Salmonella Enteritidis Risk Assessment: A Kinetic Analysis

S. Almonacid, J. Gutierrez, A. Jaques, and R. Simpson

ABSTRACT: Egg and egg preparations are important vehicles for *Salmonella enteritidis* infections. The influence of time-temperature becomes important when the presence of this microorganism is found in commercial shell eggs, particularly in countries where refrigeration is not mandatory as in Chile. The objective of this research was to develop a mathematical model to analyze the *Salmonella enteritidis* risk under variable ambient temperatures. Breakdown of vitelline egg membrane was assumed to be required for initiation of bacterial growth. When the critical factor concerning safety is the vitelline membrane breakdown, 15 °C was found to be the storage threshold temperature for a 30-d shelf life. This computer based tool can be used as a contribution in current regulation adjustments or modifications.

Keywords: egg safety, *Salmonella enteritidis,* risk assessment

Introduction

SALMONELLA ENTERITIDIS (SE) SPREADING, AND THE growing number of infections due to this microorganism have motivated research programs in several countries. Investigations of outbreaks of SE infection have shown that its most frequent source is shell eggs (Humphrey 1994). More than 46 billion eggs are distributed and sold annually in the United States. An estimate points out that 2.3 million eggs contain SE which results in 637000 cases of human illness per year (FSIS 1998). A recent report from The American Heart Association states that healthy people - about 75% of the world population - could consume an average of 1 egg per d without concerns of an increase in both cholesterol level or cardiovascular diseases (Osorio 2001). This statement could enhance egg consumption, and then, its associated infections. In Chile, the risk of infection is greatly enhanced during the summer season because refrigeration is not a common practice in minimarkets and supermarkets; and also because full shelf life is arbitrarily assumed to be 30 d (Gutierrez 2000). At present, Chilean regulation is changing towards mandatory refrigeration, but without a clear scientific background. The number of SE infection cases per year in Chile has increased from 7 in 1990 to 954 in 1998. In this latter year, 91% of them occurred during the warmer months, November through May, and 9% in the low temperature months, June through October. Strict and systematic monitoring of SE started in 1994, however, 1000 infection cases are concretely reported per year (ISP 1998). This high figure partially reflects the increase in egg consumption, a constant rate of 2% per year since 1991. It has been reported that almost 96% of the Chilean population considers eggs to be a vital food for health and life, the same as vegetables, milk, and fruits (Osorio 2001). Many authors have reported SE growth in eggs, affected by temperature. Therefore, it shows that the temperature could be of extreme importance, especially when eggs are stored at the retail outlets (Humphrey and others 1989; Kim and others 1989; Bradshaw and others 1990; Humphrey 1994; Schoeni and others 1995). There has been much debate however on the advisability of holding eggs under refrigeration in retail outlets (Humphrey 1994). Recent outbreaks in Chile have opened this debate, and more scientific support is needed to collaborate intellectually in the modification of current regulations. A research study is being conducted at Universidad Técnica Federico Santa María, Valparaiso, Chile, specifically, in the 2 cities which have the largest concentrated population in the region: Viña del Mar and Valparaiso. This region receives large quantities of tourists during the summer season, as this activity is one of the main sources of income for the area. The local Provincial Government, knowing the risk of salmonellosis, supports this research study. The objective of this research was to analyze the SE risk of infection from a kinetic point of view, which will be affected by the common practice of egg storage in minimarkets and supermarkets. A coupled mathematical model which considers a heat transfer simulator that accounts for the actual egg shape and composition, and a biological model to estimate the vitelline membrane degradation and SE growth under different temperature profiles, was implemented.

Materials and Methods

Mathematical model background

A SE risk assessment tool is needed to estimate rationally both the risk and the impact of refrigeration on the reduction of cases per year. The U.S. Department of Agriculture, Food Safety and Inspection Service USDA (1999) prepared a SE risk assessment computer tool based on 5 modules ranging from an Egg production Module to a Public Health Module. The risk was assessed by the incidence's probabilistic distribution for each module. This tool also includes a Storage and Distribution module which uses an empirical formulation for eggs cooling with uniform initial temperature. Therefore, it cannot be used for variable ambient temperature. Mathematical models are used in the phenomenological and kinetic simulation of food processes. In this particular case the problem includes a heat transfer phenomena—cooling and heating from environment to egg package and egg contents—along with a kinetic model of vitelline membrane breakdown and SE growth as a function of temperature at different egg locations.

