The Gamma concept approach as a tool to predict fresh produce supporting or not the growth of *L. monocytogenes*

Marisa Gomez-Galindo [a], Cristina Serra-Castelló [b,1], Sara Bover-Cid [b], Pilar Truchado [a], Maria I. Gil [a], Ana Allende [a,*]

[a] *Research Group on Microbiology and Quality of Fruit and Vegetables, CEBAS-CSIC, Murcia, Spain*
[b] *IRTA, Food Safety and Functionality Program, Finca Camps i Armet s/n, 17121, Monells, Spain*

ARTICLE INFO

ABSTRACT

Challenge tests are commonly employed to evaluate the growth behavior of *L. monocytogenes* in food matrices; they are known for being expensive and time-consuming. An alternative could be the use of predictive models to forecast microbial behavior under different conditions. In this study, the growth behavior of *L. monocytogenes* in different fresh produce was evaluated using a predictive model based on the Gamma concept considering pH, water activity (a_w), and temperature as input factors. An extensive literature search resulted in a total of 105 research articles selected to collect growth/no growth behavior data of *L. monocytogenes*. Up to 808 *L. monocytogenes* behavior values and physicochemical characteristics were extracted for different fruits and vegetables. The predictive performance of the model as a tool for identifying the produce commodities supporting the growth of *L. monocytogenes* was proved by comparing with the experimental data collected from the literature. The model provided satisfactory predictions on the behavior of *L. monocytogenes* in vegetables (>80% agreement with experimental observations). For leafy greens, a 90% agreement was achieved. In contrast, the performance of the Gamma model was less satisfactory for fruits, as it tends to overestimate the potential of acid commodities to inhibit the growth of *L. monocytogenes*.

1. Introduction

Listeria monocytogenes, a Gram-positive bacterium, poses a persistent risk throughout the food supply chain, frequently found in fresh fruits, vegetables, and environments associated with perishable product processing (Gil et al., 2024; Spanu and Jordan, 2020; de Simón et al., 1992; Townsend et al., 2022; Zhu et al., 2017). Contamination can occur at any stage from cultivation to consumer preparation, making it a significant challenge for the fresh produce industry (Buchanan et al., 2017; EFSA and ECDC, 2018; IFSAC, 2021; Townsend et al., 2022). Foodborne outbreak reports highlight fresh produce as a potential source of listeriosis, although many cases go unrecorded in outbreak databases, potentially leading to an underestimation of its impact. This emphasizes the need for improved diagnosis and surveillance tools (Desai et al., 2019; EFSA and ECDC, 2018; FAO/WHO, 2022; Magdovitz et al., 2020).

It has been demonstrated that *L. monocytogenes* can actively proliferate in many fruits and vegetables, such as lettuce and cabbage, while others are poor substrates for its growth (Botticella et al., 2013; Girbal et al., 2021). The behavior of *L. monocytogenes* in different fresh produce matrices is affected by numerous factors, including their composition and structure (e.g. natural antimicrobial compounds, crevices or cracks on surfaces), pH, type of packaging (e.g. modified atmosphere packaging (MAP)), and storage temperature (Berrang et al., 1989; Fulano et al., 2023; Jovanovic et al., 2016; Nyarko et al., 2016a; Truchado et al., 2020; Ukuku and Fett, 2002). Therefore, knowing that *L. monocytogenes* growth, during the stages of retail and consumer handling, contributes to elevate the risk of listeriosis (Ricci et al., 2018; EFSA and ECDC, 2018), it's evident that not all fruits, vegetables, and their respective processing and storage methods will yield equivalent levels of contamination upon consumption. Consequently, they present varying degrees of risk to public health.

To optimize resources while reducing risk, it is necessary to identify the most susceptible fruits and vegetables and conditions that pose a higher risk. However, a proper classification of *L. monocytogenes* risk in

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* Corresponding author.
E-mail address: aallende@cebas.csic.es (A. Allende).
[1] Present address: Cristina Serra-Castelló, Laboratory of Food Microbiology, Wageningen University, PO Box 17, 6700 AA Wageningen, The Netherlands.

fresh produce, including the identification of the most relevant fruits and vegetables which support the growth of *L. monocytogenes*, as well as the identification of factors that might enhance survival and growth of *L. monocytogenes* in fresh produce, is still lacking.

Challenge tests are commonly used to study the growth potential of *L. monocytogenes* in a food matrix. However, challenge tests with foodborne pathogens are expensive, time-consuming, impossible to implement in real facilities, and challenging to simulate real conditions in laboratories and pilot plants. One alternative is to use the vast amount of worldwide available data regarding the growth of *L. monocytogenes* in fresh fruits and vegetables (Farber et al., 2021), in conjunction with the application of mathematical predictive models to simulate the behavior of the pathogen as a function of the most critical factors. Several mathematical models have been developed that can describe the combined effect of different factors (i.e. temperature, pH, a_w, and gas composition) on the growth of *L. monocytogenes* (te Giffel and Zwietering, 1999; Girbal et al., 2021). The models based on the Gamma concept can be used to characterize the effect of different factors on the behavior of microorganisms in food. This approach relies on the hypothesis that measurable intrinsic characteristics (e.g. pH, a_w) and extrinsic environmental factors (e.g. temperature) affect the growth rate of microorganisms independently. The growth inhibition effect is represented by dimensionless Gamma factors that can be combined as multiplicative terms, consisting of the ratio between the observed growth rate (at the given environmental factor) and the reference growth rate when the environmental factor is at the optimum level (Ross and Dalgaard, 2003; Zwietering et al., 1996). The Gamma concept modeling approach has been mostly applied to estimate the growth rate of *L. monocytogenes* for meat and meat products, seafood, and cheese (Augustin et al., 2005; Mejlholm et al., 2010; Serra-Castelló et al., 2022; Zwietering et al., 1996). However, this model has been scarcely applied to estimate the effects of intrinsic factors such as a_w, pH, and lactic acid and extrinsic ones such as storage temperature on the growth/no growth behavior of *L. monocytogenes* in fruits and vegetables.

