Time-temperature profiles of chilled ready-to-eat foods in school catering and probabilistic analysis of Listeria monocytogenes growth.

Philippe Rosset, Marie Cornu, Véronique Noël, Elisabeth Morelli, Gérard Poumeyrol

TIME-TEMPERATURE PROFILES OF CHILLED READY-TO-EAT FOODS IN SCHOOL CATERING AND PROBABILISTIC ANALYSIS OF *LISTERIA MONOCYTOGENES* GROWTH.

Philippe ROSSET[*] , Marie CORNU, Véronique NOËL, Elisabeth MORELLI, Gérard POUMEYROL

Agence Française de Sécurité Sanitaire des Aliments, LERAC, 22 rue Pierre Curie, BP 332, 94709 Maisons-Alfort cedex, F.

Abstract

The purpose of this study was to evaluate the chill chain in school catering by monitoring time/temperature profiles. Chilled ready-to-eat foods have been chosen as subject of this study because of their high risk due to a fabrication and a distribution without any thermal sanitation treatment. In order to integrate the effects of storage duration and storage temperature, a quantitative criterion, was calculated. To show the sanitary consequences of these data *Listeria monocytogenes* growth was predicted. The study of 5 centralized kitchens and 11 school lunch canteens demonstrated in general a satisfactory respect of the chill chain. However, the coincidence of extend storage duration (due to week-ends) and abuse temperature was observed and could lead to a significant microbial development.

1. INTRODUCTION

Food safety is primordial in catering because of the high number of meals served every day. Foodborne outbreaks resulting from such mass catering facilities have been reported worldwide, notably in elementary and nursery schools. These school-lunch systems are of particular interest, as young children are at relatively high risk of developing serious complications from exposition to foodborne hazards.

The Japanese school lunch program constitutes an interesting case-study, intensively investigated by Michino and Otsuki (2000) and Lee et al. (2001). From 1981 through 1995, 533 outbreaks of food poisoning in Japan occurred at schools, and concerned over 100.000 people (Lee et al., 2001). Michino and Otsuki (2000) identified the causative factors for 62 outbreaks of foodborne illnesses that occurred in Japanese school lunch facilities, from 1987 to 1996. They were: storage of foods for an excessive period of time before serving (29/62), raw material contamination (29/62), inadequate cooking (21/62), cross contamination (21/62), and contamination by infected employees (9/62).

In France, almost 3.500 cases of foodborne illnesses linked to school lunch facilities were registered in 2000. Major factors contributing to foodborne outbreaks were attributed to: equipment contamination (55%), raw material contamination (50%), non-hygienic operations during preparation (46%), abuse temperature of refrigerated storage (43%), abuse duration of refrigerated storage (35%) (Gallay, 2002). Non-respect of the chill chain (abuse temperature and/or abuse duration) appears then as an important reason for these foodborne outbreaks in school catering.

Centralized kitchens are mainly used in school catering. In these kitchens a very high number of meals (from a few hundreds to many thousands per day and per kitchen) are prepared and then delivered at short notice (1 to 3 days) to the canteens (quite often located in schools) where they are served to children. The meal fabrication can be laid on the use either of a warm chain - the meals are kept hot

---

[*] Corresponding author. Tel: +33 (0)1 49 77 26 36; Fax: +33 (0)1 49 77 38 88; E-mail: p.rosset@afssa.fr

from cooking until eating - or of a chill chain - the cooked food is quickly cooled as soon as cooking ends and then kept cold, if necessary the food is rethermalized just before serving.

The purpose of this study was to evaluate the chill chain in school catering, by monitoring time/temperature profiles. Chilled ready-to-eat foods have been chosen as subject of this study because of their high risk due to a fabrication and a distribution without any thermal sanitation treatment.

In order to show the sanitary consequences of the time/temperature profiles - and more particularly the impact on potential growth of psychotrophic bacteria as *Listeria monocytogenes* - we integrated the effects of storage duration and storage temperature, through a quantitative criterion, the TTE ("time-temperature equivalent"). The quality of the chill chain was assessed using this criterion

Finally, to illustrate the potential use of these data for a quantitative microbial risks assessment, *Listeria monocytogenes* growth was predicted using standard predictive microbiology models (van Gerwen and Zwietering, 1998). Probabilistic modeling was conducted to incorporate variability (and uncertainty) of input data (time-temperature conditions and growth ability of *Listeria monocytogenes*) (Vose, 2000).

