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Effect of stabilizers, oil level and structure on the growth of *Zygosaccharomyces bailii* and on physical stability of model systems simulating acid sauces

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ABSTRACT

The effect of xanthan gum, guar gum, oil and the structure promoted by these compounds on the growth of *Zygosaccharomyces bailii* and on physical stability of emulsified systems simulating acid sauces was studied. Furthermore, the effect of yeast growth on physical stability of emulsions was also evaluated.

Yeast growth was evaluated by plate count and modeled by the modified Gompertz equation. Emulsions characteristics and their stability were determined by droplet size, zeta potential and rheological measurements. The latter was also used to evaluate structure's effect on yeast growth.

Physical characteristics of emulsions depended on system composition. Yeasts slightly affected droplet size. *Z. bailii* growth was satisfactorily modeled by the modified Gompertz equation. The specific growth rate (μ_m) and the asymptotic value (A) obtained depended on xanthan gum, guar gum and oil content. Furthermore, the structure promoted by these compounds exerted a significant effect on growth. In general, an increase in the solid character and yield stress through the addition of xanthan gum promoted a decrease in A parameter. On the contrary, a decrease in the solid character through the addition of guar gum promoted an increase in the A parameter. The results obtained stressed that stabilizers, oil and their structuring ability play an important role on *Z. bailii* growth.

1. Introduction

Foods are in general dispersed systems and most of them exhibit a structure. The latter is provided by the presence of plant or meat tissues or by the inclusion of hydrocolloids and/or lipids in order to get viscous, gelled or emulsified food products (Brocklehurst, 2004; Walstra and van Vliet, 2008).

The ability of microorganisms to grow in foods depends on storage conditions, food composition, presence of additives and food structure (Wilson et al., 2002). The latter modifies water mobility and distribution of solutes such as acidulants, water activity depressors and preservatives (Brocklehurst, Parker, Gunning, Coleman, and Robins, 1995; Castro, Garro, Gerschenson, and Campos, 2003; Wimpenny et al., 1995). Moreover, in structured products, microorganisms are immobilized and therefore they are forced to grow in colonies (Theys et al., 2008).

The effects of structure on microbial growth had been evaluated in gels applying mainly models of growth/no growth or by absorbance readings (Mertens et al., 2009, 2011; Theys et al., 2008; Theys, Geeraerd, Devlieghere, and Van Impe, 2010). Trends reported about the effect of structure on microbial growth are diverse. Many studies postulated that structure acts as an additional stress factor and therefore lower growth is expected. As an example, Theys et al. (2008) reported that growth rate of *Salmonella typhimurium* is decreased when 1% of gelatin was added to broth. However, an increase in gelatin concentration to 5% had no effect on growth rate. Conversely, other studies showed that structure increases growth. Boons et al. (2015) reported that an increase in growth rate and maximum cell density of *Saccharomyces cerevisiae* when gelatin or dextran were added to broth. Furthermore, this trend was enhanced when both polymers were combined. It must be highlighted that information about the effect of structure in dispersed opaque systems is scarce, particularly when dealing with emulsions (Boons, Van Derlinden, Mertens, Peeters, and Van Impe, 2013; Boons et al., 2014, 2015; Brocklehurst, Parker, Gunning, and Robins, 1993; Brocklehurst et al., 1995; Parker, Brocklehurst, Gunning, Coleman, and Robins, 1995).

Acid sauces are dispersed systems whose structure is provided by thickening, gelling and emulsifier agents, they included concentrated suspensions and salad dressings (Mertens et al., 2009). Furthermore, their physical stability depends on the right selection of mentioned agents (Sikora, Badrie, Deisingh, and Kowalski, 2008). Microbial stability is based on the use of high concentration of an organic acid,

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depression of water activity, addition of preservatives and the use of an impermeable packaging (Sikora et al., 2008). The low pH prevented the growth of food-borne pathogens. However, microbial spoilage of these products can take place conducting to important economic losses for the food industry. The microflora that causes spoilage comprises *Lactobacillus*, *Saccharomyces* and *Zygosaccharomyces*, *Z. bailii* being the main responsible for spoilage (Kurtzman, Rogers, and Hesseltine, 1971; Smittle, 2000).

Agents such as stabilizers and emulsifiers added to build up the structure of acid sauces can modify the microbial stability. On the one hand, the presence of a structure affects microbial growth and the effectiveness of stress factors as it was previously commented. On the other hand, according to the chemical structure of the agent, it can be metabolized by the microorganism promoting its growth or it can form complexes with preservatives decreasing their availability (Boons et al., 2015; Castro et al., 2003; Kurup, Wan, and Chan, 1991; Wedzicha, Zeb, and Ahmed, 1991).

The objective of this work is to evaluate: i) the effect of different levels of xanthan gum, corn oil and the presence of guar gum on the physical stability and on the growth of *Z. bailii* in dispersed systems modeling acid sauces; ii) the effect of the rheological characteristics of the studied model systems on *Z. bailii* growth, and iii) the effect of the yeast on the physical stability of the emulsified systems.

2. Materials and methods

2.1. Materials

Reagent grade citric acid was from Merck Química (Argentina, Argentina), xanthan and guar gum were from Cargill (Argentina) and corn oil was from Refinerías de Maiz (Argentina). They were Food grade. All media used were from Biokar (Biokar Diagnostics, Beauvais, France).

2.2. Model system preparation

Model systems were formulated in Sabouraud broth (SB) with the addition of different concentrations of xanthan gum, guar gum and corn oil as it is mentioned in Table 1. All the ingredients, with the exception of oil, were suspended in distillated water and poured into glass flasks. Xanthan gum was finely dispersed and agitated for 24 h at 25 °C to assure complete hydratation. Then, the systems were sterilized for 30 min at 100 °C. Guar gum was added after sterilization in sterile conditions. The pH was adjusted to 3.50 by adding some drops of sterilize citric acid solution (250 g/L). Oil in water emulsions were obtained by aseptically adding the corresponding amount of oil to the aqueous phase and mixing with an Ultra-turrax homogenizator (IKA, Germany) for 1 min at 13,500 rpm and then for 3 min at 24,000 rpm. This procedure was undertaken under laminar flow and onto ice to dissipate the heat generated by the emulsification. In order to compare aqueous systems with the emulsions, xanthan and guar concentrations in the aqueous systems B, C and I were the same as the aqueous phase of emulsions F, G and J. It must be mentioned that it was not possible to generate stable emulsions without the addition of xanthan or guar gum. The latter only produces stable emulsion at 1.000%.