The biological problem indicated that microbial growth accelerates when the vitelline membrane breaks down and that this breakdown is temperature dependent (Humphrey and others

1989; Kim and others 1989; Bradshaw and others 1990; Humphrey 1994; Schoeni and others 1995; Whiting and others 2000).

Egg contents could be contaminated with SE by migration through the shell and associated membranes, the so-called horizontal way, or as a result of infection of the reproductive tissue of laying hens, the so-called vertical way. Research studies strongly suggest that eggs are vertically contaminated (Humphrey 1994; Dolman and Board 1992), based on the following facts:

Eggs are infected from the reproductive tissue of the hen.

SE becomes entrapped in the eggs albumen near vitelline membrane.

SE in albumen does not grow, as long as the vitelline membrane is able to keep them separated from egg yolk nutrients.

Breakdown of vitelline membrane is temperature dependent and at some point will allow: (a) egg yolk nutrients to diffuse through egg white, (b) bacteria to penetrate egg yolk, or (c) both mechanisms acting together.

When the vitelline membrane is broken down, SE starts exponential growth.

The numbers of SE per egg can ranges from 1 to 400 microorganisms (Aserkoff and others 1970; Humphrey 1994).

A kinetic model for vitelline membrane breakdown and SE exponential growth was developed by the USDA Food Safety and Inspection Service (FSIS) in its farm-to-table assessment of the risk of human illness from SE in eggs and reported by USDA (1999) and Whiting and others (2000). The model was validated with experimental data provided by Dr. T. Humphrey to the FSIS team (USDA 1999). In his study, intact eggs with shells free of fecal contamination were obtained within 2 h of being lain and were stored, at 8 to 37 °C. At time intervals of about 3 d, 10 eggs were removed and broken, using aseptic techniques. The contents of each egg were inoculated with approximately 500 cells of SE into the albumen next to the vitelline membrane. This site was chosen because studies with eggs from artificially (Gast and Beard 1990) and naturally infected hens (Humphrey and others 1991b) have shown it to be an important site of contamination with SE. The inoculated eggs were held at 20 °C for 5 d. If more than 2 of 10 eggs sampled at a storage time had SE exceeding 10^4 cfu/egg, estimated with standard laboratory techniques, that time-temperature period was designated as growth permitting.

The average time for vitelline membrane breakdown is estimated by the following exponential expression:

$YMT = 10^{(2.1-0.043T)}$
YMT: Yolk membrane time (d)
T: Temperature °C (1)

Two studies reporting the growth rates of SE in blended whole eggs provided the best estimate of SE growth after YMT is exceeded. These results are reported by Whiting and others (2000).

The average exponential growth rate as a function of temperature is estimated by:

$EGR = (0.026T-0.14)^2$
EGR: Exponential growth rate (1/h)
T: Temperature °C (2)

The heat transfer problem in solids for irregular shape has been investigated by many authors and applied for different food systems. A rather complete list of cases can be found in literature (Holdsworth 1992). Although irregular and oval shapes are considered; egg shaped solids with multicomponent conformation has been studied only recently (Gutierrez 2000; Almonacid and others 2000). In the aforementioned studies a computer aid mathematical tool was validated to estimate temperature at different egg locations under variable ambient temperatures.

A mathematical model that accounts for both the shape and the complex nature of the egg system was fed with a finite difference algorithm written in C++. Two extreme cases were simulated: a) low temperature with forced air, and b) ambient temperature with stagnant air. Parameters of interest, such as local heat transfer coefficient (h) and global heat transfer coefficient (U considering package material), were estimated and adjusted by an inverse procedure using experimental data.