2. MATERIALS AND METHODS

2.1. KITCHEN SAMPLING

Public centralized kitchens and school canteens in the suburbs of Paris were subject to a telephone survey to evaluate preparation of meals in the chill chain (see Table 1). They were divided among two groups according to the number of meals prepared per day. Two centralized kitchens (A and E) preparing under 5000 meals per day and three others ones (B, C and D) preparing over 5000 meals per day were randomly selected. For each one, 2 (or 3) school canteens were selected according to the air temperature measured inside the cold cabinets (the best and the worst). If no significant difference among the air temperatures was noticed, the nearest and the farthest school canteens were selected. The study was conducted from November 2001 to June 2002.

2.2. SELECTION OF FOOD PRODUCTS

Chilled entrées, prepared with cooked or uncooked ingredients, were selected according to the methods (cutting, slicing, grating, mixing,...).Chilled entrées which did not undergo any transformation in the centralized kitchen, were eliminated (e.g. green salad with French dressing: the green salad packages were delivered unpacked to the school canteen, and then opened and mixed with some French dressing in the school canteen just before consumption by children).

2.3. TEMPERATURE MEASUREMENT

Food temperature was measured by inserting time temperature indicators (TTIs) (Proges Plus, Willems, France) into foods. Time and temperature were monitored every 3 minutes during all the stages of the fabrication and distribution: packaging, cold storage in centralized kitchen, transport to school canteens, and cold storage in canteens. Measuring started as soon as the last food transformation and/or mixing was completed. Measuring stopped at 12 A.M., at the day the chilled entrées were served to the children.
 If possible foods were studied in the worst conditions (fail-safe choice): e.g. studied foods were transferred to cold storage only when the fabrication of this meal was completely finished.

2.4. TIME-TEMPERATURE EQUIVALENT (TTE) CALCULATION

The interest of time-temperature profiles was to show their sanitary impact on growth of pathogen bacteria. This modeling was based on simple and standard predictive microbiology models. The chosen primary model is the exponential growth, without lag phase nor stationary phase (fail-safe choice):

$$N_{i+1} = N_i \exp(\mu_i \, t_i) \Leftrightarrow \ln\left(\frac{N_{i+1}}{N_i}\right) = \mu_i \, t_i \tag{1}$$

where N_i (resp. N_{i+1}) is the number of microorganisms (CFU/g) at time i (resp i+1), μ_i is the growth rate (day^{-1}) assumed constant between i and i+1, and t_i is the time duration (days) between i and i+1.

For n successive steps, the total growth increment is:

$$\ln\left(\frac{N_n}{N_0}\right) = \sum_i \mu_i \, t_i \tag{2}$$

where N_0 is the initial number of microorganisms (CFU/g) at time 0, N_n is the final number of microorganisms (CFU/g) after n steps.

The chosen secondary model is the widely-used square-root model (Ratkowsky *et al.*, 1983; Zwietering et al., 1996):

$$\mu_i = \mu_{réf}\left(\frac{T_i - T_{min}}{T_{réf} - T_{min}}\right)^2 = \frac{\mu_{réf}}{(T_{réf} - T_{min})^2}(T_i - T_{min})^2 = b^2(T_i - T_{min})^2 \tag{3}$$

and $b = \sqrt{\mu_{réf}}/(T_{réf} - T_{min})$.

where T_i is the temperature (°C) assumed constant between i and i+1, T_{min} is the theoretical minimal temperature (°C) of the species, and μ_{ref} is the growth rate (day^{-1}) of the species in the food at temperature T_{ref}.

From (2), and (3), the logarithmic microbial increment can be deduced:

$$\ln\left(\frac{N_{i+1}}{N_i}\right) = b^2 \, t_i \, (T_i - T_{min})^2 \Rightarrow \ln\left(\frac{N_n}{N0}\right) = b^2 \sum_i t_i \, (T_i - T_{min})^2 \tag{4}$$

From equation 4, we defined a quantitative criterion, the TTE ("time-temperature equivalent"):

$$TTE = \sum_i t_i \times (T_i - T_{min})^2 \tag{5}$$

where t_i is the time between 2 measurements (days), T_i is the temperature (°C) measured at time i and T_{min} (°C) is the minimum growth temperature.

Thus, the TTE is linked to time, temperature and the studied bacterium, or more exactly its minimum growth temperature. The theoretical minimal temperature of *Listeria monocytogenes* was estimated at -2,7°C on the basis of 1865 growth curves by Augustin and Carlier (2000). Though uncertain and variable, this value was assumed to be fixed. Table 2 shows a few equivalent values between TTE and reference time-temperature couples. It is quite important to note that TTE does not depend on the type of food. A potential growth increment can be predicted as the product of a TTE and a parameter b^2, specific of the pathogen and the food product.

