Food Microbiology 57 (2016) 71–80

Contents lists available at ScienceDirect

Food Microbiology

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

Effect of zinc formulations, sodium chloride, and hydroxytyrosol on the growth/no-growth boundaries of table olive related yeasts

V. Romero-Gil [a], L. Rejano-Zapata [b], A. Garrido-Fernández [a], F.N. Arroyo-López [a, *]

[a] Food Biotechnology Department, Instituto de la Grasa (Agencia Estatal Consejo Superior de Investigaciones Científicas, CSIC), Campus Universitario Pablo de Olavide, Building 46, Ctra. Utrera, km 1, 41013 Seville, Spain
[b] AgroSevilla SCA, Paseo de Castellmadama s/n, 41590 La Roda de Andalucia, Seville, Spain

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
2 December 2015
Accepted 22 January 2016
Available online 23 January 2016

Keywords:
Growth/no-growth interfaces
Logistic regression
Zinc interaction
NaCl
Hydroxytyrosol
Preservatives

ABSTRACT

This study uses a mathematical approach to assessing the inhibitory effect of Zn^{2+} (0–10 mM, obtained from $ZnCl_2$ and $ZnSO_4$) in presence of NaCl (0–8%) and hydroxytyrosol (0–2588 mg/L), on a yeast cocktail formed by species *Pichia galeiformis, Pichia kudriavzevii, Pichia manshurica* and *Candida thaimueangensis* obtained from spoilt green olive packages. The logistic/probabilistic models were built in laboratory medium using a total of 1980 responses (1188 for NaCl and 792 for hydroxytyrosol). $ZnCl_2$ showed significantly higher inhibitory effect than $ZnSO_4$ in the presence of both NaCl ($p < 0.033$) and hydroxytyrosol ($p < 0.009$). NaCl did not interfere the effect of Zn^{2+} while hydroxytyrosol, at high levels, had a slight antagonistic effect. According to models, Zn^{2+} inhibits ($p = 0.01$) the yeast cocktail in the range 4.5–5.0 mM for $ZnCl_2$, or 8.5–9.5 mM for $ZnSO_4$. Therefore, this work confirms the fungicidal activity of zinc compounds (mainly $ZnCl_2$) in synthetic medium, and also shows that the loss of zinc effectiveness in real green Spanish-style olive packaging is not due to the presence of NaCl or hydroxytyrosol, two of the most abundant chemical compounds in the product.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The production of table olives (nowadays >2.5 million tons/season) represents an important economic resource for producing countries (Spain, Turkey, Egypt, Italy and Greek among the most important). Green Spanish-style constitutes the most popular table olive presentation in the markets (Garrido-Fernández et al., 1997). Lactic acid bacteria (LAB) and yeasts have an important role during processing of this specific type of table olive elaboration (Arroyo-López et al., 2012a; Hurtado et al., 2012). In particular, yeasts can act as desirable microorganisms (due to both technological and probiotic characteristics) but also may cause the product spoilage through production of CO_2, unwanted odors/flavors, the consumption of lactic acid, the softening of fruits, and the clouding of olive brines (Arroyo-López et al., 2012a). These drawbacks are particularly relevant when a residual sugar concentration (or nutrients in general) is present in the packaged product. Usually, product stabilization, in absence of thermal treatment, requires preservatives to control microbial growth. However, the usual chemical additives currently used by industry (sorbic and benzoic acids or their respective salts) may produce diverse negative effects such as brine browning, development of off-flavour or accumulation in the oily phase and other tissue components of fruits (Brenes et al., 2004; Garrido-Fernández et al., 1997). This absorption reduces their level in brines and consequently the efficacy for the product preservation. Sorbic and benzoic acids proved to be effective in laboratory medium to control the growth of a native yeast cocktail isolated from seasoned cracked Manzanilla-Aloreña olives (Arroyo-López et al., 2008). However, in real olive packaging, sorbic contents decreased with time causing progressive product instability (Arroyo-López et al., 2009; Bautista-Gallego et al., 2013). Similar problems can also occur in non-thermally treated partially fermented (cured) green Spanish-style olives (Garrido-Fernández et al., 1997). These circumstances make the investigation of new alternative packaging procedures and preservatives a priority for non-pasteurized table olive presentations.

