@LIMENTECH CIENCIA Y TECNOLOGÍA ALIMENTARIA
ISSN Impreso 1692-7125/ ISSN Electrónico 2711-3035. Volumen 21 No. 2, p. 57 - 68, año 2023
Facultad de Ingenierías y Arquitectura
Universidad de Pamplona

EFFECT OF HIGH AND LOW ACYL GELLAN ON GROWTH PARAMETERS OF *LACTOBACILLUS DELBRUECKII*

EFECTO DEL ACILGELLAN ALTO Y BAJO EN LOS PARÁMETROS DE CRECIMIENTO DE LACTOBACILLUS DELBRUECKII

Villegas Duran, Mari[1*], González Cuello, Rafael[2], Taron Dunoyer, Arnulfo[3*]

[1]University of Cartagena. Faculty of Engineering. Biotechnology, Food and Education Research Group (GIBAE). MSc. Food Engineering Program. Cartagena. Colombia. Correo electrónico: mvillegasd@unicartagena.edu.co
[2]University of Cartagena. Faculty of Engineering. Food Packaging and Shelf Life research group (FP&SL). PhD. Programa de Ingeniería de Alimentos. Cartagena. Colombia
Orcid: https://orcid.org/0000-0002-2674-2876 . Correo electrónico: rgonzalezc1@unicartagena.edu.co
[3*]University of Cartagena. Faculty of Engineering. Biotechnology, Food and Education Research Group (GIBAE). MSc. Food Engineering Program. Cartagena. Colombia.
Orcid: https://orcid.org/0000-0002-6942-4480. Correo electrónico: atarond@unicartagena.edu.co

Received: May 2023; Accepted: October 2023

ABSTRACT

The aim of this study was evaluate the growth parameters of *Lactobacillus delbrueckii* in viscoelastic system made from gellan gum. Initially dispersions of high (HAG), low acyl gum (LAG) and their mixture HAG/LAG at 5 % w/v were incorporated with inulin and gelled by calcium ions addition. The viscoelastic study of these systems was performed through dynamic oscillatory. The counts of *L. delbrueckii* grown on gellan systems were adjusted to Huang and Gompertz models in order to determinate the growth parameters. All gellan systems studied showed a predominantly elastic behavior, where the elastic modulus (G') was greater than viscous modulus (G''). G' is inversely proportional to maximum growth rate (μ). HAG and LAG had significant effect mainly on latency phase (λ) and μ, while no influence was observed for initial count cells (Y_0) and final count cells (Y_{max}) parameters. The high value of μ found on HAG system indicate that *L. delbrueckii* grows faster at HAG than LAG and HAG/LAG systems. These findings can be useful to develop new alimentary products.

Keywords: High and low acyl gellan, Huang model, Gompertz model, Viscoelasticity.

ABSTRACT

El objetivo de este estudio era evaluar los parámetros de crecimiento de Lactobacillus delbrueckii en un sistema viscoelástico elaborado a partir de goma gelan. Inicialmente se incorporaron dispersiones de goma de alto (HAG), bajo acilo (LAG) y su mezcla HAG/LAG al 5 % p/v con inulina y se gelificaron mediante la adición de iones de calcio. El estudio viscoelástico de estos sistemas se realizó mediante oscilación dinámica. Los recuentos de L. delbrueckii cultivados sobre los sistemas gelan se ajustaron a los modelos de Huang y Gompertz para determinar los parámetros de crecimiento. Todos los sistemas de gelan estudiados mostraron un comportamiento predominantemente elástico, donde el módulo elástico (G') fue mayor que el módulo viscoso (G"). G' es inversamente proporcional a la velocidad máxima de crecimiento (μ). HAG y LAG tuvieron un efecto significativo principalmente en la fase de latencia (λ) y μ, mientras que no se observó ninguna influencia en los parámetros de recuento inicial de células (Y0) y recuento final de células (Ymax). El alto valor de μ encontrado en el sistema HAG indica que L. delbrueckii crece más rápido en el sistema HAG que en los sistemas LAG y HAG/LAG. Estos resultados pueden ser útiles para desarrollar nuevos productos alimenticios.

[3*]***Corresponding author***:
atarond@unicartagena.edu.co

Keywords: Goma de alto y bajo, modelo de Huang, modelo de Gompertz, viscoelasticidad.