2.5. STATISTICAL ANALYSIS OF TTE

Statistical analysis was conducted with Statgraphics Plus 5.1. (Manugistic™, Maryland, USA) to evaluate the impact of each of six factors (centralized kitchens, school canteens, process, air temperatures, week-end, initial food temperature) on the TTE. The factor "School canteen" was embedded in the factor "centralized kitchen", with 2 or 3 canteens per kitchen: A1, A2, B1, B2, C1, C2, D1, D2, D3, E1, and E2. For each "process" parameter, 4 possibilities were defined: T, M, TM, and O. According to recipes, the process was able to follow 4 different actions: transformation (T) as in cutting, slicing, crushing..., mixing (M), transformation and mixing (TM) or depackaging (D).
Median external air temperature was calculated every day with minimum and maximum air temperatures measured by the meteorological office (Météo France, Toulouse, France) in the kitchen surroundings. The reference day being the day the studied food was delivered to the school canteens. Averages and 95% Bonferoni confidence intervals were calculated for every parameter.

2.6. PREDICTION OF POTENTIAL *LISTERIA MONOCYTOGENES* GROWTH

To predict the potential growth of *Listeria monocytogenes*, the next step in the model development was to take into account the impact of the food products. A literature survey was conducted to determine reference growth parameters of *Listeria monocytogenes* in chilled ready-to-eat entrees similar to those encountered in the present study. For each published challenge test, the maximal growth rate (μ_{max}) was if necessary estimated by fitting an exponential model to the growth curve. The parameter b^2 was calculated on the basis of equation (2). When different growth curves were available for the same food product, the median of all calculated b^2 values was selected.

2.7. PROBABILISTIC SIMULATIONS

A probabilistic analysis of potential *Listeria monocytogenes* growth was performed, taking into account "real world" variability of time-temperature profiles and food products. Measuring and sampling uncertainty were assumed to be negligible in comparison with variability. The potential growth increment of *Listeria monocytogenes* in a given food product along a given time-temperature profile is the product of the parameter b^2, characterizing the food product, and the TTE, characterizing the time-temperature profile. Thus, a distribution of potential growth increments of *Listeria monocytogenes* could be obtained by multiplication of both distributions of b^2 and TTE. Note that Monte Carlo simulations were not necessary, thanks to the simplicity of the chosen model.

3. RESULTS AND DISCUSSION

3.1. TIME-TEMPERATURE PROFILES

Were collected 287 time-temperature profiles (kitchen A : 62; kitchen B : 60; kitchen C : 54; kitchen D : 52; kitchen E : 59). Figure 1 shows that each time-temperature profile was based on four successive periods with different durations: a period (a) with preparation and packaging in centralized kitchen, a period (b) with cold storage in centralized kitchen, a period (c) with delivering to school canteen, a period (d) with cold storage in school canteen.

Period (a) consequences: As the temperature increases during this period, the cooling down period will last longer. This effect was according to initial food temperature.

Period (b) consequences: During this period (see period a) food was cooled and stored at a temperature lower than 4°C. Generally the refrigerating system of centralized kitchens was correctly adjusted to obtain a temperature lower than 4°C. Time-temperature profiles could be unsatisfactory when this period was reduced for the benefit of a longer period (d), e.g. when ready-to-eat foods were delivered to canteens on Friday, before the week-end, for being served on Monday, see period (d).

Period (c) consequences: Food was delivered on the day of serving. The refrigerating system of trucks was correctly adjusted to obtain a temperature of < 4°C and was on the whole time during delivery. Transfer time between lorry/cold cabinet in canteen was quite reduced, delivery time was limited (under 2 h 30).

Period (d) consequences: Generally this period was limited to a specific time, ready-to-eat foods were delivered on the day the food was to be served. Time-temperature profiles could be unsatisfactory when ready-to-eat foods were stored in incorrectly adjusted chill cabinet (sometimes temperature close to 6/8°C) or during a longer period d, e.g. during week-end, see period (b).

3.2. EVALUATION OF SANITARY CONSEQUENCES

Time-temperature profiles were expressed by a single quantitative criterion, integrating both effects of temperature and duration on potential microbial growth: the time-temperature equivalent (TTE). Note that this TTE is a generalization of tTT, a criterion used by Nauta (in press), who assumed $T_{min}=0°C$ for the spoilage microbial population.