The main assumptions for the model were:

Heat transfer within egg content is totally by conduction.

Egg content is made of egg yolk and egg white.

Thermal properties do not change within the simulated temperature range (5 to 37 °C).

The heat transfer mechanism up to the egg's surface is convective.

Good agreement was found between experimental and theoretical data, mean temperature errors were in the range of 0.19 through 0.32 °C. Global heat transfer coefficients were found to be 4.4 through 15 (W/m^2 K) and 2.4 through 8.0 (W/m^{2}K) when eggs were stored in a typical cardboard package.

Simulation scenarios

As previously stated the kinetic problem of SE growth in egg contents encompasses 2 phases, which take place sequentially. First, the time calculation for vitelline membrane breakdown

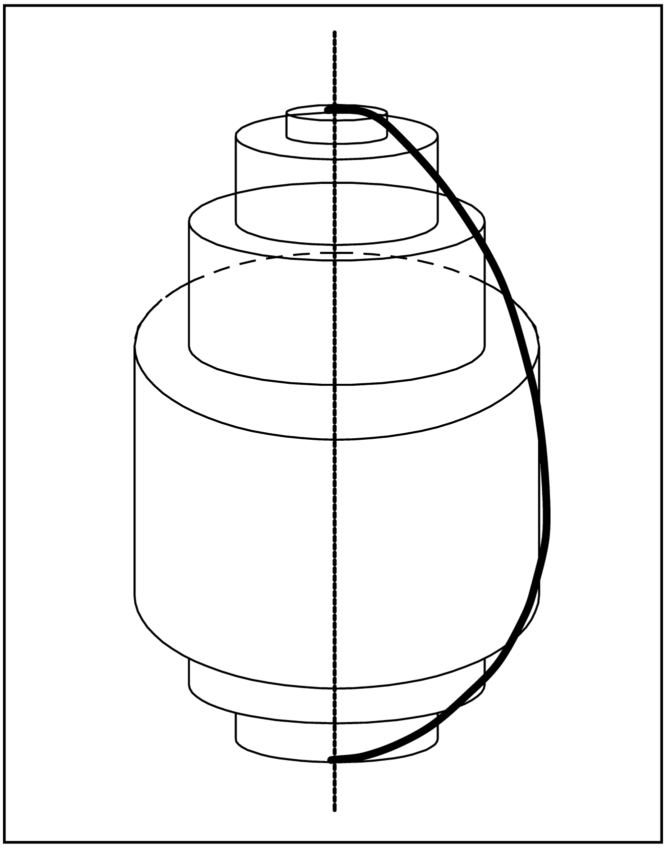


Figure 1—Three-dimensional view of the egg shape as modeled by Finite difference

which is temperature dependent, as shown by equation 1; and 2nd, the exponential growth rate shown in equation 2. The bacteria concentration can be expressed as a dimensionless number by the following expression:

$$\frac{N}{N_0} = 10^{(0.026T - 0.14)^2 \Delta\theta} \tag{3}$$

where, N is the microbial concentration in (cfu/g) after a $\Delta\theta$ hours held at a constant temperature T (°C), and N_0 is the initial concentration.

This 2-phased-kinetic problem was analyzed under several different time-temperature scenarios. Computer experiments were divided into the following expected and temperature controlled situations:

1. Expected situation 1: Winter average conditions.

24 h at a constant temperature (15 °C): Eggs after being lain, selected and packed remain 24 h at constant room temperature before transportation to the supermarket.

30 d at a sinusoidal temperature regime (24 h period) with a maximum of 20 °C at 3 PM and a minimum of 15 °C at 3 AM.

2. Expected situation 2: Summer average conditions 24 h at a constant temperature (20 °C): Eggs after being lain, selected and packed remain 24 h at constant room temperature before transportation to the supermarket.

30 d at a sinusoidal temperature regimen (24 h period) with a maximum of 30 °C at 3 PM and a minimum of 20 °C at 3 AM.

3. Simulation 1: Controlled temperature at 20 °C

This situation would simulate an average air conditioning temperature of 20 °C.

