Food Microbiology 38 (2014) 143–150

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Effects of pH and sugar concentration in *Zygosaccharomyces rouxii* growth and time for spoilage in concentrated grape juice at isothermal and non-isothermal conditions

M.C. Rojo [a, c], F.N. Arroyo López [b], M.C. Lerena [a, c], L. Mercado [c], A. Torres [a, d], M. Combina [a, c, *]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Ciudad Autónoma de Buenos Aires C1033AAJ, Argentina
[b] Food Biotechnology Department, Instituto de la Grasa (CSIC), Av. Padre García Tejero 4, 41012 Sevilla, Spain
[c] Wine Research Center, Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA Mza INTA), San Martín 3853, Luján de Cuyo, Mendoza 5507, Argentina
[d] Microbiology and Inmunology Department, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36, Km 601, Argentina

ARTICLE INFO

Article history:
Received 25 April 2013
Received in revised form
5 August 2013
Accepted 29 August 2013
Available online 11 September 2013

Keywords:
Response surface methodology
Zygosaccharomyces rouxii
Concentrated grape juice
pH
Sugar concentration
Spoilage

ABSTRACT

The effect of pH (1.7–3.2) and sugar concentration (64–68 °Brix) on the growth of *Zygosaccharomyces rouxii* MC9 using response surface methodology was studied. Experiments were carried out in concentrated grape juice inoculated with *Z. rouxii* at isothermal conditions (23 °C) for 60 days. pH was the variable with the highest effect on growth parameters (potential maximum growth rate and lag phase duration), although the effect of sugar concentration were also significant. In a second experiment, the time for spoilage by this microorganism in concentrated grape juice was evaluated at isothermal (23 °C) and non-isothermal conditions, in an effort to reproduce standard storage and overseas shipping temperature conditions, respectively. Results show that pH was again the environmental factor with the highest impact on delaying the spoilage of the product. Thereby, a pH value below 2.0 was enough to increase the shelf life of the product for more than 60 days in both isothermal and non-isothermal conditions. The information obtained in the present work could be used by producers and buyers to predict the growth and time for spoilage of *Z. rouxii* in concentrated grape juice.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Grape juice and its by-products represent an important part of the food industry in the world. Argentina grape production is mainly industrialized, where wine and concentrated grape juices are the two mayor types of commercial products. Mendoza and San Juan provinces (West of Argentina) are the main manufacturers of concentrated grape juices in the country, with 75% of their production mainly exported to United States, Japan, Russia and México (Bruzone, 1998; INV, 2013). Concentrated grape juice represents a critical additive in several mass consumption products. Due to their natural qualities, concentrated grape juice is employed to manufacture baby foods, pharmaceutical products, foods and drinks (Bruzone, 1998). Concentrated grape juices are microbiologically more stable than other fruit products due to the high sugar concentration and usually are stored at room temperature without any additional treatment (ICMSF, 1980; Splittstoesser, 1987). However, these products are not free of microbiological spoilage problems. The combination of high concentration of sugar and low pH still support the development of a reduced number of microorganism species. Osmophilic yeasts represent the primary spoilage cause in high sugar food and drink industries, with the genus *Zygosaccharomyces* as the most frequent described spoilage microorganism (ICMSF, 1980; Deák and Beuchat, 1993; Worobo and Splittstoesser, 2005; Martorell et al., 2007).

The genus *Zygosaccharomyces* has a long history of spoilage in the food industry. Three *Zygosaccharomyces* species, *Z. bailii*, *Z. bisporous*, and *Z. rouxii*, have been associated with the spoilage of grape must, concentrated grape juice and wine (Loureiro and Malfeito-Ferreira, 2003; Fugelsang and Edwards, 2007; Deák, 2008). Spoilage by *Zygosaccharomyces* species can be categorized

* Corresponding author. Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA Mza INTA), San Martín 3853, Luján de Cuyo, Mendoza 5507, Argentina. Tel.: +54 261 4963020x295.
E-mail addresses: mcombina@mendoza.inta.gov.ar, maricombina@yahoo.com (M. Combina).

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.08.017

into two groups: i) visible growth on the surface of the product, and ii) fermentative spoilage manifested by alcoholic, esteric or other types of odours and/or visible evidence of gas production, leading to bubbling of the product and/or packaging expansion (Legan and Voyset, 1991; Smith et al., 2004).

In a previous study from our group, the osmotolerant and osmophilic yeast population in concentrated grape juice from Argentina was characterized, with *Z. rouxii* being the only yeast species isolated from spoilt products. Moreover, in other samples without visible evidence of spoilage, *Z. rouxii* was also frequently isolated representing 76% of the total yeast population (Combina et al., 2008). The unique physiological characteristics of *Z. rouxii* are largely responsible for their ability to cause spoilage. These include resistance to low-acid preservatives, extreme osmotolerance, ability to adapt to high glucose concentrations, or low activity water (a_w) values and high temperatures, ability to ferment glucose, and ability to growth at low pH values (Emmerich and Radler, 1983; James and Stratford, 2003; Martorell et al., 2007).