The gamma concept approach characterizes growth or lack thereof based on the growth rate, regardless of the presence of a lag time. This approach, considered conservative or the worst-case scenario, is commonly employed in food safety assessments, particularly when *L. monocytogenes* is already adapted to the specific food and conditions, resulting in no lag time. Consequently, the capability for growth or lack thereof is solely determined by the influence of environmental factors on the growth rate of *L. monocytogenes*.

The first objective of the present study was to perform an extensive literature search to collect representative data on *L. monocytogenes* growth, survival and inactivation on different fresh produce matrixes as affected by intrinsic and extrinsic factors. Based on the retrieved data, the second objective was to assess the performance of the Gamma concept approach as safety predictor tool to prioritize the risk of different types of commodities, allowing the identification of the most critical factors to be considered in fresh-cut produce supply chain. This information will provide the fresh produce industry with relevant data to enforce their Food Safety Management Systems, including the definition of the most adequate consumer handling practices for ensuring food safety. While the proposed model may assist in reducing the number of challenge tests required, it cannot entirely replace them. This is because the model does not account for all the factors that influence *L. monocytogenes* ability to grow in each particular case.

2. Materials and methods

2.1. Literature search, data collection and synthesis

A comprehensive literature search and review were conducted to gather information on the growth of *L. monocytogenes* in fresh produce. Original research papers addressing the growth of *L. monocytogenes* on fresh produce from January 1986 to January 2022 were retrieved from

the Web of Science™ Core Collection. Review papers, book sections and books summarizing relevant information were also considered to complement the data from research papers. The search and review were conducted following the recommendations previously described (Marik et al., 2020; Pautasso, 2013). The selected strings used for the search are included in Table S1. Records were screened in three steps: (i) titles, (ii) abstracts and (iii) full-text documents to identify eligible papers on the following criteria: 1) quantitative data on *L. monocytogenes* behavior (growth/survival or inactivation) obtained from challenge tests on fresh produce was provided, either as kinetic parameter (e.g. lag, growth rate, inactivation rate) or as a series of concentration data along time for a constant temperature storage conditions; 2) the final format of the fresh produce (e.g. shredded, diced, whole) was described; and 3) information about the temperature of storage was reported. Data from studies in which the control group met the selection criteria were included for review. The limit of 2 $\log_{10}$ CFU/g was selected to consider the European regulations for ready-to-eat products. The European Commission established a limit of 100 CFU/g for *L. monocytogenes* in ready-to-eat foods till the end of shelf-life (Bergis et al., 2023).

Eligible full-text documents were reviewed to extract the relevant information. Data were collected into an Excel file (Microsoft Corp., Redmond, WA) and classified into the following categories: (a) product characteristics, including produce category (e.g. fruit, leafy greens, other vegetables), type of commodity, format (e.g. fresh-cut, fresh-whole), pH, water activity (a_w); (b) study characteristics (challenge test): *L. monocytogenes* strain, single/cocktail inoculation, inoculum level, inoculation method, storage conditions regarding gas composition within packages (if applicable), temperature, time (if applicable); (c) *L. monocytogenes* behavior: qualitative behavior (e.g. growth, survival, inactivation, see below), measured *L. monocytogenes* concentration (in $\log_{10}$ CFU/g or piece or whole produce), growth rate (GR in $\log_{10}$/h), lag time, inactivation rate; and (d) scientific reference.

In cases where selected studies did not report specific physico-chemical characteristics, average data for pH and a_w, from the literature, for each specific commodity, were used (Bridges and Mattice, 1939; Brochier et al., 2019; Colás-Medà et al., 2015; Chirife and Fontan, 1982; Culliney and Schmalenberger, 2020; CFSAN, 2024; Feng et al., 2015; Galdino et al., 2016; Manjunatha and Raju, 2013; Molinos et al., 2008; Penteado and Leitão, 2004; Salazar et al., 2017; Scolforo et al., 2017; Székely et al., 2016; Ziegler et al., 2019). Data on certain categories provided by commodity and reference sources from the selected studies can be found in Table S3.

The behavior of *L. monocytogenes* during the storage of fresh produce was classified into three qualitative categories based on the considerations of the NACMCF (2010), which is the basis for the US used by the US regulations to refer to intrinsic and extrinsic factors aiming to reduce or limit the growth of *L. monocytogenes* in ready-to-eat foods (CFSAN/FDA, 2017):

- Growth: increase of *L. monocytogenes* concentration higher than 1 $\log_{10}$.
- No-growth-Inactivation: reduction of *L. monocytogenes* concentration higher than 1 $\log_{10}$.
- No growth-Survival: no significant change (rate not statistically different than zero) in the concentration of *L. monocytogenes*.

Survival and inactivation cases have been considered as no-growth cases. The distinction was made for discussion section. In cases where *L. monocytogenes* behavior was classified as survival, the inoculum level and the duration of the study were checked to identify if the observation of inactivation or growth of the pathogen could be affected by these factors. In these cases, the behavior of *L. monocytogenes* was (re)classified as growth or inactivation, if it followed a consistent growth or inactivation trend, respectively, along the storage, even if the total change was <1 $\log_{10}$. Inoculum was qualitatively classified as low when inoculum levels were <2 $\log_{10}$ CFU/g and high when inoculum levels

were ≥ 2 $\log_{10}$ CFU/g (Carlin et al., 1995; Flessa et al., 2005; Koutsoumanis and Sofos, 2005; Sinigaglia et al., 2006).

