Predictive microbiology and table olives

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The table olive is probably one of the most important and most recognized fermented vegetable in the food industry. Basically, the elaboration of this food is constrained to Mediterranean countries, but there are also well established production regions in Australia, USA and South-America. Thus, table olive elaboration is widespread around the world and represents an important economic source for the producing countries. Microorganisms play an important role in the production of table olives. Diverse groups are involved through olive fermentation determining the quality and flavor of the final product, but *Enterobacteriaceae*, lactic acid bacteria and yeasts are the most relevant. As in other food fermentations, it is necessary to favor the growth of desirable microorganisms and inhibit pathogen and spoilage microorganisms. Specifically, the levels of microorganisms in table olive fermentation and packaging can be controlled by diverse factors such us temperature, pH, water activity or additives. Predictive microbiology is a multidisciplinary area (statistical, microbiology, chemistry, food technology, etc.) which is devoted to quantitatively studying the effects of environmental factors on microbial growth in foods. The response is evaluated objectively by means of mathematical models, which can be later used as a useful tool to predict microbial response under different combinations of the variables. In this review, the different steps for the successful development and implementation of a predictive model in the specific case of table olive matrices will be discussed. It includes planning and experimental design, collection and analysis of data, model development, and, finally, validation and maintenance of the model. The most important predictive models (probabilistic, polynomial, neural networks, etc.) which have been developed in the last decade in table olive fermentation and packaging for different environmental variables and microorganism, as well as their immediate applications (food safety and quality), will also be treated.

Keywords: Table olives; Mathematical modeling; Vegetable fermentations; Predictive Microbiology

1. Table olives

The olive, the fruit of cultivated *Olea europaea* tree, is a drupe which contains a bitter component (oleuropein), a low sugar concentration (usually between 2.5-6.0%) and a high fat content (between 9-30%), although these values can differ with maturity degree and olive variety. Such characteristics prevent olives from being consumed directly from the tree and it has promoted a series of processes to make them edible, which can differ considerably from region to region.

Table olive is probably one of the most important and most widely recognized fermented vegetable of the food industry. Its importance was already mentioned in the first century by Lucius Columela in *De Re Rustica* (42 BC), which is the first reference on how to prepare table olives. The International Olive Oil Council (IOOC) estimates that the table olive's world production reached approximately 2,153,500 tones in the 2007/2008 season [1], with Spain (475,000 tones) and Turkey (390,000 tones) as the main producers. Basically, the elaboration of table olives is constrained to the Mediterranean countries, but there are also well established production regions in Australia, USA and South-America. Thus, table olive elaboration is widespread around the world and represents an important economic source for the producing countries. Fruits for production are chosen according to their volume, maturation, shape, flesh-to-stone ratio, fine flesh taste, firmness and ease of detachment of flesh from the stone [2]. There are several ways to elaborate table olives, but the most prominent industrial elaborations are: a) the green Spanish style (the most widespread process because olives may be subjected to very diverse conditioning operations and may be offered as many commercial presentations), b) ripe olives by alkaline oxidation (the so-called Californian style), and c) naturally black olives (also known as Greek style) [3].

Briefly, the procedure for preparing green Spanish-style olives consists of treating the fruits with a dilute NaOH solution (2-3%). The alkali is used to penetrate the olive skin and to destroy the glucoside oleuropein, which reduces the natural olive bitterness. Lye treatment not only has the effect of removing bitterness but also markedly increases skin permeability which, in turn, favours the release of nutrients. Then, one or two water washes are carried out to remove the excess of alkali and the fruits are immediately immersed in brines (water with salt, usually NaCl in concentrations between 9-12%), where olives undergo a lactic acid fermentation. Finally, when substrates are exhausted, the fruits are stored, graded, sorted and conditioned (pitted, stuffed, etc) before packaging.

Olives for producing ripe olives (by alkaline oxidation) are previously preserved in an aqueous solution (brine, acidic water, etc.) and darkened throughout the year according to demand. Darkening consists of several treatments of dilute NaOH solutions and water washes between them. During the oxidation process, air is passed throughout the suspension

of the olives in the liquid. Once the olives obtain the proper colour ring around the outer surface, this is fixed by immersion in a lactate or gluconate iron solution. These olives are usually packed in light brine.