Few studies have been carried out to assess the effect of limiting factors on the growth *Z. rouxii*. The vast majority of these studies were performed in culture media and some of them assessed each variable independently (Kalathenos et al., 1995; Praphailong and Fleet, 1997; Membré et al., 1999). Conversely, the response surface (RS) methodology is a very useful tool which has been previously applied to estimate the combined effects of different environmental variables on yeast growth (Arroyo-López et al., 2006; D'Amato et al., 2006). The RS methodology has been widely used in predictive microbiology as a secondary polynomial model to predict the microorganism response as a function of environmental changes determining at the same time the interaction among them (McMeekin et al., 1993).

In this work, the combined effect of the two limiting factors (pH and sugar concentration) on the growth parameters of a native strain of *Z. rouxii* (MC9) previously isolated from spoilt concentrated grape juice was assessed. This task was accomplished using RS methodology, as secondary model. In an effort to provide useful and practical considerations to producers and buyers, the time for spoilage (TFS) was also determined in natural substrate under the standard storage (isothermal) and shipping (non-isothermal) temperature conditions normally found in Argentinean concentrated grape juices.

2. Materials and methods

2.1. Yeast strain

The strain *Z. rouxii* MC9, previously isolated from spoilt concentrated grape juices, was used in the present study. The strain was identified by molecular sequencing of the D1/D2 domain of the 26S ribosomal gene and registered at the Wine Research Centre Microorganism Collection from INTA, Argentina (GenBank Accession Number KF002711). This strain was selected from a previous study among several native *Z. rouxii* strains because of its better adaptation to concentrated grape juice and fast growth (Rojo et al. unpublished data).

2.2. Media and growth conditions

Z. rouxii MC9 was previously grown on YPD broth (40 g/L glucose, 5 g/L bacteriological peptone, 5 g/L yeast extract, 20 g/L agar) during one day at 28 °C. Before inoculation in natural substrate (concentrated grape juice), the strain was adapted to osmotic shock by growing in a medium (MYGF) with an intermediate concentration of sugar (195 g/L glucose, 195 g/L fructose, 20 g/L malt extract, 5 g/L yeast extract) with pH adjusted to 4.5 by the addition of citric acid. This last medium was incubated during three days at 28 °C without shaking until the highest possible population was reached (10^7 CFU/mL) right at the end of exponential growth phase. Experiments were finally performed in concentrated grape juice provided by a local company located in Mendoza (Argentina).

Table 1
Biological growth parameters of *Zygosaccharomyces rouxii* MC9 (μ_{max}, potential maximum growth rate; λ, lag phase duration) obtained at isothermal conditions (23 °C) for the different treatments (combinations of pH and °Brix) included in the experimental design.

Run	°Brix	pH	μ_{max} (log_{10} CFU/mL days^{-1})	λ (days)
1	64	1.7	0.000	>60
2	64	1.7	0.000	>60
3	64	1.7	0.000	>60
4	64	2.5	0.360	2.23
5	64	2.5	0.530	1.39
6	64	2.5	0.199	3.07
7	64	3.2	0.417	0.00
8	64	3.2	0.404	0.00
9	64	3.2	0.434	0.00
10	66	1.7	0.000	>60
11	66	1.7	0.000	>60
12	66	1.7	0.000	>60
13	66	2.5	0.287	4.14
14	66	2.5	0.285	1.99
15	66	2.5	0.262	6.29
16	66	3.2	0.455	0.88
17	66	3.2	0.392	1.59
18	66	3.2	0.422	0.17
19	68	1.7	0.000	>60
20	68	1.7	0.000	>60
21	68	1.7	0.000	>60
22	68	1.9	0.058	17.38
23	68	1.9	0.085	17.28
24	68	1.9	0.066	17.33
25	68	2.1	0.140	11.97
26	68	2.1	0.132	8.42
27	68	2.1	0.115	15.52
28	68	2.5	0.181	6.73
29	68	2.5	0.165	12.1
30	68	2.5	0.168	9.37
31	68	3.2	0.185	0.95
32	68	3.2	0.182	2.29
33	68	3.2	0.192	0.00

Note: Values were obtained with the Baranyi and Roberts' model (1994) using DMfit 2.1. program. In the case of no growth, μ_{max} and λ were set to 0.000 log_{10} cfu/mL days^{-1} and 60 days, respectively, for modelling purposes.

2.3. Experimental design

The different runs (a total of 66) were carried out in 1-L of concentrated grape juice placed in sterile bag-in-box with a Vitop® valve. Bags were placed in metal containers to reproduce the standard storage (isothermal) and overseas shipping (non-isothermal) conditions and monitored for 60 days. The experimental design was obtained from the combination of two variables (pH and sugar concentration) with 3 levels for each variable (Table 1). Variables levels were established taking into consideration a range of conditions usually found in Argentinean concentrated grape juices. The pH values were 1.7, 2.5 and 3.2 and the sugar concentration (expressed as °Brix) were 64 °Brix (779 g/L reducing sugar; a_w: 0.778 ± 0.003), 66 °Brix (810 g/L; a_w: 0.767 ± 0.003) and 68 °Brix (842 g/L; a_w: 0.744 ± 0.003). Two intermediate pH values (1.9 and 2.1) were also evaluated at the sugar concentration condition more frequently required by the market (68 °Brix), yielding a total of eleven different treatments, each performed in triplicate. To achieve the different pH values, grape juices were passed through ion exchange column to obtain the desired pH prior to concentration in order to reproduce industrial

Download English Version:

https://daneshyari.com/en/article/4362929

Download Persian Version:

https://daneshyari.com/article/4362929

Daneshyari.com