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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