Untreated olives (green, turning colour or naturally black olives) are directly brined after picking. In brine, fruits undergo a fermentation whose characteristics depend on the physicochemical conditions, cultivar, temperature and salt content. These preparations do not include the lye treatment of fruits and thus they are characterized by a slow diffusion of olive compounds into brines. Usually, the lactic acid process is more difficult to complete due to the presence of high concentrations of polyphenols. During fermentation, the diffusion of oleuropein to brines occur and, consequently, the natural debittering of fruits progresses. In fact, the fruits are maintained in this solution until they lose their natural bitterness, at least partially. As the market demands, olives are sorted, graded and packed. In some commercial presentations, they can be cracked or cut along their longitudinal diameter and/or seasoned with natural products (garlic, peppers, thyme, etc.).

Therefore, and as can be easily deduced, the preservation and preparation of table olives are carried out by a combination of salting, natural fermentation and acidification [3]. These systems of processing have numerous adventges, but fundamentally offer an easy and economic way to preserve the raw material over extended periods of time. In the case of olive packaging, the application of heat treatments or the addition of preservatives (sorbate, benzoate, etc.) is also very common. In the present review, we discuss the perspectives that predictive microbiology can offer for the future development of the table olive industry.

2. Role of microorganisms in table olives

As was commented above, olives need to be fermented, so the study of the processes ocurring during this elaboration phase is fundamental to improve the preparation, storage and safety of the final product. Microorganisms play an important role in the production of table olives. Diverse groups are involved in both table olive fermentation and packaging, determining the quality and flavor of the final product, but *Enterobacteriaceae*, lactic acid bacteria (LAB) and yeasts are the most relevant microorganisms.

During spontaneous Spanish-style green table olive fermentations, there is a succession of the microorganism species present, which depends on their respective nutritive requirements and physiology. Gram-negative bacteria, mainly belonging to the *Enterobacteriaceae* family, appear in the early stages of the process (2-3 days) because of the high level of pH reported after the lye treatment (alkaline conditions) and their low nutrient demand. However, as fermentation progresses, these microorganisms are quickly inhibited by the reduction in pH originated by LAB growth. Lactic cocci of the genera *Pediococcus* and *Leuconostoc* are the first LAB species to appear (about 15 days), followed by the strong homofermentative species *Lactobacillus pentosus* and *Lactobacillus plantarum*. Diverse yeast species (among the most important *Pichia anomala, Pichia membranifaciens, Saccharomyces cerevisiae, Debaryomyces hansenii* and *Candida boidinii* can be mentioned) coexist with LAB practically throughout the entire process. Moreover, these microorganisms are especially relevant in directly brined green and black natural olive fermentations, where fruits are not treated with NaOH and LAB are partially inhibited due to the presence of phenolic compounds [3]. The fermentation period can be considered finished when sugars are completely exhausted by microorganisms; then, the storage period begins. The pH reached at the end of fermentation, originated by the production of lactic acid by LAB, must be below 4.5, otherwise the growth of undesirable microorganisms could occur. During storage, one may observe the growth of species of the *Propionibacterium* genera which increase the pH because of the production of acetic and propionic acids using the lactic acid produced during the previous phase of active fermentation. This implies a considerable microbiological risk because such changes may facilitate the growth of spoilage or pathogen microorganisms. In fact, there are several serious poisoning cases caused by the growth of *Clostridium botulinum* type B in Italian table olive fermentations [4,5]. The growth of *Propionibacterium* species can be inhibited by increasing the salt level up to above 9%.

Thus, LAB species, specifically *L. plantarum* and *L. pentosus*, are very important because they use the sugars present in olive flesh (glucose, fructose and sucrose) to produce lactic acid and bacteriocins that originate the rapid and safe acidification of brines [3,6,7]. Yeasts are also beneficial in many cases because these microorganisms possess many interesting technological properties. Diverse authors have studied, among others, the lipolytic, β-glucosidase, catalase, and killer activities of the yeast species related to table olives for their potential use as starters [8]. In addition, they also consume the sugars present in brines and produce diverse types of organic acids (citric or acetic), vitamins and aromatic compounds. However, in its negative aspect, yeasts can produce spoilage of fruits during table olive storage or packaging. If these microorganisms become dominant, they can originate a product with a milder taste and a limited self preservation [3]. Fermentative yeasts can also produce a vigorous production of CO_2 that may penetrate olives and damage the fruits, as well as lead to swollen containers, clouded brines, or the production of off-flavors. Other unfavorable properties of some yeast are their polysacchrolytic activity (which cause the softening of fruits) and their ability to consume, under aerobic conditions, the produced lactic acid [8]. Finally, *Enterobacteriaceae* can also cause spoilage through the formation of gas pockets in the olive surface or due to the production of metabolites that affect the aroma of the product [3]. In order to prevent this, it is crucial to control the growth of *Enterobacteriaceae* at the first stages of fermentation by rapidly dropping the pH to below 4.6.