Table 1
Concentrations of corn oil, xanthan and guar gum in model systems.

System	Xanthan gum (wt.%)*	Guar gum (wt.%)*	Corn oil (wt.%)
A	0.000	0.000	0.0
B	0.448	0.000	0.0
C	1.818	0.000	0.0
D	0.250 (0.282)	0.000	11.0
E	1.000 (1.136)	0.000	11.0
F	0.250 (0.448)	0.000	44.0
G	1.000 (1.818)	0.000	44.0
H	0.625 (0.870)	0.000	27.5
I	0.000	1.818	0.0
J	0.000	1.000 (1.818)	44.0

* between parenthesis is given the concentration in aqueous phase for comparison purposes.

2.3. Physical characterization of studied systems

2.3.1. Droplet size

Droplet size of emulsions was determined by light scattering using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit (Malvern Instruments, Worcestershire, United Kingdom). A refractive index of 1.473 for the corn oil phase and its absorption parameter (0.001) was used. Droplet size is reported as the Sauter diameter ($D_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$) and the De Broucker diameter ($D_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$), n_i being the number of droplets of diameter d_i (McClements, 2007). Determinations were made after 24 h of emulsification and after 7 days of storage. Data reported were the mean of ten determinations made on two different emulsions of identical composition.

2.3.2. Zeta potential measurements

The electrical charge measurements were carried out using a particle electrophoresis Nanoseries ZS instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Before analysis, the systems were diluted. Three readings were made per sample and each measurement was repeated on at least two separately prepared samples.

2.3.3. Rheological measurements

Dynamic oscillatory measurements were performed to assess the viscoelastic behavior of the samples. Therefore, the frequency dependence and magnitude of the storage modulus G' and the loss modulus G" were evaluated. Also the phase angle, δ, was calculated from measurements of G' and G" as tan δ = G"/G'. Frequency sweep tests were performed from 0.1 to 100 rad/s. Prior to measurements, samples were tested over a range of strains to determine appropriate conditions for non-destructive testing. For this purpose, strain sweeps at a frequency of 10 rad/s were performed to determine the linear viscoelastic range.

Similarly to Mertens et al. (2009), the yield stress (σ) of the samples was determined by using the tangent crossover method (Mezger, 2006). It was determined as the shear stress value at which the range of reversible elastic deformation behavior ends and the range of the irreversible deformation behavior begins. In this study, yield stress measurements were performed by increasing shear stress from 0 Pa up to values of 150 Pa, depending on the sample under study, with a time interval of 60 s between each measuring point.

Oscillatory shear experiments were conducted with a tangential controlled stress rheometer (Paar Physica MCR 300, Antón Paar GMBH, Germany) using a cone (24.94 mm diameter, 2° angle) and plate geometry (CP25-2 sensor).

2.4. Inoculum preparation, storage systems and sampling

Zygosaccharomyces bailii NRRL 7256 was stored at −20.0 ± 0.5 °C in SB broth plus 10.0 g/100 g glycerol. Before its use, the strain was grown twice in Sabouraud broth at 25.0 ± 0.5 °C for 24 h. After that, the inoculum was diluted in peptone water (1.5 g/100 g) to reach 0.5 McFarland units, corresponding to a population of approximately 10^6 CFU/mL A suspension of the yeast was added to the model systems in order to have an initial population of 10^4 CFU/g. To assure the inoculum homogeneous distribution, the system was mixed with an Ultra-turrax homogenizator (IKA, Germany) for 1 min at 13,500 rpm. Then, aliquots of 30 g of each inoculated system were placed in sterile glass flasks in duplicate and stored at 25.0 ± 0.5 °C for 7 days. At selected times, viable yeast counts were determined by surface plate with SB agar. For that purpose, samples of 2.5 g of system and 22.5 mL of 0.1% peptone water (Biokar Diagnostics, Beauvais, France) were placed into

stomacher bags and homogenized for 1 min in a stomacher (Seward Medical, London, UK). All plates were incubated at 25.0 ± 0.5 °C and growth was inspected after 5 days.

2.5. Use of xanthan and guar gum as carbon source for Z. bailii growth

Some model systems containing casein meat peptone (base broth) as nitrogen source were prepared in order to evaluate the use of xanthan and guar gum as carbon source (Table 2). For comparison purposes, systems formulated using Sabouraud broth were also reevaluated (systems A, C and I, Table 1). Systems preparation and inoculation were made as previously mentioned. The systems were stored at 25.0 ± 0.5 °C and yeast population was evaluated at initial time and after 24 and 72 h. Selected times corresponded to exponential and stationary yeast growth phase, respectively.

2.6. Combined effect of oil and xanthan gum on yeast growth

To evaluate the combined effect of xanthan gum and oil concentrations on *Z. bailii* growth, a full factorial design with two variables at two levels (2^2) and a central point was applied. Variables and their levels are shown in Table 3. The complete design consisted of 5 experimental treatments with 3 replicates each, resulting in a total of 15 experimental units. An additional emulsion containing xanthan gum (0.375 wt.%) and corn oil (27.5 wt.%) was also analyzed for validation purposes.

2.7. Experimental design and data analysis

Assays of physical characterization of systems were performed in duplicate independent samples and parameters obtained were evaluated through an ANOVA and the Tukey test.

Z. bailii population counts–obtained from three replicate samples–were modeled using the modified Gompertz equation (Zwietering, Jongerburger, Rombouts, and van'tRiet, 1990):

$$Ln\left(\frac{N}{N_0}\right) = A\exp\left\{-\exp\left[\mu_m \frac{e}{A}(\lambda - t) + 1\right]\right\}$$

which expresses the logarithm of the ratio between microbial count (N) at a specific time (t) and initial microbial count (N_0) vs the time. The growth parameters are the specific growth rate (μ_m), the lag phase time (λ) and the asymptotic value (A). Values of μ_m and A obtained for studied systems were analyzed by ANOVA and the Tukey's test searching for significant differences. Mean values of μ_m and A represented in graphs are the average of three replicates together with their standard errors.

Regarding the use of xanthan and guar gum as carbon source for *Z. bailii* growth, the value of the ratio between log of population at time t and initial population obtained were also analyzed by ANOVA and the Tukey's test.

Regarding the combined effect of oil and xanthan gum, values of μ_m and A obtained for emulsions (systems D to H) were subjected to a multiple regression analysis to fit the following first-order regression model:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2$$

Table 2
Model system composition to study the metabolization of gums by *Z. bailii*.

