ELSEVIER

Thermochimica Acta 370 (2001) 73–81

thermochimica acta

www.elsevier.com/locate/tca

Isothermal calorimetry approach to evaluate shelf life of foods

Marco Riva, Dimitrios Fessas, Alberto Schiraldi*

DISTAM, University of Milan, Via Celoria 2, 20133 Milano, Italy

Received 30 July 2000; accepted 8 December 2000

Abstract

Isothermal calorimetry (IC) traces were obtained at three temperatures for industrial whole-eggs, fresh milk and carrot convenience-salads to assess their durability when stored at various temperatures. According to the nature of the degradation process (microbial, metabolic (aerobic, or anaerobic), enzymatic), the order of magnitude of the exothermic signal recorded changed. The present work mainly aimed at determining the onset time of the calorimetric signal which was related to the stability (i.e. safe shelf life) of the food investigated. The higher the storage temperature, the earlier was the onset of the calorimetric signal: the temperature effect on the stability time could be, therefore, determined. This piece of information was used to choose time–temperature–integrators suitable for the products considered.

Stability times for the three products were also evaluated with other approaches (microbial plate counts, pH variation, development of turbidity). The comparison between the results of these traditional techniques and the calorimetric monitoring supported the reliability of the latter, which offers some peculiar advantages, like better temperature control, continuous follow up, easier mathematical description, overall energy balance of the degradation process.

Keywords: Food shelf life; Microbial growth; Isothermal calorimetry

1. Introduction

Foods are thermodynamically metastable systems, as long as their properties change over time depending on the intrinsic nature (chemical composition, endogenous enzymatic endowment, etc.) of the system and the storage conditions (temperature, relative humidity, available oxygen, etc.). At given storage conditions, one can define the shelf life of a food product as the maximum time span across which the progress of any reaction produce changes that escape sensory perception and are not safety threatening. The quality of stored foods changes mainly as a consequence of chemical reactions [1], whereas endo- or exo-microorganisms and enzymatic activity are the main responsible for the quality decrease of shelved fresh or fresh-like foods.

The shelf life span can be predicted either controlling the driving agents (growth of microbial populations, enzymatic activities, concentration of reactive compounds) or monitoring their effects, like changes in pH, aroma, texture, nutritional value, and presence of peculiar compounds, mainly in the early stages [2]. Although the analytical methods generally used to detect quality changes can nowadays be well refined, they only provide indirect information about the transformation mechanisms and may be related to effects (e.g. acidification sustained by the microbial growth) which are delayed with respect to the main

* Corresponding author. Tel: +39-2-70602063; fax: +39-2-70638625.
E-mail address: alberto.schiraldi@unimi.it (A. Schiraldi).

0040-6031/01/$ – see front matter
PII: S0040-6031(00)00782-6

degradation process. When so, the kinetic parameterization of the food quality decline can be misleading [3].

In this work, isothermal calorimetry (IC) is proposed as an appropriate technique to directly and continuously monitor the kinetics of microbial growth or enzymatic activity in fresh-like foods, namely, industrial whole-egg, fresh milk and convenience carrots, at well controlled temperatures, as the degradation rate sharply increases following any temperature upraise during the storage of fresh products [4].

Pasteurized milk is the most diffused fresh food and its shelf life (4–7 days in the cold chain) is limited by the growth of mesophilic and psycrotrophic bacteria which are resistant to the pasteurization treatment. The main sensory property acquired because of this spoilage is the acidification of the food. Pasteurized whole-egg is a novel product, proposed as a substitute of fresh eggs for artisan or home pastry use, which maintains its freshness for 2–3 week when stored in cold. This product is exposed to an easy contamination: the pasteurization treatment allows destruction of pathogenic bacteria and preservation of some functional properties like the foaming attitude. Packaged fresh carrots are an example of a successfully ready-to-use vegetable: their preparation includes washing, cutting and packaging in plastic films which allow the natural respiration of the food. During the storage (maximum shelf life 5–7 days) they undergo the effects of microbial spoilage and related enzymatic degradation which induce tissue shrinkage and color change.

The calorimetric results are correlated with those of microbiological and analytical observations traditionally used for these products, like microbial counts, changes of pH, color and/or texture, which were performed in batch samples with a classical shelf life test at three different temperatures.