Therefore, as in other food fermentations, it is necessary to favour the growth of desirable microorganisms and inhibit pathogen and spoilage microorganisms. Specifically, the levels of microorganisms during table olive fermentation and packaging can be controlled by diverse factors such us temperature, pH, water activity or the addition of additives (sorbic acid, benzoic acid, etc.). In this way, predictive microbiology could be a very useful tool.

3. Predictive microbiology and table olives

3.1 History and definition

Methods such as drying, salting and fermentation have been used by humans for thousand of years to unconsciously preserve foods, representing an empirical approach to the control of microbial populations. However, predictive microbiology currently offers a quantitative and objective approach to the solution of this problem, emerging as a new and crucial element of food microbiology.

Predictive microbiology probably arose in 1922 with the appearance of the first model that described the thermal inactivation of *Clostridium botulinum* type A spores [9]. References to the potential use of predictive microbiology to describe microbial growth can also be found in the 1930s, when Scott understood the benefit of accumulating kinetic microbial growth data to predict the shelf life and safety of foods [10]. However, it was not until the 1980s, with the development of computer technology and statistical software (which considerably simplified the calculus necessaries to build the models), and with the appearance of diverse outbreaks of food poisoning, when this discipline experienced an important expansion. In fact, in the last 20 years, hundreds of papers have been published with the keyword 'predictive microbiology' in the ISI Web of Knowledge.

Predictive microbiology is an interdisciplinary area where statisticians, food microbiologists, mathematicians, food technologists and computing scientists all collaborate. It is based on the premise that microorganism response as a function of environmental factors can be estimated and reproduced. In the first book on the subject, published in 1993 by McMeekin et al. [11], predictive microbiology was defined as a quantitative science that enables users to objectively assess the effect of processing, distribution and storage on the microbiological safety and quality of foods. A later book on the field published in 2003 by McKellar and Lu [12], define it as the quantitative description of the microbial response in food environments by mathematical models. Thus, as can be directly deduced from both definitions, a first and important step in the development of a predictive model is the accumulation of data on the microbial behaviour in foods. The increasing importance and utility of predictive microbiology in the food industry has favoured the recent publication of a third book on the matter, edited in 2007 by Brul et al. [13]. Predictive models can be used to assess the risks of food processing and consequently to implement control measures in order to protect the microbiological quality and safety of foods, anticipating the behaviour of pathogen and spoilage microorganisms. But it can also be used to optimize fermentative conditions by favoring the growth of desirable microorganisms. It is clear that predictive microbiology has a major role to play for industry, government and consumers as a modern and essential element of food microbiology.

3.2 Phases in the development of a predictive model in table olives

The successful development and implementation of a predictive model in the specific case of table olives involves, as in other foods, a series of steps that include, in this order, a study of the matrix, an experimental design, data collection, model development and finally model validation. The final result is obtaining a safe and useful tool to evaluate the applications of corrective actions during olive processing and packaging.

3.2.1. Study of the matrix

The olives are characterized by a high fat and low sugar concentration. When the fruits are brined, there is a diffusion of nutrients from the flesh into the liquid, which progressively becomes an appropriate medium for the growth of microorganisms. On the contrary, salt penetrates into the flesh. The kinetic of such exchanges is faster in lye treated olives than in directly brined olives. Moreover, in the specific case of table olives, the presence of oleuropein or its derivates (hydroxityrosol) is also a determining factor because these compounds are supplied by olives and they can potentially inhibit the growth of fermentative microorganisms [3]. Industries can manipulate the levels of salt and pH in brines by the addition of NaCl and diverse types of acids (HCl, acetic or lactic) during both fermentation and packaging. However, it is more difficult to control the content in sugars and polyphenols because they could change depending on olive varieties, maturity degrees and seasons. Thus, three alternatives appear when we have to choose the medium for model development. The first option is to use a standard laboratory medium where components and constants are known, which is modified by the factors that the industry can govern (temperature, pH, salt, additives, etc.). The second alternative, and a better approximation to authentic conditions, is to obtain the juice of olives [14] or industrial brines [15], which are sterilized, modified and later used as medium culture in the laboratory. A third and more laborious option is to sterilize olives by immersion in sodium hypochlorite, and then transfer them to sterilized-modified brines to

carry out small controlled fermentations. In any case, it is important to analyze the matrix to determine the concentration in the olives of the natural compounds (nutrients) mentioned above.

