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Evaluation of different chemical preservatives to control *Zygosaccharomyces rouxii* growth in high sugar culture media

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ABSTRACT

Zygosaccharomyces rouxii is an osmophilic yeast responsible for a large amount of economic loss in high sugar food production. Statistical modelling techniques were used in the present study to assess the individual effects of different chemical preservatives (potassium sorbate, sodium benzoate, dimethyldicarbonate, vanillin, ferulic, *p*-coumaric and caffeic acids) to control the growth of a cocktail of five yeast strains belonging to this species and isolated from spoilt concentrated grape juices. None of the preservatives assayed were able to completely inhibit the *Z. rouxii* growth. However, the mathematical models obtained in a high sugar culture media showed that especially four preservatives (potassium sorbate, sodium benzoate, dimethyldicarbonate and vanillin) were the best options to control the growth of this microorganism, obtaining a maximum reduction on yeast growth of approximately 40%. On the contrary, *p*-coumaric and caffeic acids were the preservatives with the lower effects, which only showed a maximum growth reduction percentage of approximately 15%. Results obtained in this paper could be very useful for industry for a better control of this spoilage yeast in concentrated grape juice.

1. Introduction

Osmotolerant and osmophilic yeasts are the most common spoilage agents of sugar-rich foods, where water activity (a_w) is the main limiting factor for microbial growth (Deak & Beuchat, 1994). Among all yeast species recognized, only a small fraction (about 10 yeast species), are responsible for major losses in processed foods around the world (Pitt & Hocking, 1997). The importance of these spoilage yeasts is increasing, because in the modern world a great proportion of foods are being processed, preserved in some form, and stored or transported over long distances before consumption. Spoilage yeasts most frequently described in sugar food and drink industries are those belonging to *Zygosaccharomyces* genus. Spoilage resulting from growth of the yeast *Zygosaccharomyces* is widespread and has caused considerable economic losses in the food industry (Fleet, 2011). Food containing high concentrations of sugar (40–70%) includes sugar cane, sugar syrups, honey, concentrate fruit juices, jams, jellies and dried fruits. Their spoilage by yeasts is not uncommon, with *Zygosaccharomyces rouxii* being most frequently implicated because of its unique ability to tolerate the high osmotic stresses and low a_w conditions of these products, and also due to its resistance to different preservatives (Fleet, 2011; Martorell, Stratford, Steels, Fernández-Espinar, & Querol, 2007; Stratford et al., 2013). Occasionally, other yeasts are also found and these include *Zygosaccharomyces bailii*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces mellis*, *Schizosaccharomyces pombe*, *Torulasopora delbrueckii* and various *Candida* species (Combina et al., 2008; Martorell et al., 2007; Stratford, 2006). Spoilage activity by *Zygosaccharomyces* can lead to excessive gas production in foods. The amount of CO_2 generated can be sufficient to distort packaging, and break cans or kegs (Grimbaum, Ashkenazi, Treister, Goldschmied-Reouven, & Block, 1994).

In a previous study, we have characterized the osmophilic and osmotolerant yeast species present in Argentinean concentrated

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grape juice. *Z. rouxii* was the only yeast species isolated from spoiled concentrated grape juices and it was the main yeast species present in unspoiled samples followed by lower proportions of other yeast species (Combina et al., 2008). We have also shown that the spoilage depends on the size of the initial inoculum as well as the strain ability to grow under certain conditions (Combina et al., 2008). We confirmed that the visual sign of spoilage become noticeable when yeast growth reaches approximately 10^4–10^5 CFU/g and are clearly evident at 10^6–10^7 CFU/g (Combina et al., 2008; Fleet, 2011). In a recent work performed by our group, the microbiological stability of the concentrated grape juice according their pH and sugar concentration was established. Results allowed us to propose a model system to determine the intrinsic stability of the product and to define the juice parameters (pH and a_w) for increasing microbial stability (Rojo et al., 2014). However, when the product has a high population of osmophilic yeast, the adjustment of these parameters is not enough to inhibit microbial growth and to extend the shelf life long enough to allow the exportation of the product. Thus, it is necessary to develop other technological tools to obtain a reduction in this population, without affecting the sensorial characteristics of the concentrated grape juice.

The control of spoilage yeasts is one of the most important aspects in food preservation. Factors such as low temperature, reduce a_w, addition of preservatives and low pH, are all used to inhibit yeast species and other microorganisms (Hidalgo Togores, 2002; Marechal, Martinez de Marnanon, Poirier, & Gervais, 1999). Moreover, the addition of preservatives in foods has been used for centuries. The most commonly used food preservatives are weak acids, such as sorbic, benzoic, propionic, and acetic acids and sulfur dioxide (sulphite), some of which occur naturally in foods (fruits and vegetables) (Piper, 2011). Weak acid preservatives are widely used in sugar containing low-pH foods such as fruit juices, beverages, wine, dressings and sauces, in which spoilage is most often caused by yeasts, moulds and lactic acid bacteria (Beuchat, 1982; Vermeulen et al., 2008). Yeast resistance and even metabolism of such preservatives raises problems for the food industry; causing a requirement for increased preservative levels in low-pH foods to prevent yeast spoilage (Piper, 2011).

