Listeria which is rod-shaped and gram-positive. Listeria monocytogenes, due to ability to grow at refrigerator temperature, ability to grow at relatively low pH and tolerate large quantities of salt, is considered as a food pathogenic bacteria. So that, according to researches that have been done, importance of role of pH and importance of amount of producted lactic acid in prevention growth of Listeria monocytogenes in feta cheese, have been proven [2 ,1], and has been reported that with decreasing in pH to below 4/6, the growth will be stopped [2 ,1].

The growth of Listeria monocytogenes in pH < 5 is very low and also this bacteria will reproduce at pH > 5 quickly. Maximum of pH for growth, has been reported 9/5 [3].

Poisonings caused by this bacteria is called listeriosis. Listeriosis occurs due to consumption of cheese and meat and its products. The risk of listeriosis in people with weakened immune systems, is far more.

Listeria species, are sensitive to number of antibiotics. In the past decade, many researchers have studied on anti-listerial property of antibiotics such as nisin and pediocin, which has had favorable results [10-4].

Thus, given the nature of pathogenic of listeria monocytogenes and its wide distribution in nature, in this study a model is presented by using cellular automata to evaluate the population of listeria monocytogenes in feta cheese during its ripening. On this basis, by taking the parameters, rules and conditions, we simulated systems in artificial environment by using software Netlogo.

In this study, we used the laboratory results obtained from the New Technologies Laboratory, department of Food Science and Technology, Ferdowsi University of Mashhad [11]. However, this research has not been raised so far and documentation of this research has not been registered.

The purpose of this paper is to present a model by using cellular automata to evaluate the population of listeria monocytogenes in feta cheese during its ripening.

Materials and Methods

1) Performed Experiments

Necessary data for assessment and checking of authenticity of this model is provided from experiments performed in the New Technologies Laboratory, Department of Food Science and Technology, Ferdowsi University of Mashhad. Part of these experiments are as follows [11]:

During stages the preparation of iranian white cheese, at temperature of 34-37 °C and pH = 6/4, DVS starter bacteria and rennet were added and mixture entered inside the molds. then 10^6 CFU/ml of listeria monocytogenes was added to the milk. Then the samples crossed from coagulator for 25 minutes at 30 °C until curd be created. After these process, cheese samples were kept for 28 days at 4 °C and the microbiological experiments were done in 20 to 24 hours after the production and once every 7 days. Then, in order to microbiological analysis of Listeria monocytogenes in cheese, 10 grams of cheese sample with 90 milliliter of solution of 2% of sterile sodium citrate were homogenized for 3 minutes and cheese samples were diluted in sodium citrate of 2% from 10^1 to 10^9. Then, appropriate dilutions were added into plates containing listeria selective medium (SR140E from Merck, Germany) in three duplicate and surface inoculation was performed. The number of colonies were counted after 24-48 hours incubation at 37 ° C [12] .

Listeria monocytogenes population changes in feta cheese during its ripening:

pH at the beginning of cheese production is 6/4 and then the pH has downward trend. So that, in the weeks of after production, the pH reach 4/96, 4/93, 4/85, 4/78 respectively [11].

According to researches that have been done, importance of role of pH and importance of the amount of produced lactic acid in prevention the growth of Listeria monocytogenes in Iranian white cheese, have been proven [2 ,1]. So, by decreasing of pH, population of Listeria monocytogenes decrease in feta cheese during its ripening.

So that, after 20 hours of production time, population of Listeria monocytogenes has decreased by about log 0/9. In the first week of the ripening, downward trend has continued with a log 0/3 reduction and then remains constant. At the end of the fourth week of ripening, population of Listeria monocytogenes reach 10^4 * 5/5 CFU/ml [11].

2) Modeling and model description:

In fact, artificial life according to definition of christopher Langton, is the study of human-made systems that exhibit behaviors and characteristics of natural life systems [13].

In this study, we use from two-dimensional cellular automata with Moore neighborhood structure, to provide a model to estimate the population of Listeria monocytogenes in feta cheese during its ripening. In fact, cellular automata is discrete dynamical system whose behavior entirely is based on local communication. Cellular automata [15 ,14] consists of two parts: the cell space and transfer rule. Space is defined as a network where each home is called a cell. Time moves forward in discrete mode and rules are to global form. Through which, each cell obtains its new status at each step, with respect to status of its adjacent neighbors.