3.2.2. Experimental design

The choice of the experimental design will determine the number of experiments to carry out, the combination of factors and how data will be analyzed and processed. In this step, one must establish the range and number of environmental factors to study. In many cases, the levels of additives or preservatives are determined by industry practices or legislation, which may constrain our design. Obviously, the experimental design must be chosen as a function of our final objectives. For instance, if we can build a polynomial model to explain the effects of the environmental variables on microorganism response, the application of central composite or D-optimal designs will be useful. These designs considerably reduce the total number of experiments to be performed, and consequently costs and time. They have already been satisfactorily applied to model different *Lactobacillus* and yeast species isolated from table olive fermentations [7, 14, 16]. On the contrary, a complete factorial design will result most appropriate in order to estimate the growth-no growth limits of microorganisms, as was proved recently for *Saccharomyces cerevisiae* and *Issatchenkia occidentalis* in packaging brine [15, 17]. A third type of design with a great application in table olive fermentations are the mixture designs, where the response is associated to the proportion of the components in the mixture. In this way, Arroyo López et al. [18] used a simplex-centroid mixture design to evaluate the combination of different proportions of NaCl, ascorbic acid and sodium metabisulphite on the microbiological profile of Manzanilla-Aloreña green cracked table olive fermentations. This type of design is also useful when one wants to prove different combinations of compounds while always keeping a determined restriction or constraint [19].

3.2.3. Primary modelling

Microbial curves can be segmented into four phases: lag phase, growth phase, stationary phase and death phase. While many primary models have been developed for microbial growth (which include the first three phases: see Fig 1a), few have been built in the case of inactivation or survival (death phase: Fig 1b). The function of a primary model is essentially to obtain the growth/inhibition parameters for each of the treatments established by the experimental design under well-defined and controlled environmental conditions. Firstly, the response of the microorganism versus time is determined by means of plate count or optical density (OD) measurements. This is a laborious and expensive step which can be facilitated by the use of an automatic apparatus (spiral plate maker, spectrophotometer, etc).

In the case of microbial growth, diverse sigmoid equations (modified Gompertz, Baranyi-Roberts, Logistic, etc) are used to fit the experimental data and obtain the growth parameters (lag phase duration, maximum specific growth rate and maximum population level reached), which is accomplished by curve-fitting with appropriate software. A non-linear regression procedure is usually used for this purpose. Recently, Arroyo et al. [20] compared diverse primary models to fit the response of the olive yeast *Pichia anomala* as a function of diverse combinations of environmental variables. The reparametrized Gompertz primary model was also used to study the interactions between the species *L. pentosus* and *S. cerevisiae* in reused alkaline washing waters treated with ozone [21]. As a graphic example of a primary model fit for the growth phase, Fig 1a shows the yeast population evolution during table olive packaging. On the contrary, in the case of the inactivation, microorganisms may reflect an initial "shoulder", a linear reduction or sometimes the presence of a tail. Van Boekel [22] developed an inactivation model based on the Weibull distribution which can be used to determine the shape of the inhibition curve as well as the time necessary to take the first decimal reduction. A graphic fit of this model can be observed in Fig 1b, which shows the inhibition of the yeast population during storage in presence of high salt concentrations. The Weibull model was also recently applied to determine the inactivation of the olive spoiling yeast *Issatchenkia occidentalis* as a function of citric and sorbic acids [15], as well as to model the first phase of inhibition suffered by *L. pentosus* in alkaline wastewaters of the olive industry [23]. In another inactivation survey, the survival of the pathogen microorganism *Escherichia coli* O157:H7 during the fermentation of *Conservolea* green olives was studied by Spyropoulou et al. [24] by calculating the rate of death of the bacteria. Their work checked the effect of the addition of two different carbon sources (glucose and sucrose) and the use of LAB starter on this parameter. They found that *E. coli* O157:H7 was inhibited in all fermentation procedures, but inactivation was higher in treatments supplemented with starter cultures and sugars.