Calculations were in a first step applied to the specific case of *Listeria monocytogenes*, as its growth at low temperature and its potential presence in ready-to-eat chilled foods have been widely recognized (e.g. Farber et al., 1998; Aureli et al. 2000; Farber et al., 2000; Pingulkar et al., 2001).
Figure 2 presents the empirical histogram of the 287 calculated TTE. All of them were below 500 day.°C², this limit corresponding to potential growth of *Listeria monocytogenes* after 267 hours (11.1 days) at 4°C, or 105 hours (4,4 days) at 8°C, or 56 hours (2.3 days) at 12°C (see Table 2). The majority of TTE (158 among 287, 55%) was under 100 day.°C², this limit corresponding to potential growth of *Listeria monocytogenes* after 53 hours at 4°C, or 21 hours at 8°C, or 11 hours at 12°C. Note that those TTE can not be converted into growth increments without taking into account the behavior of the micro-organism in the food product (namely through the parameter b2).

To determine conditions having the biggest effect upon TTE (and in fact upon the quality of the chilled chain), a one-way analysis of variance was performed. Significant factors were: the centralized kitchen (p<0.0001), the school canteen (p=0.0001), and the presence or absence of a week-end between preparation and consumption (p<0.0001). The between-kitchen and within-kitchen between-school variabilities are demonstrated in table 3.

No significant differences were shown for processes (p=0.25), meteorological conditions (p=0.25) and initial food temperature (p=0.25). It was also demonstrated that neither the season nor the recipe have a significant effect on TTE (data not shown).

Tessi et al. (2002) highlighted the importance to cool boiled ingredients (rice, cereals, vegetables...) before their integration into mixed salads. In absence of this cooling step, they observed that salads were sometimes kept at temperatures between 28 and 32°C, enabling both spore germination and fast

microbial growth. In the surveyed kitchens, this cooling step was satisfactory as the initial temperature of salads at the time of mixing was always below 22°C (75% studied foods had an initial temperature between 6°C and 11°C, with an average of 8°C). The absence of significant effect of initial temperature on the TTE confirms that this potential risk factor was well controlled.

The highest values of TTE were explained by a combination of long storage time (during week-end, preparation on Friday and service on Monday) and high storage temperatures (in school canteens chilled cabinets incorrectly adjusted). So we can consider that food storage during the week-end was better managed in centralized kitchen than in school canteens.

The integration of time and temperature effect in a single quantitative criterion appears to us as a useful way to assess the quality of the chill chain, especially since miniaturized, simple and inexpensive temperature captors have appeared. This general field of temperature function integration and time-temperature integration was at the basis of early works in predictive microbiology and is still in great development (Olley and Ratkowsky, 1973; Daud *et al.*, 1978; Gill *et al.*, 1991; Dickson *et al.*, 1992; Snyder, 1998; Giannakourou *et al.*, 2001).

3.3. PROBABILISTIC ANALYSIS OF POTENTIAL LISTERIA MONOCYTOGENES GROWTH.

Reference growth rates of *Listeria monocytogenes* in different ready-to eat chilled foods, similar to those of the present study, were calculated from literature, see Table 4. Thus, 16 values of b^2 were obtained, and they were considered as equiprobable. This empirical distribution of b^2 was taken as representative of the variability (and uncertainty) of *Listeria monocytogenes* growth ability in these different foods. Combining distributions of TTE and b^2, 16x287=4592 potential growth increments were obtained. Their distribution (Figure 3)characterized the variability (and uncertainty) of *Listeria monocytogenes* growth increments in these different foods along with these different time-temperature courses.

For most combinations (70%), the growth increment would be less than 0.69 ln(CFU/g) or $0.3\log_{10}$(CFU/g), i.e. the growth would be either null (case of carrots and tomatoes) or negligible (less than one doubling of the population). Only 4% of combinations lead to more than 3 doublings, i.e. a multiplication of the microbial population by more than 8. The highest growth predicted, corresponding to the worst time-temperature profile and the fastest growth (in chicken salad), would lead to a growth increment of 8 ln(CFU/g) or 3.5 $\log_{10}$(CFU/g), i.e. a multiplication of the microbial population by 3.000.

This probabilistic analysis illustrates the application of field data to the development of quantitative predictions, taking into account variability (and uncertainty) of the food service industry. A previous study concerned the warm chain and the potential growth of *Clostridium botulinum* (Fazil *et al.*, 2002) and, to our best knowledge, our study is the first one applied to the chill chain, even if numerous data are still lacking to fully characterize both uncertainties and variabilities on each input factor for a complete microbial risk assessment.