4. Simulation 2: Controlled temperature at 15 °C

This situation would simulate an average air conditioning temperature of 15 °C

5. Simulation 3: Refrigerated temperature at 5 °C

Situations 1 to 4 considered eggs packed in a typical cardboard package in stagnant air, therefore, the global heat transfer coefficient U was assumed to be 4.5 (W/m²K) (Gutierrez 2000). Situation 5 considered eggs in a typical cardboard package in forced refrigerated air, therefore, the global heat transfer coefficient U was 9.4 (W/m²K) (Gutierrez 2000).

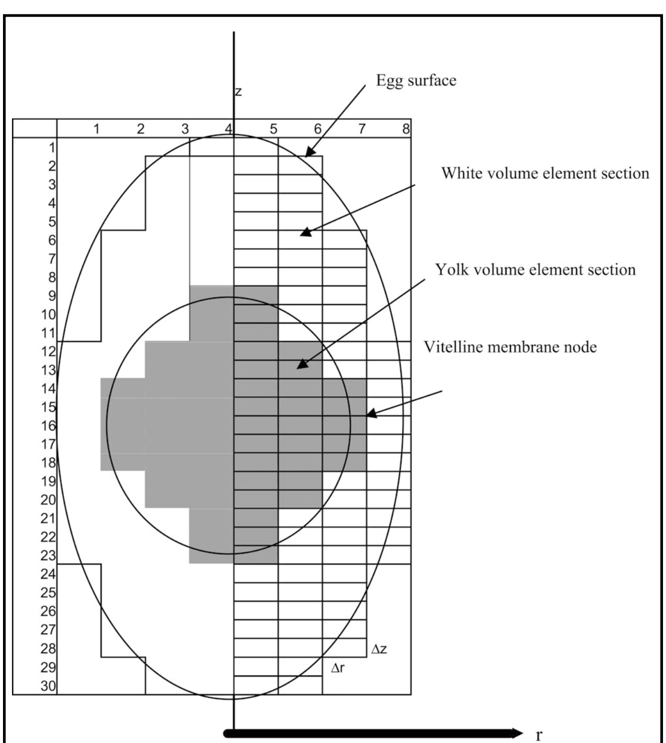


Figure 2—Schematic representation of the numeric method for heat transfer calculations

Yolk membrane time (YMT) calculations under variable temperature

The total Yolk membrane time (YMT_{Total}) under fluctuating conditions was calculated by noting that, if YMT_i, $\Delta\theta_i$ are the YMT and incubation period at temperature Ti, respectively, then:

$$\Delta\theta_i \left(\frac{1}{YMT_i} \right) =$$

in the fraction of time accumulated to attain yolk breakdown during incubation period $\Delta\theta_i$

Therefore:

$$\frac{\Delta\theta_1}{YMT_1} + \frac{\Delta\theta_2}{YMT_2} + \frac{\Delta\theta_3}{YMT_3} + \ldots\ldots + \quad \text{==> Yolk breakdown completed}$$

YMT_i is related to temperature (Ti) according to the following expression:

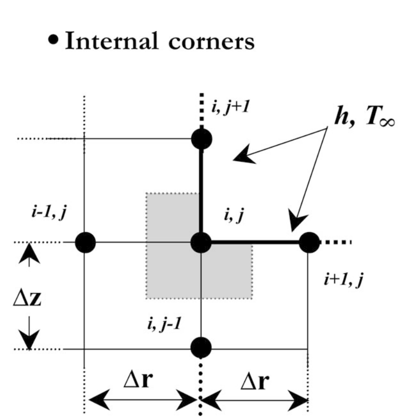
$$YMT_i = 10^{(2.1 - 0.043\,Ti)} \tag{4}$$

After time $YMT_{Total} = \sum\Delta\theta_i$, vitelline membrane breakdown is completed, then microorganisms begin to grow exponentially. The concentration of cells can be calculated using equation 3.