2. Materials and methods

Pasteurized whole milk and pasteurized whole-egg were packaged in 1 l carton boxes. Fresh carrots (cut "*à la julienne*") were packaged in PP trays wrapped in cling PVC film with high gas permeability. All the products were sampled after packaging at the production site, stored in a cold bag (4–6°C) and rapidly (less than 1 h) transferred to our laboratory: the arrival time was considered the zero time for all the kinetic evaluations.

Isothermal calorimetry (IC) was performed with a differential flux calorimeter SETARAM C80 hosting 10 ml cells. Five grams of each product were sampled just at the arrival of the relevant lot in our laboratory and sealed in a previously sterilized calorimetric cell. The reference cell was filled with inert material. Isothermal traces were recorded at 10, 15 and 20°C for whole-egg and at 15, 20 and 25°C for the other two products: the run time ranged between 1 and 10 days. Two or three replicas were performed for each experiment.

The raw trace was analyzed with a specific software (PEAKFIT, Jandel Sci., Germany) in order to smooth electronic noise, define the baseline and express data ($mW\,g^{-1}$) versus days units [5]. This treatment allowed the recognition of an early stability phase followed by a large release of heat, related to the metabolic activity, which attained a maximum value when the growth rate was the highest. The same software was also used for the kinetic parameterization.

Classical shelf life studies were performed in batch, by storing the fresh original products in thermostatic cells at constant and controlled temperatures (5, 10, 15, 20 and 25 ± 1°C, according to the specific product). For each observation, three replicas were considered. The following parameters were considered for each single product:

- Milk: microbial counts (total bacterial count-TBC-using plate count agar [6]), pH (with digital pH meter Gibertini);
- Whole-egg: microbial counts [6], pH;
- Carrots: microbial counts [6], turbidity (aqueous suspension of a 16.66% (w/w) carrot sample sieved through a 100 mm nylon mesh), the results of turbidity measurements were expressed in NTU units, by the Ratio Turbidimeter HACT mod. 18900/00 [7]).

3. Results and discussion

The main scope of this research was the assessment of a reliable stability time on the basis of some

experimental evidences. The principle chosen was that the rates of the microbial and enzymatic degradation had to remain of the same order of magnitude as at the zero time of the shelf life. It was found that this condition was no longer met when the microbial growth or the enzymatic degradation attained a maximum acceleration, since beyond such a threshold the system underwent very fast changes and quickly lose the generally accepted safety or quality requirements. The formal definition of this stability time was, therefore, related to the kind of the quantity measured, namely, plate count, heat flux, metabolite concentration. This principle seemed more reliable than the current practice that defines food stability according to the ratio between attained and starting microbial population levels.

The calorimetric traces obtained for the three products at various temperatures were reported in Fig. 1. Previous investigations dealing with model microbial systems [8–10] allowed interpretation of the traces observed in this work, these should be related to the thermal effects of microbial growth and metabolism, the amplitude of the peak signal and the following plateau being usually matched with the maximum growth rate and the metabolic activity, respectively.

The trace of pasteurized whole-egg (Fig. 1A) showed a main peak followed by a plateau trend which was well above the starting base line and could be attributed to the metabolic activity of the microbial population [8]. In the case of fresh milk (Fig. 1B), at the highest temperature considered, no plateau followed the main peak, which would likely imply an abrupt decline of any metabolic activity. A similar behavior was observed for fresh carrots (Fig. 1C), where a splitting of the calorimetric signal was also apparent.

The shoulders observed in the calorimetric traces of pasteurized eggs and milk and the split of the calorimetric signal in the trace relevant to fresh carrots could be explained taking into account that microbial spoilage in fresh foods can be sustained by organisms of different species with different metabolism rates [11], although lactic bacteria were the main species producing the spoilage in all the three products.

For every product considered, higher storage temperatures produced earlier development of the microbial population which corresponded to a sharper and narrower peak in the calorimetric trace. With decreasing temperature this signal was broadening with reduction of its amplitude, as expected for a reduced and slower expansion of the microbial population.

These considerations were left at this qualitatively stage and no detailed investigations were undertaken to achieve a quantitative interpretation of the calorimetric traces, since the scope of the present work was the definition of a reliable system stability which did not deserve further interpretation details.

The stability time was chosen as the point where the time-derivative of the calorimetric trace attained its maximum, namely when the signal underwent a upward flexus (Fig. 2). This condition was easily identifiable and corresponded to the early sprout of the microbial population, just after the relevant lag-phase. Coupling the calorimetric trace with the relevant plate count (at the same temperature) allowed us to recognize that the signal was strictly related to the increase of the microbial population [8]: accordingly, the stability time assessed as above could be considered as a microbial safety parameter.