4. CONCLUSION

Refrigerated storage of ready-to-eat foods has often been targeted as a potential risk factor for development of microbial hazards, and foodborne illnesses. The present survey of 5 centralized kitchens and 11 school lunch canteens demonstrated in general a satisfactory respect of the chill chain. However, the coincidence of extend storage duration (due to week-ends) and abuse of refrigeration temperature was observed and could lead to a significant microbial development. A modeling approach, based on predictive microbiology and probabilistic simulations, was proposed for a simple quantitative assessment of the quality of the chill-chain, and the associated microbial risks. This preliminary approach should then be included in a whole microbial risk assessment.

REFERENCES

Augustin, J.C.,Carlier, V., 2000. Modelling the growth rate of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. Int. J. Food Microbiol. 56, 53-70.

Aureli, P., Fiorucci, G.C., Caroli, D., Marchiaro, G., Novara, O., Leone, L., Salmaso, S., 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. N. Engl. J. Med. 342, 1236-1241.

Berrang, M.E., Brackett, R.E., Beuchat, L.R. ,1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. 52,702-705.

Beuchat, L.R., Brackett, R.E., 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55, 755-758, 870.

Carlin, F., Nguyen-The, C., 1994. Fate of *Listeria monocytogenes* on four types of minimally processed green salads. Lett. Appl. Microbiol. 18, 222-226.

Carlin, F., Nguyen-The, C., Morris, C.E., 1996. Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Chichorium endivia var. latifolia*). J. Food Prot. 59, 698-703.

Daud H.B., McMeekin, T.A., Olley J., 1978. Temperature function integration and the development and metabolism of poultry spoilage bacteria. Appl. Environ. Microbiol. 36, 650-654.Delaquis, S., Stewart, S.,

Delaquis, S., Stewart, S., Cazaux, S.Toivonen, P., 2002. Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. J. Food Prot. 65, 459-464.

Dickson, J.S., Siragusa, G.R., Wray, J.E., 1992. Predicting the growth of *Salmonella typhimurium* on beef by using the temperature function integration technique. Appl. Environ. Microbiol. 58, 3482-3487.

Erickson, J.P., McKenna, D.N., Woodruff, M. A.Bloom, J.S., 1993. Fate of *Salmonella* spp., *Listeria monocytogenes*, and indigenous spoilage microorganisms in home-style salads prepared with commercial real mayonnaise or reduced calorie mayonnaise dressings. J. Food Prot. 56, 1015-1021.

Farber, J.M., Wang, S.L., Cai, Y., Zhang, S., 1998. Changes in populations of *Listeria monocytogenes* inoculated on packaged fresh-cut vegetables. J. Food Prot. 61, 192-195.

Farber, J.M., Daley, E.M., MacKie, M.T., Limerick, B., 2000. A small outbreak of listeriosis potentially linked to the consumption of imitation crab meat. Lett. Appl. Microbiol. 31, 100-104.

Fazil, A..M., Ross, T., Paoli, G., Vanderlinde, P., Desmarchelier, P., Lammerding, A.M., 2002. A probabilistic analysis of *Clostridium perfringens* growth during food service operations. Int. J. Food Microbiol. 73, 315-329.

Gallay, A., 2002. Toxines microbiennes dans les toxi-infections alimentaires collectives déclarées en France. Congress of the French Society of Microbiology. November 2002, Paris.

Giannakourou, M.C., Koutsoumanis, K., Nychas, G.J., Taoukis, P.S., 2001. Development and assessment of an intelligent shelf life decision system for quality optimization of the food chill chain. J. Food Prot. 64, 1051-1057.

Gill, C.O., Jones, S.D.M., Tong, A.K.W, 1991. Application of a temperature function integration technique to assess the hygienic adequacy of a process for spray chilling beef carcasses. J. Food Prot. 54, 731-736

Glass, K.A., Doyle, M.P., 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol., 55, 1565-1569.

Lee, W.C., Lee, M.J., Kim, J.S., Park, S.Y., 2001. Foodborne illness outbreaks in Korea and Japan studied retrospectively. J. Food Prot. 64, 899-902.

McKellar, R.C., Moir, R., Kalab, M., 1994. Factors influencing the survival and growth of *Listeria monocytogenes* on the surface of Canadian retail wieners. J. Food Prot. 57, 387-392.

Michino, H., Otsuki, K., 2000. Risk factors in causing outbreaks of food-borne illness originating in schoollunch facilities in Japan. J. Vet. Med. Sci. 62, 557-560.

