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Uma abordagem do uso de microbiologia preditiva para formação de biofilmes

An approach on the use of predictive microbiology for biofilm formation

Un enfoque al uso de microbiología predictiva para la formación de biofilms

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Resumo

É necessário garantir a qualidade e a segurança dos alimentos durante todas as etapas da produção de alimentos. O grande desafio no setor de alimentos é o controle da multiplicação microbiana, pois os microrganismos estão buscando alternativas, que envolvem o seu desenvolvimento tanto na forma livre quanto em biofilmes, para sobreviver a ataques ambientais. Devido a essa preocupação, os pesquisadores usam novas estratégias para entender a dinâmica do crescimento microbiano. Nesse contexto, a microbiologia preditiva está ganhando espaço na microbiologia de alimentos. O objetivo do estudo foi verificar se os atuais modelos preditivos são adequados para prever também o crescimento de células sésseis além das planctônicas. Realizou-se um levantamento bibliográfico sobre a aplicação da microbiologia preditiva na avaliação do controle de segurança alimentar e concluiu-se que, devido à escassez de estudos, não foi possível afirmar a adequação de modelos terciários no controle de biofilmes durante a produção de alimentos. Destaca-se a necessidade de estudos que possam modelar a formação de biofilme de patógenos sob diferentes fatores ambientais.

Palavras-chave: Análise bibliométrica; Células planctônicas; Células sésseis; Controle de qualidade; Predição microbiana.

Abstract

It is necessary to ensure food quality and safety during all stages of food production. The major challenge in the food sector is the control of microbial multiplication, as microorganisms are increasingly looking for alternatives, which involve their development, both in free form as in biofilm, to survive environmental attacks. Due to this concern, researchers use new strategies to understand the dynamics of microbial growth. In this context, predictive microbiology is gaining space in food microbiology. Therefore, the objective of the study was to verify whether the current predictive models are adequate to predict the growth of sessile cells, as well as planktonic cells. A bibliographic survey on the application of predictive microbiology in the evaluation of food safety control was carried out, and we concluded that, due to the scarcity of studies, it was not possible to state the adequacy of tertiary models in the control of biofilms during food production. We highlight the need for studies that can model the formation of biofilm of pathogens under different environmental factors.

Keywords: Bibliometric analysis; Microbial prediction; Planktonic cells; Quality control; Sessile cells.

Resumen

Es necesario garantizar la calidad e inocuidad de los alimentos durante todas las etapas de la producción de alimentos. El principal desafío en el sector alimentario es el control de la multiplicación microbiana, ya que los microorganismos buscan alternativas, que implican su desarrollo tanto en forma libre como en biofilms, para sobrevivir a los ataques ambientales. Debido a esta preocupación, los investigadores utilizan nuevas estrategias para comprender la dinámica del crecimiento microbiano. En este contexto, la microbiología predictiva está ganando espacio en la microbiología de los alimentos. El objetivo del estudio fue verificar si los modelos predictivos actuales son adecuados para predecir el crecimiento de células sésiles, así como se usan para planctónicas. Se realizo una búsqueda bibliográfica sobre la aplicación de la microbiología predictiva en la evaluación del control de seguridad alimentaria. Con base en la investigación realizada, se concluyó que, debido a la escasez de estudios, no fue posible afirmar la idoneidad de los modelos terciarios en el control de las biopelículas durante la producción de alimentos. Destacamos la necesidad de estudios que puedan modelar la formación de biopelículas de patógenos bajo diferentes factores ambientales.

Palabras clave: Análisis bibliométrico; Células planctónicas; Células sésiles; Control de calidad; Predicción microbiana.

1. Introduction

Food safety is becoming one of the main concerns of the food sectors every day. Its relevance is attributed to the constant search of consumers for quality products free of physical, chemical and biological contaminants. Amid this problem, the focus of the industry and researchers is the control of microbial dissemination, considering that microorganisms are the main types of contaminants that cause damage to public health worldwide.

Contamination by both planktonic and sessile cells causes deeply worrying during all stages of the production chain. This involves the food industry since most microorganisms are resistant to environmental attacks. They have easy adhesion to contact surfaces and, consequently, form biofilm communities that are difficult to control (Xu et al., 2019). Predictive microbiology arises to prevent this problem, which is a new approach applied to understand the dynamics of biofilm formation, gaining space in food microbiology.