System*	Xanthan gum (wt.%)	Guar gum (wt.%)	Casein meat peptone (wt.%)
K	0.000	0.000	10.0
L	1.818	0.000	10.0
M	0.000	1.818	10.0

* K: base broth, L: base broth plus xanthan gum, M: base broth plus guar gum.

Table 3
Two-level full factorial design used to evaluate the effects of xanthan gum and oil time on *Z. bailii* growth.

Independent variable	Variable code	Levels (wt.%)		
		Minimum	Center point	Maximum
Levels of oil	x_1	11.0	27.5	44.0
Levels of xanthan gum	x_2	0.250	0.625	1.000

where Y are biological parameters (μ_m and A); x_1 and x_2 are studied variables (Table 3); β_0, is the independent term; β_1 and β_2, the linear coefficient and β_{12}, the interaction coefficient.

The adequacy of the regression model generated by the factorial design was examined by analysis of variance (ANOVA), the adjusted correlation coefficients (R^2adj.) and the absolute average deviation (AAD) (Bas and Boyaci, 2007). Also, ANOVA and p-value were used to evaluate the significance of the linear and interaction terms of each model. Furthermore, as previously mentioned, an external validation of the regression models was performed through the formulation and the analysis of an emulsion containing xanthan gum (0.375%) and corn oil (27.5%). Experimental values of μ_m and A obtained were compared with the values predicted by the models and their adequacy was estimated through the root mean square error (RMSE).

In all cases, statistical significance was evaluated at a 5% level using Statgraphics Plus for Windows, version 5.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.).

3. Results and discussion

3.1. Physical characterization of studied systems

3.1.1. Droplet size distribution

As it can be seen in Fig. 1, emulsions containing 11.0% oil presented a monomodal distribution. On the contrary, emulsions containing 44.0% oil presented a bimodal distribution and a higher mean diameter. It must be mentioned that food emulsions usually contain a range of different droplet sizes and therefore are polydisperse (McClements, 2007). The D_{32} provides a measure of the mean diameter where most of the particles fall in between while the D_{43} is related with changes in particle size involving destabilization processes so it is more sensitive to fat droplet aggregation than D_{32} (McClements, 2007). The D_{32} and D_{43} significantly

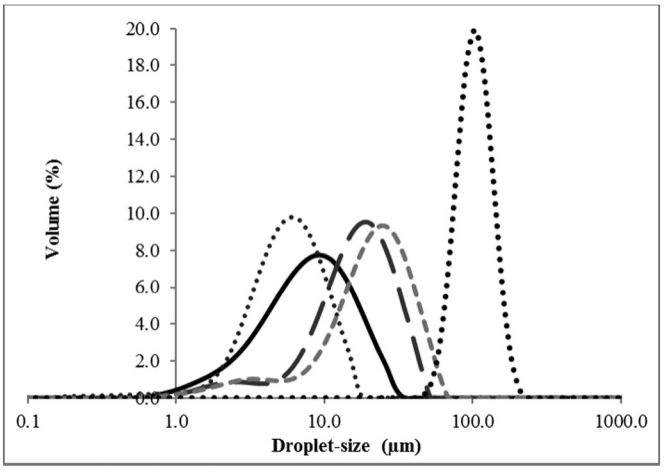

Fig. 1. Droplet-size distribution (μ_m) in non-inoculated emulsions at initial time. (——) D: 0.250% xanthan gum and 11.0% oil; (·····) E: 1.000% xanthan gum and 11.0% oil; (— —) F: 0.250% xanthan gum and 44.0% oil; (- - - - -) G: 1.000% xanthan gum and 44.0% oil and (•••••) J: 1.000% guar gum and 44.0% oil. *Zygosaccharomyces bailii* cell size was within the range of (3.5–6.5) × (4.5–11.5) μm.

decreased with the increase in xanthan gum concentration for the 11.0% oil level (Table 4). This effect might be due to the increase in the continuous phase viscosity and this trend had been previously reported by others authors (Qian and McClements, 2011; Sun, Gunasekaran, and Richards, 2007). On the contrary, in emulsions containing 44.0% oil, the increase in xanthan gum level did not modify D_{32} and promoted an increase in D_{43} (Table 4). Probably, at this oil level, it would be necessary for a higher xanthan concentration to stabilize the droplets and to prevent aggregation. According to Parker, Gunning, Ng, and Robins (1995) xanthan gum stabilizes dressings due to repulsive interaction between emulsion droplets and xanthan molecules and by the formation of a weak network around the droplets which prevents flocculation. Furthermore, the use of 44.0% oil and 1.000% xanthan gum conducted to a more viscous system – as it will be mentioned later – and as a consequence the efficiency of homogenization diminished conducting to the formation of droplets with a higher D_{43} (Huang, Kakuda, and Cui, 2001).

The use of 1.000% of guar gum instead of 1.000% of xanthan gum produced an increase in D_{32} and D_{43} independently of storage time (Table 4, system J vs. G). According to Huang et al. (2001), guar gum presented lower ability to decrease interfacial tension than xanthan gum. The storage of these emulsions for 7 days at 25 °C did not modify either D_{32} nor D_{43} with the exception of the emulsion with guar gum where D_{43} showed an increase (Table 4, system J) suggesting the occurrence of aggregation. As it will be discussed later, this gum exhibited a lower solid character which can facilitate aggregation.

Yeast inoculation only promoted a slight increase in D_{32} for the emulsion containing 0.250% xanthan gum and 44.0% oil (system F). Moreover, a decrease in D_{32} was observed for the guar gum emulsion. In the case of 44.0% oil emulsions, an increase for xanthan gum emulsions and a decrease for guar gum emulsion were observed. These trends can be linked with the changes in Zeta potential (Zp) induced by the addition of the yeast as it will be commented in the next section. After 7 days of storage, only the emulsion with guar gum showed an increase in D_{32} and D_{43}. However, the latter parameter was lower than the one observed for the non-inoculated emulsion, suggesting that in this case inoculation acted against droplet aggregation.