Nauta, M., Litman, S., Barker, G.C., Carlin, F., in press. A retail and consumer phase model for exposure assessment of *Bacillus cereus*. Int. J. Food Microbiol. in press.

Nguyen The, C., Lund, B.M., 1991. The lethal effect of carrot in *Listeria* species. J. Appl. Bacteriol. 70, 479-488.

Nguyen The, C., Halna du Fretay, B., Abreu da Silva, A., 1996. The microbiology of mixed salad containing raw and cooked ingredients without dressing. Int. J. Food Sci. Technol. 31, 481-487.

Olley, J, Ratkowsky, D.A. 1973. The role of temperature function integration in monitoring of fish spoilage. Food Technol. NZ. 8, 13-17.

Papageorgiou, D.K., Marth, E.H. 1989. Fate of *Listeria monocytogenes* during the manufacture and ripening of feta cheese. J. Food Prot. 52, 82-87.

Pingulkar, K., Kamat, A.Bongirwar, D., 2001. Microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads: an evidence of inhibition of *Listeria monocytogenes* in tomatoes. Int J. Food Sci. Nutr. 52, 15-23.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154, 1222-1226.

Rosenow, E.M., Marth, E.H., 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8,13, 21 and 35°C. J. Food Prot. 50:452-459.

Sizmur, K., Walker C. W., 1988. *Listeria* in prepacked salads. The Lancet 331, 1167.

Snyder, O.P., 1998. Updated guidelines for use of time and temperature specifications for holding and storing food in retail food operations. Dairy Food Environ. Sanit. 18, 574-579.

Stecchini, M.L., Aquili, V., Sarais, I., 1995. Behavior of *Listeria monocytogenes* in mozzarella cheese in the presence of *Lactococcus lactis*. Int. J. Food Microbiol. 25, 301-310.

Steinbruegge, E.G., Maxcy, R.B., Liewen, M.B., 1988. Fate of *Listeria monocytogenes* on ready to serve lettuce. J. Food Prot. 51, 596-599.

Thomas, C., Prior, O., O'Beirne, D., 1999. Survival and growth of *Listeria* species in a model ready-to-use vegetable product containing raw and cooked ingredients as affected by storage temperature and acidification. Int. J. Food Sci. Technol. 34, 317-324.

Thomas, C., O'Beirne, D., 2000. Evaluation of the impact of short-term temperature abuse on the microbiology and shelf life of a model ready-to-use vegetable combination product. Int. J. Food Microbiol. 59, 47-57.
van Gerwen, S.J., Zwietering, M.H., 1998. Growth and inactivation models to be used in quantitative risk assessments. J. Food Prot. 61, 1541-1549.

Vose, D., 2000. Risk Analysis, a quantitative guide, 2nd ed. Wiley and Sons, Chichester, pp. 418.
Wederquist, H.J., Sofos, J.N., Schmidt, G.R., 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packed turkey bologna by chemical additives. J. Food Sci. 59, 498-500, 516.

Zwietering, M.H., de Wit, J.C., Notermans, S., 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. Int. J. Food Microbiol. 30, 55-70.

Figure 1: Example of a typical time-temperature profile, with four successive periods. a: preparation and packaging in the centralized kitchen; b: refrigerated storage in the centralized kitchen; c: transfer to school canteen; d: refrigerated storage in the school canteen
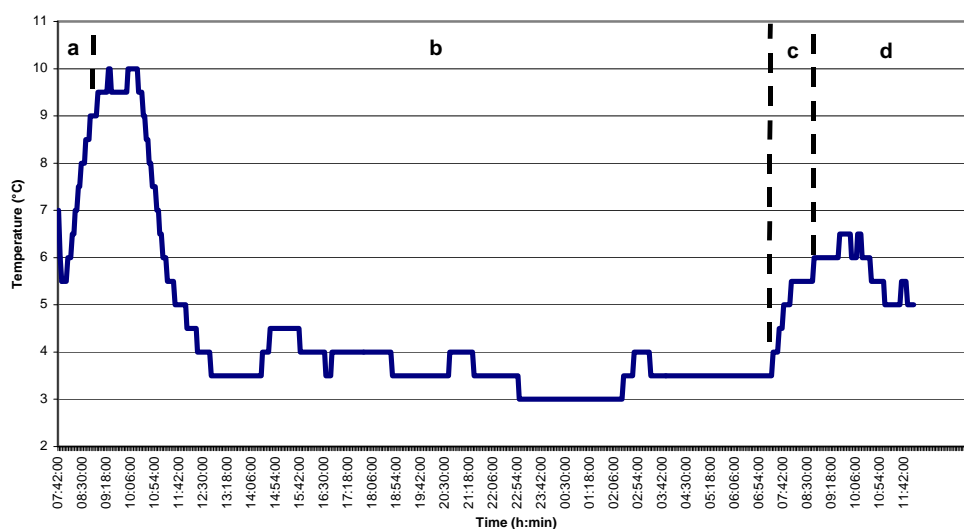