Recently, diverse primary models have started to be used in table olive fermentations to fit simultaneously both the growth and decay phases of microorganisms during table olive fermentation and storage, such as the quasi-chemical primary model [25], the Peleg model [18], or the Churchill and two-term Gompertz equations [26]. Finally, for cases in which the probability of growth is the only relevant factor, is useful to determine the growth/no growth boundaries of microorganisms as a function of environmental factors, and data may be scored simply as growth or no-growth.
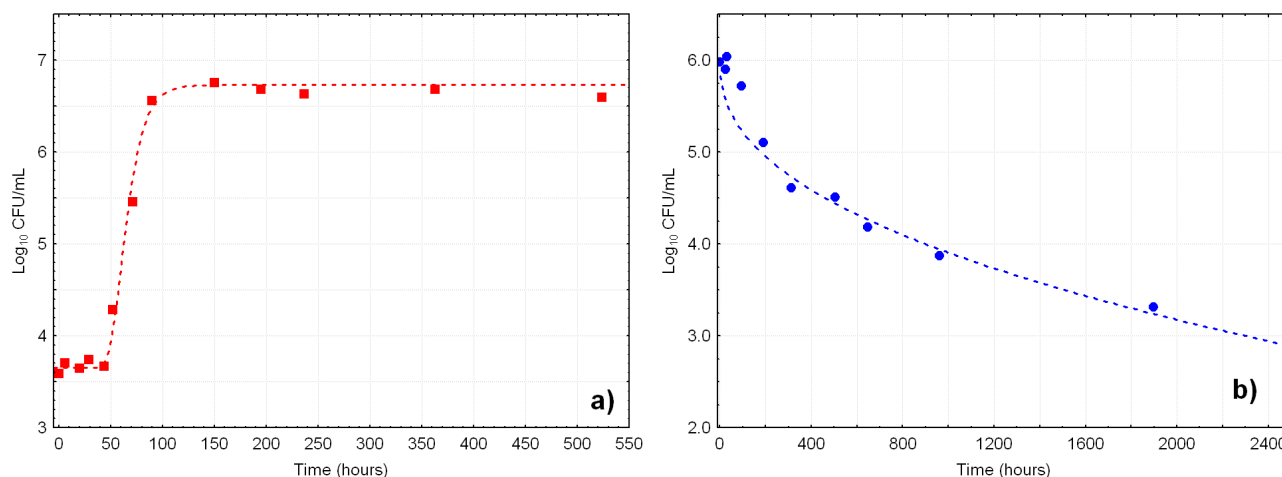
Fig. 1 Two example of primary modelling for a) yeast growth phase during packaging conditions (4.0% NaCl, 0.02% potassium sorbate, 0.1% citric acid), and b) yeast death phase during storage conditions (11.0% NaCl). Fits were carried out using the modified Gompertz equation and the Weibull distribution, in the cases of growth and inactivation, respectively.

3.2.4. Secondary modelling

Secondary models are built with parameters obtained from primary modelling (for instance lag time, growth/inactivation rate, growth/no growth data, etc.) and they are used to quantitatively characterize these parameters as a function of environmental conditions. Sometimes, primary model parameters need to be transformed (ln, $\log_{10}$, square root, etc.) before the modelling process in order to homogenise the variance of data and to improve the quality of the secondary model. Once the secondary model has been built, it can be used to predict the response of microorganisms against new combinations of environmental variables not included originally in the experimental design. These predictions may have obvious advantage in product development or stabilization. The most common variables used to build secondary models in olive fermentations have been the temperature, pH, salt and diverse types of acids and preservatives (lactic, citric, HCl, acetic, sorbic, benzoic, etc), because they can be easily modified by industry during elaboration. Secondary models can be simply non-linear regression [27], or more complex polynomial, probabilistic or artificial neural network models that require sophisticated computational software for data processing and analysis (briefly described below). By means of the mathematical analysis of secondary models, one is able to estimate in many cases the linear, quadratic and interactive effects of the environmental variables, and identify those with the highest influence on the response. As was mentioned previously, the type of secondary model used is closely related to the experimental design chosen.

3.2.5. Validation of the model

It is necessary to corroborate that the model makes good predictions before it can be used for food safety and quality decisions. Sometimes, the model is built under laboratory conditions, so validation in real food is essential. A set of new experiments, not included originally in the experimental design, are carried out and the observed responses are compared with those predicted by the models. In the case of table olive fermentations, the accuracy (A) and bias (B) factors [28] have been satisfactorily used for polynomial model validations [16, 20]. The accuracy (A) is the sum of absolute differences between predictions and observations and measures the overall model error. On the contrary, bias (B) is a multiplicative factor that is used to determine whether the model over- or under-predicts the growth response. In the case of probabilistic models, validation can be carried out determining the growth-no growth limits and formulating diverse combinations of the variables where the microorganism is not able to grow [15]. In any case, the model is valid only in the environmental region where it was built, and cannot be used to extrapolate the response of the microorganisms outside these limits.