Neighbors of each cell, are the adjacent cells that interact with it. From common types of neighborhood in cellular automata can noted into von Neumann neighborhood and the Moore neighborhood (Figure 1). So that, in von Neumann neighborhood, for each central cell there is four neighboring cells and in Moore neighborhood, for each central cell there is eight neighboring cells.

In this paper, an agent-based modeling is used. So that, the term of agent means an the element that does the work. Thus, the model consists of Listeria agent with the following characteristics:

Characteristics of Listeria:

Listeria is a living organism (a bacterial cell) that can reproduce or die. Listeria can also move randomly. In this model, we consider age as a variable for each Listeria. so that, initial value of this variable is equal to one, and after each run, one unit is added to it.

a)Moor neighborhood b)von Neumann neighborhood

Figure 1. Two types of neighborhoods in cellular automata

This model includes rules that Listeria agent must follow these rules. The rules are the following:

a) Move rule:

Each Listeria randomly selects one of its eighboring cells that is lacking of Listeria. then moves to this cell.

b) Reproduction rule:

Each Listeria can reproduce with a probability. So that, parent listeria generates a new Listeria with an age equal to one (age = 1), and puts the child Listeria in one of the neighboring cells that is lacking of Listeria.

c) Death rule:

ach Listeria dies due to reduction of environment pH with a probability.

It is noteworthy that, the value of reproduction probability and value of death probability for each Listeria are determined by considering pH of environment and the number of Listeria, in the real world and in the desired time. Then, we run the model and compare results of running of model with the laboratory results (the actual numbers). If necessary, we attempt to change probabilities and then re-run the model.

After modeling and determining of characteristics and rules, we write programming codes for the proposed model in software netlogo and then we run the model. The model is runed in five steps of time. Results in the form of controls, diagrams and values can be observed and measured and analyzed.

Figure (2), shows how pH effects on population of Listeria monocytogenes in feta cheese. In this figure, the initial color of listeria is red and with implementation of model, the bacteria that are born with pink and bacteria die due to reduction of pH are shown in blue. In addition, concurrently with implementation of the program, diagram of pH changes and diagram of Listeria population changes are drawn in Netlogo.

Since 10^6 CFU/gr Listeria monocytogenes are added to the cheese, and these number are very much, therefore, in this model is used 10 times of the logarithm of this number of bacteria ($10 * \log 10^6 = 60$). At the beginning of production, pH is equal to 6/4. The initial population consists of 60 Listeria agents. so that, age of each one is equal to one and are distributed randomly in environment (cheese) (view 1 of figure 2).

With implementation of model and elapse 20 hours of production time, population of Listeria monocytogenes have downtrend and reache 50. this decrease in population of Listeria monocytogenes is due to reduction of pH and therefore the deaths some of bacteria (view 2 of figure 2). After this step and elapse a week after cheese production, the pH is equal to 4/96. As a result, population decrease and reache to 47 (view 3 of figure 2). By continuing of implementation of model and elapse 2 weeks of production time, the pH is equal to 4/93. So, the population reache 44 (view 4 of figure 2). Finally, from the end of the second week until the end of the fourth week after production, pH is equal to 4/85 and 4/78 respectively and there is a constant trend in the population of Listeria monocytogenes (view 5 and 6 of figure 2). According to Figure (2), to indicate the pH reduction during the implementation of the model, is used from the change in the background color. so that, whatever the environment becomes more acidic, then the background color will be darker.

