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Description and importance of some predictive models that are used as tools in food conservation

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ABSTRACT

Predictive Microbiology is a branch of Food Microbiology that studies the microbial responses against several environmental factors that can be controlled, giving as a result responses that are quantified and summarized using mathematical equations. This study makes a review of the description and importance of some predictive models used as a tool in food conservation. Due to the fact that the application of mathematical models to the growth of microorganisms allows us to predict microbial behavior in storage conditions, its application may give to the establishment of the product´s shelf life a greater precision.

Key words: predictive microbiology, microbial recount methodology, microbial growth models.

INTRODUCTION

Microbial diagnosis is based in laboratory techniques that require time associated to the growth of microorganisms, which has a detrimental impact on making decisions, especially in the industry [32]. The require time for cell revitalization and cell recount through CFU (Colony Forming Units) is at least 48 hours. Moreover, for pathogen identification it is common to turn to biochemical tests or selective mediums, this leads to wait for days or even weeks to get results. Because of this, quicker methods have been developed to obtain results (30), including the ones developed by Predictive Microbiology.

Predictive Microbiology has become a worldwide cutting edge tool that allows us to evaluate, through suitable mathematical models, the response of microorganisms to environmental factors. This discipline has been applied in great measure to pathogenic bacteria and microorganisms associated to food spoilage. However, Predictive Microbiology can extend its reach to other fields such as Medicine, Biology and even Pharmacy, where it studies the growth and inactivation of microorganisms [32].

Predictive microbiology can be used to model the growth, survival and death of microorganisms in terms of the main factors of food preservation, especially when these factors are used together in so-called combined methods of preservation.

Nowadays, given the fact that predictive models have evolved from the basic research labs to being used in the industry and in regulatory agencies they must be consider as initial estimates of microbial behavior and serve as guidance to asses potential problems [7 & 46].

Predictive models do not replace completely the evaluation of microbial behavior neither the judgment of an experimented microbiologist. However, they may provide useful information to make a decision in the manufacture and conservation of food [46].

Hence, the goal of this review describe some of the models used in Predictive Microbiology and highlight their importance and application as a tool for food preservation.

LITERATURE REVIEW

Definition and history of Predictive Microbiology

Predictive Microbiology is the detailed knowledge of microbial responses to certain environmental conditions, which allows an objective evaluation of the effect that processing and storage parameters have on the food quality and safety. This response includes the accumulation of a series of knowledge of the behavior of microorganisms found in food, consequently resulting in the development of mathematical models [22].

Predictive Microbiology is based on using mathematical modeling to describe microbial behavior and predicting growth. With this technique, problems with food spoilage caused by microorganisms and food safety can be resolved through an objective analysis based on scientific knowledge [32]. This is remarkable since the increase of infections caused by toxins present in food in many countries.

It has been recognized that the origin of predictive models in food was a model developed by [12] used to describe the thermal treatment needed to destroy 10^{12}*Chlostridium botilinum* type a spores [46 & 24]. This model described a process with a broad margin of safety and probably its continuous use prevented it from being recognized at that time as a predictive model.

Other references place the beginning of Predictive Microbiology in the 1930´s, when Scott established that knowing the growth rate of certain microorganisms at different temperatures was vital for fresh meat spoilage studies. Once this information was obtained one could predict the relative influence exerted by various organisms on spoilage at each storage temperature. Scott understood the potential of gathering kinetic data about the response of microorganisms, to predict useful life and food safety[24].

After a long time of silence from Predictive Microbiology in the scientific literature, in the 1960´s and 1970´s predictive models were used to resolve food poisoning problems, particularly botulism [26, 35& 39].

However, it was not until the 1980´s that the interest in Predictive Microbiology started as a result of a massive outbreak of food poisoning and therefore the public need to provide healthy and safe food. Two pathogenic microorganisms transmitted in food, one traditional as Salmonella and one emergent, *Listeria monocytogenes* of psychotropic origin favored placing food research as a priority for the United States, United Kingdom, Australia and New Zealand governments [22].

During the 1980´s and part of the 1990´s various approaches of growth kinetic models dominated the Predictive Microbiology scene, but nowadays the return of the probability models for growth is clear, which can be attributed to the following:

a) Recognizing that the variability of responses in an estimated time period (generation time and latent period) does not show a normal distribution, but it is commonly described by an inverse Gaussian distribution, where the response´s variance is directly proportional to the square or cube of the mean response time [34].

b) In the case of potentially dangerous pathogens (*Escherichia coli* O157:H7), in situations when the microorganism is found in a low infectious dose, it is require to know it, in order to recommend conditions to avoid its

multiplication, thus the probability of finding this kind of organism is more important than knowing its growth rate, maximum population density or generation time.