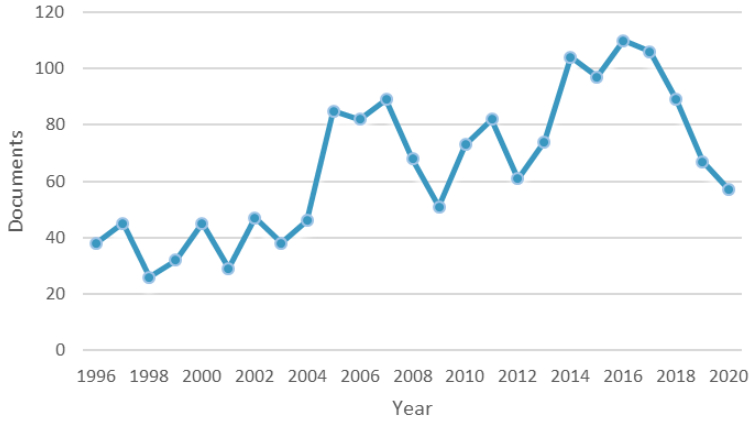
In predictive microbiology, several mathematical models are applied to study the dynamics of microbial growth and inactivation. The application of these tools is important because they contribute to the prevention of food risks and bring a more precise answer concerning food safety and quality (Akkermans et al., 2018). Although there are several predictive models available for free access that model the growth of planktonic cells, still, there are few studies that address modelling for the formation of biofilm in food matrices, surfaces, and industrial equipment.

Given the challenge in the search for new approaches for the prevention of microbial contamination and improvement in the quality of food, the objective of the study was to verify whether the current predictive models are adequate to predict the growth of sessile cells, as well as planktonic cells.

2. Bibliographic Survey on Predictive Microbiology Studies

Regarding the importance of studying the dynamics of microbial behavior using predictive microbiology, it's verified the relevance of analyzing the degree of impact that this subarea of traditional microbiology is causing in research. Given this, according to a survey carried out in the main ScienceDirect and Scopus databases that are available at Capes Journal System, there are many articles involving the keyword predictive microbiology. It was found a total of 1,750 results on ScienceDirect between the years 1996 to 2020, highlighting the year 2016 that obtained more publications involving this research line, according to Figure 1.

Figure 1: Publication data by year.

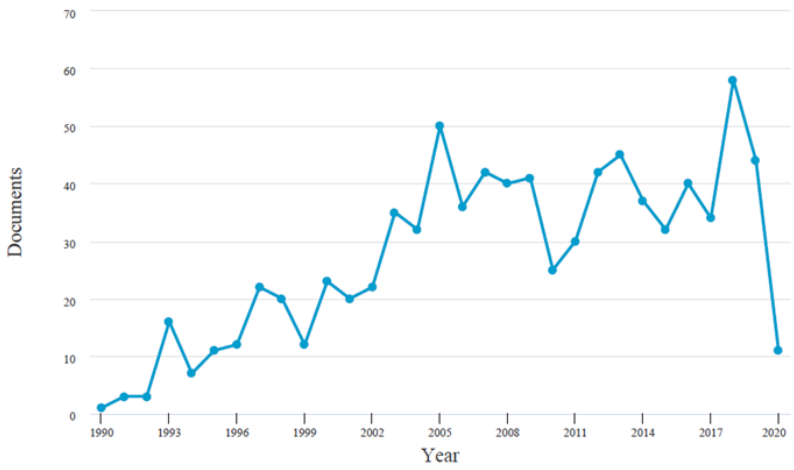


Source: ScienceDirect database.

According to data collected on ScienceDirect, the main journals that received the most publication between 1996 and 2020 were, International Journal of Food Microbiology, Food Microbiology, Food Control, Food Research International, Journal of Food Engineering, Trends in Food Science & Technology, LWT, Encyclopedia of Food Microbiology, Meat Science and Innovative Food Science & Emerging Technologies.

Regarding the results of the Scopus database, 867 documents were found between the years 1990 to 2020, with the highest publication in the year 2018 and the lowest in the year 1990 according to Figure 2.