The stability times evaluated according to the different experimental approaches considered in this work were reported in Table 1.

Plate count data (see Fig. 3 for milk) were fitted, as in previous works [8], with a modified Gompertz function [12–14] which reproduced the sigmoid trend of the experimental data (Colony Forming Units, CFU)

$$N = N_0 \exp\{a \exp[-\exp(b - ct)]\} \quad (1)$$

The conventional parameters, namely, microbial population, N, lag-phase, $\lambda = (b - 1/c)$, maximum growth rate, μ, and plateau level, $N_{\max} = N_0 \exp(a)$ (where N_0 is the starting microbial population level), were accordingly determined. Table 2 reports the Gompertz parameters a, b and c, the maximum growth rate (μ) and the starting microbial population (No) for the three products at $T = 20°C$.

The stability time based on these data was related to the maximum of the second time-derivative of the population level, d^2N/dt^2, namely to the maximum acceleration of the microbial growth (see Table 1). To match this choice with the one used for the calorimetric traces, one has to remind that the calorimetric signal corresponds to a heat flux, which is proportional to dN/dt.

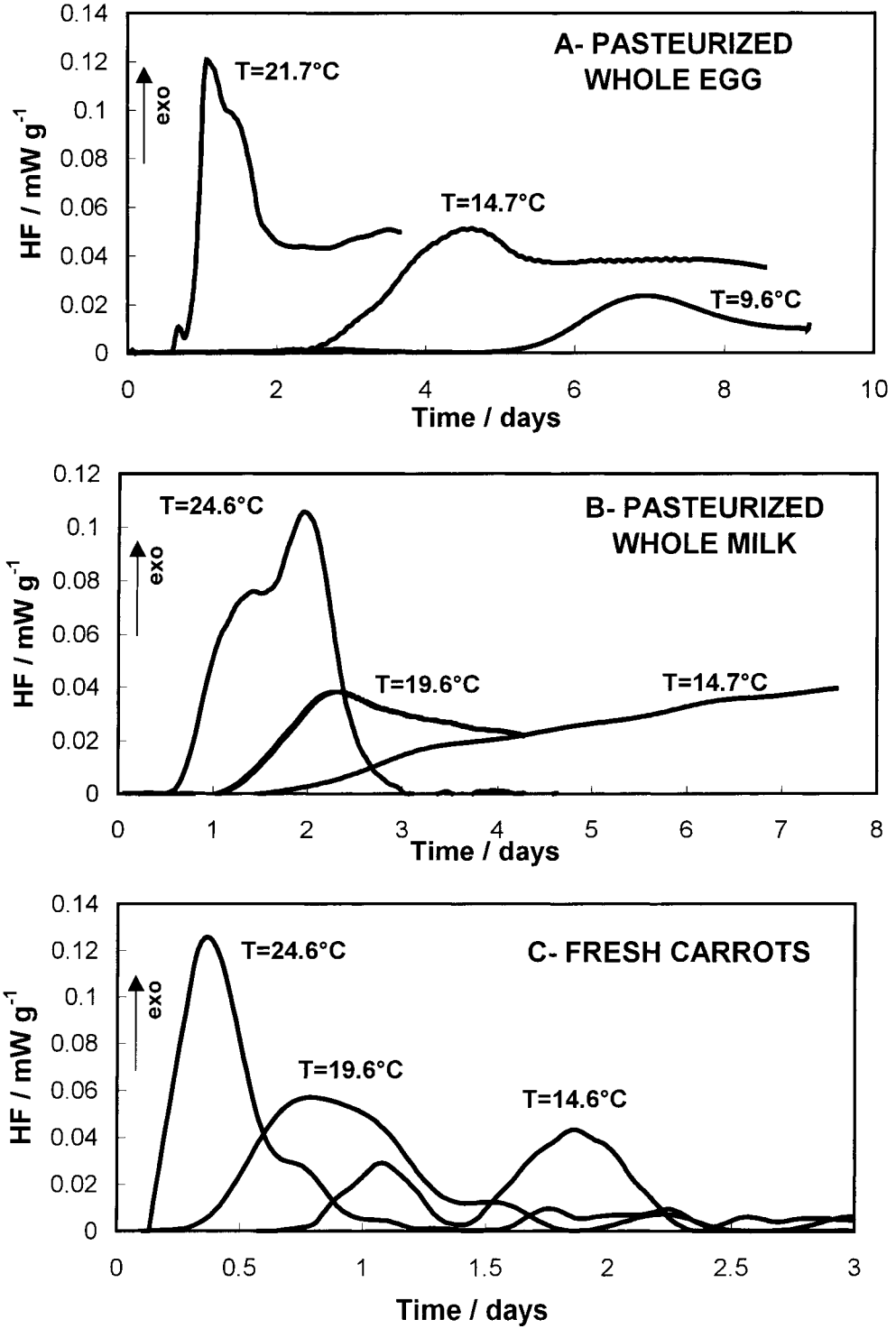

Fig. 1. Isothermal DSC traces at different temperatures.

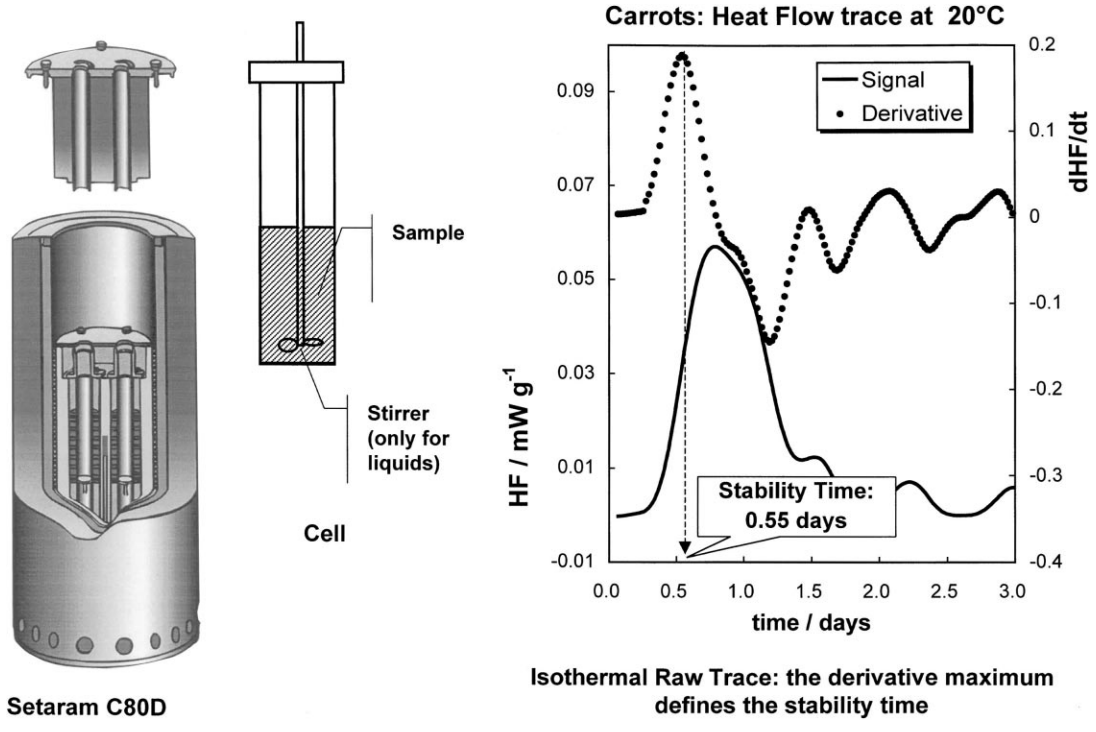

Fig. 2. Instrumental apparatus used in the present work and typical trace of an isothermal calorimetric investigation.

Fig. 4 shows the results of pH variation during storage at different temperatures of the milk. Similar trend were observed for whole-egg. A sigmoid transition function was used to fit the pH decrease, produced by the microbial metabolic activity (i.e. organic acid or CO_2 production) in these products. In the case of carrots, turbidity was considered as an index of metabolic activity. This index increases during storage as a consequence of tissues degradation sustained by the enzymatic activity of microorganisms. Also in this