Figure 2: Empirical distribution of 287 time-temperature equivalents (day.$°C^2$). Reading key : 27% of time-temperature profiles are characterized by a TTE between 75 and 100 day.$°C^2$ and would allow a potential growth of *Listeria moncocytogenes* equivalent to 40-53 hours at 4°C, 16-21 hours at 8°C or 8-11 hours at 12°C.
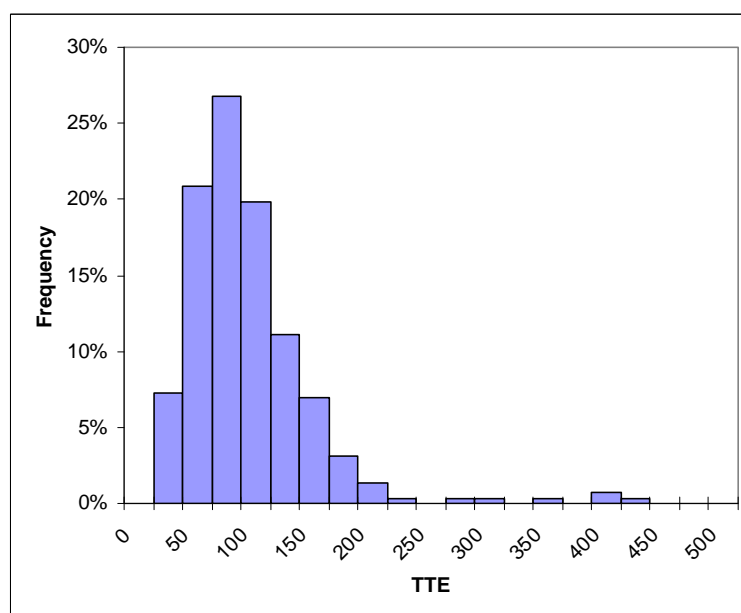
Figure 3: Simulated distribution of 287x16=4592 predicted growth increments. Reading key : 20% of combinations are characterized by a growth increment comprised between 0.69 and 1.38 ln (UFC/g), and would allow a potential growth of *Listeria moncocytogenes* between one and two doublings.
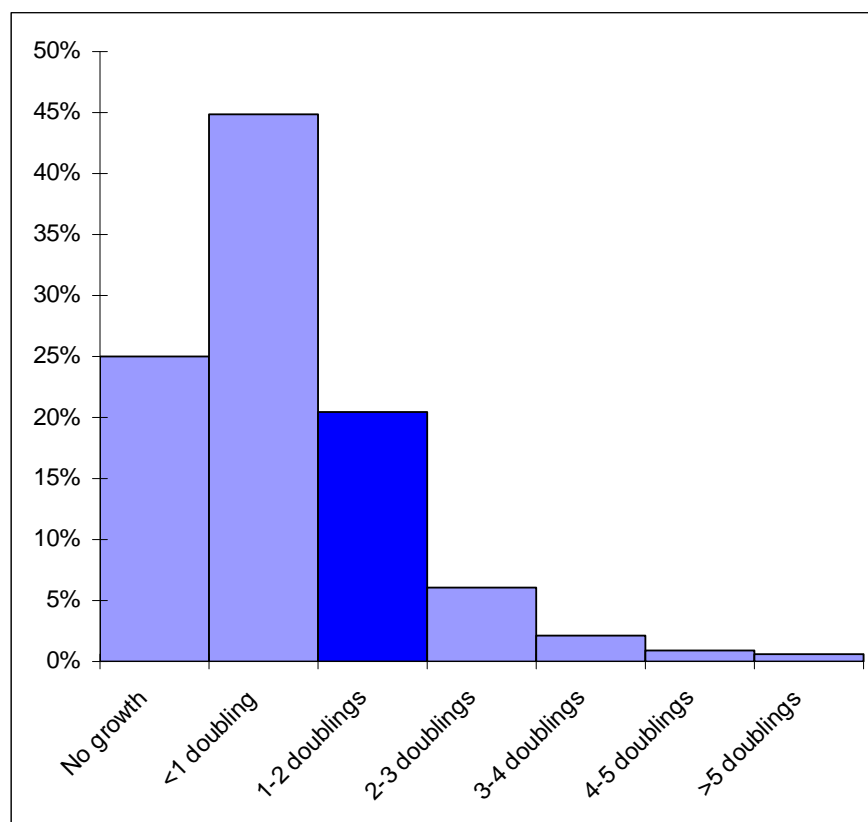
Table 1: Centralized kitchens repartition