Quantitative data on the concentration of *L. monocytogenes* along the storage time and/or the growth kinetic parameters were extracted from article text, tables, and graphics. For the latter, the Web Plot digitizer (Ankit Rohatgi, San Francisco, CA; https://apps.automeris.io/wpd/) tool was used. When the growth rate (GR) was not reported, the MS-

$$\gamma_X(X_i) = \begin{cases} 0 & , \text{if } X_i \leq X_{min} \\ \dfrac{(X_i - X_{max}) \cdot (X_i - X_{min})^n}{(X_{opt} - X_{min})^{n-1} \cdot \left((X_{opt} - X_{min}) \cdot (X_i - X_{opt}) - (X_{opt} - X_{max}) \cdot ((n-1) \cdot X_{opt} + X_{min} - n \cdot X_i) \right)} & , \text{if } X_{min} < X_i < X_{max} \\ 0 & , \text{if } X_i \geq X_{max} \end{cases} \qquad 3$$

Excel add-in DMFit (https://www.combase.cc/images/cbtools/DMFit3_5.zip) was used to estimate it by fitting the Baranyi primary model to the *L. monocytogenes* concentration data along time (Baranyi and Roberts, 1994). The mCurv and nCurv (i.e. h_0) values were not manually fixed, but the values provided by the tool were used. When the reported GR was estimated with the Gompertz primary model, a correction factor of 0.852 was applied as it is known that this model overestimates the growth rate compared to that of the Baranyi model (Duan et al., 2016). The use of the Gompertz model was done when the data provided by the research papers used this model or when enough data was missing to fit it using the Baranyi model.

2.2. The Gamma concept approach

The evaluation of the ability of *L. monocytogenes* to grow in fruits and vegetables as a function of the most relevant physicochemical characteristics and storage conditions was performed using a predictive modeling approach based on the Gamma concept (Zwietering et al., 1992, 1996). This approach quantifies the growth inhibition effect of environmental factors through a dimensionless Gamma factor (Γ, Eq. (1)). The Gamma factor is represented by the ratio between the observed growth rate at the given combination of measured environmental factors (GR_{obs}) to the reference growth rate when the considered environmental factors are at the optimum level (GR_{ref}).

$$\Gamma = \frac{GR_{obs}}{GR_{ref}} \qquad 1$$

where the GR_{obs} was estimated by primary model fitting to the collected experimental data with the DMFit tool as described above (section 2.1).

Within the overall Gamma factor, the relevant environmental (intrinsic and extrinsic) factors such as pH, a_w and temperature are introduced as individual terms with microbe-dependent parameters. To assess whether the combination of these factors would support or not the growth of the *L. monocytogenes* in the specific fresh produce, the overall Gamma product (Γ, Eq. (2)) was calculated including the interaction factor as described in Serra-Castelló et al. (2022).

$$\Gamma = \prod_{i=1}^{k} \gamma_X(X_i) \cdot \xi \qquad 2$$

Where X_i is the environmental factor influencing the growth of *L. monocytogenes* (i.e. pH, a_w and temperature). The individual effect of each environmental factor is described by the individual gamma factor γ_X, and ξ is the interaction between factors. Both γ_X and ξ values vary from 0 (when growth is inhibited totally by the factor) to 1 (when the individual factor or the interaction does not affect the growth rate, i.e. growth potential is optimal for the factor). Based on this model, if $\Gamma = 0$, the model predicts no growth of *L. monocytogenes*; if $1 \geq \Gamma > 0$, the model

predicts growth of *L. monocytogenes*. Both γX and ξ values vary from 0 (when growth is inhibited totally by the factor) to 1 (when the individual factor or the interaction does not affect the growth rate, i.e. growth potential is optimal for the factor).

To describe the individual effect of each environmental factor (storage temperature, a_w and pH) on *L. monocytogenes* growth rate, the cardinal model (Ross and Dalgaard, 2003) was used (Eq. (3)).

Where X_i is the value of the environmental factor and X_{min}, X_{opt} and X_{max} are the minimum, optimum and maximum values for the growth of *L. monocytogenes*, respectively (Coroller et al., 2012). The n value was set up to 2 for temperature and to 1 for pH and a_w (Couvert et al., 2010).

The interaction between the environmental factors (ξ) was defined as Le Marc et al. (2002), Eq. (4):

$$\begin{aligned} &\text{If } \psi \leq 0.5, \xi = 1 \\ &\text{If } 0.5 < \psi < 1, \xi = 2(1-\psi) \\ &\text{If } \psi \geq 1, \xi = 0 \end{aligned} \qquad 4$$

Where, following the approach of Coroller et al. (2012), ψ value was defined and calculated differently according to the environmental factor:

$$\psi = \sum_i \frac{\varphi(X_i)}{2 \prod_{j \neq i} (1 - \varphi(X_j))} \qquad 5$$

$$\begin{aligned} \varphi(T) &= \left(1 - \sqrt{\gamma_T(T)}\right)^2 \\ \varphi(pH) &= \left(1 - \gamma_{pH}(pH)\right)^2 \\ \varphi(a_w) &= \left(1 - \gamma_{a_w}(a_w)\right)^3 \end{aligned} \qquad (6)$$

Besides pH, a_w and temperature, the growth of *L. monocytogenes* in fresh produce can be affected by other intrinsic (e.g. organic acids, phenolic compounds of the produce, etc.) and extrinsic (e.g. CO_2 in the packaging) factors. However, these factors were hardly ever measured and quantitatively reported in the retrieved articles. Therefore, to understand the overall impact of these factors, the growth rates observed for each product with its specific combination of pH, a_w and temperature (GR_{obs}) were divided by the overall gamma factor (Γ) to derive the reference growth rate (GR_{ref}) (Eq (3))

$$GR_{ref} = \frac{GR_{obs}}{\Gamma} \qquad 7$$

In general, GR_{ref} is higher when the Γ value is lower and/or when GR_{obs} is higher. The higher the GR_{ref} the more important the impact of factors other than pH, a_w and temperature on lowering the growth rate of *L. monocytogenes*.

