Predicting lactic acid bacteria population in a functional yoghurt

Elena Hadjimbei, George Botsaris, and Vassilis Gekas

***Abstract*—** Lactic Acid Bacteria (LAB) are technologically necessary to manufacture yoghurt. Functional yoghurts contain not only *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, but also probiotic LAB strains which are associated with certain human health benefits. Therefore, total LAB population is an essential quality criterion, as their viability should be retained at the highest level possible, throughout the commercial life of the yoghurt. The aim of the present study was the modeling of the survival of LAB in a functional goats' milk yoghurt, enriched with *Pistacia* resin extracts and *Saccharomyces boulardii*. Novel modeling approaches were adopted, by the authors, which coined the concept of "thermal action" (Temperature x logarithm of Time). The modeling results provided a very promising approach not only in death kinetics but also in the case of moderate and low microbial population reductions as in the case of LAB survival in functional yoghurts. A modification of Bayes approach was also applied, which could provide an alternative way of modeling LAB growth and survival.

***Keywords*—**Thermal Action, Bayes approach, LAB bacteria, functional yoghurt

I. Introduction

THERE are different ways of coping with microorganisms, as there are different categories of microorganisms according to their impact on our health. Foodborne pathogens pose a significant health hazard and should be eliminated or reduced to acceptable levels, by the application of food preservation methods. In the thermal processing methods this implies the exposure to high temperatures for a specific time, depending on the kind of the microorganism and the population reduction degree is expressed logarithmically. Temperature affects the population linearly and Time affects logarithmically. Death kinetics is typically viewed in diagrams with Temperature in the X-axis whereas the logarithm of time is presented in the Y-axis.

In a previous communication we provided an alternative way through the use of the, by us coined, concept of Thermal Action, defined as the product of Temperature times the logarithm of Time [1]. In this methodology the microbial population is plotted vs. thermal action. It is understood that temperature-time combinations which produce same values of thermal action, iso-drastic values (from the Greek word δράσις, meaning action), result in same microbial population values. Examples of such diagrams are given later in the text (see Fig.1).

Beneficial (probiotic) microorganisms which are associated with documented health benefits, should be able to survive or at least retain high values, in functional food products. The functional food market is constantly growing in the developed world and yoghurts are rapidly gaining space as they provide ideal vehicles to deliver bioactive nutrients to humans [2]. The majority of yoghurts are characterized by the limited viability of probiotics towards the end of their shelf-life [3, 4]. The aim of this work was to predict LAB population in a functional goats' milk yoghurt. The yoghurt was developed in our Laboratory of Food Science and Technology at the Cyprus University of Technology, and it contained both LAB and *Saccharomyces boulardii*; a probiotic yeast. The yoghurt was manufactured from goats' milk. The yoghurt underwent different treatments, using *Pistacia atlantica* resin extracts and/or *Saccharomyces boulardii* aiming at the enhancement of its functional properties. One of the most important quality parameters is the survival of the LAB. It is a system, by far more complicated than simple thermal death of harmful bacteria but we wanted to see how our Thermal Action modeling approach could be applied in such complicate systems.

Furthermore we attempted a Bayes approach for assessing the effect of the various treatments on the initial expectation of quality preservation of the product.

II. THEORY

A. Thermal Action

The rationale for the definition of the quantity Thermal Action, is based on the fact that Time, t, and temperature, Θ are main factors influencing many phenomena in Food Technology and Preservation, upon thermal treatment. Temperature is dimensionally the concentration of heat that is of the thermal energy, under constant pressure, according to the dimensional analysis equation (1)

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$$[Q] = [\rho][C\rho][\Theta] \quad (1)$$

Where Q: being the heat; ρ: the product of the density; Θ: Temperature; Cp: is the specific heat capacity

Since the product ρ times Cp remains a constant during many processes, temperature instead of heat could be used in the definition of Thermal Action. There is an overwhelming evidence that the effect of the temperature on the result of the thermal treatment is linear, whereas the effect of time is not linear but a logarithmic one (the logarithm with the base of ten is traditionally used). Therefore we coin the concept of Thermal Action S_Θ as shown in equation (2).

$$S_\Theta = \Theta \log t \quad (2)$$

In a plot of t versus Θ the obtained curves are, thus, isodrastic curves (from Δράσις, pronounced drasis, and the Greek word for Action). Furthermore in a plot of logt versus Θ the obtained isodrastic curves become parallel straight lines.