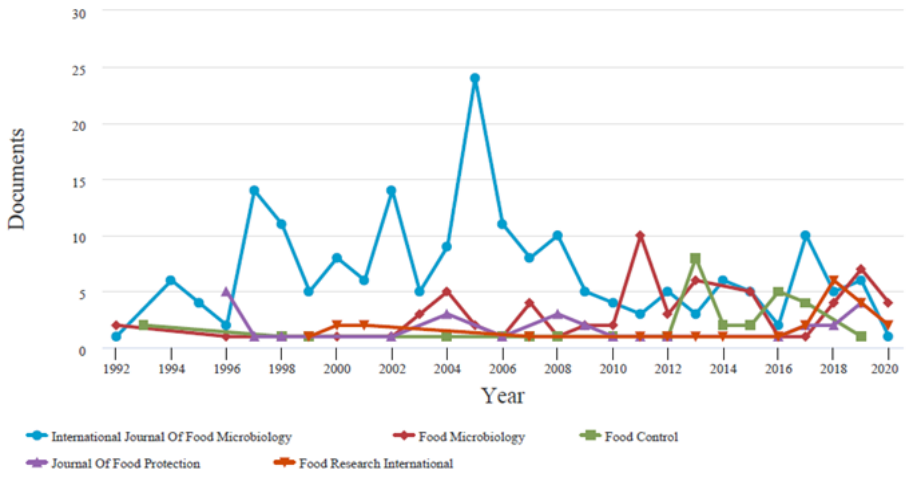
Figure 2: Publication data by year.



Source: Scopus database.

According to the data collected on Scopus, the main journals that received most publication between the years 1990 to 2020 were, International Journal of Food Microbiology, Food Microbiology, Food Control, Journal of Food Protection and Food Research International, as presented in Figure 3.

Figure 3: Published documents by year and source.



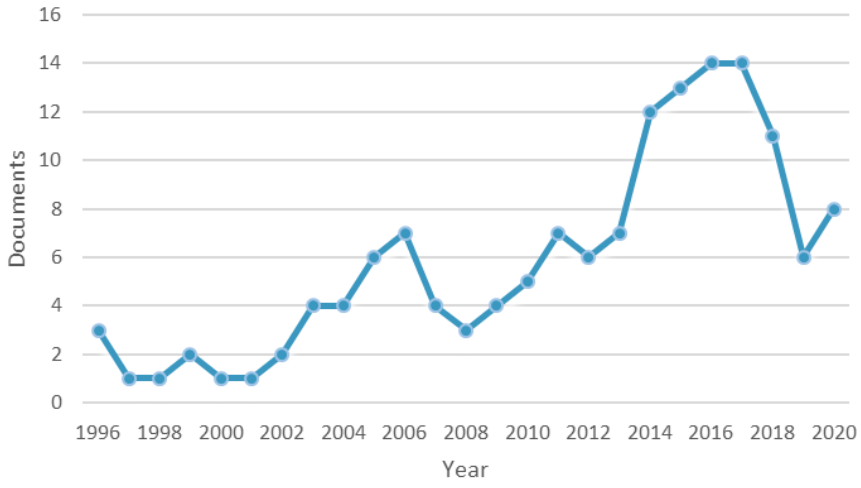
In addition to these journals found on both Scopus and ScienceDirect, there are many others available for submitting research, and among these published documents on predictive microbiology, the main types are research articles, review articles, encyclopedia and book chapters.

2.1. Bibliographic survey on biofilms prediction

In addition to the survey on the amount of publication using the keyword predictive microbiology, another collection of information about studies that addresses the application of these predictive models in biofilms was also carried out, with the result that the keywords used were predictive microbiology and biofilms. A total of 8 documents were found in the Scopus database between the years 2007 to 2020 with the highlight of more publications in 2018, and the journals that received such publications were Food Microbiology, Food Control, Journal of Applied Microbiology and Water Practice and Technology.

In ScienceDirect, 151 publications were found between 1996 to 2020, with more publications in the years 2016 and 2017 highlighted, as shown in Figure 4.

Figure 4: Published data of biofilms prediction by year.



Source: ScienceDirect.