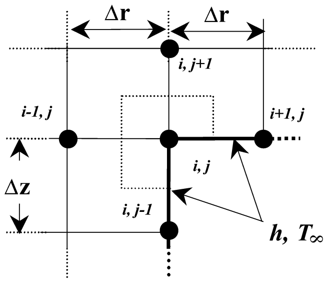
Heat transfer model

Temperature profiles within eggshells with variable environmental temperature can be calculated numerically, based on an energy balance (Gutierrez 2000). Assuming heat transfer symmetry along the major axis, and expressing mathematical model in cylindrical coordinates, a space grid in terms of r and z was generated with one half of the product (Figure 1 and Figure 2).

Temperatures at the generated nodes were predicted using an energy balance for a control volume surrounding each node, for example:



$$T_{i,j}^{n+1} = \frac{\mathrm{a}\ \Delta t}{(\Delta r)^2(\Delta z)^2}\left\{\begin{array}{l}\dfrac{2h(\Delta z)(\Delta r)(\Delta z+\Delta r)}{3k}T_\infty + \dfrac{2}{3}(\Delta z)^2\left[T_{i+1,j}^n + 2T_{i-1,j}^n\right] + \dfrac{2}{3}(\Delta r)^2\left[T_{i,j+1}^n + 2T_{i,j-1}^n\right] + \\ \left[\dfrac{(\Delta r)^2(\Delta z)^2}{\mathrm{a}\ \Delta t} - \dfrac{2h(\Delta z)(\Delta r)(\Delta z+\Delta r)}{3k} - 2(\Delta r)^2 - 2(\Delta z)^2\right]T_{i,j}^n\end{array}\right\}$$



$$T_{i,j}^{n+1} = \frac{\mathrm{a}\ \Delta t}{(\Delta r)^2(\Delta z)^2}\left\{\begin{array}{l}\dfrac{2h(\Delta z)(\Delta r)(\Delta z+\Delta r)}{3k}T_\infty + \dfrac{2}{3}(\Delta z)^2\left[T_{i+1,j}^n + 2T_{i-1,j}^n\right] + \dfrac{2}{3}(\Delta r)^2\left[T_{i,j-1}^n + 2T_{i,j+1}^n\right] + \\ \left[\dfrac{(\Delta r)^2(\Delta z)^2}{\mathrm{a}\ \Delta t} - \dfrac{2h(\Delta z)(\Delta r)(\Delta z+\Delta r)}{3k} - 2(\Delta r)^2 - 2(\Delta z)^2\right]T_{i,j}^n\end{array}\right\}$$

With the aforementioned procedure, temperatures can be calculated for each node. Also global heat transfer coefficients including package resistance can be estimated when required (Almonacid and Torres 1993).

Combined heat transfer and biological model

Heat transfer equations were solved using an explicit finite difference scheme with time increments of 5 s. Microbial growth was calculated using kinetics constants, which ones were assessed using an average temperature along the time interval, as follows: If at a given point (node), which also happens to be considered the center of a very small volume element (relative to the whole egg), the temperature is known, it can be assumed that this temperature is representative of the whole element. This principle also applies to the dimensionless relative concentration (N/N_0). Using this concept, the egg was virtually divided into volume elements in the shape of concentric ring layers having rectangular cross sections. This idea is illustrated in Figure 2. This one shows the right hand side of an egg, where the egg yolk and egg white were considered with individual thermophysical properties. *Salmonella enteritidis* growth calculations, would proceed as follows, with the aid of a PC computer: For the 1st time increment, an average temperature T, over the time interval $\Delta\theta_1$ at the center of each element, is supplied. Consequently, the final concentration N_1 at that time interval is calculated by equation 3.

$$\frac{N_1}{N_0} = 10^{(0.026T-0.14)^2\Delta\theta_1}$$

where N_0 is the known initial concentration in the respective volume element.