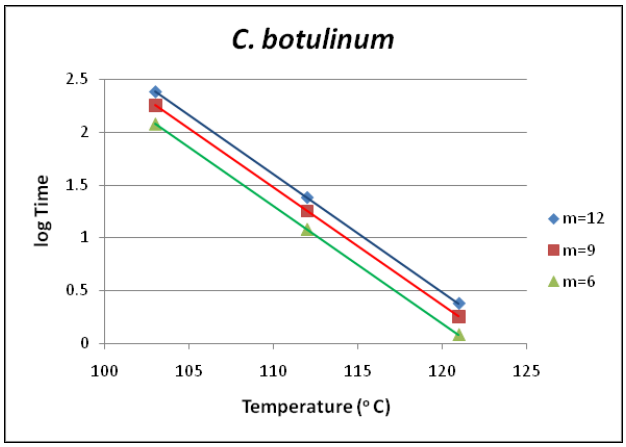

Fig.1 Isodrastic curves of *C. botulinum* for various values of m, i.e. population reduction degrees expressed logarithmically.

Consequently if we plotted the population vs thermal action the curves obtained should be straight lines, if the only parameters affecting are time and temperature.

B. Bayes approach

The Bayesian approach is one of the significant tools of prediction also in the Science and Technology (3). There are four basic elements in the Bayesian approach:

1. The prior probability of a hypothesis p(H)
2. The posterior probability given an evidence E, p (H/E)
3. The likelihood i.e. given the hypothesis is fulfilled what is the probability this to be owed to the presence of the evidence E
4. The normalization constant p(E)

Then, the well known Bayes formula connects these four elements:

$$p(H/E) = p(H).\ p(E/H)\ /\ p(E) \quad (3)$$

It is possible that we have more pieces of evidence E1, E2…and the equation can be easily generalized to take in to account all those evidences.

Another useful relation derived from the one above gives the ratio of the two probabilities the posterior and the prior as the so called Bayes factor which equals the ratio of the likelihood to the normalization constant [5].

III. EXPERIMENTAL

.A. Yogurt manufacture process

Fresh batches of yoghurt were made using goats' milk and total resin extract. For the manufacture of the yoghurt, a volume of 500 ml of fresh goat milk was poured in a sterilized 1 L Scott Duran® bottle and preheated at 55 ^{o} C. Calcium caseinate (2% w/w) was then added to the milk and thoroughly homogenized (IKA ®T25 digital Ultra Turrax) with a rotating speed of 12000 rpm for 3 min. A heat treatment at 90 ^{o} C for 5 min was carried out, and then the bottle containing the milk was cooled to 46 ^{o} C in a water bath. 2 gr of the starter culture and 300 mg of total resin extract were then added. The bottle was incubated at 46 ^{o} C for 15 min. The milk was then poured into twelve sterile plastic pots and sealed. All pots were then incubated at 46 ^{o} C for 3 h and were then transferred and stored at three different temperatures. Four pots stored at 6 ^{o} C, four pots stored at 10 ^{o} C and four pots stored at 14 ^{o} C. A total of three replicates were made, and starting from Day 0 (Yoghurt manufacture day), one pot from each of the three different temperatures was analyzed microbiologically every one week, until day 28, which was the maximum storage period tested.

B. Enumeration of total lactic acid bacteria

Plating and enumeration were performed using a modification of the methods described by Lourens-Hattingh and Viljoen (2001), [6]. From each pot of yoghurt of the three different temperatures, 10 g was aseptically weighted and transferred into sterile stomacher bags, where mixed with 90 ml sterile Maximun Recovery Diluent (MRD; Oxoid, UK) and homogenized in stomacher for 1 min. Serial dilutions were prepared (1:10) using MRD down to 10^{-6}. A volume of 100μL from each dilution was inoculated and dispersed onto MRS agar (Oxoid, UK) for the enumeration of LAB in duplicates. To create microaerophilic conditions, the MRS agar was overlaid with molten MRS agar at 48 ^{o} C. MRS plates were incubated at 37 ^{o} C for 3 days. This procedure was repeated every 7 days, for 28 days, and the total LAB were enumerated.

C. Statistical analysis

ANOVA methods were applied to evaluate differences in mean values of continuous variables as appropriate. Pairwise comparisons of the group means performed using Duncan test. A two-sided p value of less than 0.05 was considered statistically significant.

IV. RESULTS

Total LAB were enumerated for all three different batches of yoghurt, stored at 6 ^{o} C, 10 ^{o} C and 14 ^{o} C respectively from day 0 to day 28. The initial number was 8.76 $\log_{10}$cuf/g for the three batches.