Microorganisms need zinc (Zn) for the proper functioning of their metabolism due to the element participation as cofactor of diverse enzymes in numerous biological reactions. Nevertheless, above a threshold concentration, it may act as cytotoxic due to its competition with other metal ions for intracellular transport

* Corresponding author.
E-mail address: fnarroyo@cica.es (F.N. Arroyo-López).

http://dx.doi.org/10.1016/j.fm.2016.01.007
0740-0020/© 2016 Elsevier Ltd. All rights reserved.

proteins or the active sites of enzymes (Walker, 2004). Above such levels, Zn cation could be used as preservative agent in food processing provided its addition be in agreement with the conditions required for the mineral fortification of products. This possibility is supported by previous studies which have shown that $ZnCl_2$, in laboratory medium, has a strong inhibitory effect against a considerable number of table olive related yeasts (Bautista-Gallego et al., 2012). Also, in cracked (directly brined) Aloreña de Málaga table olives, the presence of $ZnCl_2$ led to a more marked reduction in the yeast population during shelf life than the traditional preservatives (Bautista-Gallego et al., 2011). However, the inhibitory effect of $ZnCl_2$ was not observed in green Spanish-style Manzanilla olive packaging (Bautista-Gallego et al., 2015). Apparently, the species of yeasts present in this product could be resistant to Zn, or alternatively, a potential interference of the chemical compounds present in the presentation could be responsible for the activity decrease. NaCl and hydroxytyrosol (Hy) are among the most abundant chemical compounds present in this type of olive preparation (Blekas et al., 2002; Garrido-Fernández et al., 1997; Romero et al., 2004). Disclosing the causes of the different inhibitory behaviour of Zn in laboratory medium and in real green olive packaging has then an unquestionable scientific interest.

In this survey, the yeast population present in green Spanish-style table olive plastic pouches with evidence of spoilage were isolated and molecularly identified. Then, the effect of Zn (from $ZnCl_2$ and ZnSO4), and the possible interference with NaCl and Hy, on a cocktail formed with the most representative isolated species, was evaluated using a probabilistic/logistic model. Predictive microbiology is a valuable tool to describe quantitatively the response of microorganisms as a function of environmental variables or preservatives (McMeekin et al., 1993). By using this mathematical approach, it is possible: i) to determine the sensitivity of native yeasts to the assayed chemical compounds, evaluating the applicability of Zn as a new preservative in olive packaging, ii) to study the potential interference of NaCl and Hy on the inhibitory effect of the diverse Zn formulations, and iii) to estimate the corresponding growth/no growth (G/NG) boundaries of microorganisms.

2. Material and methods

2.1. Yeast isolation, characterization, and identification

A total of 20 yeast isolates were obtained from diverse plastic pouches of commercial Manzanilla green Spanish-style table olives stuffed with red paper paste and with spoilage signs (clouding of brines and swelling). Firstly, yeast isolates were genotypically characterized by RAPD-PCR with primer M13 (Tofalo et al., 2009). The resulting fingerprints were digitally captured and analysed with the BioNumerics 6.6 software package (Applied Maths, Kortrijk, Belgium). The similarity among digitalized profiles was calculated using the Pearson product–moment correlation coefficient. Dendrogram was obtained using the Unweighted Pair Group Method with the Arithmetic Average (UPGMA) clustering algorithm. *Candida boidinii* TOMC-Y5 was used as internal control to determine the reproducibility of the technique. Then, one representative isolate from different clusters was selected for molecular identification, which was achieved using an RFLP analysis of the 5.8S-ITS rDNA region (Esteve-Zarzoso et al., 1999) and the sequencing of D1/D2 domains of the 26S rDNA gene (Kurtzman and Robnett, 1998).