Table 1
Stability time vs. storage temperatures

Product index	Stability time (days) at different temperatures				
	5°C	10°C	15°C	20°C	25°C
Milk					
pH	–	–	4.65 (±0.51)	2.88 (±0.21)	1.42 (±0.14)
Plate Count	–	–	2.96 (±0.47)	1.07 (±0.34)	0.55 (±0.12)
Calorimetry	–	–	2.74	1.59	0.85
Eggs					
pH	31.7 (±0.55)	5.75 (±0.55)	2.74 (±0.25)	1.24 (±0.12)	–
Plate count	⩾40	8.32 (±0.85)	3.10 (±0.52)	1.73 (±0.28)	–
Calorimetry	–	6.11	2.98	0.95	–
Carrots					
Turbidity	6.39 (±0.75)	4.0 (±0.37)	–	0.96 (±0.16)	0.30 (±0.11)
Plate Count	4.25 (±0.49)	3.17 (±0.29)	–	0.48 (±0.11)	–
Calorimetry	–	–	1.61	0.55	0.15

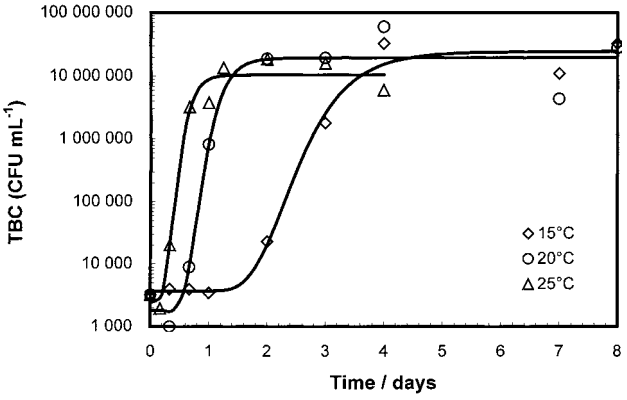

Fig. 3. TBC trend in milk stored at different temperatures. Fitting curves correspond to the relevant Gompertz functions.

Table 2
Gompertz coefficients (a, b, c, see Eq. (1)), maximum microbial growth rate (μ), and starting microbial population (N_0)

Product	a	b	c	μ (CFU per g per day)	N_0 (CFU per gram)
Eggs	5.91	2.39	2.31	1.31×10^8	2×10^2
Milk	4.03	3.46	4.34	2.94×10^7	1.8×10^3
Carrots	1.73	2.25	5.34	4.65×10^9	5×10^7

case, transition functions were used to fit the experimental data.

The stability times related to pH and turbidity were singled out at the minimum and maximum, respectively, of the second time-derivative of the relevant transition function (see Table 1), since both are proportional to the microbial population level, N.

Fig. 5 shows the case of carrots stored at 20°C: the turbidity changes took place with some delay after the attainment of the calorimetric maximum and plate count plateau level. The delay could be explained taking into account that the enzymatic activity underlying a reliably detectable tissue transformations has to be sustained by a more developed microbial population. Analogous behavior was observed for milk, where the pH trend was delayed with respect to the maximum calorimetric signal and attainment of TBC plateau level.

In the case of eggs, the mismatch between the calorimetric and the other two stability times was

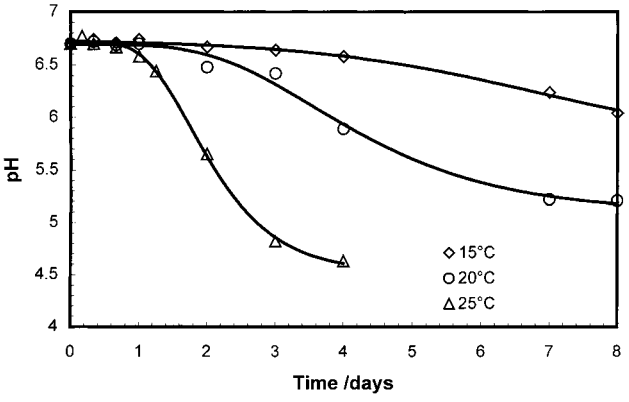

Fig. 4. pH trend in milk stored at different temperatures. Fitting curves correspond to sigmoid functions.