Total LAB numbers remained significantly higher and steady at about 8 $\log_{10}$cuf/g in yoghurt stored at 6 ^{o} C for 14 days compared with the other two batches. Similarly total LAB numbers remained significantly higher and steady at about 7 $\log_{10}$cuf/g in yoghurt stored at 10 ^{o} C for 14 days compared with yoghurt stored at 14 ^{o} C.

At the last day of storage the values of total LAB were decreased in all three batches. However it seems that values did not decrease below a critical value of 3.5 $\log_{10}$cuf/g for all three different batches. Although the decrease of total LAB numbers in yoghurts stored at 14 ^{o} C was faster until the 14 days, this trend did not last until the 28 days, where the decrease in values was very small.

The results are depicted graphically in Fig.2 below:

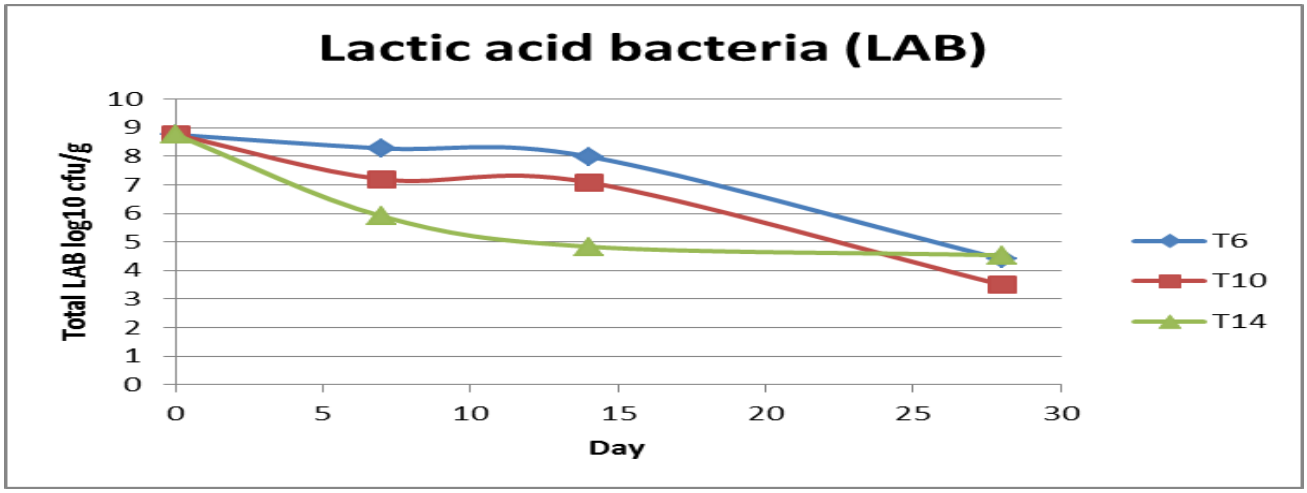

Fig.2 LAB population vs. Storage time for three different temperatures 6, 10 and 14^{o}C