Comparisons between observed data and fitted data with the model has been conducted. When predictions did not align with the reality they were classified as "fail-safe predictions" when growth was predicted but no growth was observed, and "fail-dangerous" predictions, when growth was not predicted but growth was observed. The terms "fail-dangerous" and "fail-safe" has been chosen to facilitate readers comprehension. However, it is crucial to highlight that classification has been performed qualitatively. In real-world scenarios involving the growth of *L. monocytogenes*, the health risk is not solely tied to its mere presence or absence. Therefore, while these terms offer insight, their application

should be nuanced, considering other factors influencing health risks.

3. Results and discussion

3.1. Database

A total of 778 articles were initially retrieved from the Web of Science Core Collection database. After duplicate removal, 743 articles remained. The process of screening titles and abstracts selected 122 papers for a full-text review, resulting in 105 articles eligible for data extraction. Table S2 summarized the selected articles. A database was created including 808 primary growth rates, with 320 values corresponding to fruits (126 to acid fruits and 194 to low acid fruits), 257 behavior data points to leafy greens, and 231 to other vegetables. A total of 184 behavior data points were directly extracted from studies (Castillejo Rodríguez et al., 2000; Corbo et al., 2005; Koseki and Isobe, 2005; Leong et al., 2013; Omac et al., 2015, 2018; Pinton et al., 2020; Salazar et al., 2017, 2020; Scolforo et al., 2017; Sinigaglia et al., 2006; Szewczuk et al., 2016; Tucci et al., 2019; Uchima et al., 2008; Vandamm et al., 2013; Wang et al., 2013; Yin et al., 2018; Yoon et al., 2014), while 624 behavior data points were estimated from the original Log count versus time data. extracted from each article and fitted using the Baranyi primary model with the DMFit tool. Figs. 1–3 show the square root growth rate values collected and estimated (DMfit) from the literature search for the different fresh product categories versus the temperate of storage (i. e. fruits, leafy greens, and vegetables). Most growth rates overlap in the lower-left quadrant, which indicates the low growth of *L. monocytogenes* at low temperatures, representing an example of temperature-dependent growth. Many studies use temperatures between 0 and 25 °C and growth rates generally remains <0.6 $(\mathrm{log10/h})^{1/2}$
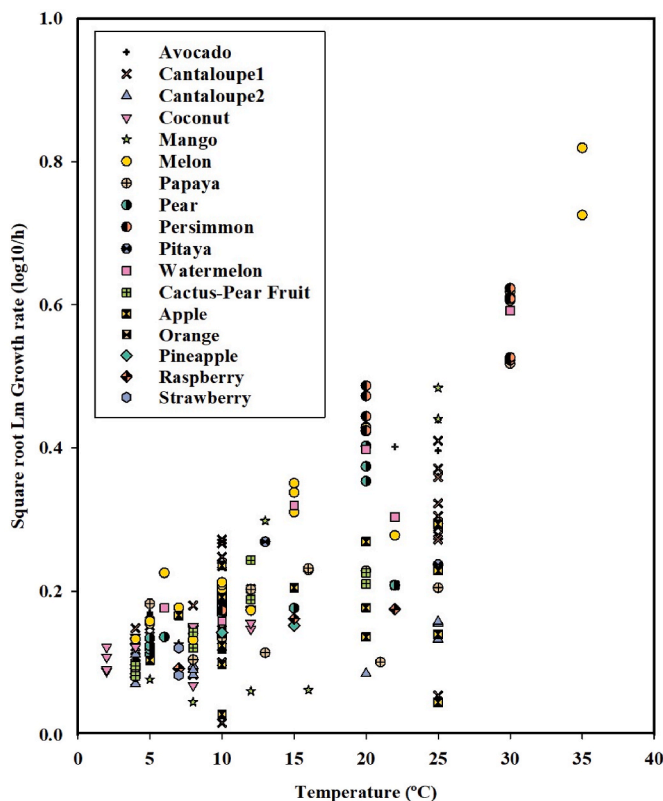

Fig. 1. Scatter plot representing the *L. monocytogenes* growth rate (GR) (positive results) in different fruits at different storage temperatures obtained from the selected studies.. (given the substantial volume of behavior data points associated with cantaloupe, it has been depicted using two distinct symbols to mitigate excessive overlapping.)
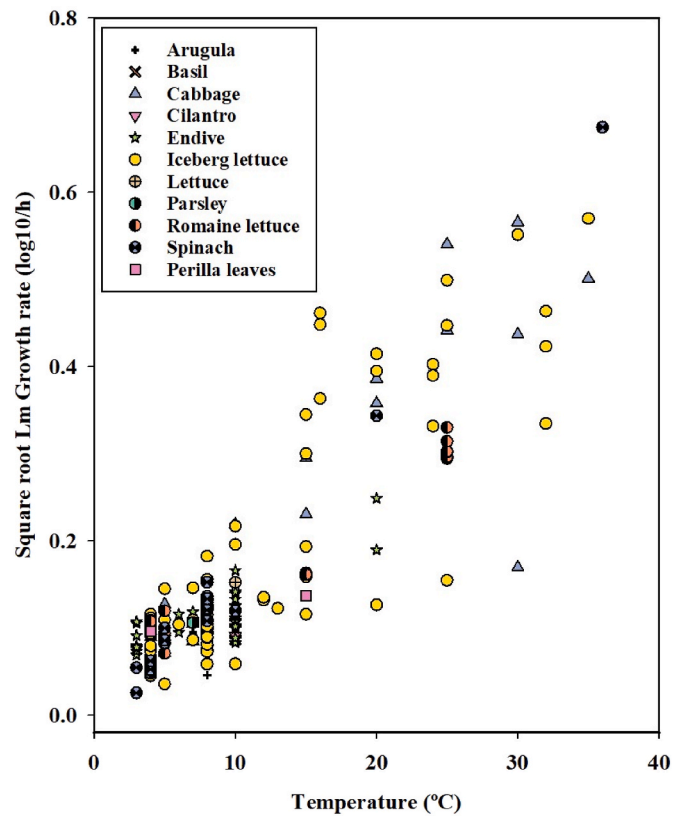

Fig. 2. Scatter plot representing the *L. monocytogenes* growth rate (GR) (positive results) in different leafy greens at different storage temperatures obtained from the selected studies.