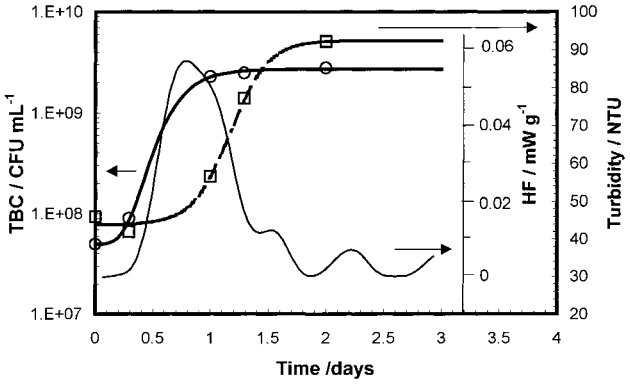

Fig. 5. Kinetics indices (heat flow, turbidity and Plate count) for packaged fresh carrots stored at 20°C.

attributed to some process occurring when the microbial growth is still very slow. It was indeed observed that pH trend and thermal effect indicate close stability time values, which were smaller than the one drawn from plate counts. Parallel investigations on foam stability [15] supported this finding, as long as a decline of foam stability was observed before the attainment of the microbial growth maximum. As a tentative explanation, some enzymatic reactions might be supposed to be still active at the zero time of the shelf life in spite of the previous pasteurization treatment.

Fig. 6 shows the dependence of stability time from temperature in the case of milk: the error bars are related to the three replicas. Taking into account the error range, calorimetric and plate count stability times were practically coincident, both depending on temperature according to a pseudo-exponential trend, which was observed also for the other two products. To this respect, the calorimetry-based stability times allowed evaluation of the following Q_{10} (increase of the reaction rate for a 10°C temperature increase) in per day units: 4.7 (eggs), 3.2 (milk), and 7.3 (carrots).

These data were turned into a time–temperature–tolerance (TTT) chart (Fig. 7), which is the best practical picture of the shelf life of a given product.

In the last few years the use of devices for monitoring thermal history (TTI: time–temperature–integrators) in the form of labels applied to the wrapping has been proposed both for research and application [16]. They are structured such in a way that they can show a chromatic variation proportional to time–temperature exposure. These devices, programmed according to

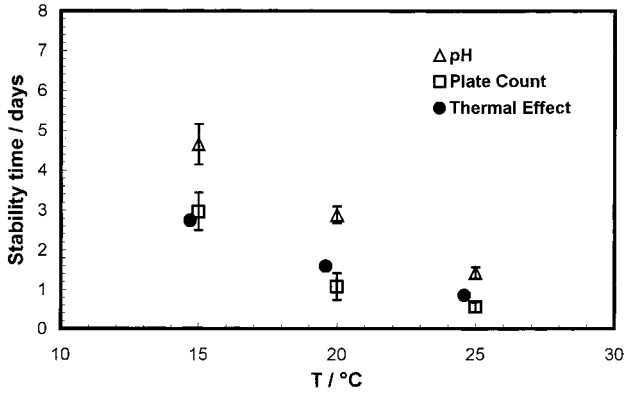

Fig. 6. Stability time vs. temperature for milk samples according to pH, Plate count and calorimetric determinations.

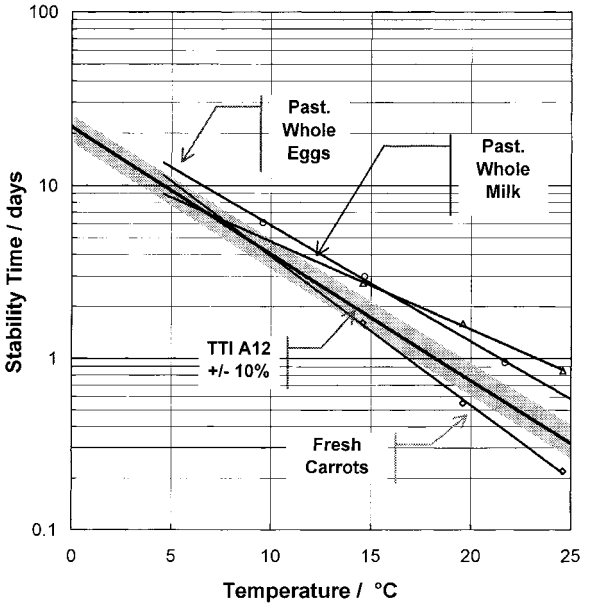

Fig. 7. Time–temperature–tolerance (TTT) plot of stability time according to IC for pasteurized whole-eggs, pasteurized whole milk and packaged fresh carrots. The response of TTI Lifelines A12 device is represented by the shaded region.

the kinetics of the loss of a quality index (that is the loss of food freshness), can be used to predict the shelf life of a product and to give final information to consumers.