Prediction models allow us to interpolate experimental data points, helping predict responses for unstudied conditions [13]. Moreover, predictive growth models give us the chance to estimate food´s useful life, evaluate hygienic conditions in which the food was processed, to identify critical points in the process, to guide on how environmental variables affect the pathogen´s behavior and to determine the microbiological safety of food [23].

Kinetic parameters of the models

In Microbiology, growth refers to an increase of cells per unit of time in a microbial population [20]. In this case, it is important to consider the presence of coenocytic microorganisms i.e. multinucleated, where the nuclear division is not followed by cell division, whereby, growth causes an increase in size but not in cell number. Growth causes an increase in the number of cells when microorganisms reproduce by binary fission or budding [31]. The growth curve of a microorganism can be divided in four different phases: *lag phase, exponential phase, stationary phase and death phase (Fig. 1).* The lag phase is the period of time when cells adapt to a culture medium before starting to grow. The logarithmic or exponential phase is when the microorganisms grow and divide themselves until a maximum is reached, all of this as a function of the medium, growth conditions and genetic potential. In this period there is a linear relation between the logarithm of the number of cells and the incubation time. Since every cell divides at slightly different rates, the growth curve increases slowly instead of having big fluctuations. The stationary phase is when the growth rate slows down, as a result of the decrease in nutrients available or the effect of toxic metabolic products accumulating. Finally, the death phase is a consequence of various factors; the most important is the depletion of the microbial cell´s energy reserves. In this phase there is also a linear relation between viable cells that decrease as time goes by [20 & 31].

Along the growth process, there are various factors for the microorganism to adapt, such as the composition of the medium or the physical state of the strain. Thus, the variability of microbial responses influences in different ways the prediction of the microorganism´s kinetics. Said responses can be reflected on growth parameters, such as: latent period, generation time and growth rate (Figure 1)

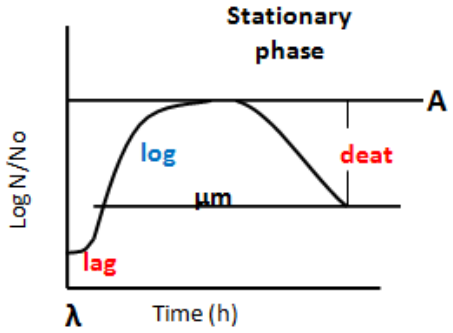

Fig 1.Phases of microbial growth function of time [20]

Latent period (λ). During this period there is anadjustment in which the microbial cells take advantage of the new environment and start an exponential growth period [7]. The growth and multiplication of pathogenic or food spoilage microorganisms is not desired. Therefore it is important to accurately estimate their latent period, which in most cases is not achieved with a good trust level [23]. In the matter of microbial inactivation kinetics, the latent period is observed as a “shoulder” in the logarithmic inactivation curve [15].

Generation time. Generation time is the time needed to double the microbial population. The generation time varies depending on the type of microorganism. A great number of bacteria have a generation time of 1 to 3 hours; it is very hard to find microorganisms that reproduce in 10 minutes. Nonetheless, this parameter is useful to indicate the physiological state of a microbial population. With the generation time one can evaluate the positive and negative effect of a particular treatment upon a microbial population [20].

Growth rate (µm). The growth rate is defined as the pendant in the exponential growth phase and it is related to the generation time. The growth rate is the increase in cell mass per unit of time and it is influenced by environmental factors (temperature, composition of the medium, and others) as well as the microorganism genetic characteristics [20].

Maximum population density (A). It is the logarithmic growth curve´s asymptote (stationary phase), it is presented in Fig.1.
Types of models. Predictive models are classified by their complexity as primary, secondary and tertiary. Here is presented a brief description of these three types of models.

Primary models. Primary models deal with the description of the changes of the microbial number (growth, multiplication, and inactivation) over time. For quantifying microorganisms one can use Colony Forming Units (CFU), biomass, absorbance or measuring produced metabolites [17]. Most of the primary models that have been developed so far determine the amount of microbial population. In these models, the development of a total number of cells from a population is described by a simple group of parameters: maximum population density (A), growth rate (µm) and latent period (λ) [41].

The literature suggests that the sum of single cell´s behavior is the same as the population´s. This is what leads to the development of more mechanical approaches for Predictive Microbiology [2]. This leads to probabilistic modeling techniques, in which the model parameters are casually distributed within the total population. This means that the parameters of the model are part of a random distribution, which may represent the biological variability between single cells. Probability models become more useful when the amount of inoculum is small and the individual latent period within this small population is highly variable [41]. An example of primary models is the Gompertz equation (Ec. 1), [2 & 35] equation and the three-phase linear model.