(square root function of growth rate). The influence of temperature in *L. monocytogenes* growth could be observed in commodities such as melon or spinach, where placing the temperature (35 °C) at an optimal level for this pathogen growth increases the growth rate by 0.6 $(\mathrm{log10/h})^{1/2}.$"

The number of selected studies and behavior data points extracted from the literature is above the average of previously published research studies. Marik et al. (2020) selected a total of 29 articles from an initial search retrieval of more than 3000 studies focusing on *L. monocytogenes* behavior on whole fresh produce, while Hoelzer et al. (2012) selected 61 articles focusing on *L. monocytogenes* growth on fruits and vegetables, where not only fresh, but cooked and dried products were included. In both cases, authors extracted data from the articles to estimate the growth rates.

3.2. The Gamma concept model

The Gamma concept model was applied to predict the growth/no growth behavior of *L. monocytogenes* in various commodities based on the information collected in the database, i.e. the overall gamma was calculated for the specific pH and a_w of the commodity at the storage temperature applied to perform the challenge test. The predictions obtained from the Gamma concept model were qualitatively compared with observed results published for each individual condition (observed) and summarized in Fig. 4. Overall, among the 808 behavior data points included in the database, 636 (79%) predictions by the Gamma concept model agreed with the observed data. This included 552 cases where growth was predicted and observed, and 84 cases where no growth was predicted and observed (Fig. 4). The number of predictions that did not align with the observed data reached a total of 173 (21%), of which 84 (Table 1) results predicted no growth of *L. monocytogenes* while growth was observed (fail-dangerous predictions) and in 89 cases (Table 1), the
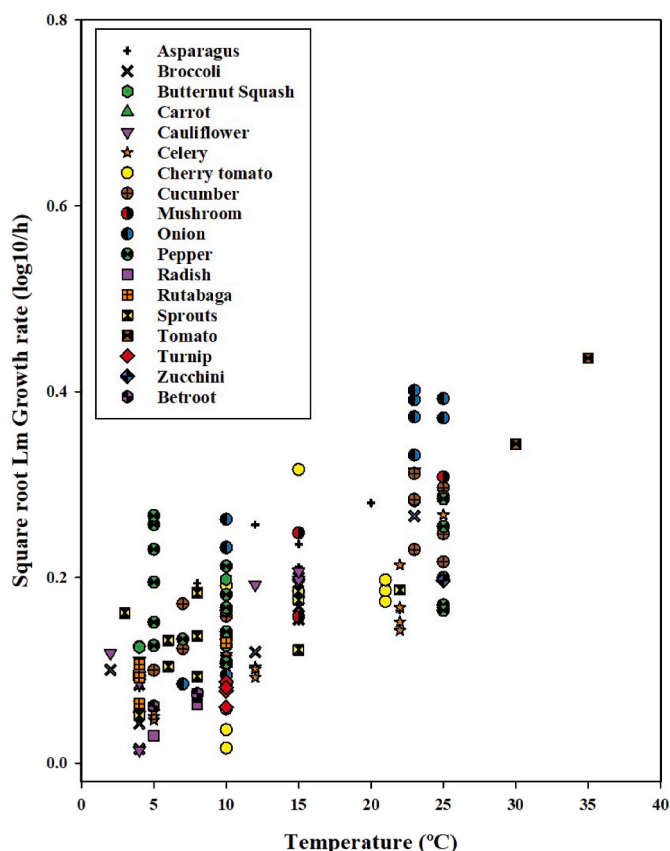
Fig. 3. Scatter plot representing the *L. monocytogenes* growth rate (GR) (positive results) in different vegetables at different storage temperatures obtained from the selected studies.

Gamma concept model predicted growth where no growth was observed (fail-safe predictions).

Within the fruit category, a total of 320 predicted Gamma factors were assessed. Among these, 230 cases (71.8%) corresponded to correct predictions, where 152 cases predicted growth and the remaining had no growth predictions (78 cases). All growth predictions were associated with low-acid fruits (pH > 4.6), whereas no growth predictions were associated with acid fruits (pH < 4.6), emphasizing the critical role of pH within the Gamma concept model. Among the 90 incorrect predictions, 77 were classified as fail-dangerous predictions (90.6% of the total): 49 corresponded to acid fruits and 28 to low acid fruits. On the other hand, 13 cases of fail-safe predictions were associated with low acid fruits (14.6% of the total).

It is important to highlight that for leafy greens (n = 257), most predictions aligned with observations (87.5%, n = 225) and the rest of the predictions (n = 32) were within the fail-safe predictions (36% of total fail-safe predictions), which means the model always predicted growth where observation demonstrated no growth of *L. monocytogenes*. Therefore, according to the Gamma concept model, the pH and the a_w of the produce, along with the temperature conditions during storage of leafy greens were always compatible with the growth of *L. monocytogenes,* but some unmeasured factors might exert an additional inhibitory effect.

In the case of other vegetables, 78% (181/231) of the predictions matched the observed data. Among these correct predictions, 96% (175/ 181) were cases where growth was predicted, and 4% were cases where no growth was predicted. The wrong predictions (22%, 50/231) comprised 16% (8/50) fail-dangerous predictions (9.4% of the total) and 84% (42/50) fail-safe predictions (49.4% of the total).

This analysis signifies the first extensive application of the Gamma

concept model as a predictive tool for the growth/no growth behavior of *L. monocytogenes* in fruits and vegetables, revealing that while the model provides satisfactory predictions for vegetables, including leafy greens, its performance for fruits necessitates further refinement.