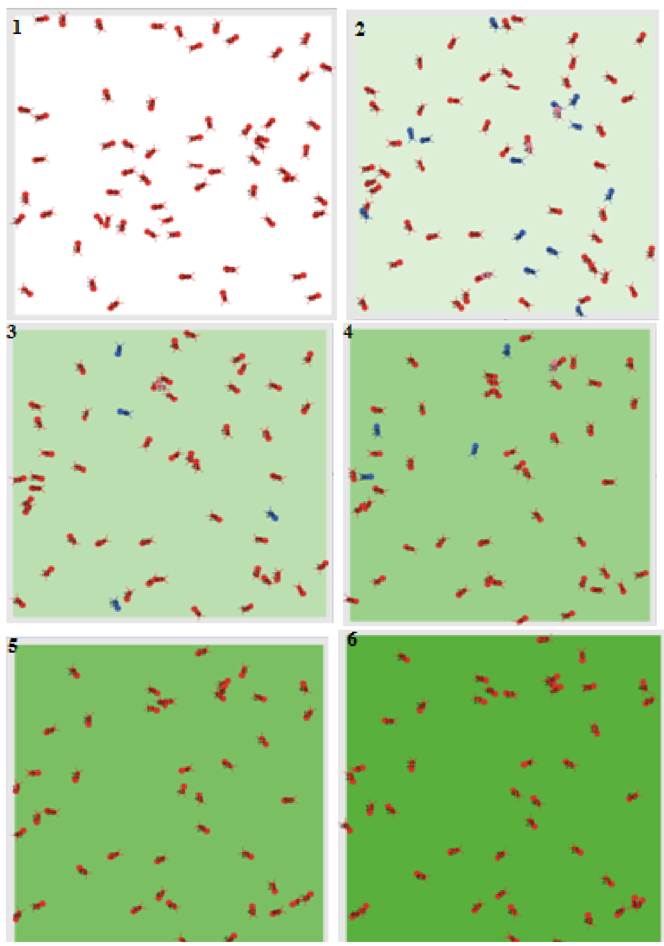

Figure 2. Effect of pH on population of Listeria monocytogenes in feta cheese

Thus, the results indicate that population of Listeria monocytogenes decreased gradually. this population reduction is due to pH reduction in cheese during its ripening. So that, at the end of the simulation time, the pH is equal to 4/78 and as a result, number of Listeria in model reached 44. this problem can be seen with checking of diagram of pH changes in figure (3) and diagram of listeria population changes in figure (4).

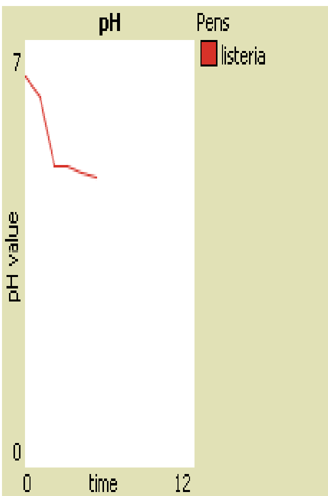

Figure 3. Diagram of pH changes during ripening of cheese

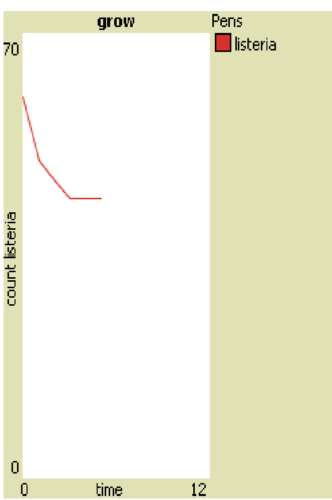

Figure 4. Diagram of Listeria monocytogenes population changes during ripening of cheese

Results and discussion:

In order to evaluation and comparison of the behavior of the model with the behavior of real world, we used from laboratory results conducted in the laboratory of New Technologies, Department of Food Science and Technology, Ferdowsi University of Mashhad [11].

Figure (5), shows diagram of Listeria population changes resulting from implementation of model and also diagram of Listeria population changes resulting from laboratory results.

As is observed, the convergence between the two diagrams is acceptable (given the large number of Listeria monocytogenes, this results are presented in 10 times the logarithm).

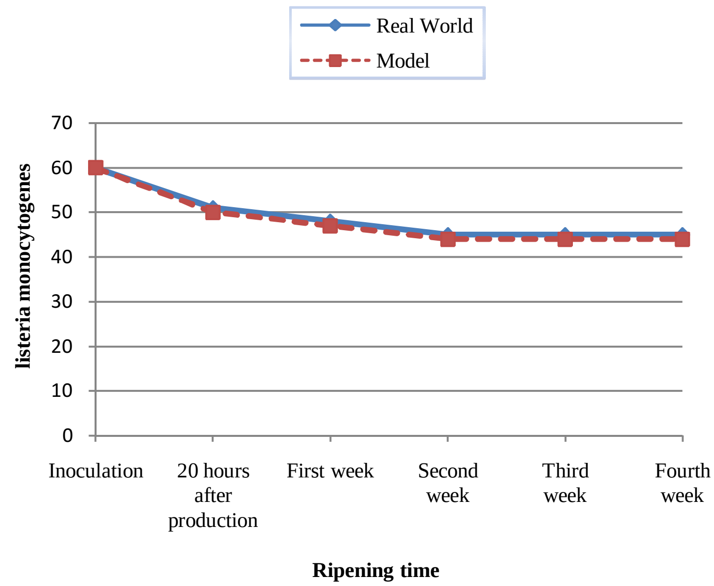

Figure 5. Comparison of behavior of model with behavior of real world

Conclusions:

in this paper, we used cellular automata for modeling of the Listeria monocytogenes population changes in feta cheese during its ripening.