3.1.2. Zeta potential

All the emulsions exhibited negative values of Zp (Table 5) suggesting the presence of negative particles. It is reported that a positive or negative Zp greater or lower than +30 mV and −30 mV suggests that the particles will repel each other and the dispersion will be stable (Carneiro-da-Cunha, Cerqueira, Souza, Teixeira, and Vicente, 2011). Moreover, the larger the absolute magnitude of Zp, the greater is the electrostatic repulsion between droplets, and therefore the stability obtained (Dickinson, 2009). Some differences were observed in Zp absolute values of emulsions according to system compositions. In the presence of 11.0% oil, an increase in xanthan gum from 0.250 to 1.000% promoted a slight decrease in Zp (Table 5, system D vs. E). However, no effect was observed for 44.0% oil level. The emulsion containing guar gum (system J) presented the lower Zp absolute value being less stable than xanthan gum emulsions. Probably, this can be linked with the neutral behavior of guar gum (BeMiller and Huber, 2008). Furthermore, the lower Zp absolute value is correlated with the larger values of D_{32} and D_{43} reported previously (Table 5).

Table 5
Zeta potential (Zp) of studied emulsions and *Z. bailii* cells.

System	Zp ± standard deviation
F	−49.60 ± 0.76 a
G	−47.60 ± 1.65 ab
D	−42.30 ± 4.62 b
E	−32.60 ± 4.57 c
J	−22.80 ± 0.86 d
Z. bailii	−18.80 ± 5.80 d

Z. bailii exhibited a negative Zp value as well as the emulsions, therefore no electrostatic attraction forces are expected between cells and emulsion droplets. The Zp value of *Z. bailii* is within the range of Zp values informed for other spoilage yeasts (Brugnoni, Lozano, and Cubitto, 2007). Probably, inoculation of guar gum emulsion with *Z. bailii* promoted an increase in Zp rendering a more negative Zp value and therefore increasing its stability.

3.1.3. Rheological properties

Mechanical spectra depended on system composition. The aqueous system containing 0.448% xanthan gum (system B) and the 44.0% oil emulsion with an equal amount of xanthan gum in the aqueous phase (system F) exhibited a behavior similar to weak gels since G' was higher than G" almost in all the range of frequencies and a crossing at high frequencies (Fig. 2, panel I). However, a decrease in the oil level to 11.0% (system D) promoted a change of rheological properties since this system behaved as a polymeric dispersion where G' and G" curves presented two crossings, one at low (0.322 s^{-1}) and the other at high frequencies (14.8 s^{-1}) (Fig. 2, panel I). It must be stressed that in polymeric dispersions macromolecules exhibited more mobility since there are no specific interaction between them (Da Silva and Rao, 1992).

Systems containing the highest levels of xanthan gum (systems C, E, and G) behaved similar to strong gels since G' changed slightly with the

Table 4
Sauter (D_{32}) and de Broucker (D_{43}) diameters of inoculated and non-inoculated emulsions after one day and seven days of storage at 25 °C.

System	D_{32} ± standard deviations (μm)			
	Non-inoculated		Inoculated	
	Day 1	Day 7	Day 1	Day 7
D	5.91 ± 0.04 A, a	5.95 ± 0.04 A,a	6.00 ± 0.02 A, a	7.11 ± 0.11 A, b
E	4.62 ± 0.38 B, a	4.93 ± 0.04 B,a	4.91 ± 0.09 B, a	4.70 ± 0.12 B, a
F	9.63 ± 0.09 C,a	9.89 ± 0.03 C,a	10.44 ± 0.07 C,b	10.47 ± 0.13 C,b
G	10.13 ± 0.10 C,a	9.45 ± 0.14 C,a	9.82 ± 0.93 C,a	10.73 ± 0.01 C,a
J	93.31 ± 0.51 D,a	95.51 ± 0.52 D,a	89.75 ± 0.13 D,b	93.16 ± 0.13 D,a
System	D_{43} ± standard deviations (μm)			
	Non-inoculated		Inoculated	
	Day 1	Day 7	Day 1	Day 7
D	9.90 ± 0.09 A, a	11.52 ± 1.32 A, a	10.13 ± 0.02 A, a	11.42 ± 0.11 A, a
E	6.77 ± 0.01 B, a	6.89 ± 0.03 B, a	6.83 ± 0.03 B, a	6.82 ± 0.01 B, a
F	16.91 ± 0.12 C,a	17.46 ± 0.12 C, ab	18.07 ± 0.09 C,bc	18.29 ± 0.27 C,c
G	20.89 ± 0.53 D,a	21.01 ± 0.37 D, a	22.67 ± 0.18 D,b	23.08 ± 0.38 D,b
J	99.95 ± 0.32 E,a	103.14 ± 0.93 E,b	97.43 ± 0.41 E,c	99.78 ± 0.15 E,a

Upper case letters within the same column or lower case letters with the same row show parameters that are not significantly different ($p \leq 0.05$).