To prevent any risk, it is important to determine the reasons why the Gamma concept model cannot provide satisfactory predictions, particularly regarding the fail-dangerous predictions. Several hypotheses were suggested and discussed in the following sections through the evaluation of each situation.

3.3. Intrinsic parameters impacting the growth/no growth behavior of *L. monocytogenes*

The Gamma concept model elucidates the quantitative impact of various intrinsic and extrinsic factors on the growth of select microorganisms. This section examines environmental factors included and excluded in the model utilized in this study, formulating specific hypotheses to interpret the observed data.

Water activity (a_w) significantly influences microbial growth. Several studies have assessed its impact on *L. monocytogenes* growth. For instance, Lieberman and Harris (2019) showed that the low a_w of intact onion skin limited *L. monocytogenes* growth. Similarly, Nyarko et al. (2016a) concluded that while *L. monocytogenes* could grow on the stem scar of cantaloupes, it did not proliferate when inoculated on the rind surface, primarily due to the low a_w in that part of the fruit. In the case of beetroot, a fail-dangerous prediction scenario, the physicochemical properties were not reported (Ziegler et al., 2019), and thus the a_w value used for the model was sourced from a different study, and it was below the minimum a_w value required for *L. monocytogenes* growth (<0.92) (FAO/WHO, 2004). The assumption of the a_w value from a different study might have been wrong leading to an erroneous prediction output. The same situation occurred for coconut (assumed a_w = 0.926).

The pH greatly affects the capacity of microorganisms to grow in a food matrix. However, it is often the combination of factors, rather than the impact of a single factor, that determines microbial behavior. For example, even though the Gamma concept model was expected to predict pathogen growth in cherry tomatoes due to the pH value used as an input for the model (4.6) was within the range of *L. monocytogenes* growth (FAO/WHO, 2004), the combination of this factor with the applied storage temperature (10 °C) resulted to be a limiting factor for *L. monocytogenes* growth. The maturity or ripeness stage of the commodity has an impact on its physicochemical composition, mainly pH, and, consequently, might impact the growth behavior of *L. monocytogenes*. However, exceptions exist, such as a study by Colás-Medà et al. (2015) that did not find a general correlation between ripeness stage and *L. monocytogenes* behavior.

Moreover, the mode of produce inoculation influences the outcomes. In some studies, the whole fruit was inoculated as were the cases of cherry tomato (Beuchat and Brackett, 1991; Kim et al., 2021; Yoon et al., 2014), blueberry, sweet cherry, mandarin orange, lemon (Girbal et al., 2021), strawberry, raspberry (Molinos et al., 2008; Siro et al., 2006), grapes (Kim et al., 2021) and apples (Rodgers et al., 2004; Salazar et al., 2016; Trias et al., 2008). The skin and peel of fruits usually have higher pH values than the flesh, but the pH values of the skin are not commonly reported, while the flesh pH is usually used as a reference (Irkin et al., 2015). This could be one of the reasons why, in these cases, the Gamma concept model predicted no growth while growth was observed. For fruits like kiwi and navel orange, despite no significant change in growth obtained by DMfit, authors noted inactivation dynamics primarily due to the low pH of the produce (Jang et al., 2021). In cases where the pH of the fruit was below or close to a minimum limit for *L. monocytogenes* growth, the Gamma concept model predicted no growth, while the empirical results showed growth. All these cases corresponded with fresh-cut fruits. In these cases, the impact of other relevant factors, such as the format (cut) of the product could have a considerable influence. When produce is cut, it can indeed provide more surface area for
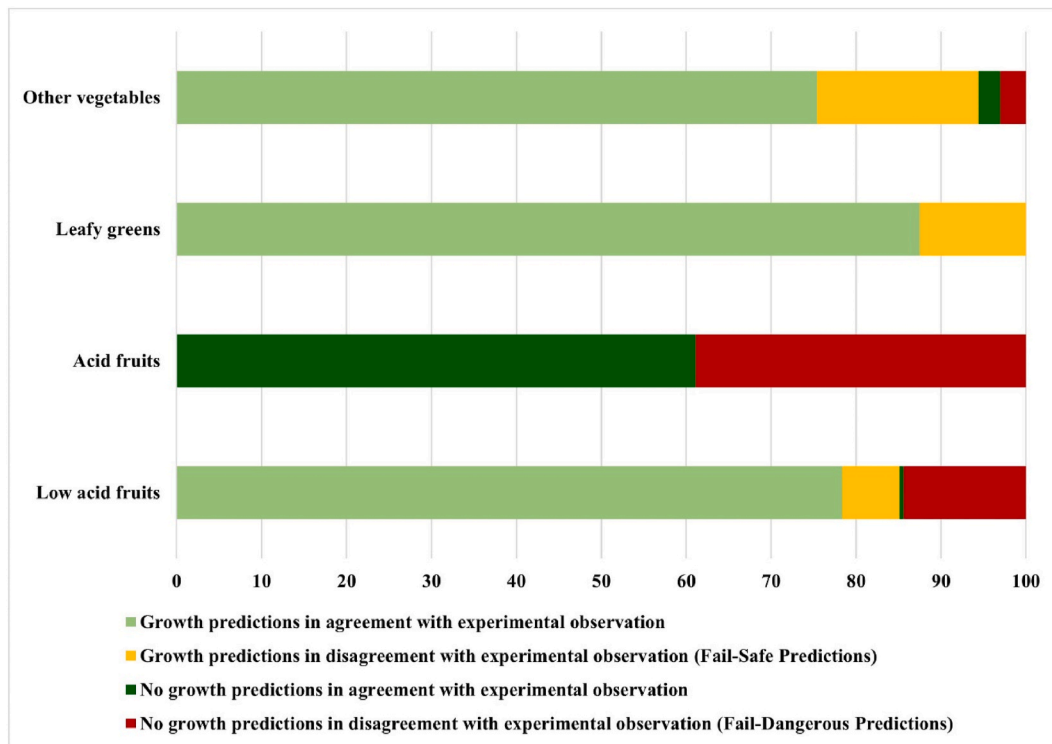
Fig. 4. Proportion of correct (light and dark green) and fail predictions (orange and red) obtained from the Gamma concept model for each product type included in the study (fruit, vegetables, and leafy greens). Fail predictions have been divided in fail-safe and fail-dangerous predictions. In the fail-safe predictions the Gamma concept model predicted growth while observations indicated no growth. In the fail-dangerous predictions, the Gamma concept model predicted no growth while growth was observed in the experimental data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Summary of the growth/no growth predictions compared with the observed behavior.