INTRODUCTION

The probiotics are bacteria which when are administered in precise quantity exert a beneficial effect on consumer's health (Tripathi and Giri, 2014). It is recommended a daily intake around of 10^6–10^9 CFU/g to perceive and ensure the health benefits. Some benefits associated with the probiotics ingestion are the following: stimulation of the immune system, reduction of lactose intolerance, modulation of the gastrointestinal system and reduce irritable bowel symptoms (Sanders *et al.,* 2013). In last years, there has been an increasing demand for food products with functional characteristics. One way to expand the tender of functional products is to incorporate probiotic bacteria into different food matrices. Nevertheless, diffusional limitations of nutrients and oxygen along with accumulation of metabolic products in the food matrix could affect the probiotic growth (Aspridou *et al.,* 2014). Studies have been carried out for describing the growth of probiotic microorganisms by means of mathematical models (Gonzalez *et al.,* 2020).

Predictive microbiology is a multidisciplinary area, where statisticians, food microbiologists, mathematicians and computer scientists work together. In general terms, predictive models are built and used for improving food quality anticipating the microorganism's behavior on certain foods matrixes (Szczawinski 2012, Arroyo-Lopez, *et al.,* 2012). Considering the above-mentioned, it is import develop experiments estimating the probiotic growth on systems that simulate different kinds of food. In this sense, polysaccharides have been used at industrial level mainly to modify food texture. Gellan gum is an anionic extracellular heteropolysaccharide produced by the bacterium *Sphingomonas paucimobilis,* which consist of repeating units of a tetrasaccharide (1,3-β-D-glucose; 1,4-β-D glucuronic acid; 1,4 β-D-glucose; and 1,4-α-L-rhamnose). It is available in two presentations: high acyl gellan (HAG) and low acyl gellan (LAG). HAG form soft elastic gels; whereas LAG forms strong, brittle gels. Mixtures of the two gellans can produce gels with a varied properties depending on the each gellan proportions (Huang *et al.,* 2003).

Although, most of the studies have been focus on systems composed by gelling agents such as gelatin, agar, xanthan gum, and κ-carrageenan containing nutrient broth (Aspridou *et al.,* 2014; Velliou *et al.,* 2013; Wang *et al*., 2017) or protein gelling agent (Boons *et al.,* 2014, Léonard *et al.,* 2015). Nowadays, there is not research about modeling probiotic growth on a food system model with different structural complexity. It has been observed that the concentration and the kind of polysaccharide modifies the rheological properties of food systems (González *et al.,* 2012).

Dynamic rheological studies are key considering that they provide information respect to the gelation process of polysaccharides. Besides they may be used to improve the textural attributes delivered by gellan gum to food products (Nickerson *et al.,* 2004). In rheological dynamics analysis, the main parameters employed to characterize a system are: the storage modulus (G'), the loss modulus (G'') and the phase angle (δ), being G' a measure of the energy temporarily stored in a material, G'' a measure of the energy used to activate a flow and δ indicate the quantitative relationship between the viscous and the elastic components of a system (Jiménez-Avalos *et al.*, 2005). Hence, the main goal of this paper was study the growth parameters of *Lactobacillus delbrueckii* in viscoelastic system made from mixtures of gellan gum.

MATERIALS AND METHODS

Inoculum preparation

Lactobacillus delbrueckii was incorporated in 10 mL of nutrient broth at 37°C for 24 hours. Then, the microorganisms were cultivated until the exponential growth phase was reached. The count of bacterial cells was carried out by surface plating counting method using agar MRS. The initial bacterial count was adjusted to 2 log colony-forming units (CFU)/mL. Each analysis was performed in triplicate.

Preparation of viscoelastic systems

Initially, dispersions of LAG and HAG at 0.5 % (w/v) were prepared separately in distilled water at 90°C under agitation during 10 min. For binary system, a mixture of LAG and HAG (1:1) ratio was prepared into test tube. The mentioned dispersions were added with inulin. To start the gelation process, calcium ions were incorporated in order to promote the self-association between gellan helix. Then, 2 log CFU of *L. delbrueckii* was inoculated for each preparation. For obtaining the microbial growth curve, a sample was processed every 40 minutes. Finally, the enumeration of viable count cells was carried out at 37 °C in petri dishes containing agar MRS, reporting the amount of bacteria in CFU/g. Each analysis was performed in triplicate.