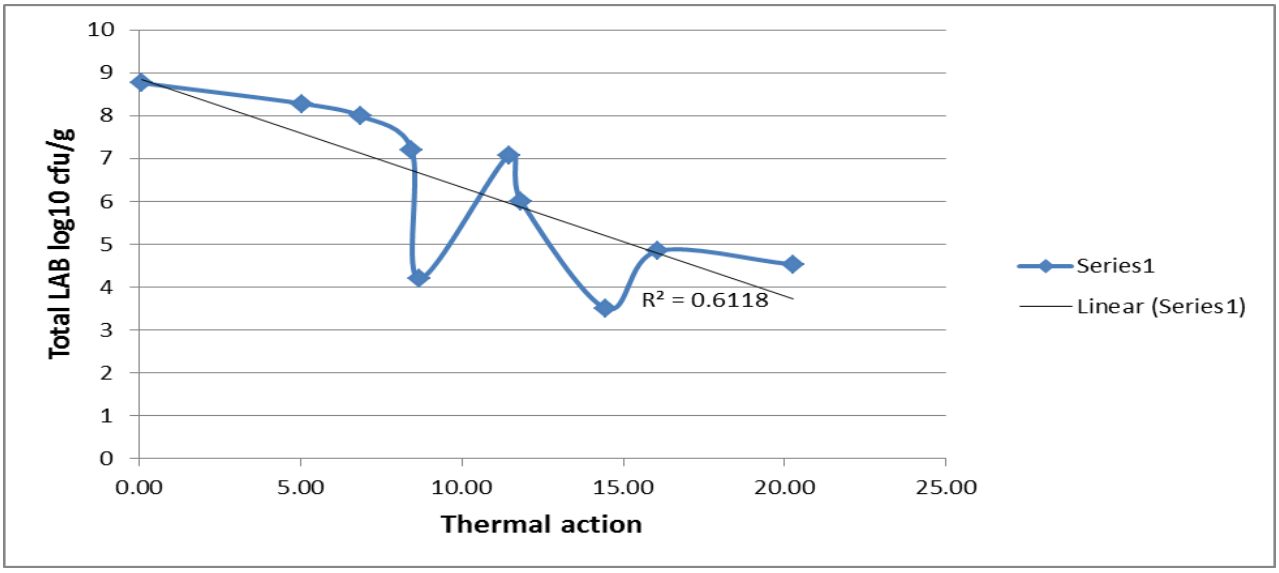

Fig.3 LAB population plotted vs. Thermal Action

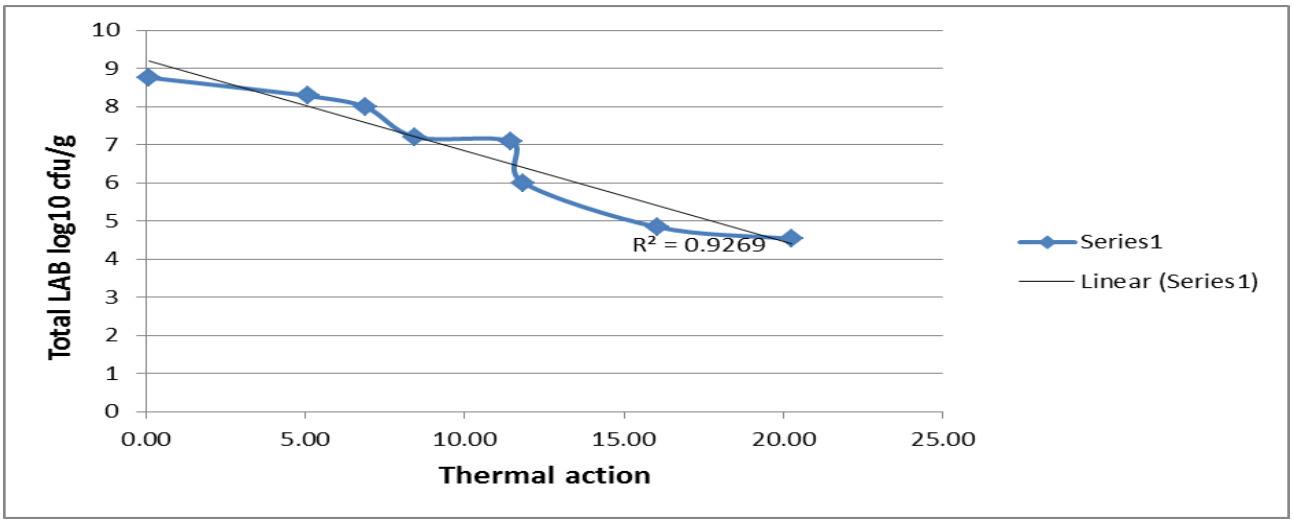

Fig.4 The diagram LAB population vs. Thermal Action omitting the two outliers

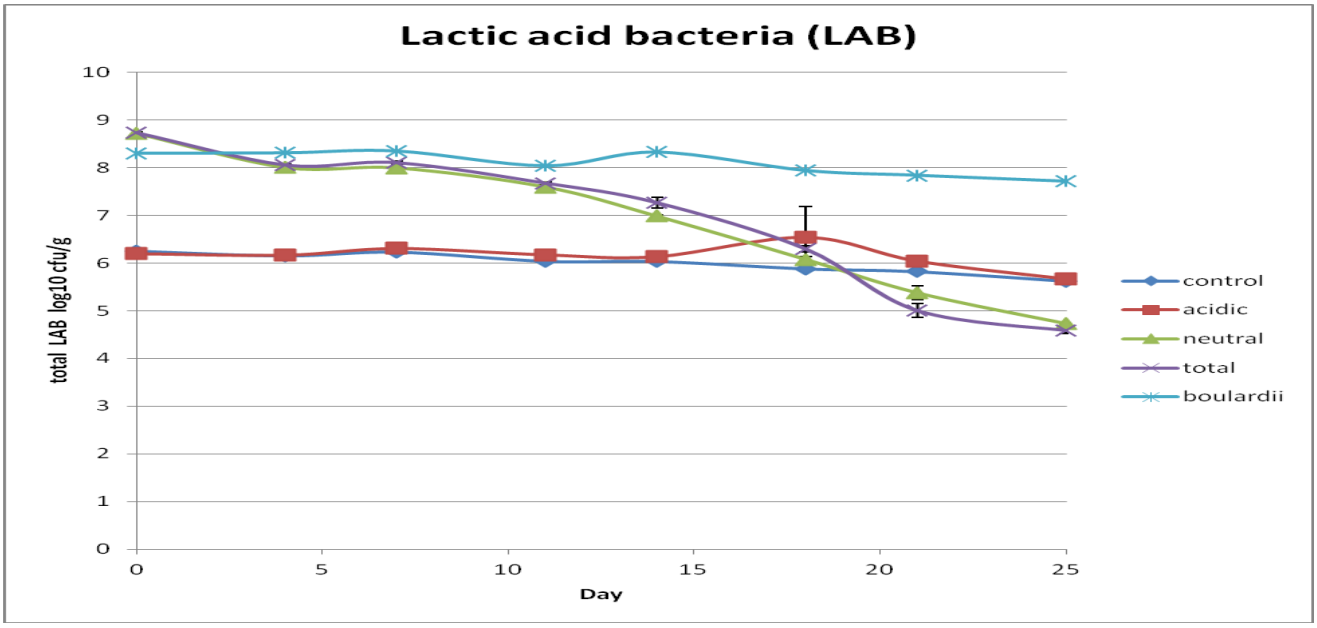

Fig.5 Survival of total lactic acid bacteria (LAB) in yoghurt formulations. Means of duplicates. Day 0 = day of inoculation.

V. DISCUSSION

A. *Thermal Action*

The data in Fig.2 were replotted as Total LAB population vs. Thermal Action (Fig.3) and the linearity of the curve was estimated.

The two obvious outliers correspond to the higher 14°C temperature for times 14 days and 28 days, storage time for which the yoghurt, as more or less was expected was rather deteriorated. Despite that the coefficient R^2 was 0.6118, which is not too bad for a complex biological system to show a tendency to linearity. If the two outliers are removed an even better coefficient is obtained (Fig. 4)

The R^2 obtained is a 0.9269 which is a very good one to show that the above studied complex system responds to positively to the thermal action approach.

B. Bayes approach

Figure 5 refers to the first series of experiments described elsewhere [7]. A description however of these experiments is given below with the caption of Figure 5:

Here the temperature was 6^o C for all samples. Control is the sample of yoghurt without any treatment. The acidic sample was the yoghurt containing the acidic portion of the mastic extract. Neutral, respectively, the neutral portion of the extract, total the total acidic plus the neutral portions of the extract and boulardii refers to the sample in which also the *Saccharomyces boulardii* yeast was added together with the total extract.