Type of product		Fail predictions	
		Fail-safe[a] (n = 89)	Fail-dangerous[b] (n = 84)
Fruits		13	77
	Low acid fruits[c]	13	28
	Acid fruits[d]	0	49
Other vegetables		44	7
Leafy greens		32	0
TOTAL		89	84

[a] 'Fail-safe predictions': model predicted growth, but the observations showed no growth of *L. monocytogenes*.
[b] 'Fail-dangerous predictions': model predicted no growth, but the observations showed growth of *L. monocytogenes*.
[c] Low acid fruits: pH > 4.6.
[d] Acid fruits: pH < 4.6.

microorganisms to access nutrients and water compared to whole produce. This increased surface area can potentially lead to faster microbial growth and spoilage. Additionally, cutting produce can also compromise its protective outer layer, making it more vulnerable to microbial contamination.

Fresh-cut operations, as it has been described, cause changes in the tissues, which might affect the physicochemical properties of the fruits, including pH and a_w, and consequently, changes in the *L. monocytogenes* growth potential. These changes were observed for pears (Colás-Medà et al., 2015, 2017), mango (Feng et al., 2015; Lokerse et al., 2016; Luciano et al., 2022), strawberry (Flessa et al., 2005; Lokerse et al.,

2016; Siro et al., 2006), pineapple (Kim et al., 2021; Lokerse et al., 2016) and apple (Alegre et al., 2011; Conway et al., 2000; Kim et al., 2021; Leverentz et al., 2006; Lokerse et al., 2016; Martinez et al., 2020; Rodgers et al., 2004). The impact of the processing operations on the physicochemical characteristics of fruits and vegetables such as the soluble solids and sugar content has been also reported. Abadias et al. (2014, 2012) reported that cutting breaks the tissue, making sugar more available to microorganisms, which might enhance microbial growth even at low pH values (Abadias et al., 2014). Other factors such as *L. monocytogenes* strains used in the experiments, should be considered to explain contradictory results as described below, but its influence is smaller.

Another intrinsic parameter that might have an impact on microbial growth/inactivation is the presence of antimicrobial compounds in the food matrix. The presence of antimicrobial compounds in fruits and vegetables is not considered by the Gamma concept model, but they might contribute to explaining some contradictory results between the predictions and the empirical (observed) results. Several authors have already described the presence of antimicrobial compounds in different fruits and vegetables such as brussels sprouts (Jacxsens et al., 1999), garlic (Salazar et al., 2020), lamb's lettuce (Carlin and Nguyen-The, 1994). One of the most well-known cases of anti-listerial activity is the case of carrots (Noriega et al., 2010). Similar results were also reported for coconut (Collu et al., 2021) and eggplant (Salazar et al., 2020), where the anti-listeria activity was attributed to the presence of specific components but also to the competition dynamics between *L. monocytogenes* and the natural microbiota of these matrixes (Salazar et al., 2020). The impact of these microbial related factors is described in the following section.

3.4. Microbial related factors impacting the growth/no growth behavior of L. monocytogenes

The type of *L. monocytogenes* strain used in the experiments as well as the competition between the *L. monocytogenes* and the natural microbiota present in the produce might play a relevant role. González-Fandos et al. (2001) reported that the initial *L. monocytogenes* growth observed in mushrooms during the first days of storage corresponded to the lag phase of the natural microbiota, but as soon as competitors reached high levels, *L. monocytogenes* levels declined. Similar interactions were described between the background microflora such as lactic acid bacteria (LAB) and *L. monocytogenes* growth (Aytac and Gorris, 1994; Francis and O'Beirne, 2001a). However, the effect of microbial competition is not captured in the Gamma concept model, although could contribute to explain some of the discrepancies between the model predictions and the observed empirical results.

The variability in growth rates among different *Listeria* strains, due to their sensitivity to various external factors, might also impact the predictions made by the Gamma concept model, explaining some of the fail-dangerous predictions. Francis and O'Beirne (2005) demonstrated significant differences among various *L. monocytogenes* strains in terms of their survival in acidic conditions on packaged vegetables. In addition to strain type, the physiological state of the microbial strains also plays a critical role. Several studies have highlighted the advantages of using cold-adapted strains for inoculation when refrigerated storage conditions are employed (Miller et al., 2009). For example, Ramos et al. (2020) showed that cold-adapted strains increase the probability of growth in fresh produce stored at refrigerated temperatures. Other studies reported fluctuations in *L. monocytogenes* growth during storage, which might be misinterpreted as inactivation but usually corresponds to a decrease in *L. monocytogenes* concentration after an initial phase of growth. Examples of these studies can be found in coconut (Collu et al., 2021), green leaf lettuce (Rodgers et al., 2004), artichoke (Sanz et al., 2003), asparagus (Castillejo-Rodríguez et al., 2000), pitaya (Feng et al., 2015), cauliflower (Berrang et al., 1989), honeydew melon (Collu et al., 2021) and cantaloupe (Zhang et al., 2020).