The purpose of this model is the design of a software package to evaluate population of Listeria monocytogenes in feta cheese during its ripening. In addition, this model can be used as an educational tool to better understand of this system.

Comparison of the results of the model with the results of experiments conducted at the University of Mashhad, showed that the model is able to evaluate the population of Listeria monocytogenes in feta cheese during its ripening. Also, results of running of the model, such as performed laboratory results witness against is that with reduction in cheese pH from 6/4 (beginning of cheese production) to 4/78 (end of forth week), Listeria population reduce. But since the final pH of cheese is more from 4/6, the growth of Listeria monocytogenes does not stop.

Given the results of running of the model, durability and quality stability in feta cheese during its ripening, are not too much.

REFERENCES:

[1] L. J. Pearson and E. H. Marth, " Listeria monocytogenes–Threat to a Safe Food Supply: A Review," *Journal of dairy science,* vol. 73, pp. 912-928, 1990.

[2] Y. Le Marc*, et al.*, "Modelling the growth kinetics of Listeria as a function of temperature, pH and organic acid concentration," *International Journal of Food Microbiology,* vol. 73, pp. 219-237, 2002.

[3] D. Liu, *Handbook of Listeria monocytogenes*: CRC, 2008.

[4] R. Laridi*, et al.*, "Liposome encapsulated nisin Z: optimization, stability and release during milk fermentation," *International dairy journal,* vol. 13, pp. 325-336, 2003.

[5] K. Naghmouchi*, et al.*, "Class I/Class IIa bacteriocin cross-resistance phenomenon in Listeria monocytogenes.," *Food microbiology,* vol. 24, pp. 718-727, 2007.

[6] J. Delves-Broughton*, et al.*, "Applications of the bacteriocin, nisin," *Antonie van Leeuwenhoek,* vol. 69, pp. 193-202, 1996.

[7] L. J. Arauz*, et al.*, "Nisin expression production from Lactococcus lactis in milk whey medium," *Journal of Chemical Technology and Biotechnology,* vol. 83, pp. 325-328, 2008.

[8] C. Aranha*, et al.*, "Contraceptive efficacy of antimicrobial peptide Nisin: in vitro and in vivo studies," *Contraception,* vol. 69, pp. 333-338, 2004.

[9] T. Abee*, et al.*, "Bacteriocins: modes of action and potentials in food preservation and control of food poisoning," *International Journal of Food Microbiology,* vol. 28, p. 169, 1995.

[10] R. O. Benech*, et al.*, "Antibacterial activities of nisin Z encapsulated in liposomes or produced in situ by mixed culture during cheddar cheese ripening," *Applied and environmental microbiology,* vol. 68, pp. 5607-5619, 2002.

[11] E. Zaerzadeh*, et al.*, "Antibacterial Effect of Nano-Encapsulated Nisin in Liposoms In Contrast to Free Nisin in Control of Listeria monocytogenes in Iranian Feta Cheese (UF)," *Iranian Food Science and Technology Research Journal,* vol. 7, pp. 191-199, 2011.

[12] R. O. Benech*, et al.*, "Antibacterial activities of nisin Z encapsulated in liposomes or produced in situ by mixed culture during cheddar cheese ripening," *Applied and environmental microbiology,* vol. 68, pp. 5607-5619, 2002.

[13] R. Pfeifer*, et al.*, "Artificial life," *Institute for Informatics, Zurich University, Germany,(June 2001),* 2000.

[14] A. Langlois and M. Phipps, "Automates cellulaires: application à la simulation urbaine," *Recherche,* vol. 67, p. 02, 1997.

[15] T. Ostoma and M. Trushyk, "Cellular Automata Theory and Physics: A new Paradigm for the Unification of Physics," *Arxiv preprint physics/9907013,* 1999.