The main journals that received the most publication between 1996 and 2020 were International Journal of Food Microbiology, Food Microbiology, Food Control, Encyclopedia of Food Microbiology, Food Research International, Innovative Food Science & Emerging Technologies, LWT, Food and Bioproducts Processing and The Microbiological Quality of Food. The increase in publications, especially in the last years, shows that the scientific community is increasingly committed to the search for methodologies that can offer reliable responses in the dynamics of microbial multiplication in food.

According to the documents analyzed, it was found that they address the importance of applying predictive microbiology in improving food quality and safety. Microbial prediction is applied in several studies, in general, the common points showed in these studies are related to the assessment of growth dynamics and microbial inactivation.

3. Food Safety

Ensuring food safety must be a priority in all industrial sectors, as the safety and quality of products intended for human consumption have significant implications for nutrition and

public health, in addition to contributing to domestic and international economic development (Boatemaa et al., 2019; Zhang et al., 2019).

Despite being a priority, the food sector still faces a major challenge to become free from contaminations, which are most often caused by microorganisms (Fukuda, 2015). Microorganisms have a fast multiplication speed that makes it increasingly difficult to control in the food processing industries. Therefore, they can be the main responsible for foodborne outbreaks and food deterioration during all stages of production and cause a serious public health problem.

In addition to foodborne diseases, microorganisms also cause serious economic problems due to the deterioration of food products, where it is estimated that around 1.3 billion tons of food can be wasted worldwide each year (Bräutigam, Jörissen, & Priefer, 2014). For this reason, food safety worldwide gains more attention, especially in developed countries because the food supply is abundant (Marino et al., 2018).

Control over microbial multiplication is the food-sector major challenge, as microorganisms are increasingly looking for alternatives to survive environmental attacks. The formation of organized communities called biofilms is one of these strategies for microorganisms to survive due to adhesion on surfaces with the formation of a microenvironment capable of protecting them against antimicrobial agents (Gutiérrez, 2016).

Hence, the microbiological community responsible for food safety applies several security systems, such as: Standard Sanitation Operating Procedures, Good Manufacturing Practices, Hazard Analysis of Critical Control Points, as well as alternative ways for the prevention of risks that affect the quality of food. These alternative tools are predictive modelling that can be used in all food sectors to prevent microbial risk, both in planktonic cells and in the sessile form (Plaza-Rodríguez et al., 2018).

The use of mathematical modelling grows in the field of food microbiology, being a subarea of traditional microbiology. Despite the various limitations of a predictive model, it provides an objective and quick means of estimating the degree of food safety. For this reason, more research addresses the importance of studying microbial behavior using predictive microbiology as a means that will respond to the environmental variables responsible for microbial multiplication and contamination.

4. Predictive Models

Predictive microbiology is a science that studies the application of mathematical models to describe the dynamics of the development of microorganisms in surfaces exposed to different environments. The use of predictive microbiology to quantify the risks associated with food products use tools such as software and online platforms that aim to predict the ideal conditions in the microorganism's development, inactivation, and survival. This area of microbiology provides quantitative information on risk analysis and the probability of a hazard occurring; however, this study is used as an alternative to conventional microbiological analyses, through a more detailed approach on microbial behavior and possible ways of inactivation (González et al., 2019).

Most of the current models used in predictive microbiology are continuous models that describe the microbial population using equations capable of observing and evaluating safety in guaranteeing the quality of a product or technological process in the food industry. Table 1 shows the definition of some of these computational tools used in microbiological predictions.

Table 1: Description of the main tertiary models used in predictive microbiology.

SOFTWARE PACKAGE	DESCRIPTION
ComBase	ComBase provides a database with more than 50,000 records to describe the evolution (growth and inactivation) of various microorganisms in culture media or different foods. It also provides some assembly and simulation modules for growth and inactivation. Twenty microorganisms are considered.
PMP (Pathogen Modelling Program)	PMP is a package of models that can be used to predict the growth and inactivation of foodborne bacteria, especially pathogens, under various environmental conditions.
MicroHibro	MicroHibro evaluates and validates the parameters of a primary model based on experimental data. The implementation of risk assessment models, followed by a model sensitivity analysis, is also possible based on the combination of several basic processes and the use of the database of predictive models provided.