Dynamic rheological test

All tests were performed with an MARS 60 control stress rheometer using a parallel plate geometry of 20 mm of diameter. An amplitude scan was performed to determine the linear viscoelasticity zone (LVZ), which is obtained when the storage modulus (G') and the loss modulus (G'') are independent of amplitude. G′ represents the elastic portion of the material response (its means the stiffness of a gel), while G″ represents the viscous response. The loss tangent (tan δ=G″/G′) provides an indication of whether the material is closer to an elastic solid (tan δ < 1), or a viscous fluid (tan δ > 1).

Mathematical modelling

Experimental data obtained from growth curves of *L. delbrueckii* were fitted to the Gompertz and Huang models (Baranyi Roberts, 1994; Huang, 2013; Zwietering *et al.,* 1997).

The Gompertz model is the following:

$$\left[Y = y_0 + (y_{max} - y_0) * exp\left\{-exp\left[\frac{\mu^e_{max}}{y_{max} - y_0}(\lambda - t) + 1\right]\right\}\right]$$

Where y_0, y_{max}, and $y_{(t)}$ are the bacterial concentration in natural logarithm at initial, maximum, and time t; μ_{max} is the specific growth rate; and λ is the duration of the latency phase.

The Huang is the following:

$$\left[Y(t) = y_0 + y_{max} - In\{e^{y_0} + [e^{y_{max}} - e^{y_0}]\, e^{-\mu maxB(t)}\}\right]$$

$$\left[B(t) = t + \frac{1}{\alpha} In \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}\right]$$

Where y_0, y_{max} and $y_{(t)}$ are the bacterial concentration in natural logarithm at initial, maximum, at time t; μ_{max} is the specific growth rate and λ is the duration of the latency phase. The latency phase coefficient is α 4.

Statistical analysis

Growth parameters of *L. delbrueckii* obtained through Huang and Gompertz models were analyzed employing ANOVA (one way) in order to determine significant differences (p<0.05). This was accomplished, using the software SPSS (version 23.0 for Windows).

RESULTS AND DISCUSSION

Dynamic rheological analysis

Changes in viscoelastic parameters, storage (G′), loss moduli (G″), and phase angle (tan δ) are presented in Table 1, where the elastic behavior was greater than viscous behavior (G'>G'') for all gellan systems studied, indicating that these systems have viscoelastic properties. Likewise, statistical differences were appreciated in G' and G'' moduli among HAG, LAG and HAG/LAG systems. A loss tangent tan δ < 1 was gotten, signifying that elastic component dominates the flow properties. This characteristic behavior indicates that all prepared systems behave like strong gels according to Mounsey & O'Riordan, (1999).

For LAG system G' was 1,90 times greater than G'', while for HAG system this difference was 6,86. It must be highlighted that the mixture HAG/LAG had the highest G' value (2400) regarding to the pure gellan (HAG and LAG), this is likely caused by an interaction between both gellan as a result of hydrogen bonds formation. These findings are in agreement with those stated by González *et al*., (2012) who found higher values of G' in mixtures of HAG/LAG than pure gellan.

Table 1. Behavior of G', G'' and tan δ in viscoelastic system based on gellan gum

samples	G'	G''	tan δ
HAG	84[a]	12,23[a]	0,14[a]
LAG	270[b]	142[b]	0,52[b]
HAG/LAG	2400[c]	118[c]	0,04[c]

Rows with no common letter showed statistically significant difference (significance level<0.05)

With respect to tan δ, values between 0,04 and 0,52 were attained, being 0,04 the lowest tan δ values that corresponds to HAG/LAG mixture; whereas the highest value was 0,52 for LAG system. The value for HAG was 0,14. Likewise this behavior coincides with results reported by Gonzalez *et al.,* (2012) who observed a strong gel behavior for gels made from gellan gum. Applying statistical analysis ANOVA (one-way) to all viscoelastic parameters (G', G'' and tan δ), significant differences (p<0,05) were reveled between HAG, LAG and its mixture HAG/LAG. As was above-mentioned rheological analysis being key because they provide information about gelation mechanisms of hydrocolloid. Moreover, it is well known that hydrocolloids have various rheological properties which could affect the microbial growth (Kapetanakou *et al.,* 2011). Likewise, a viscoelastic environment is considered a stress factors (Costello *et al.,* 2018).

Growth curve fitting

The CFU log of *L. delbrueckii* obtained from each viscoelastic system were adjusted to primary models as can be seen in figure 1; where three trends guided more by the gellan ratio than by the model used to predict the data are appreciated. The adjustment to each model allow determinate growth parameters (Y_0, Y_f, μ_{max} and λ). Table 1 shows the growth parameters of the Huang and Gompertz models used to assess the behavior of *L. delbrueckii* grown in viscoelastic materials based on gellan gum, where it is valued the influence of the HAG and LAG on some growth parameters of *L. delbrueckii* indicating that gellans proportions had effect on microbial growth.

