LWT - Food Science and Technology 84 (2017) 338–343

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

A new seasoning with potential effect against foodborne pathogens

Javier García-Lomillo, María L. González-SanJosé*, Raquel Del Pino-García, María Dolores Rivero-Pérez, Pilar Muñiz-Rodríguez

Department of Biotechnology and Food Science, Faculty of Science, University of Burgos, Plaza Misael Bañuelos, 09001, Burgos, Spain

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form
19 May 2017
Accepted 23 May 2017
Available online 26 May 2017

Keywords:
Seasoning
Natural
Antimicrobial
Wine pomace
Foodborne pathogen

ABSTRACT

The food industry is constantly looking for natural alternatives to chemical additives. Recently, a new red wine pomace seasoning (RWPS) with flavoring, antioxidant, and antimicrobial activities has been obtained from red wine pomace, one of the most important byproduct from winemaking process. This study focused on the antimicrobial activity of a RWPS against three foodborne pathogens: *Staphylococcus aureus*, *Listeria innocua*, and *Escherichia coli*. The microbial growth was assessed in the absence and the presence of RWPS for 34 h at 37 °C. RWPS (40 g L^{-1}) presented bactericidal effects against *S. aureus* and *L. innocua*. Bacteriostatic effects were observed against *E. coli* with RWPS at 40 g L^{-1} and against *S. aureus* with RWPS at 20 g L^{-1}. Furthermore, RWPS at 40 g L^{-1} also slowed the growth of *E. coli* and *L. innocua*, extending the duration of the lag phase and decreasing the maximum growth rate of these two microorganisms. In conclusion, the studied seasoning reasonably inhibits the growth of the studied foodborne pathogens. The present study may improve the efficacy of the food chain system by extending the shelf-life of food products and reducing the amount of food wasted.

1. Introduction

Foodborne pathogens cause more than 320,000 cases of human infection each year in the European Union (EFSA & ECDC, 2015). They are caused by the consumption of food and water contaminated with pathogenic microorganisms and their toxins. Therefore, additives are widely applied by the food industry to ensure the food safety by controlling the growth of pathogens (Davidson & Branen, 2005). However, the rejection of chemical additives by consumers has led the food industry to seek other alternatives. Different natural products with a high potential to inhibit microbial growth, such as spices and products derived from agricultural byproducts, have been proposed to extend the shelf-life of food products (Souza, Stamford, Lima, Trajano, & Barbosa Filho, 2005). In this sense, natural seasonings with effective preservative capacity have been recently obtained from red wine pomace (RWPS) (García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014; González-SanJosé, García-Lomillo, Del Pino-García, Dolores-Rivero, & Muñiz-Rodríguez, 2015). The patented process to obtain these natural seasonings enables the re-utilization of an agricultural byproduct by using only sustainable techniques. These seasonings showed satisfactory antimicrobial effects against total aerobic mesophilic bacteria, lactic acid bacteria, and Enterobacteriaceae in beef homogenates, and presented excellent properties to be used as food ingredients as well as high levels of fiber, protein, and minerals, mainly potassium with low level of sodium (García-Lomillo et al., 2014).

Different microorganisms may be involved in foodborne outbreaks with different grades of frequency and severity. Listeriosis is the primary cause of death by foodborne pathogens, presenting a very high mortality rate (15.6%), with a relatively large number (1,763) of confirmed cases of listeriosis occurred in the European Union in 2014 (EFSA & ECDC, 2015). Listeriosis is linked to the presence of *Listeria monocytogenes*, a Gram-positive bacterium, in smoked fish, cheeses, and ready-to-eat meat products.

Escherichia coli,a common Gram-negative bacterium in the human gastrointestinal, also caused over 6,000 confirmed cases and 13 deaths in the EU in 2013. Pathogenic strains of *E. coli* may cause urinary tract infections, diarrhea, respiratory problems, and other illnesses associated with the consumption of food or water contaminated with feces (EFSA & ECDC, 2015).

Staphylococcal intoxication is a less common cause of bacterial food poisoning in comparison to listeriosis and *E. coli*. However, the presence of heat stable enterotoxins produced by *Staphylococcus aureus* is responsible for almost 300 foodborne outbreaks every

* Corresponding author.
E-mail address: marglez@ubu.es (M.L. González-SanJosé).

http://dx.doi.org/10.1016/j.lwt.2017.05.053
0023-6438/

year in a wide range of foods such as cheese, meat, and bakery products (EFSA & ECDC, 2015). *S. aureus* is an ubiquitous Gram-positive bacterium that survives well in food factory environments (Le Loir, Baron, & Gautier, 2003).