4. Most important predictive models developed for table olives

4.1. Polynomial or response surface (RS) models

The RS models are capable of dealing with the effects of complex environmental factors on primary model parameters without any prior knowledge. They are usually used to estimate the linear, quadratic, cubic and interactive effects of environmental variables on microorganism response in term of kinetic data. The structure of an RS model is enough flexible to incorporate even very strong interactive effects by stating the order of the model. Despite their complexity, in

combination with primary models, RS models can provide reasonable predictions of the behaviour of microbes in food systems and for this reason they have been widely used in table olives to optimize the growth of both LAB and yeasts [14, 16, 20]. Table 1 shows the microorganisms and variables studied in table olive fermentations with this type of secondary model. RS models are purely empirical and have certain limitations. A higher order polynomial model, such as the third order, with a great number of coefficients, can be expected to show a better fit to primary model parameters, but it often produces greater topographic complexity with the presence of unrealistic hills and valleys. According to the principle of parsimony, a model should contain as few terms as possible. The decision to remove or include a term in the polynomial model only depends on whether or not the regression coefficient for the term has a significant effect on the predictive capability of the model. The sign and value of regression coefficient show the correlation and influence on the modelled parameter. Due to their complexity for a high number of variables, normally this type of model is only used to estimate the effects of no more than three environmental variables. For this reason, a screening experimental design (as Plackett-Burman design) is recomended in a first step to limit the number of environmental factors to study in detail, using then a central composite or D-optimal experimental design for the 2-3 variables with the highest effects. This approach was satisfactorily applied to model the production of bacteriocins and growth in the LAB strains *L. plantarum* 17.2b and *L. pentosus* B96 [7, 29]. An RS secondary model based on a factorial design was used by Panagou et al. [30] to determine the combined effect of temperature, pH and NaCl on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table olives, while an RS based on a simplex-centroid mixture design was applied by Echevarria et al. [25] to determine the effects of sodium metabisulphite, sodium chloride and ascorbic acid on the growth and survival of yeasts and LAB during the spontaneous fermentations of green cracked Manzanilla-Aloreña olives. This was possible because the authors used for the first time in table olive fermentations a Quasi-chemical primary model, which integrates the four phases of microbial life into a series of chemical reaction steps, with associated rate constants, which are then solved by means of a system of ordinary differential equations. As an example, Figs 2a and 2b show the graphical representation of a polynomial secondary model obtained for the growth parameters of *L. pentosus* and *P. anomala*.
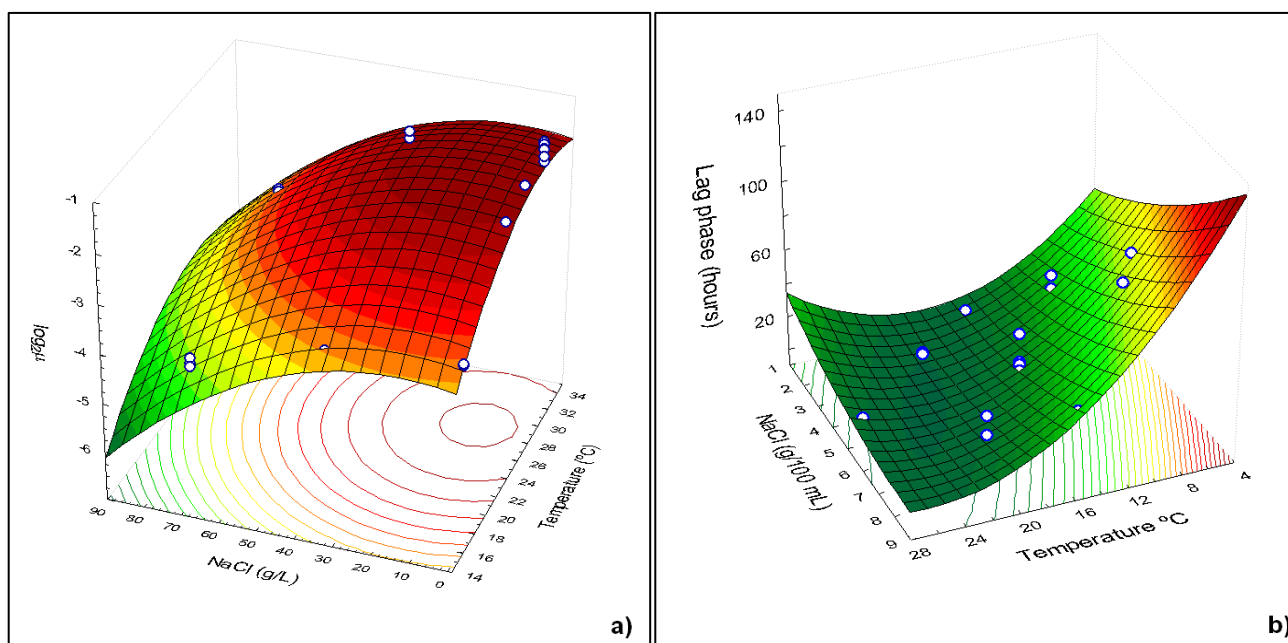

Fig. 2 Response surface secondary models for the growth parameters a) maximum specific growth rate of *Lactobacillus pentosus*, and b) lag phase of yeast *Pichia anomala*, as a function of temperature and NaCl concentration (in physical values). The presence of curvature in the temperature and NaCl axes is indicative of a quadratic effect for both environmental variables.