However, the initial count cells (Y_0) values did not varied significantly ($p<0,05$) with the presence of HAG, LAG or their mixture HAG/LAG in both models applied (Huang and Gompertz), showing that this parameter might be controlled by the number of probiotics bacteria incorporated into the gellans' systems. Values of Y_0 were similar for both models, being 2,300 for Huang model and 2,280 for Gompertz model in the HAG system. For the mixture based on HAG/LAG a value of 2,313 in the Huang model was obtained, while for Gompertz model a value of 2,202 was reached. Similar behavior was also observed for both models in the LAG system.

The highest latency phase (λ) value in Huang model was obtained for HAG/LAG system with 86,903 followed by LAG and HAG systems with 33,620 and 30,602 respectively. When the Gompertz model was applied, λ values were 86,350 (HAG/LAG), 36,046 (LAG) and 24,444 (HAG). A related performance was achieved when Huang model was employed. It means, that statistical differences ($p<0,05$) on λ were appreciated among all systems. Interestingly, the lowest values of λ correspond to bacteria grown on viscoelastic system with the low G' values and vice versa, the highest values of λ was for the bacteria grown on systems with high G' value. The λ is an adaptation phase of the microorganisms to the culture medium.

Therefore, it is important consider this parameter to reduce the fermentation time of some fermented foods, which implies that low values of λ are needed for improving fermentation time. Thus, the system based only in HAG is more efficient to make functional foods, due to it had the lower λ and G' values in comparison with others viscoelastic systems.

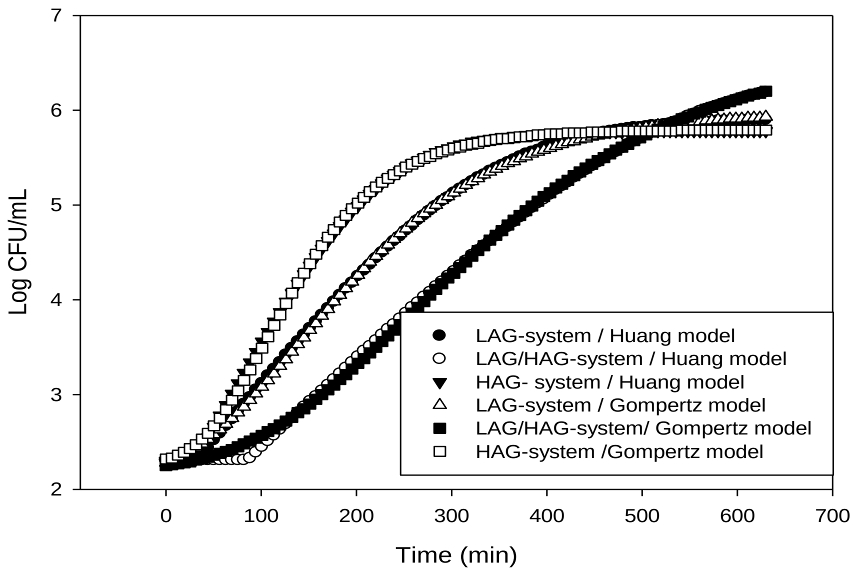

Figure 1. Predicted values of *L. delbrueckii* growth applying Huang and Gompertz models

Regarding to the maximum growth rate (μ); in the Huang model, the highest value was obtained for HAG system with 0,019 followed by LAG system with 0,013 and HAG/LAG system with 0,010. These μ values are higher than those reported by Antunes-Rohling *et al.,* (2019) on foodborne microorganisms meaning that *L. delbrueckii* quickly overtook the others bacteria. The low values of μ probably are caused by limitations of nutrients, oxygen and metabolites produced by the cell immobilization which resulting in an environmental stress (Jeanson *et al.,* 2015; Skandamis and Jeanson, 2015).

When Gompertz model was applied, the μ values calculated for HAG, HAG/LAG and LAG systems were 0,019, 0,010 and 0,012 respectively. It means, a behavior similar to rheological parameters. It must be recorded that low G' values were presented for HAG system. The higher μ values, the lower G' module. In general term, μ was significantly ($p<0.05$) affected by the gellan gum ratio. These results are contrary to those reported by Boons *et al.,* (2013) who stated an insignificant effect of the structured system based on Xanthan gum, carrageenan and gelatin on the microbial growth rate.