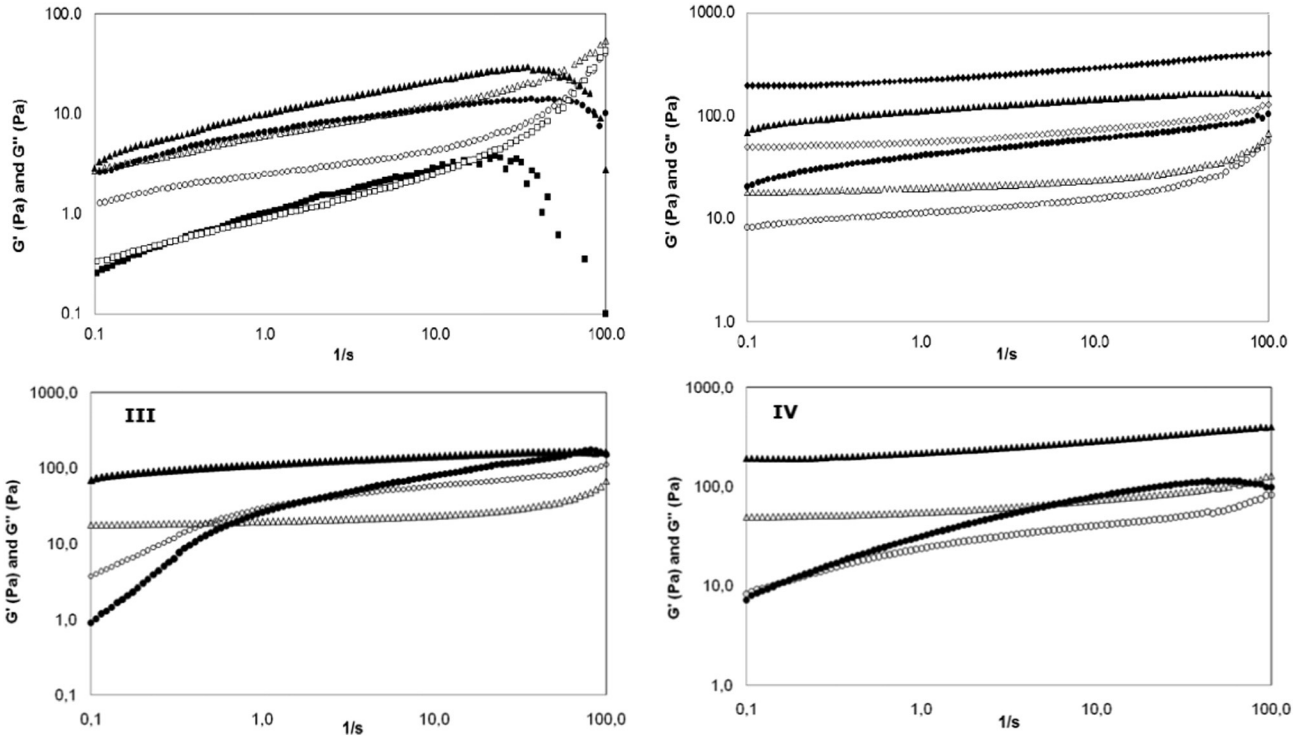

Fig. 2. Mechanical spectra of studied systems. Panel I: systems containing low xanthan gum levels: (●) G', (○) G" for system B (0.448% xanthan gum); (■) G', (□) G" for system D (0.250% xanthan gum and 11.0% oil); (▲) G', (△) G" for system F (0.250% xanthan gum and 44.0% oil). Panel II: systems containing high levels of xanthan gum: (▲) G', (△) G" for system C (1.818% xanthan gum); (●) G', (○) G" for system E (1.000% xanthan gum and 11.0% oil); (◆) G', ( ) G" for system G (1.000% xanthan gum and 44.0% oil). Panel III: effect of stabilizer used in aqueous media: (▲) G', (△) G" for system C (1.818% xanthan gum); (●) G', (○) G" for system I (1.818% guar gum). Panel IV: effect of stabilizer used in 44.0% oil emulsions: (▲) G', (△) G" for system G (1.000% xanthan gum and 44.0% oil); (●) G', (○) G" for system J (1.000% guar gum and 44.0% oil).

frequency and there were significant differences between G' and G" (Fig. 2, panel II). However, tan δ was not lower than 0.1, condition needed to be a strong gel (Da Silva and Rao, 1992). This trend is linked with a low macromolecular mobility.

The existence of a yield stress (σ) was also verified. Yield stress is the minimum shear stress needed to star flow and it is related to the internal structure of the material that can be destroyed before flow takes place (Mertens et al., 2011). As expected yield stress increased with the increase in xanthan gum or oil level (Table 6, systems B vs C, D vs E, and F vs G; systems D vs F and E vs G, respectively). A decrease in the oil level from 44.0 to 11.0% promoted a decrease in G' and G" which conducted to an increase in tan δ. This trend is linked with the decrease in the amount of oil droplets which conducted to a less packed structure.

The aqueous systems containing guar gum showed the behavior of concentrated polymeric dispersions (Fig. 2, panel III). The emulsion exhibited a cross at low frequencies being G' higher than G" in almost all the range of frequencies therefore it can be approximated to a weak gel (Fig. 2, panel IV).

Table 6
Flow behavior of the systems studied. Summary of storage modulus (G'), loss modulus (G") and tangent of the phase shift angle δ (tan δ = G"/G') values at 0.1 rad/s and 25 °C. Summary of yield stress (σ), values determined by the tangent crossover method at 25 °C. In all cases the ± is the standard deviation.

System	G'	G"	Tan δ	σ
B	2.26 ± 0.034 a	1.34 ± 0.155 a	0.61 ± 0.161 a	3.31 ± 0.66 a
C	100.20 ± 2.545 b	24.00 ± 0.710 b	0.24 ± 0.01 b	44.71 ± 3.31 b
D	0.23 ± 0.001 c	0.31 ± 0.007 c	1.34 ± 0.056 c	1.49 ± 0.04 c
E	20.40 ± 0.001 e	8.19 ± 0.014 e	0.40 ± 0.001 e	18.89 ± 0.70 d
F	2.90 ± 0.26 g	2.68 ± 0.15 g	0.93 ± 0.02 g	4.5 ± 0.32 f
G	190.00 ± 5.65 h	48.90 ± 0.56 h	0.26 ± 0.004 b	57.76 ± 1.85 g
J	6.37 ± 1.20 i	8.22 ± 0.177 e	1.32 ± 0.219 c	49.28 ± 1.33 b
I	1.03 ± 0.185 j	3.94 ± 0.255 i	3.88 ± 0.445 h	56.32 ± 3.34 g

Guar systems showed a greater dependency of module values with the frequency, higher tan δ and lower G' values than 1.000% xanthan gum systems (Fig. 2, panels III and IV, Table 6). However, σ of both systems was in the range of 44–58 Pa (Table 6, systems C vs I and G vs. J).

3.2. Development of *Z. bailii* in studied systems

3.2.1. Growth curves

Growth curves obtained were satisfactorily modeled by the modified Gompertz equation as it was proved throughout an ANOVA, by R^2 adj. which showed values of 0.98–0.99 for all systems and by the low values of AAD and RMSE. Mentioned parameters suggested a good correlation between observed and model estimated values. Experimental data and modeled curves for the aqueous system B and for the emulsion F are shown in Fig. 3 as an example. Few experimental points were within the lag phase and as a consequence its estimation presented high errors being this parameter not considered.

3.2.2. Effect of the concentration of xanthan gum, the presence of 1.000% of guar gum and/or the presence of 44.0% of oil on *Z. bailii* growth

Addition of 0.250% of xanthan gum promoted an increase in μ_m. This trend is enhanced by the use of 1.000% gum (Fig. 4, panel I). Regarding the A value, the addition of 0.250% produced an increase but no significant differences were observed when the concentration of the gum increase to 1.000% (Fig. 4, panel II). This trend is related with the possibility that xanthan gum could provide a more favorable media for yeast growth as it will be discussed in the next section. However, the presence of a structuring agent could decrease microbial growth since microorganisms can grow as colonies instead of planktonically (Antwi et al., 2006). Probably, a level of 0.250% xanthan gum acted meanly as nutrient and when it increased to 1.000%, it acted mainly as structuring agent.

