ABSTRACT

The aim of this study was evaluate the growth parameters of \textit{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 \textit{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 ($\mu$). HAG and LAG had significant effect mainly on latency phase ($\lambda$) and $\mu$, while no influence was observed for initial count cells (Y$_0$) and final count cells (Y$_{max}$) parameters. The high value of $\mu$ found on HAG system indicate that \textit{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.

**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 (Szcawinski 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 matrix...
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 (it 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:

\[ Y(t) = y_0 + y_{max} \] 
\[-ln\left(e^{y_0}\right) + \left[e^{y_{max}} - e^{y_0}\right] e^{-\mu_{max}B(t)}\] 
\[ B(t) = t + 1 + e^{-\alpha(t-\lambda)} \] 

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

The Huang is the following:

\[ Y(t) = y_0 + y_{max} \]
\[-ln\left(e^{y_0}\right) + \left[e^{y_{max}} - e^{y_0}\right] e^{-\mu_{max}B(t)}\] 
\[ B(t) = t + 1 + e^{-\alpha(t-\lambda)} \] 

Where \( y_0, y_{max}, \) and \( y(t) \) are the bacterial concentration in natural logarithm at initial, maximum, at time t; \( \mu_{max} \) is the specific growth rate and \( \lambda \) is the duration of the latency phase. The latency phase coefficient is \( \alpha \).

**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 $\tan \delta < 1$ 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 $\tan \delta$ in viscoelastic system based on gellan gum

<table>
<thead>
<tr>
<th>samples</th>
<th>$G'$</th>
<th>$G''$</th>
<th>$\tan \delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAG</td>
<td>84$^a$</td>
<td>12.23$^a$</td>
<td>0.14$^a$</td>
</tr>
<tr>
<td>LAG</td>
<td>270$^b$</td>
<td>142$^b$</td>
<td>0.52$^b$</td>
</tr>
<tr>
<td>HAG/LAG</td>
<td>2400$^c$</td>
<td>118$^c$</td>
<td>0.04$^c$</td>
</tr>
</tbody>
</table>

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

With respect to $\tan \delta$, values between 0.04 and 0.52 were attained, being 0.04 the lowest $\tan \delta$ value 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 $\tan \delta$), significant differences ($p<0.05$) were revealed 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

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gellan ratio than by the model used to predict the data are appreciated. The adjustment to each model allow determinate growth parameters ($Y_0$, $Y_f$, $\mu_{\text{max}}$ and $\lambda$). 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 ($\lambda$) 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, $\lambda$ 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 $\lambda$ were appreciated among all systems. Interestingly, the lowest values of $\lambda$ correspond to bacteria grown on viscoelastic system with the low G’ values and vice versa, the highest values of $\lambda$ was for the bacteria grown on systems with high G’ value. The $\lambda$ 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 $\lambda$ 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 $\lambda$ and G’ values in comparison with others viscoelastic systems.
Figure 1. Predicted values of \textit{L. delbrueckii} growth applying Huang and Gompertz models

Regarding to the maximum growth rate (\(\mu\)); 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 \(\mu\) values are higher than those reported by Antunes-Rohling \textit{et al.}, (2019) on foodborne microorganisms meaning that \textit{L. delbrueckii} quickly overtook the others bacteria. The low values of \(\mu\) probably are caused by limitations of nutrients, oxygen and metabolites produced by the cell immobilization which resulting in an environmental stress (Jeanson \textit{et al.}, 2015; Skandamis and Jeanson, 2015).

When Gompertz model was applied, the \(\mu\) 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 \(\mu\) values, the lower \(G'\) module. In general term, \(\mu\) was significantly (p<0.05) affected by the gellan gum ratio. These results are contrary to those reported by Boons \textit{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 (\(Y_{\text{max}}\)) 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 ($Y_{\text{max}}$) depend on nutritional composition of the culture medium.

The highest $Y_{\text{max}}$ 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<sup>a</sup> | 2.313<sup>a</sup> | 2.310<sup>a</sup> |
| | $\lambda$ (min) | 30.602<sup>a</sup> | 86,903<sup>b</sup> | 33,620<sup>c</sup> |
| | $Y_{\text{max}}$ (log CFU) | 5.780<sup>a</sup> | 6.472<sup>b</sup> | 5.921<sup>b</sup> |
| | $\mu_{\text{max}}$ (min<sup>-1</sup>) | 0.019<sup>a</sup> | 0.010<sup>b</sup> | 0.013<sup>b</sup> |
| Gompertz | $Y_0$ (log CFU) | 2.280<sup>a</sup> | 2.202<sup>a</sup> | 2.142<sup>a</sup> |
| | $\lambda$ (min) | 24,444<sup>a</sup> | 86,350<sup>b</sup> | 36,046<sup>c</sup> |
| | $Y_{\text{max}}$ (log CFU) | 5.791<sup>a</sup> | 6.671<sup>b</sup> | 5.985<sup>a</sup> |
| | $\mu_{\text{max}}$ (min<sup>-1</sup>) | 0.019<sup>a</sup> | 0.010<sup>b</sup> | 0.012<sup>b</sup> |

*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 ($\mu$) and latency phase ($\lambda$), while no significant differences ($p>0.05$) were noted for $Y_0$ and $Y_{\text{max}}$. The highest $\mu$ 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 $\lambda$ 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 $Y_{\text{max}}$ parameters. The behavior of these parameters ($Y_0$ and $Y_{\text{max}}$) 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.

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