With respect to maximum cells population (Y_{max}) which is the maximum microbial concentration achieved by the bacteria at the end of the logarithmic phase. This parameter also is relevant for optimizing functional foods due to the probiotics range inside foods matrices must be between 10^6 and 10^7 CFU/g to consumer perceive beneficial effects associated to probiotic ingestion (Tripathi and Giri, 2014). The values of this parameter (Y_{max}) depend on nutritional composition of the culture medium.

The highest Y_{max} value was 6,472 for HAG system followed by 5,921 for LAG system and 5,780 for HAG system when Huang model was used to calculated them. Similar values were gotten when Gompertz model was applied. Costello *et al.,* (2018) found that little differences on growth parameters of *Listeria innocua* growing on viscoelastic system based on xanthan gum may be attributed to kind of hydrocolloid and its high concentration.

Table 2. Growth parameters of *Lactobacillus delbrueckii* at different systems based on gellan gum

Model	Parameters	HAG	HAG/LAG	LAG
Huang	Y_0 (log CFU)	$2,300^a$	$2,313^a$	$2,310^a$
	λ (min)	$30,602^a$	$86,903^b$	$33,620^c$
	Y_{max} (log CFU)	$5,780^a$	$6,472^b$	$5,921^a$
	μ_{max}(min^{-1})	$0,019^a$	$0,010^b$	$0,013^b$
Gompertz	Y_0(log CFU)	$2,280^a$	$2,202^a$	$2,142^a$
	λ (min)	$24,444^a$	$86,350^b$	$36,046^c$
	Y_{max} (log CFU)	$5,791^a$	$6,671^b$	$5,985^a$
	μ_{max} (min^{-1})	$0,019^a$	$0,010^b$	$0,012^b$

Rows with no common letter showed statistically significant difference (significance level < 0.05)

To sum up, gellans proportions (HAG and LAG) affect mainly maximum growth rate (μ) and latency phase (λ), while no significant differences ($p>0.05$) were noted for Y_0 and Y_{max}. The highest μ values found on viscoelastic system made from HAG indicate that *Lactobacillus delbrueckii* grows faster into HAG with respect to LAG and the mixture HAG/LAG. Likewise, the system based on HAG had the lowest λ value; whereby it could be considering to modify or create new alimentary products containing probiotic bacteria.

CONCLUSIONS

The analysis of growth parameters of a probiotic bacteria as *L. delbrueckii* grown on viscoelastic system based on gellan gum is reported for first time. The Huang and Gompertz Models were employed to calculate the growth parameters indicated that high and low acyl gellan gum had significant effect mainly on latency phase and maximum growth rate, while no influence was noted for Y_0 and Y_{max} parameters. The behavior of these parameters (Y_0 and Y_{max}) suggest that they can be controlled by the nutritional composition and initial number of probiotic bacteria incorporated to the viscoelastic system. All analyzed systems behaved as materials predominantly elastic. The elastic modulus (G') is inversely proportional to maximum growth rate. The high value of maximum growth rate found on HAG system indicate that *L. delbrueckii* grows faster in HAG than LAG and HAG/LAG systems. It should be noted that the results obtained herein can be useful to develop new alimentary products.

REFERENCES

Antunes-Rohlinga A., Artaiza, A., Calero, S., Halaihel, N., Guillén, S., Raso, J., Álvarez, I., Cebrián, G. (2019). Modelling microbial growth in modified-atmosphere-packed hake (*Merluccius merluccius*) fillets stored at different temperatures. Food Res Int., 122, 506–516

Arroyo-López, F., Bautista-Gallego, J., Garrido-Fernández, A. (2012). Role of Predictive Microbiology in Food Preservation. Progress in Food Preservation, First Edition. Edited by Rajeev Bhat, Abd Karim Alias and Gopinadhan Paliyath. Published by John Wiley & Sons, Ltd.

Aspridou, Z., Moschakis, T., Biliaderis, C.G., Koutsoumanis, K.P. (2014). Effect of the substrate's microstructure on the growth of *Listeria monocytogenes*. Food Res Int, 64, 683–691.

Baranyi, J. and Roberts, T.A. (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol, 23, 277–294.

Boons, K., Noriega, E., Van den Broeck, R., David, C.C., Hofkens, J., Van Impe, J.F. (2014).