$y = a \exp(-\exp(b-cx))$ Ec.1

Where: A is themaximum population density, µm=b is the growth rate and

λ = c is the latent period.

Secondary models. Predictive secondary models describe the parameters that can appear in the primary models, as a function of the environment conditions such as temperature, pH, a_w, etc., observing the interaction between two or more factors with microbial growth.

Formerly, secondary models for the lag phase referred only to the effect that incubation temperature had; however, nowadays new models have emerged, and they include other important factors such as the pre-enrichment conditions.

Other authors have developed secondary models independent from generation time and latent period, e.g. polynomial approaches [8, 18 & 49] and low complexity models of artificial neuronal networks[14, 16 & 51]. Other examples of secondary models are the Arrhenius equation (Ec. 2), square root models and the response surface model (8).

$\mu = \mu\infty \exp(- Ea/ RT)$ Ec.2

Where: µ is defined as the reaction rate constant, Ea is the activation energy, R is the gas constant, T is absolute temperature and µ∞ is the pre-exponential factor.

Tertiary models. Tertiary models can have different forms, starting by combining the first two models (primary and secondary) based on laboratory experiments. A representative example of this kind of models is the "Pathogen Modeling Program", created and put at the scientific community´s disposal for free by the USDA; said model allows us to import a series of temperature data in order to predict the useful life. Another example is the "Seafood Spoilage Predictor" (10), which includes seafood specific deteriorative microorganisms. Finally, tertiary models enable to incorporate predictive models into a microbiological risk evaluating network, like SERA ("*Salmonella enteritidis* Risk Assessment") by the USDA. This kind of models are computer based [17].

Building predictive models.
Building a predictive model involves the following stages: selecting the strains, generating the data, applying a primary, secondary or tertiary model and validating such model. The initial stages of this process are fundamental for its success, therefore they are described here:

Strain selection. There are various criteria that are used when choosing the strain for building a model. The strain can be chosen isolated or mixed (cocktail). Before selecting a strain it is very important to have a clear idea to where the model is aimed, for example: Is the model being used to predicting possible growth of a specific pathogenic species? Or is it a model for deteriorative microorganisms in a specific food?

To use a strain that has been previously studied in other scientific research or even for creating other models gives and advantage of having knowledge on this specific strain. On the other hand, selecting an isolated strain from food you want to create a model for, gives the advantage of knowing the product [33]. The hypothesis that variation between strains could be equal or less than the experimental statistical variation was studied by [47]. They studied the growth, survival, inactivation and production of toxins from 17 different *E. coli* strains and observed that the variations between strains were greater than the uncertainties related to the experimental error.

[37]Compared the growth of non-pathogenic *E.coli* M23 with the growth of different pathogenic *E.coli*species; they only found little differences in the growth responses between the strains. The generated model turns out to be of great help, since several researchers do not have access to adequate laboratory facilities to work with the pathogenic strain of *E.coli* and the model that appears to predict the behavior of *E.coli* M23 is also capable of describing the behavior of pathogenic *E coli* strains including *E.coli* O157H:7.

Mixed strains are being broadly used in predictive models, because they have a more real picture of the situation in food.

Viable cells recount method. The most used method to monitor the growth of a bacterial population is recounting viable cells. However, as it has been discussed previously, the conventional microbiological analysis has different limitations such as the time required torevitalize, enrich and incubate the samples. Probably the most important limitation is that for identifying a microorganism one requires selective mediums and biochemical tests, which can delay the results by days or even weeks. Due to these limitations, it has been necessary to turn to the development of quick methods that provide results in hours [30].

For the exact estimation of the growth curve´s parameters it is important the number and the quality of the recounts performed by the technicians [5 & 29]. [45]recommend having 10 values per point, minimum. [3]Compared the adjustment of three models with groups with few values per point and groups with several values per point, indicating that the inaccuracy in the estimated parameters was clearly related to the amount of data.

In order to facilitate the recount of microbial growth, alternative methods such as flux cytometry, turbidimetry, impedanciometry among others, have been developed [4].

When comparing with viable recounts, turbidimetry and impedanciometry are considered automatic methods, which allow analyzing a high number of experiments, while flux cytometry and microscopy allow us to get additional information, such as the physiological state of the cells [33].

Flux cytometry. Flux cytometry allows us to measure different physical and chemical features of singles cells in suspension, providing an indication on the heterogeneity of eucariotic and procariotic cell populations in a matter of minutes [1, 11 & 27].