Dimethyldicarbonate (DMDC) is a chemical preservative that has recently been approved for the control of spoilage yeasts in wines (Martorell et al., 2007; OIV, 2013). It has been demonstrated that more than 3 mM DMDC is necessary for a complete inhibition of alcoholic fermentation conducted by different yeast species (Costa, Barata, Malfeito-Ferreira, & Loureiro, 2008; Delfini et al., 2002). Also, Renouf, Strehaiano, and Lonvaud-Funel (2008) have suggested that DMDC should not be used as a preventive agent but only as curative agent against unwanted populations already present in wine.

Nowadays, modern consumers prefer high quality foods that are more natural, minimally processed and preservative free. This latter along with stricter legislation regarding current preservatives has challenged the food industry leading to increased research into the use of "naturally derived" antimicrobials. Over the last two decades, other preservatives from plant, animal and microbial origins have been intensely investigated for practical applications (Pozo-Bayón, Monagas, Bartolomé, & Moreno-Arribas, 2012). Thereby, vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major constituent of vanilla beans and is the main flavouring compound used in numerous foods such as ice cream, chocolate and confectionary products. Moreover, recent reports have shown that vanillin can be an effective inhibitor of yeasts and moulds when tested in fruit purees and fruit based agar systems (López-Malo, Alzamora, & Argaiz, 1995; López-Malo, Alzamora, & Argaiz, 1998). Additionally, different studies have demonstrated the potential application of phenolic extract as antimicrobial and antioxidant agents in order to prevent food spoilage and to prolong the shelf life of final products (García-Ruiz et al., 2012). Hydroxycinnamic acids (HCAs) are endogenous components of grapes and are considered natural food preservatives (Ou & Kwok, 2004; Smid & Gorris, 1999). The most abundant HCAs in grapes are caffeic, *p*-coumaric and ferulic acids, in decreasing order (Flanzy, 2000). HCAs have been reported to inhibit growth of a variety of organisms including fungi and bacteria (Campos, Couto, & Hogg, 2003; Ravn, Andary, Kovacs, & Moelgaard, 1989; Stead, 1993; Van Sumere, Cottenie, De Gref, & Kint, 1971; Walker, Bais, Halligan, Stermitz, & Vivanco, 2003). In particular, the growth of a number of yeast species is inhibited by HCAs in concentrations over 1 mM, with ferulic and *p*-coumaric acids being generally found to be the most inhibitory (Harris, Jiranek, Ford, & Grbin, 2010; Ou & Kwok, 2004; Pastorkova et al., 2013).

In the present work, seven chemical preservatives were assessed to control the growth of *Z. rouxii* in a high sugar culture media, in order to find a new strategy for preservation of concentrated grape juices. The study was carried out by using a modelling approach, obtaining diverse mathematical equations useful to determine the percentage of growth reduction of this microorganism as a function of preservative concentration.

2. Material and methods

2.1. Yeast strains

Five strains belonging to *Z. rouxii* species (MR4, MT6, MC8, MC9 y MC10) previously isolated from spoilt Argentinean concentrated grape juices were used in the present study (Combina et al., 2008). Strains were previously identified by molecular sequencing of the D1/D2 domain of 26S ribosomal gene and registered at the Wine Research Centre Microorganism Collection from INTA (Mendoza, Argentina).

2.2. Yeast cocktail preparation

First, the five *Z. rouxii* strains were independently grown during 24 h at 28 °C on YPD broth (40 g/L glucose (Biopack Co.), 5 g/L bacteriological peptone (Britania Co.), 5 g/L yeast extract (Britania Co.) and 20 g/L agar (Britania Co.). Then, a high sugar culture media MYGF 195 g/L glucose (Biopack Co.), 195 g/L fructose (Biopack Co.), 20 g/L malt extract (Britania Co.), 5 g/L yeast extract (Britania Co.) adjusted to pH 4.5 by citric acid addition) was prepared and inoculated with the different strains to form a cocktail of approximately 10^4 CFU/mL. Finally, yeasts were incubated in this high sugar culture media during 48 h at 28 °C without shaking until the yeast population reached 2×10^7 CFU/mL.

2.3. Modelling of the individual effects of preservatives on *Z. rouxii* growth

The effect of preservatives on yeast growth cocktail was studied using MYGF as basal medium supplemented with different doses of the following chemical compounds: potassium sorbate (Sigma–Aldrich Co., St. Luis, USA), sodium benzoate (Sigma–Aldrich Co.), dimethyldicarbonate (Velcorin®), vanillin (Sigma–Aldrich Co.) and different hidroxy-cinnamic acids such as ferulic, *p*-coumaric and caffeic acids (Sigma–Aldrich Co.). The first three preservatives were selected considering the products approved by the International Organization of Vine and Wine (OIV) and the National Viticulture Institute (INV, Argentina), whereas the last preservatives were selected by their natural character and its promising antimicrobial activities in food industry. Chitosan (Lallemand Co.) was also evaluated, but no effect on *Z. rouxii* growth was detected (data not