This value is then multiplied by the volume fraction represented by the element to obtain the weight of the dimensionless relative concentration represented by the volume element Vj. The mass average of the dimensionless relative concentration, for the time interval, is finally obtained as follow:

$$\left\langle \frac{N_1}{N_0} \right\rangle = \sum_{j=1}^{n} \frac{V_j}{V_{Total}} x \left(\frac{N_1}{N_0}\right)_j \tag{5}$$

Where V_{Total} is the total volume considered in the analysis and n the numbers of elements in the total volume. Repeating this process up to the storage time under study, allows obtaining the final mass average relative concentration for each element. The accumulated time effect is evaluated by equation

$$\left\langle \frac{N_f}{N_0} \right\rangle = \left\langle \frac{N_1}{N_0} \right\rangle x \left\langle \frac{N_2}{N_1} \right\rangle x \left\langle \frac{N}{N} \right\rangle \tag{6}$$

where m is the number of time intervals

In which the final average SE concentration N_f can be calculated by multiplying by the known homogeneous initial concentration N_0:

$$\left\langle N_f \right\rangle = \left\langle \frac{N_f}{N_0} \right\rangle x \left\langle N_0 \right\rangle \tag{7}$$

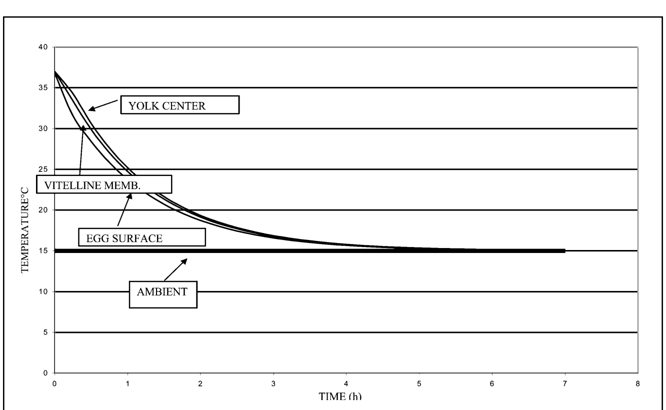


Figure 3—Temperature gradient in egg contents as affected by a constant temperature of 15 °C

Model application for SE risk assessment

Regular egg's average physical dimensions, were used:

Width: 4.5×10^{-2} (m) (at the widest point)

Height: 6.0×10^{-2} (m)

Total volume: 63×10^{-6} (m^3)

Total mass: 57.9×10^{-3} (kg) (11% of shell weight was not considered)

Yolk mass: 13.8×10^{-3} (kg)

White mass: 44.1×10^{-3} (kg)

Initial egg contamination, N_0 = 400 (cell/egg)

For simulation accomplishment of the 5 detailed experiments, 3 different initial SE concentration were used, considering the time when vitelline membrane is permeable to material exchange (YMT completed):

i. It is assumed that all cells penetrate into the yolk and nutrients remain in it, so that, N_0 is uniform in the volume of yolk and its value is:

Table 1—Time in d for vitelline membrane breakdown and growth of *Salmonella enteritidis*. Effect of the 5 different scenarios and microorganism's initial concentration.

	Vitelline membrane breakdown time (day)	Exponential growth, time to $N=10^6$ (CFU/g)(day)		
		$(N_0)_{Whole}$	$(N_0)_{Yolk}$	$(N_0)_{White}$
Winter	22	2.25	1.88	2.17
Summer	10	0.88	0.79	0.88
20 °C	17	1.54	1.33	1.50
15 °C	28	3.54	3.08	3.46
5 °C	>>30	-	-	-

$$(N_0)_{Yolk} = \frac{400cell}{13.8g} = 29.01(cfu$$

ii. It is assumed that nutrients migrate to egg white and all cells remain in egg white, so that, N_0 is uniform in the volume of egg white and its value is:

$$(N_0)_{White} = \frac{400cell}{44.1g} = 9.07(cfu$$

iii. It is assumed that both, cells and nutrients are uniformly distributed in the whole egg, so that, C_0 is uniform in the volume of the egg and its value is:

$$(N_0)_{Whole} = \frac{400cell}{57.9g} = 6.91(cfi$$

Simulations were stopped, as an end of process criterion, when SE concentration was 10^6 (cfu/g), which is considered hazardous for human consumption. In general, minimum numbers for gastroenteritis caused by *salmonella* range between 10^5-10^6/g for *S. bareilly* and *S. newport* to 10^9 to 10^{10}/g for *S. pullorum* (Jay 2000; Gutierrez 2000).