Single cells go through a measurement window; in which different parameters of millions of cells can be measured per second can be measure at the same time with high accuracy [43]. The dispersion of light measures the size and structure of the cells, while fluorescent measurements determine cell content of any component that can be marked with a fluorescent dye [44]. This way, flux cytometry combines the advantage of being a technique for single cells, with the power to measure millions of cells in a short amount of time. The resulting data is not only an average of the cell measurements but also a distribution of the assessed cell parameters.

With flux cytometry the possibility of measuring a distribution of data gives an estimation of the microbial population´s heterogeneity and this way, also the possibility of detecting subpopulations that for example are resistant to a treatment under research conditions. The use of flux cytometry in Predictive Microbiology is limited by the cost of equipment. Such is the case, that the research of [38], who used flux cytometry to enumerate viable cells of *Debaryomyceshansenii*in different environmental conditions. Growth data was used to model the latent period (λ) and the maximum growth rate (μmax) as a function of temperature, pH and NaCl concentration.

Turbidimetrymethod. Turbidimetry is a method used to study bacterial growth through optical density measurements, which allows us to have a sequence of microbial growth in real time [4 & 9]. Optical density (OD) or absorbance has been used for several years to measure concentration, which can be expressed in cell mass, number or mean length of bacterial suspensions [25].

Absorbance (*A*) is defined as the decimal logarithm of the quotient between incident light on the suspension (lo) and the transmitted light from the suspension (l) (Ec. 3) [40]

$A=log(Io)/(I)$ Ec. 3

This technique is based on the fact that small particles diffract light within certain limits in proportion to their concentration. Measurements are made with a photometer or spectrophotometer.

According to [23], in turbidimetry, microbial growth is related to the turbidimetry in the medium. These authors highlighted the limitations of the method, being the most important one that the vitality recount can only be made if the equipment is calibrated to link absorbance to a given number of microorganisms [23]. However, it is possible to identify the growth parameters when the inoculum size is below the detection threshold. For this, it is necessary to know the initial cell recount and the calibration equation [6].

TTD Method (time to detection). This method consists in measuring, after an established thermal treatment, the probability of a population of microorganisms not growing in a suspension at certain culture conditions. It is an analysis that allows us to evaluate the most likely number of survivors (indirect method). This method depends on the temperature and the time of treatment in order to achieve a physiological effect [33].

Microscopy method. Microscopy allows direct study of single cells, which makes it possible to trace that same cell for large periods of time. Microscopy has gained interest with the development of computer programs for optic interferometry and image analysis. One advantage of this method is that it allows us to study solid systems whose situation is similar to which food systems present [33]. There are few reports on the use of microscopy in predictive modeling. However, there are comparisons with the TTD method (time to detection) for determining the latent period of *Listeria monocytogenes*cells, having microscopy an advantage over TTD, since it is a direct method that allows visual observation of the first cell division, whilst TTD depends on time for detection, growth rate and regressive extrapolation to the single cell. Moreover, any treatment that results in the absence of cell division will not be detectable through the TTD method [48].

Model validation

Validation of predictive models can be done in two ways:

a) Mathematical validation that verifies the accuracy of the generated models.
b)
c) Validación en el alimento (sistema real), en la cual lo que se requiere es demostrar que el modelo predice con exactitud el comportamiento de los microorganismos durante el procesado, almacenamiento y distribución [17].
d)

It is important to validate a model in order to evaluate its predictive ability [17]. The accuracy of the models is evaluated graphically when data obtained in the lab is compared to the predictions of the model. Moreover, the correlation coefficient values (R^2), the mean square error and the slant and precision factors are used as confidence indicators of the models when applied to food [19].

However, it is important to mention that even when a model has proved to be adequate to predict experimental data, applying those models to food is still questionable [50].

Importance of predictive models

It is interesting to learn how predictive models can be used permanently in scientific research, the industry and even in everyday life. Here are some of the most important applications of predictive models:

- Predictive models help make immediate decisions on the re-process of food, for example, in events outside of the process such as lack of salt in the product or inadequate food refrigeration.
- Predictive models help predict the degree of growth and/or survival of some microorganisms of interest (pathogenic or deteriorative) under normal storage conditions, thereby detecting any flaw in the storage and/or distribution process, as well as estimating expiring dates in terms of microbial decomposition [28].
- Predictive models allow us to identify critical control points in a process in which a Hazard Analysis and Critical Control Point system (HACCP) has been implemented.
- Predictive models may be a teaching tool, especially for people with no training in Food Microbiology; through them one can demonstrate the importance of keeping appropriate storage conditions [42].

CONCLUSION

Progress in Predictive Microbiology in the last few years has been impressive in such a way that it is being used in a great variety of research and industry operations. Predictive models are now acommon tool when doing research and a valuable aid to assess and design food conservation processes. However, it is not possible yet to depend only on these models to determine unequivocally growth, survival and death of microorganisms present in food.

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