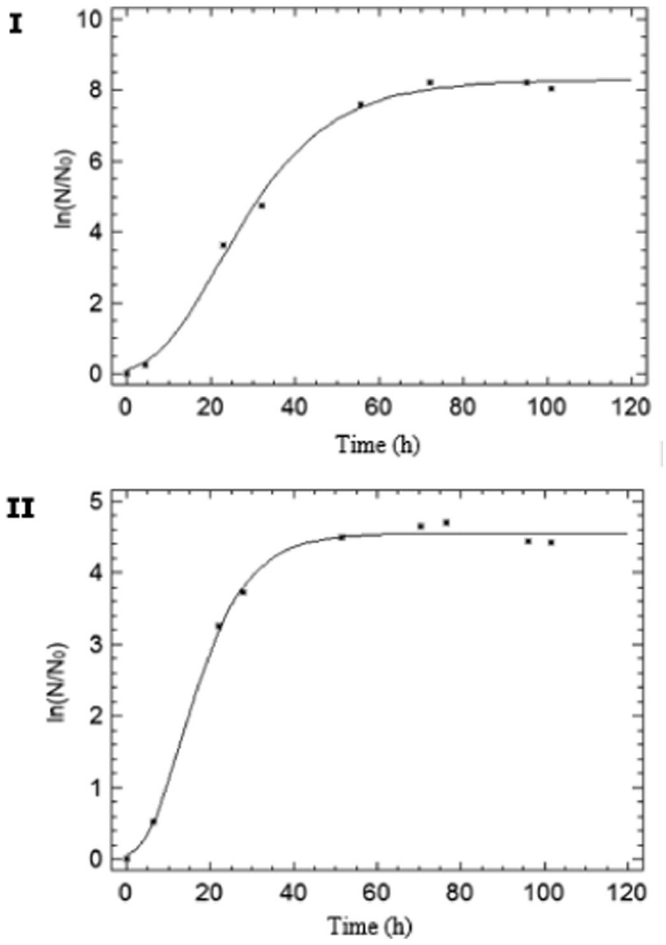

Fig. 3. Modeled growth curves of *Z. bailii*. Panel I: aqueous system containing 1.818% xanthan gum. Panel II: emulsion containing 0.250% xanthan gum and 11.0% corn oil. Experimental points (*), adjusted model (—).

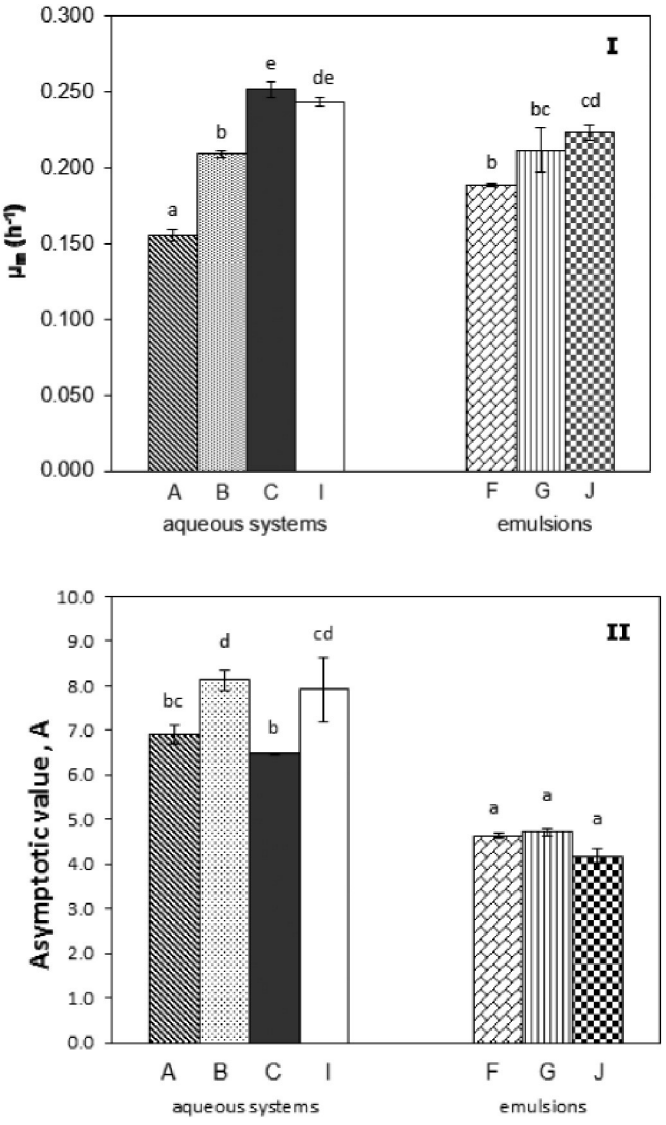

Fig. 4. Effect of xanthan gum content, use of guar gum and addition of 44.0% w/w oil on the growth parameters of *Z. bailii*. Panel I: specific growth rate μ_m (h^{-1}). Panel II: Asymptotic value (A). Bars with the same letter within each panel are not significantly different ($p \leq 0.05$). Error bars represent the standard error. ▧: System A (Sabouraud broth); ▨: System B (0.448% xanthan gum); ■: System C (1.818% xanthan gum); □: System I (1.818% guar gum); ▨: System F (0.250% xanthan gum and 44.0% w/w oil); ▥: System G (1.000% xanthan gum and 44.0% oil) and ▩: System J (1.000% guar gum and 44.0% oil).

The use of 1.000% of guar gum also promoted an increase in μ_m in relation to the system free of stabilizers. In comparison with xanthan gum, no change in μ_m was observed but guar gum promoted an increase in A (Fig. 4, panel I and II, systems I vs C). The latter trend could be linked to the different rheological characteristics of these gums, as it will be discussed in Section 3.3.

The addition of 44.0% oil decreased A value for both xanthan levels and for 1.000% guar gum. However, μ_m value was only decreased for 1.000% xanthan gum concentration (Fig. 4, panels I and II). Different trends were reported about the effect of oil on microbial growth and they were related to the structure of system, the diameter of oil droplets and the characteristics of the microorganism (Campos, Gliemmo, Zalazar, Castro, and Schelegueda, 2015). However, high oil levels promoted a decrease in growth parameters as a result of the limited space available for microbial growth (Brocklehurst and Wilson, 2000; Castro et al., 2003; Castro, Rojas, Campos, and Gerschenson, 2009).