We were able to obtain an estimation of the Bayes factor for the various treatments. Our hypothesis is that a high and stable LAB population should occur during the 25 first days of storage. Because of the shape of the curves a compromise for taking both properties, high values, stable values was followed by assuming the probabilities posterior and prior as proportional to the areas under the curves to the axis of X.

If the hypothesis applies to the total treatment then

$$p(H/E) = c.\ A_{total}$$

and

$$p(H) = c.\ A_{control}$$

and

$$\text{Bayes factor} = p(H/E)\ //p(H). = {}^{A_{total}}/_{A_{contro}}l = 1.21$$

If the hypothesis applies to the combined treatment i.e. samples containing total extract plus the *Saccharomyces boulardii* yeast , following a similar procedure as above we obtain the Bayes factor for the boulardii sample as

$$\text{Bayes factor} = p(H/E)\ //p(H). = A_{boulardii}\ /A_{contro}l = 1.33$$

VI Conclusion

In the frame of the development of a new functional product based on yoghurt, one of the most important quality parameters is the microbial population of the Lactic Acid Bacteria (LAB), which belong to the human friendly and beneficial microorganisms. *Pistacia* resins, treated in different ways were added, to the yoghurt, with or without the probiotic yeast, *Saccharomyces boulardii.* It was of essential importance to monitor the LAB population which needed to be retained at high values and for long periods of time, up to 3-4 weeks.

To such a complex system, such the functional yoghurt product developed, two novel modeling ideas were applied. The model of Thermal Action, has found to show a good agreement with the experiments i.e. the microbial population shows a linear correlation with the thermal action values.

The Bayes approach modified suitably by one of us [8], with the novel ideal of modifying the initial Bayes equation, enabled us to obtain experimentally the Bayes factors for the treatments for two kinds of yoghurt the total extract one and the combined treatment with the total extract and the *S. boulardii* yeast.

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