Effect of microstructure on population growth parameters of *Escherichia coli* in gelatin-dextran systems. Appl. Environ. Microbiol, 80, 5330–5339.

Boons, K., Van Derlinden, E., Mertens, L., Peeters, V., Van Impe, J.F. (2013). Effect of immobilization and salt concentration on the growth dynamics of *Escherichia coli* K12 and *Salmonella Typhimurium*. J. Food Sci, 78, 567–57.

Costello, K., Gutierrez-Merino J., Bussemaker M., Ramaioli M., Baka M., Van Impec J. Velliou E. (2018). Modelling the microbial dynamics and antimicrobial resistance development of *Listeria* in viscoelastic food model systems of various structural complexities. Int. J. Food Microbiol, 15–30.

González-Cuello, R.E., Ramos-Ramírez, E.G., Oruz-Orea, A. and Salazar-Montoya, J. (2012). Rheological characterization and activation energy values of binary mixtures of gellan. Eur. Food Res. Technol, 234(2012), 305–313

González Cuello, R., Morón Alcázar, L., Pedraza Galván, K. (2020). Modeling the effect of storage temperature on the growth rate of *Lactobacillus delbrueckii* in Colombian coastal cheese. Sylwan journal, 164 (3), 293 – 303.

Huang, Y., Tang, J., Swanson, G., Cavinato, A., Lin, M., Barbara, A. (2003) Near infrared spectroscopy: a new tool for studying physical and chemical properties of polysaccharide gels. Carb. Polym, 53, 281–288.

Jeanson, S., Floury, J., Gagnaire, V., Lortal, S., Thierry, A., (2015). Bacterial colonies in solid media and foods: a review on their growth and interactions with the microenvironment. Front. Microbiol. 6.

Jiménez-Avalos, H.A., Ramos-Ramírez, E.G., Salazar-Montoya, J.A. (2005) Viscoelastic characterization of gum arabic and maize starch mixture using the Maxwell model. Carb Polym 62, 11–18.

Kapetanakou, A.E., Ampavi, A., Yanniotis, S., Drosinos, E.H., Skandamis, P.N., 2011. Development of a model describing the effect of temperature, water activity and (gel) structure on growth and ochratoxin A production by *Aspergillus carbonarius* in vitro and evaluation in food matrices of different viscosity. Food Microbiol, 28, 727–735.

Léonard, L., Beji, O., Arnould, C., Noirot, E., Bonnotte, A., Gharsallaoui, A., Degraeve, P., Lherminier, J., Saurel, R., Oulahal, N. (2015). Preservation of viability and anti-Listeria activity of lactic acid bacteria, *Lactococcus lactis* and *Lactobacillus paracasei*, entrapped in gelling matrices of alginate or alginate/caseinate. Food Control, 47, 7–19.

Mounsey, J. S., & O'Riordan, E. D. (1999). Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. J. Food Sci, 64, 701–703.

Nickerson, M.T., Paulson, A.T., Speers, R.A. (2004). Time-temperature studies of gellan polysaccharide gelation in the presence of low, intermediate and high levels of co-solutes. Food Hydrocol, 18, 783–794.

Sanders, M., Guarner, F., Guerrant, R., Holt, P., Quigley, E., Sartor, R., Sherman, P., Mayer, E. (2013). An update on the use and investigation of probiotics in health and disease. Gut 62(5), 787-796.

Skandamis, P.N., Jeanson, S. (2015). Colonial vs. planktonic type of growth: mathematical modelling of microbial dynamics on surfaces and in liquid, semi-liquid and solid foods. Front, Microbiol. 6.

Szczawiński, J. (2012). Predictive microbiology – practical applications. Med Weter, 68, 540-543.

Tripathi, S. and Giri, S. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. J. Funct. Foods, 9, 225-241.

Velliou, E.G., Noriega, E., Van Derlinden, E., Mertens, L., Boons, K., Geeraerd, A.H., Devlieghere, F., Van Impe, J.F., 2013. The effect of colony formation on the heat inactivation dynamics of *Escherichia coli* K12 and *Salmonella typhimurium*. Food Res. Int, 54, 1746–1752

Wang, X., Devlieghere, F., Geeraerd, A., Uyttendaele, M. (2017). Thermal inactivation and sublethal injury kinetics of *Salmonella enterica* and *Listeria monocytogenes* in broth versus agar surface. Int. J. Food Microbiol. 243, 70–77.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., and Van'treit, K. (1990). Modeling of the bacterial growth curve. App. Environ. Microbiol, 56, 1875–1881