GroPIN	The current version of GroPIN has a total of 367 published models to predict the behavior of 29 pathogens and 43 deteriorators, including bacteria, molds and yeasts. A database of predictive models is also provided and can be implemented with new models.

Source: Adapted from Tenenhaus-Aziza and Ellouze (2015).

The various tools for food safety available on online platforms are classified into primary, secondary, and tertiary mathematical modelling software packages. The primary models are related to changes in the number of microorganisms over time, under a single set of conditions, while the secondary models describe how the primary parameters vary with environmental conditions, and the tertiary models use the primary and secondary models. For the construction of computer software or specialized systems that calculate the behavior of microorganisms under various environmental conditions (Baranyi & Roberts, 1995; Forsythe, 2013; Huang, 2014).

The software packages presented are software used as alternative prevention tools that assess microbial behavior. Their functionality occurs through the provision of data by researchers, these data are used to support the growth and inactivation dynamics of microorganisms by several professionals from food safety area. Among the most used is ComBase, which has been widely applied in different studies that model the kinetics of the behavior of microorganisms, both pathogenic and deteriorating in different foods. Some online platforms offer databases such as ComBase and programs such as the Pathogen Modelling Program (PMP), these packages can be used easily and efficiently and do not require complex mathematical skills for their use (Ferrer et al., 2009).

Predictive models are diverse and were developed by researchers involved in mathematical modelling together with microbiologists to anticipate the needs of the food industry and provide answers to the varied questions about the life of food concerning the accelerated microbial development throughout the production chain (Guillier, 2016). These models serve as answers to the hypotheses studied in each field of research under a set of circumstances with the ability to more intelligently predict, control or improve the performance of the operation of a given system (Mcmeekin et al., 2008).

4.1. Prediction of microbial multiplication

The application of mathematical models in microbial growth is a way of predicting the behavior of these contaminating agents, since the microorganisms have a capacity for rapid mutation and acquisition of new genes throughout the food production processes, thus contributing to cross-contamination and consequently in reducing product quality. Given this problem, food safety can be improved by assessing the defense and prevention mechanisms of microbial behavior concerning the intrinsic and extrinsic factors that will provide answers to all stages in the food production chain. The various predictive models used in predictive microbiology will serve to describe the behavior of contaminating agents in different physical-chemical conditions, allowing the prediction of microbial development in the lag, exponential, and decline or death phases (Akkermans & Impe, 2018).

Microbial multiplication prediction is described for the bacteria in the free form; however, new approaches appear to describe the prevention in the formation of biofilms, which involves phenomena of the environment in which the microorganisms then present, as well as their biochemical conditions. These new approaches in predictive microbiology describe the use of substrates that contribute to the dynamics of formation and the structure of the biofilm. Concerning these aspects, more studies are emerging that describe adequate conditions for the formation of biofilms under various environmental factors (Tango et al., 2018).

The meat products industry, for example, is an adequate environment where bacteria find satisfactory conditions for adhesion on product processing surfaces and biofilm formation. Also, the risk of cross-contamination during the stages is concerning because the bacterial cells detach from the biofilm and contaminate the food when it meets this contaminated equipment and surfaces (Giaouris et al., 2014). In the dairy industry, biofilm formation is also due to flaws in the hygiene processes of surfaces and equipment, both during milking and in the processing of dairy products, this contamination can lead to product deterioration as well as economic losses (Latorre et al., 2010). And the main factors that influence microbial growth and later the formation of biofilm in milk is due to its neutral pH and the many nutrients in its composition (Mogha et al., 2014).

For the industry, it is important to identify the factors involved in the formation of biofilm by pathogenic microorganisms, so that proper control can be taken in combating multiplication and, subsequently, forming these communities that are more resistant to antimicrobial agents than bacteria in its planktonic form (Ciccio et al., 2015). Therefore, the use of microbial prediction models is important in the discovery of factors that may contribute

to the prevention of the multiplication of microorganisms and, consequently, the formation of biofilms.