4.2. Probabilistic models

These types of models are used to determine the growth/no growth interface of microorganisms as a funtion of environmental variables. In other words, they determine the region where the microorganisms are able to grow and under which conditions they are not. Probabilistic models incorporate growth/no growth data (binary response) which are procceed by means of a logistic regression that relate the probability of growth (p) and no-growth (1-p) to the environmental factors assayed. A higher number of experiments are neccesary compared to polynomial models, but the great adventage is that they can be easily automatized by means of OD measurements. Moreover, these models can include a high number of environmental variables and levels to analyze. An important feature of these models is that the level of probability can be set depending on the level of stringency required, obtaining different growth/no growth

boundaries as a funtion of the risk that one sets to assume. Therefore, probabilistic models have a direct application to formulate packaging conditions that inhibit microorganism growth, optimizing the minimum levels of preservatives that guarantee their inhibition. In this way, the effects of diverse additives to inhibit olive spoilage growth have been assayed in table olive packaging using this methodology [15, 17]. A probabilistic model was also used to determine the growth-no growth boundaries of the spoilage fungus *Monascus ruber* as a funtion of temperature, pH and NaCl [31] (see Table 1).

4.3. Neural network (NN) models

A NN model is a computer program which learns or is trained from examples through iteration and automatically derives the mathematical formulae to map the relationships between the input and output data, without any prior knowledge of their relationships. The basic construction of NN consists of input, hidden, and output layers, which are composed of neuros that transmit information among the layers. NN is capable of operating with a large number of neurons at the same time, and for this reason it has been employed in predictive microbiology as an alternative to conventional regression models because of its ability to describe highly complex non-linear problems. The advantage of the use of the NN model is derived from the remarkable processing of information characteristics, such as a) nonlinearity, b) noise intensity, c) learning and adaptativity, d) high paralelism, and e) generalization. A NN model normally has no restriction on the type of relationship between the growth parameters (input patterns) and the desired outputs. Compared with RS models, the NN model is more versatile, flexible and less restrictive and it does not impose assumptions pertaining to the form of functions. When NN is trained on the appropriate data set (supervised data learning), it can then be used to predict values for unseen cases (generalization) within the experimental region assayed. In table olives, an NN model was used as a one-step procedure to determine its applicability for fitting the response-time curve for diverse LAB strains during green olive fermentations [32]. The model simulated the growth and survival of the LAB strains quite accurately throughout the fermentation process, and equally well as the logistic-Fermi and the two-terms Gompertz function.

4.4. Susceptibility and resistance models

Lambert and Pearson [33] developed a simple method for the estimation of the minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) of a determined compound using turbidimetry. The procedure relates the area under the OD/time curves to the degree of inhibition observed, using the ratio of control (absence of inhibitor) to that of the tests (progressive concentrations of inhibitor), termed as fractional area (*fa*). As the amount of inhibitor in the well increases, the effect on the growth of the organism also increases. This effect on the growth is expressed by a reduction in the area under the OD/time curve relative to the positive control (optimal conditions) at any specified time. The plot of *fa* vs log inhibitor concentration produces a sigmoid-shaped curve, which can be fit by a modified Gompertz function [33]. The great advantage of this procedure is that it permits the use of all the growth information to deduce the MIC, while the tube dilution series or its extension to the microtitre wells (based on the demarcation between growth-no growth and the concentration of inhibitor in the well with no growth) usually discard all the growth information below the MIC concentration. The whole sigmoid-shaped curve is divided into three sections: points corresponding to concentrations from zero up to the NIC (concentrations at which no effect of the inhibitor is observed), concentrations between NIC and MIC, within which growth inhibition progressively occurs, and a third section, above MIC, where no growth relative to the control is recorded. With this simple idea in mind, Bautista-Gallego et al. [27] determined the MIC and NIC values of diverse chloride salts on the widely extended table olive microorganisms *S. cerevisiae* and *L. pentosus*. They found that calcium chloride and sodium chloride showed very similar effects between them to control the growth of both microorganisms. A similar methodology was also recently used by Arroyo-López et al. [34] to estimate the MIC and NIC values of the sorbic and benzoic acids at selected pH values on a native yeast cocktail isolated from table olives (formed by species *S. cerevisiae, P. anomala, Candida diddensiae* and *I. occidentalis*).