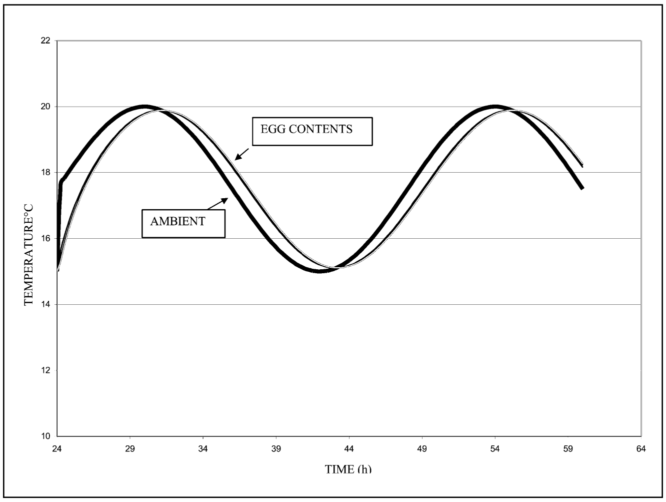


Figure 4—Temperature gradient in egg contents as affected by a sinusoidal temperature variation with a maximum of 20 °C and minimum of 15 °C. Winter situation

Results and Discussion

SIMULATIONS WERE CONDUCTED TO EVALUATE BOTH THE EFFECT OF different time-temperature records—on a 30 d period—on the safety of eggs and the effect of initial concentration of SE.

Temperature distribution within egg

When eggs are held at constant temperature, in 6 to 8 h a uniform temperature distribution is reached. This short transient effect is fully described in Figure 3, in which the temperatures of the center of the egg yolk, at the surface of the vitelline membrane and the surface of the egg are plotted against time. This short thermal inertia could severely affect the safety of the egg, especially when temperature abuse occurs. The same result is obtained when eggs are kept at a sinusoidal temperature regime. Situation 1 (winter) and situation 2 (summer) render sinusoidal pattern for the internal temperatures of the eggs, with maximum difference of approximately 1 °C with the external temperature (Figure 4). This fact illustrates the importance of using conceptual mathematical models for temperature estimations, especially when fluctuating temperature effect are being studied.

Kinetic analysis

In all situations analyzed in this research -shown in Table 1 - the required time to attain membrane breakdown was less than 30 d, except for the simulation trial carried out at refrigeration conditions (5 °C). In the latter case, simulations showed that af-

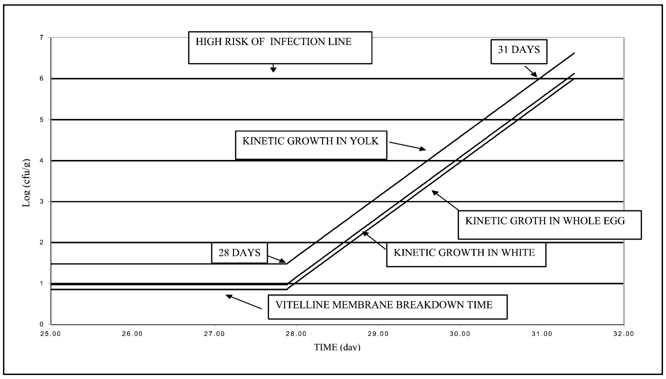


Figure 5—Microbial growth simulation under constant 15 °C temperature. Effect of initial concentration of *Salmonella enteritidis*