5. Growth Models for Sessile and Planktonic Cells

Predictive models to assess the multiplication of foodborne pathogens are developed and applied to better understand the ideal growth conditions for these contaminants. Also, they are important in risk assessment, as they determine the critical points of control throughout the food production chain. As an example, a study was carried out to evaluate the growth and inactivation dynamics of *Salmonella Typhimurium* and *Staphylococcus aureus*, where Baka et al. (2017) used the ComBase database, which is a tertiary model that served to investigate the storage temperatures of fish products. In another study, Diallo and Bogaerts (2016), also used ComBase to model the growth of *Listeria monocytogenes* in cooked and salted prawns under different temperatures, to predict the shelf life of this food.

Primary and secondary models were compared by Lee et al. (2015) to develop predictive models that describe the behavior of *S. aureus* isolated from raw pork based on the variables time and temperature during storage. These models were evaluated and compared using a new generation IPMP 2013 software that served to develop the main predictive models. The conclusion was that the development of a secondary model using other intrinsic and extrinsic factors was impossible, as the objective of developing a secondary model serves as a definition for the parameters of a primary model that was affected by temperature.

In addition to the concern with developing models capable of describing the growth of S. aureus, it is relevant to study the formation of staphylococcal enterotoxin during food production. According to this question, a dynamic predictive model was developed by Gunvig et al. (2018) to predict the formation of enterotoxins by *S. aureus* during the heat treatment and fermentation of meat products, analyzing the variables temperature, pH, nitrite, and sodium content. In conclusion, the authors affirm that the models are relevant for the prediction of the increase of S. aureus and serve to evaluate if the production of enterotoxins is possible during the processing stages of meat products.

The prediction of biofilm formation is also of great interest to the food processing industry, for this reason, some studies report on the use of predictive tools in the formation of biofilms by some microorganisms. In a study by Moraes et al. (2018), the capacity of biofilm formation by *Salmonella enterica* on stainless steel surfaces was analyzed as a function of pH, temperature and NaCl concentration using secondary models. The conclusion was that the

models showed a good fit to the experimental data and the constructed equations showed the adequate performance to predict the limits of adhesion and biofilm formation. Dimakopoulou-Papazoglou, Lianou and Koutsoumanis (2016) also evaluated the biofilm formation by *S. enterica* under conditions of pH and water activity from the development of a Cardinal Parameter Model that is part of the group of secondary models and concluded that the models constitute a good relevance for quantification of interactions of environmental factors in biofilm growth.

There are studies in the literature on the modelling of various biofilm-forming pathogens, however, concerning the prediction of biofilm formation by *S. aureus*, there are still few studies that use mathematical modelling to predict this formation. The study by Tango et al. (2018) modeled the formation of biofilm by *Staphylococcus aureus* concerning different pH ranges, water activity, and ethanol concentration, from the development and validation of a secondary predictive model that described the effects of these environmental factors on the formation of biofilm. It concluded that this model was able to evaluate the ideal rate of formation of biofilm of *S. aureus*, also, it can be applied in the assessment of risks in real food matrices, thus contributing to safety in the food industries. This study showed that although there are no data available on the influence of environmental factors on the formation of the biofilm of *S. aureus*, these models of cardinal parameters may allow describing the environmental effects on the alternation between planktonic cells and biofilm.

Predictive models are the most important and effective ways for investigating changes in microbial populations in food, in addition to contributing to the microbiological risk assessment process (Messens, Hempen, & Koutsoumanis, 2018). Because of this importance, in the last 25 years, there has been a great increase in information technologies due to the increase in the various predictive models. Through these available tools, academic researchers and the food industry can obtain knowledge from the variety of information on the multiplication, inactivation, or survival dynamics of most pathogens (Tenenhaus-Aziza & Ellouze, 2015).

6. Final Considerations

According to the research carried out, it is clear that there are many studies on predictive models applied to planktonic bacteria, but on the other hand, there are still few studies that use mathematical models to control the formation of biofilm communities.

Although the predictive models provide useful information in the control of microbial growth during food production, no attempt has been made to create a mathematical model that can be implemented on online platforms such as ComBase, to verify the dynamics of biofilm formation by microorganisms.

Therefore, there is a need to develop more studies that can assess whether these current models are adequate to model the formation of the biofilm of pathogens under different environmental factors, in addition to those that already exist, or whether it will be necessary to develop more studies for the implantation of new predictive models.

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