4.5. Models based on the gamma concept

Under completely optimal growth conditions each microorganism has a reproducible optimum growth rate. As any environmental factor becomes suboptimal, the growth rate declines in a predictive manner and the extent of that inhibition can be related to the optimum growth rate by calculating the relative rate at the test conditions compared to that of the optimum. Under the gamma concept approach, the cumulative effect of many factors at suboptimal levels can be estimated from the product of the relative inhibition of the growth rate due to each factor. The relative inhibitory effect of a specific environmental variable is described by a growth factor called gamma, a dimensionless measure that has a value between 0 and 1. The relative inhibitory effect can be determined from the distance between the optimal level of the factor and the minimum (or maximum) level that completely inhibits growth by recourse to a predictive model. Then, the combined effect of several environmental variables is determined by multiplying their respective gamma factors. Therefore, the gamma concept is based on the fact that many factors that affect microbial growth rate act independently, and that the effect of each measurable factor on growth rate can be represented by a discrete term

that is multiplied by terms for the effects of all other growth rate affecting factors. Thus, the effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate. This concept was satisfactorily applied by Panagou et al. [30] to model the maximum specific growth rate of the olive spoilage fungus *Monascus ruber* as a function of the gamma-factors temperature, pH and water activity (a_w).

Table 1 Most important predictive models recently developed for table olives

Type of model	Microorganisms	Environmental variables	Substrate	Reference
Polynomial	*Lactobacillus plantarum* *Debaryomyces hansenii*	NaCl, calcium acetate, calcium lactate, KCl	Olive juice	Tsapatsaris and Kotzekidou [14]
Polynomial	*Pichia anomala*	Temperature, NaCl, pH	Laboratory medium	Arroyo et al. [20]
Polynomial	*Lactobacillus pentosus*	Temperature, NaCl, type of acid (citric, acetic, lactic, HCl)	Laboratory medium	López et al. [16]
Polynomial	*Lactobacillus plantarum*	Temperature, NaCl, pH	Laboratory medium	Delgado et al. [7]
Polynomial	*Lactobacillus pentosus*	Temperature, NaCl, pH	Laboratory medium	Delgado et al. [29]
Polynomial	*Monascus ruber*	Temperature, pH, a_w	Laboratory medium	Panagou et al. [30]
Polynomial	Yeasts and lactic acid bacteria	Ascorbic acid, NaCl, sodium metabisulphite	Cracked green olive fermentations	Echevarria et al. [25]
Probabilistic	*Issatchenkia occidentalis*	NaCl, citric, sorbic acid	Laboratory medium and olive brine	Arroyo-López et al. [15]
Probabilistic	*Saccharomyces cerevisiae*	NaCl, potassium sorbate, type of acid (citric, lactic and acetic)	Laboratory medium and olive brine	Arroyo-López et al. [17]
Probabilistic	*Monascus ruber*	Temperature, pH, NaCl	Laboratory medium	Panagou et al. [31]
Neural Networks	Lactic acid bacteria	Fixed by fermentation conditions	Green olive fermentations	Panagou et al. [32]
Susceptibility and resistance	*Saccharomyces cerevisiae* *Lactobacillus pentosus*	NaCl, KCl, $CaCl_2$, $MgCl_2$	Laboratory medium	Bautista-Gallego et al. [27]
Susceptibility and resistance	Yeast cocktail from table olives	Sorbic acid, benzoic acid, pH	Laboratory medium	Arroyo-López et al. [34]
Gamma concept	*Monascus ruber*	Temperature, pH, a_w	Laboratory medium	Panagou et al. [30]

5. Advantages on the use of predictive microbiology in table olives

Predictive microbiology can improve table olive elaboration in many ways. Briefly, it can be used to:
  a) Increase the safety and quality of the final product, finding the combination of the environmental variables that inhibit the growth of pathogen or spoilage microorganisms (*Clostridium, Enterobacteriaceae,* etc.).
  b) Reduce the length of the fermentation period, finding the combination of factors that favour the growth of desairable microorganisms (LAB and yeasts), or the imposition of a 'starter' culture.
  c) Study the interactions between microorganisms during table olive fermentation or storage, which is very useful in the development and design of 'starters'.
  d) Optimize the concentration of preservatives during packaging that garantee the inhibition of microorganisms.
  e) Estimate the shelf life of packed table olives.
  f) Objectively evaluate the application of new processess or additives on microorganism growth.
  g) Optimize the production of desairable metabolites by microorganisms (lactic acid, bacteriocins, aromas, etc.) as a funtion of environmental variables.

Certainly, the implementation of this technology to table olive fermentation and packaging definitively will favour the elaboration of a more homogeneous and controlled product.

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