2.2. Yeast cocktail preparation

Yeast cocktail for modelling purposes was prepared using representative isolates obtained from the different clusters of the dendrogram: two isolates (MBY-14 and MBY-19) were ramdoly chosen from different sub-clusters of cluster I, which was the most numerous, one from cluster II (isolate MBY-5) and another from cluster III (isolate MBY-11).

The four mentioned yeast isolates were inoculated separately into 5 mL of a Yeast–Malt–peptone–glucose broth medium (YM, Difco™, Becton and Dickinson Company, Sparks, USA) and incubated at 28 °C for 48 h. Then, tubes were centrifuged at 9000 *g* for 15 min and the pellets re-suspended separately in 5 mL of sterile peptone water (0.1%, wt/vol). To form the yeast cocktail, 3 mL of each culture were mixed at the same proportion to reach a final volume of 12 mL with a population level of approximately 7 $\log_{10}$ CFU/mL, which was confirmed by surface spread on YM agar plates. Therefore, the composition of the cocktail was formed by approximately 25% of each strain (MBY-14, MBY-19, MBY-5 and MBY-11).

2.3. Growth media and experimental design

The basal media selected for all experiments consisted of YM broth set at pH 4.0 by citric acid addition. This value mimics the pH conditions usually found during packaging of Spanish-style green table olives. Subsequently, the media was conditioned with different concentrations of NaCl (Panreac ITW Companies, Castellar del Valles, Barcelona, Spain), $ZnCl_2$ (VWR International, Leuven, Belgium), $ZnSO_4$ (Panreac), and Hy (Extrasynthèse, Genay, France), according to treatments shown in Table 1. All reagents had >99% purity. Hy, $ZnCl_2$ and $ZnSO_4$ levels were prepared from different stock mother solutions (51,768 mg/L for Hy, 200 mM for $ZnCl_2$ and $ZnSO_4$). The Hy content was determined by HPLC at the end of the experiment to assess the stability of the phenolic compound (Romero et al., 2004). The experimental design consisted of 4 full-factorial combinations of the levels of the following couple of chemicals: $ZnCl_2$–NaCl, $ZnSO_4$–NaCl, $ZnCl_2$–Hy, and $ZnSO_4$–Hy. Six replicates of each treatment were run in parallel.

2.4. Optical density measurements

Yeast growth was recorded in a Bioscreen C automated spectrophotometer (Labsystem, Helsinki, Finland) with a wideband filter (420–580 nm) set at 28 °C for 7 days. Measurements were taken every 2 h after a pre-shaking of 10 s to avoid cell sedimentation. The wells of the micro-plates were filled with 0.02 mL of inoculum and 0.35 mL of the basal media (modified according to the experimental design), always reaching an initial optical density (OD) of approximately 0.2 units (initial inoculum level of 2×10^6 CFU/mL). This inoculum level is similar to the populations found in the packaged of Spanish-style and natural green olives (Arroyo-López et al., 2006; López-Lopez et al., 2004) or that used by Lambert and Pearson (2000) or Vermeulen et al. (2007) for the estimation of the G/NG interfaces of LAB describing the influence of pH in acidified sauces. The inoculum was always above the detection limit of the apparatus, which was determined by comparison with a previously established calibration curve (data not shown). Un-inoculated wells for each experimental series were included in the micro-plate to determine, and subtract, the noise signal due to possible chemical oxidation process. For each well, growth (coded as 1) was assumed when the OD increase with respect to the initial OD (after subtraction of the noise signal) was higher than 0.1; no-growth (coded as 0) was recorded when the initial OD remained stable or the increase was <0.1. Responses for each replicate were recorded independently, and the whole matrix was subjected to statistical analysis. After concluding the experiments, randomly selected wells (which included both growth and no-growth

Download English Version:

https://daneshyari.com/en/article/4362602

Download Persian Version:

https://daneshyari.com/article/4362602

Daneshyari.com