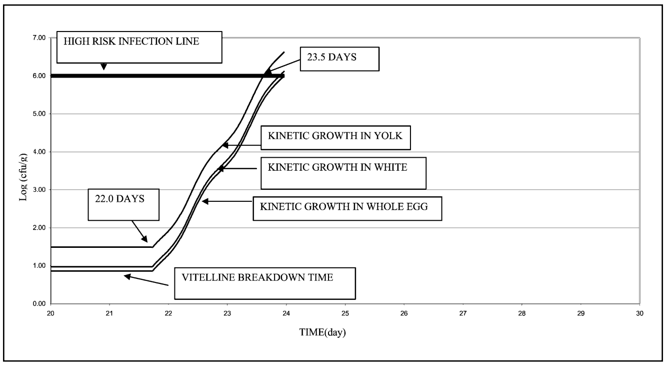


Figure 6—Microbial growth simulation under sinusoidal temperature fluctuation for the winter season. Effect of initial concentration of *Salmonella enteritidis*

ter 40 d at 5 °C, only 55% of the YMT fraction was consumed. Therefore, storage at 5 °C could be considered as an over dimensioned alternative, in terms of controlled temperature for safety reasons. In all 3 scenarios given by situations 1, 2, and 3, the total time (addition of membrane breakage time and growth time to achieve 10^6 (CFU/g)) was always less than 30 d, therefore indicating high-risk situation occurrences. On the other hand, for situation 4 (15 °C constant temperature) the total time was always longer then 30 d, regardless of the SE initial concentration used for simulations, being an indicative result of advisable storage condition. Figure 5 and 6 show the required time to achieve 10^6 (CFU/ g) as affected by initial concentration and ambient temperature profile for winter and constant temperature (15 °C) simulating experiments, respectively.

When eggs are stored under fluctuating temperature conditions (between 18 °C to 20 °C) - simulating those that might be found in a kitchen - rapid SE growth is possible after 6 to 10 d in the majority of examined eggs as stated in a research conducted by Humphrey and Whitehead (1993). Humphrey's results are in agreement with those obtained in this research for situation number 2 (summer) in which 10 d, the upper limit of the above mentioned research, was necessary to attain YMT depletion. It is presumed that insulated packaging, considered in our research, might have some effect on the results as the experiments carried out by Humphrey and Whitehead where done without taking that factor into account.

There has been much debate on the advisability of holding eggs under refrigeration in retail outlets. The data on storage-related effects on the growth rate of SE in egg contents (Humphrey and Whitehead 1993; Humphrey and others 1991b) coupled with data on naturally contaminated eggs stored at 20 °C, suggests that refrigerated storage might not be necessary provided that temperatures do not exceed 20 °C (Humphrey 1994). This recommendation may also be a result from this research study, but with a new constraint on holding time, which is no more than 17 d, the time for YMT breakdown under situation number 3 (20 °C).

Whiting generated a biological model for YMT depletion and SE growth estimation, which was used in this research (Whiting and others 2000). However, the former research used an empirical heat transfer model to estimate the temperature inside eggs which is only valid for a cooling procedure at constant ambient temperature and constant initial temperature. The error of this empirical model arose from using it for transportation or storage conditions, that consider fluctuating ambient temperatures. Their results are not comparable with the ones obtained in this research, because theirs considered eggs arranged in pallets, and ours considered packaged eggs which is the way supermarkets usually display this product.

Conclusions and Recommendations

CONCEPTUAL MATHEMATICAL MODELS COULD BE USEful tools to estimate important variables or parameters in real practical situations. Rapid progress of both software and hardware allows it to simulate real situations with fewer assumptions.

The breakdown of vitelline membrane could be used as safety criterion for shelf life estimations under different situations since its depletion time, for most cases, is more than 90% of the total safety time. In the same way, the growth phase could be considered as safety factor. In other words, the safety period for shell egg consumption is over, when vitelline membrane depletion finishes.

15 °C is the advisable control temperature to ensure consumer health when eggs are kept less that 30 d in a retail supermarket.

20 °C is the advisable control temperature to ensure consumer health when eggs are kept less that 15 d in a retail supermarket. This recommendation might be applied in those marketplaces in which rotation times of eggs are less than 15 d or generally 10 d.

The developed combined-model can be used to analyze other scenarios and offer sound scientific support for future regulation adjustments or modifications.

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