Fig. 7 includes a shaded region related to TTT response of a TTI (Lifelines A12) suitable for the products considered in the present work.

This indicator has a time tolerance of 9 days at 5°C and a $Q_{10} = 5.4$; this justifies that the indicator tends to give an overestimate of the degradation at higher temperatures: a behavior that allows a larger confidence in extreme storage conditions.

4. Conclusions

The present data show that the IC allows a reliable control of the shelf-life of three "commercially fresh" products. Because of the general character of this technique, it can be safely assumed that it might be valid for similar products undergoing enzymatic or microbial degradation. A main limitation of its practical application comes from the necessary non-easy-to-use equipment. On the other hand, calorimetry can confirm and support the response of other easy-to-use approaches, like microbial counts and change of physical properties, which are often invasive techniques that cannot be easily applied for a continuous monitoring of the product.

It must be stressed that the present work concerns a trivial use of calorimetry, which is a technique already proven suitable for much more detailed studies of food properties, not only in the field of the microbial spoilage but also in that of the molecular characterization of changes occurring in food processing and storage [5,8,9,17]. Nevertheless, the great importance of controlling the food shelf life by use of a technique that allows predictive studies justifies an oversimplified calorimetric approach, such as that used in the present work. It is, therefore, desirable that this technique may be spread in all the laboratories dealing with food quality assurance and become familiar for a larger number of food technologists.

As a future development of this calorimetry application could come from the design and production of simpler instruments, that should have an adequate sensitivity and allow the simultaneous control of various parameters in large mass samples, so as to study other preparation and storage procedures of foods, like use of inert atmospheres, various types of packaging, controlled moisture content, etc.

References

[1] R.P. Singh, Scientific principles of shelf life evaluation. In: C.M.D. Man, A.A. Jones (Eds.), Shelf Life Evaluation of Foods, Blackie, London, 1994.
[2] M. Jul, Actual shelf-life calculations. In: M. Jul (Ed.), The Quality of Frozen Foods, Academic Press, London, 1984.
[3] M. Riva, Shelf life and temperature prediction in food commercial chain with TTI devices, in: Proceedings of the 2nd Workshop on the Developments in the Italian Ph.D. in Food Biotechnology, Bertinoro (Fo), 29 September 1997, 1997, p. 51.
[4] I. Saguy, M. Karel, Food Technol. 34 (2) (1980) 78.
[5] M. Riva, A. Schiraldi, Thermochim. Acta 220 (1993) 117.
[6] C. Vanderzant, D.F. Splittsloesser (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 3rd Edition, APHA, Washington, USA, 1992.
[7] S. Odolini, Studio e verifica della shelf life di ortaggi freschi pronti per il consumo, Tesi di Laurea in Scienze e Tecnologie Alimentari, Università degli Studi di Milano, 1999.
[8] M. Riva, L. Franzetti, A. Galli, A. Schiraldi, Ann. Microbiol. Enzimol. 47 (1997) 199.

[9] M. Riva, D. Fessas, L. Franzetti, A. Schiraldi, J. Thermal Anal. 52 (1998) 753.
[10] L. Franzetti, A. Galli, A. Perazzoli, M. Riva, Ann. Microbiol. Enzimol. 45 (1995) 291.
[11] F. Nguyen, J.P. Carlin, Cr. Rev. Food Sci. Nutr. 34 (4) (1994) 371.
[12] N. Bratchell, A.M. Gibson, N. Truman, T.M. Kelly, T.A. Roberts, Int. J. Food Microbiol. 8 (1989) 47.
[13] A.M. Gibson, N. Bratchell, T.A. Roberts, Int. J. Food Microbiol. 6 (1988) 155.
[14] M.H. Zwietering, B.E. De Koos, J.C. Hasenack, De Witt, K. Van't Riet, Appl. Environ. Microbiol. 57 (1991) 1094.
[15] M. Riva, D. Fessas, Evaluation of an egg product shelf life with calorimetric and classical techniques, in: Proceedings of the VIII European Symposium on the Quality of Eggs and Egg Product, Bologna, 19–23 September 1999, p. 521.
[16] J.D. Selman, Time–temperature indicators, in: M.L. Rooney, CSIRO (Eds.), Active food packaging, Blackie, London, 1995, Chapter 10, p. 215.
[17] A. Schiraldi, L. Piazza, M. Riva, Cereal Chem. 73 (1996) 32.