3.5. Impact of extrinsic parameters on the growth/no growth behavior of L. monocytogenes

Extrinsic factors, such as packaging conditions, can influence the potential growth of *L. monocytogenes* (Ells and Hansen, 2010). Specifically, the gas composition within packages during storage, particularly concentrations of O_2 and CO_2, affects bacterial behavior. A storage environment with elevated CO_2 levels ($>15\%$) combined with very low O_2 levels ($<1\%$) has been associated with a reduction in *L. monocytogenes* concentration over 14 days of storage (Niemira et al., 2005). This trend was also observed in spinach (Lokerse et al., 2016) and iceberg lettuce (Dong et al., 2021; Francis and O'Beirne, 1997; Li et al., 2002), which may help explain the discrepancies between observed data and model predictions.

3.6. Variability in GR_ref as a function of the type of produce

Among all fruits and vegetables, pears, papaya, sprouts, cauliflower, spinach, cherry tomato, onion and peppers exhibited the highest variability in observed growth values (Hoelzer et al., 2012; Redding et al., 2023). This variability in observed growth rates could stem from differences in the input values used in the model, such as temperature. Storage temperatures varied significantly across studies and records. In the same study, a commodity could be tested in a range of temperatures from 5 to 15 °C. In these cases, despite the variability in observed growth rates, they consistently reflected temperature impact on growth rates.

The variability in growth rates has also been attributed to other intrinsic factors, such as the ripening stage and the tissue architectural structure. For example, in papayas, changes in nutritional content during ripening were identified a major source of variability in the growth potential of *L. monocytogenes* (Feng et al., 2015). Papayas, unlike other tropical fruits, accumulates sugar on their surface as they mature, providing additional nutrients for microbial growth (Dong and Li, 2021). Research involving spinach, including studies on baby and adult leaves, indicated that differences in maturity stages lead to variations in physicochemical composition (e.g. pH, a_w, concentration of constituents), thereby affecting the growth rate of *L. monocytogenes* and helping to explain observed differences (Babic and Watada, 1995; Zhao et al., 2007). Sorrells et al. (1990) noted that acetic acid has a more potent inhibitory effect on *L. monocytogenes* than other acids, such as lactic, citric, malic and hydrochloric acids. Furthermore, the variability in *L. monocytogenes* growth observed in cauliflowers could be associated to their particular micro-architectural structure (Ongeng et al., 2007).

The selection of data for peppers or sprouts, which included a wide range of cultivars and varieties with significant differences in physicochemical composition, might explain the observed variability in microbial growth (Francis and O'Beirne, 2001b). A similar hypothesis may apply to onions, where the variability in cultivars and varieties, the product type (whole or diced), and the composition of the natural microbiota could all influence *L. monocytogenes* growth rate (Lieberman and Harris, 2019).

When considering data variability, the experimental design of empirical studies (e.g. inoculum size, application method, drying time) must be taken into account (Lang et al., 2004; Gnanou Besse et al., 2006). It is crucial that the experimental design aligns with the study objectives and the scenarios being evaluated. Adhering to official technical guidelines for challenge tests (Bergis et al., 2023) is essential for obtaining reliable data that supports the validation of predictive models. Monitoring the physicochemical characteristics of food is essential for enhancing data validity.

Moreover, the application site of the pathogen inoculum may affect *L. monocytogenes* survival depending on the commodity, and should be chosen based on study objectives. Applying the inoculum to the outer skin of onions and cantaloupes has been shown to influence observed inactivation dynamics (Lieberman and Harris, 2019; Nyarko et al., 2016b; Ukuku et al., 2016; Ukuku and Fett, 2002). A similar observation was made when inoculating with cells in the stationary growth phase (Ramos et al., 2020). Demonstrating the critical role of these experimental variables, Dreux et al. (2007) found that a high initial inoculum level, close to or exceeding the carrying capacity, might reduce the estimated growth rate of *L. monocytogenes*. Similarly, using and initial inoculum near to 6 logs for inoculating endive and cantaloupe prevented *L. monocytogenes* growth during storage (Niemira et al., 2005; Zhang et al., 2020).

4. Conclusions

This study evaluates, for the first time, the use of the Gamma concept model to preliminarily assess the growth behavior of *L. monocytogenes* in various fruits and vegetables by considering their format, intrinsic physicochemical properties and storage temperature conditions. Through an extensive literature review and predictive analysis, the model's usefulness in identifying which produce commodities support or inhibit the pathogen's growth was determined.

Our results indicate that the model provides satisfactory predictions for the growth/no-growth behavior of *L. monocytogenes* in all vegetables ($>80\%$ agreement with experimental observation, with slightly conservative outputs), while for the specific group of leafy greens, a 90% agreement was achieved. However, its predictions for fruits were less consistent, primarily due to an overestimation of the inhibitory effects of acidity on pathogen growth. This discrepancy underscores the need for model refinement, particularly to better account for the influence of other factors, such as pH, a_w and specific intrinsic factors, on microbial behavior.

Food business operators and regulatory bodies are in constant search

of efficient and reliable methods to meet food safety standards and regulations, with an especial concern on *L. monocytogenes*. While challenge tests are the gold standard for assessing risks associated with this pathogen, their practical limitations highlight the value of the use of predictive models like the Gamma concept as supplementary tools. However, for these models to be accurately applied, precise measurement of the physicochemical properties of the commodities under realistic storage conditions is essential.

In conclusion, the Gamma concept model shows promise as a tool for the pre-screening of *L. monocytogenes* growth behavior in fresh produce, offering a strategic approach to food safety management. The model's continuous improvement and validation, grounded in empirical data and refined through further research, will enhance its reliability and applicability, supporting the fresh produce industry in ensuring consumer safety.

CRediT authorship contribution statement

Marisa Gomez-Galindo: Writing – original draft, Methodology, Formal analysis, Data curation. **Cristina Serra-Castelló:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Sara Bover-Cid:** Writing – review & editing, Visualization, Validation, Formal analysis, Conceptualization. **Pilar Truchado:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Maria I. Gil:** Writing – review & editing. **Ana Allende:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.fm.2024.104554.

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