	All types of chain	Chill chain
Number of districts of the studied area	123	
Number of centralized school kitchen	77	36 (47%)
Number of centralized school kitchen making during the school period:		
< 3000 meals per day	46	15 (33%)
3000 < n < 5000 meals per day	21	12 (57%)
> 5000 meal per day	10	9 (90%)
Number of public centralized school kitchen	53	16 (30%)
Number of private centralized school kitchen	24	18 (75%)
Number of school restaurants per centralized kitchen:		
< 20	46	15 (33%)
20 < n < 50	27	17 (63%)
> 50	4	4 (100%)

Table 2 : TTE and reference time-temperature couples for L. monocytogenes (Tmin=-2.7°C)

TTE (day.°C^2)	Time at 4°C (h)	Time at 8°C (h)	Time at 12°C (h)
50	27	10	6
75	40	16	8
100	53	21	11
200	107	42	22
300	160	63	33
500	267	105	56

Reading key: it is assumed that the potential growth of *Listeria monocytogenes* (or any microbial species with a minimal temperature of -2.7°C) in any food product would be equivalent during 107 hours (4.5 days) at 4°C, 42 hours at 8°C, 22 hours at 4°C or any time-temperature profile with a TTE of 200.

Table 3 : Average, minimal, and maximal TTE per kitchen (a) or per canteen (b)

a)

TTE (day.°C^2)	CENTRALIZED KITCHEN					
	A	B	C	D	E	Total
Average	127	89	130	104	78	105
Max	374	205	440	162	222	440
Mini	60	40	51	27	36	27

b)

TTE (day.°C^2)	SCHOOL CANTEEN										
	A1	A2	B3	B4	C5	C6	D7	D8	D9	E10	E11
Average	141	112	86	92	108	150	100	97	108	64	92
Max	374	172	174	205	295	440	156	131	162	118	222
Mini	78	60	53	40	55	51	27	39	43	36	45

Table 4: Reference growth curves of *L. monocytogenes* in different ready-to-eat food products

Food product	Source	T (°C)	n[*]	b^2
Chicken salad	Erickson *et al.*, 1993	4-13	4	0.018
Canned sweet corn	Thomas and O'Beirne, 2001; Thomas *et al.*, 1999; N'Guyen-The *et al.*, 1999, Carlin *et al.*, 2001	3-12	12	0.015
Cream[**]	Rosenow and Marth, 1987	4-8	2	0.010
Frankfurters	Glass and Doyle, 1989; McKellar *et al.*, 1994, Wederquist *et al.*, 1994	4-5	3	0.007
Mozarella	Stecchini *et al.*, 1995	5	1	0.007
Coleslaw	Farber *et al.*, 1998	10	1	0.007
Caesar salad	Farber *et al.*, 1998	10	1	0.006
Cauliflower	Berrang *et al.*, 1989	15	1	0.006
Mixed salad	Sizmur and Waker, 1988	4	1	0.004
Endive (broad-, curly-, and/or red-leaved)	Carlin *et al.*, 1996; Carlin and N'Guyen-The, 1994, N'Guyen-The *et al.*, 1996	3-10	6	0.003
Raw bean sprouts	Thomas and O'Beirne, 2001	3-12	7	0.002
Cut lettuce	Steinbrugge *et al.*, 1988; Beuchat and Bracket, 1990; Carlin and n'Guyen-The, 1994, Thomas *et al.*, 1999 ; Delaquis *et al.*, 2002	5-12	7	0.002
Feta	Papageorgiou and Marth	4	No growth	0[***]
Pasta salad	Erickson *et al.*, 1993	4-13	No growth	0
Carrots	Nguyen The and Lund, 1991	8	No growth	0
Tomatoes	Pingulkar *et al.*, 2001	8-10	No growth	0

[*] n is the number of growth curves published for each food product. If n>1, b^2 was estimated for each growth curve, and the median was selected.
[**] Cream is used as a dressing for cucumber salad.
[***] A null value of b^2 indicates either no-growth (survival) or inactivation.