As previously mentioned, in aqueous systems the use of guar instead of xanthan gum, did not exert a significant effect on μ_m and increased A value. In emulsions, neither μ_m nor A was modified by the use of guar gum (Fig. 4, panels I and II, systems J vs G).

3.2.3. Use of xanthan and guar gum as carbon source for Z. bailii growth

It is known that in the absence of glucose, several yeasts have the ability to metabolize different biopolymers. For example, Boons et al. (2015) reported that *S. cerevisiae* is able to metabolize gelatin when glucose was not available.

Z. bailii growth significantly increased when xanthan gum was added to a casein meat peptone broth (Fig. 5, systems L vs. K). This result suggested that xanthan gum could be use as a carbon source by the yeast promoting its growth. Yeasts incubated in Sabouraud broth reached a higher population than in base broth casein meat peptone broth plus xanthan gum. Sabouraud broth provided to yeasts a more favorable media since it contains glucose as carbon source.

Addition of guar gum to a base broth did not enhance *Z. bailii* growth, however it did when it was added to Sabouraud broth (Fig. 5, systems M vs. K). This trend suggested that guar gum was able to promote the growth if the media contained glucose. The different chemical composition of both polysaccharides could be linked with trends obtained. It can be pointed out that guar gum is composed of a backbone of a linear chain of β 1,4-linked mannose residues to which galactose residues are 1,6-linked at approximately every second. However, xanthan gum is formed by a main chain of β-(1,4)-glucose units and a side chain composed of a trisaccharide, consisting of α-D-mannose that contains an acetyl group, β-D-glucuronic acid, and a β-D-mannose terminal unit, linked to a pyruvate group (BeMiller and Huber, 2008). Probably, the

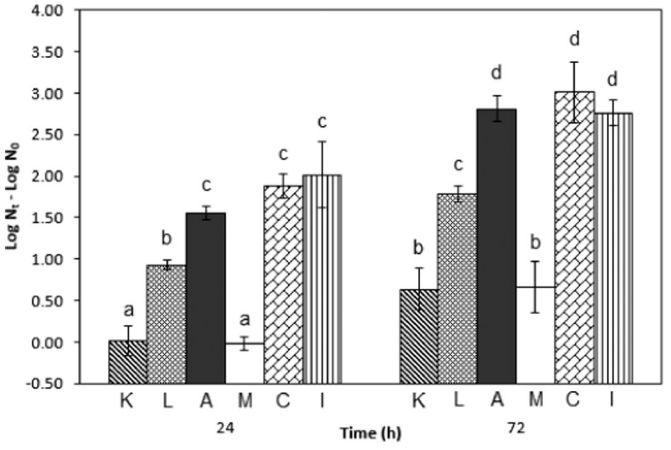

Fig. 5. Use of xanthan and guar gum as carbon source for Z. *bailii* grow thin some aqueous systems. ▧:System K (base broth, 10.0% Casein meat peptone); ▨: System L (base broth plus 1.818% xanthan gum); ■: System A (Sabouraud broth); □: System M (base broth plus 1.818% guar gum); ▨: System C (Sabouraud broth plus 1.818% xanthan gum) and ▨: System I (Sabouraud broth plus 1.818% guar gum). Bars with the same letter are not significantly different ($p \leq 0.05$). Error bars represent the standard error.

glucose chain present in xanthan gum would be more easily metabolized by the yeast.

3.2.4. Combined effect of different concentrations of xanthan gum and corn oil on Z bailii growth in emulsions

The effect of different levels of xanthan gum and corn oil on μ_m and A estimated by the modified Gompertz equation was satisfactorily adjusted to regression models (Table 7) since high values of R^2adj. and low values of AAD and RSME were obtained. Concentration of oil and xanthan influenced μ_m and A (Fig. 6). An increase in the oil level promoted a decrease in μ_m and A. On the contrary, an increase in xanthan gum level promoted an increase in μ_m and A. The latter trend was minimized at the oil level of 44.0%, probably as a result of the interaction between both factors. The conditions that maximize and minimize growth parameters were calculated using the regression model and it was found that the use 11.0% oil and 1.000% xanthan gum maximizes μ_m (0.434 h^{-1}) and A (6.72). On the contrary, the use of 44.0% oil and 0.250% xanthan gum minimizes μ_m (0.184 h^{-1}) and A (4.65).

As previously mentioned, growth of *Z. bailii* was monitored in a system containing 0.375% xanthan gum and 27.5% of corn oil to probe the performance of the model. Experimental data obtained were modeled and growth parameters obtained μ_m (0.19–0.20 h^{-1}) and A (5.25–5.27) were very close to the ones predicted by the regression models μ_m (0.22 h^{-1}) and A (5.44).

3.3. Relationship between physical characteristics of the system and yeast growth

Microbial growth is influenced by the structure of the system. In aqueous systems, maximum populations reached by *Z. bailii* decreased with the increase in xanthan gum level (Fig. 6). This trend could be linked with the more solid character of the system – similar to a strong gel – and the higher σ exhibited (Table 6). Probably, in this condition, the yeasts will be developing in the form of colonies instead of planktonically.

When guar gum is used instead of xanthan, the maximum population reached at the stationary phase was greater (Fig. 4). This trend can be explained by the fact that the system with guar behaved as a polymeric dispersion and consequently exhibited more mobility than the system with xanthan, which behaved as a strong gel (Table 6).

In emulsions with 11.0% oil, an increase in xanthan gum promoted an increase in both growth parameters (Fig. 6). Although the emulsion with 1.000% xanthan exhibited a more solid character as it was mentioned, it behaved as a strong gel while the emulsion containing 0.250% xanthan behaved as a polymeric dispersion (Fig. 2, panels I and II). Probably, in the first emulsion the effect of xanthan gum as nutrient prevailed over its effect as a structuring agent.

When the oil level increased to 44.0% the positive effect of xanthan gum on growth disappeared. As previously commented, an interaction between oil and xanthan gum was observed and the structuring action of the combination of 1.000% xanthan and 44.0% oil, which rendered the system with the highest solid character and the highest yield stress, prevailed over the nutrient action of 1.000% xanthan gum. It must be stressed that the increase in the oil level increased the number and the diameter (D_{32}) of oil droplets (Table 4) which were close packed and did not allow the yeast to grow planktonically.

The use of guar instead of xanthan gum in 44.0% oil emulsions did not show significant differences in growth parameters. Although the guar system behaved as weak gel while the xanthan system behaved as a strong gel, both systems exhibited a similar yield stress (Table 6). Probably, the great ability of xanthan gum as nutrient could compensate the negative effect of structure on growth.

Commented results suggest that the effect of oil and stabilizers on *Z. bailii* growth seems to depend on their ability to act as structuring agents and or nutrients which are influenced by oil concentration and by the system composition.

3.4. Relationship between yeast growth and physical stability

Microbial growth can promote physical instability of emulsions as a result of pH changes and by the interaction between microorganism and oil (Li, McClements, and McLandsborough, 2001). There is no information available about the effect of yeast growth on physical stability of emulsions. However, it is known that inoculation with bacteria which possess a negative Zp promoted flocculation and coalescence in emulsions prepared with cationic surfactants. On the contrary, no effect on physical stability was observed when a non-ionic surfactant such as Tween 20 was used. These trends suggest that interaction between bacteria and oil droplets is governed by electrostatic interactions which depends on the surface charge of both (Ly et al., 2006; Li et al., 2001).

Inoculation with a population of 10^4 CFU/g of *Z. bailii* only increased D_{32} and D_{43} of the emulsion containing 0.250% xanthan gum and 44.0% oil. On the contrary, inoculation promoted a slight decrease in D_{32} and D_{43} for emulsions with guar gum (Table 4). Probably the addition of

Table 7
Coefficients of regression models for yeast growth parameters, adjusted correlation coefficients (R2 adj.), absolute average deviation (AAD), root mean square error (RMSE) and probability (p) values.

Response	Coefficients of regression				R^2adj	AAD	RMSE	P-value		
	β_0	β_1	β_2	β_{12}				x_1	x_2	x_1x_2
μ_m	0.1425	0.0008	0.3676	−0.0077	98.5	2.90	0.01	0.0001	0.0001	0.0001
A	6.3655	−0.0397	1.0133	−0.0206	97.4	1.71	0.11	0.0001	0.0258	0.0627

β_0 is the independent term, β_1 is the regression coefficient for oil level, β_2 is the regression coefficient for xanthan gum level and β_{12} is the interaction coefficient; x_1: oil level; x_2: xanthan level and x_1x_2: interaction.

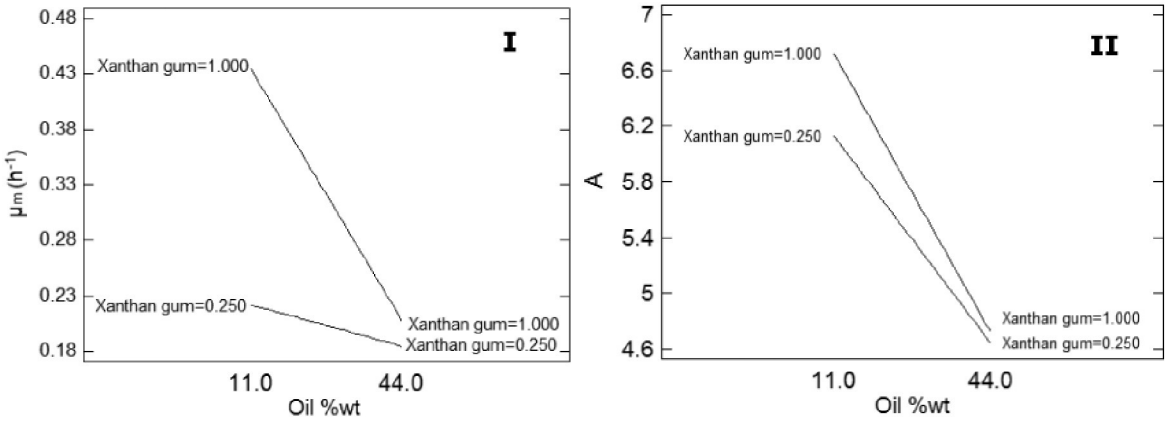

Fig. 6. Combined effect of different levels of xanthan gum and oil on growth parameters of *Z. bailii*. Panel I: specific growth rate (μ_m, h^{-1}). Panel II: Asymptotic value (A, log CFU/g).

the negative charge yeast to a system with a Zp lower than the absolute value of 30 mV exerted a stabilizing effect on this emulsion (Table 5).

Alter 7 days of storage, *Z. bailli* population increased approximately to 10^8 CFU/g and D_{32} and D_{43} also increased for the emulsions containing 0.250% xanthan gum – 11.00% oil (system D) and guar gum (system J). However, in the latter emulsion the increase in droplet size observed after storage in inoculated samples was significantly less than the increase observed for the non-inoculated samples (Table 4). This trend is coincident with the previously commented stabilizing effect. Regarding the emulsion containing 0.250% xanthan gum – 11.0%oil (system D), the increase observed could be linked with the decrease in G' and G" which can promote aggregation.

In summary, the presence of *Z. bailii*, yeast with a negative charge, only affected the stability of negative charge emulsions that were prone to destabilization.

4. Conclusions

Z. bailii growth was satisfactorily modeled by the modified Gompertz equation. Stabilizers and oil influenced growth. In aqueous systems, in general both gums promoted an increase in μ_m and this trend was related to the use of these gums as nutrients. The effect on A parameter depended on the gum, its level and its structuring ability. Addition of 44.0% oil decreased the maximum population and this behavior could be explained by the structuring effect of oil since solid character and yield stress were increased. The evaluation of different levels of oil and xanthan gum on the yeast growth reveals that an increase in the oil level promoted a decrease in μ_m and A. On the contrary, an increase in xanthan gum level promoted an increase in μ_m and A. Furthermore, an interaction between both factors was verified. As a result of the mentioned interaction, a level of 44.0% oil and 0.250% xanthan gum were the conditions that minimize μ_m and A. On the contrary, a level of 11.0% oil and 1.000% xanthan gum maximizes growth parameters.

In general, an increase in the solid character and yield stress of the system through the addition of xanthan gum promoted a decrease in the maximum population reached. Furthermore, a decrease in the solid character promoted an increase in the maximum population reached.

The inoculation with *Z. bailii*, yeast that possessed a negative Zp charge, slightly affected emulsions stability.

Results obtained highlight the importance of considering the different effects that ingredients alone or combined can exert on *Z. bailli* growth and on physical stability of dispersed systems. Furthermore, it was demonstrated that system structure, the agent used to build the structure and the interaction between them determine the development of *Z. bailii*. More studies will be necessary to confirm trends observed in real acid sauces.

Acknowledgments

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