The antimicrobial effect of natural ingredients is usually tested using endpoint methods such as agar diffusion and broth dilution (López-Malo Vigil, Palou, Parish, & Davidson, 2005, pp. 659–680). These tests provide interesting information on the antimicrobial activity and permit comparisons between antimicrobials under the same conditions, but they only provide qualitative results with sometimes contradictory conclusions (Rhodes, Mitchell, Wilson, & Melton, 2006; Xu et al., 2014). To obtain safe food products, the complete microbial inactivation may not be required, being sufficient to maintain populations at low levels (López-Malo Vigil et al., 2005, pp. 659–680). Consequently, the assessment of growth and inactivation curves is of paramount importance, in order to obtain more detailed and accurate information. Different kinetic growth models, both empirical and mechanistic, have been successfully applied to describe the inhibition exerted by natural compounds (Gupta, Cox, Rajauria, Jaiswal, & Abu-Ghannam, 2012; Jaiswal & Jaiswal, 2015). Modified Gompertz and Logistic models are empirical sigmoidal models that simply describe experimental data under certain conditions. In contrast, semi-mechanistic models, such as the Baranyi-Roberts model, are based on theoretical principles and are usually preferred over empirical methods because they are simpler and more sensible (Pérez-Rodríguez & Valero, 2013).

In view of the above, this study aimed to examine the antimicrobial potential of a new RWPS against three microorganisms (*Listeria innocua*, *E. coli*, and *S. aureus*), as well as to evaluate its inhibitory effect by using kinetic growth models.

2. Material and methods

2.1. Materials

Red wine pomace seasoning (RWPS) was obtained using the process patented by González San José et al. (2015) at the pilot plant of the Food Technology Area (University of Burgos). The seasoning used in the present work was obtained from seedless wine pomace, it was a powdered product free of microorganisms with a particle size of <250 μm that is obtained from different physical treatment including milled, sieved and heating. The main composition of this seasoning (main components, minerals, fiber, and extractable phenolic contents), as well as its antioxidant capacity and its capacity to inhibit spoilage microorganisms were described in a previous work (García-Lomillo et al., 2014).

2.2. Growth medium preparation

Tryptic soy broth (TSB) (Oxoid, Basingstoke, UK) supplemented with yeast extract (Oxoid) (6 g L^{-1}) was used in the present study. Broths containing 20 and 40 g L^{-1} of RWPS were also prepared, and their pH and water activity were measured using a Crison 2001 pH meter and an AquaLab Cx-2 water activity-meter (Decagon Devices Inc., Pullman, Wash., U.S.A). Furthermore, Folin Index and IPT were measured according to the classical Folin-Ciocalteu reaction (Singleton & Rossi, 1965) and by the absorbance to 280 nm, respectively (Ribereau-Gayón, Peynaud, Sudraud, & Ribereau-Gayón, 1982). Controls at the same pH as broths with RWPS were also prepared by adjusting their pH with tartaric acid, in order to evaluate the contribution of pH reduction on the antimicrobial effect.

2.3. Microbial culture

Strains were obtained from the Spanish Type Culture Collection (CECT) and were reconstituted according to CECT recommendations. Four strains were used in the present study: *E. coli* CECT 434 (ATCC 25922) and CECT 504, *L. innocua* CECT 910 (ATCC 33090) and *S. aureus* CECT 435 (ATCC 25923). These microorganisms were selected based on their relevance to the food industry as has been previously described. *L. innocua* was used as a surrogate of *L. monocytogenes* due to its nonpathogenic nature and their similar response to stress factors.

2.4. Microbial growth tests

Bacterial strains, taken from overnight samples, were aseptically inoculated in TBS broths at the different pHs and in the broths with the studied seasoning (at 20 and 40 g L^{-1}). Optical density measurements were used for convenient dilution of the inoculum to reach an initial level of 3 Logs (CFU mL^{-1}) using a SmartSpec Plus spectrophotometer (Biorad, Hercules, CA, USA). Broths were then incubated at 37 °C and sampled at 1, 3, 5, 7, 9, 11, 24, and 34 h. Samples were decimally diluted in peptone water (Merck, Darmstadt, Germany) and counted on pouring plates using tryptic soy agar (Oxoid) supplemented with yeast extract (6 g L^{-1}), and incubated at 37 °C for 24 h. The experiments were conducted in triplicate, on three different days, using three different batches of the seasoning.

2.5. Growth kinetic parameters

Inhibition provided by RWPS was characterized using three primary growth models, which were used to fit the experimental data. The online tool DMFit, available at http://www.combase.cc, was used to obtain lag time (λ) and maximum specific growth rate (μ), according to the equation proposed by Baranyi and Roberts (1994).

The modified Gompertz model proposed by Gibson, Bratchell, and Roberts (1987) was also applied. Data were fitted to equation (1), as proposed by the above authors.

$$Nt = A + C * \exp[-\exp(-B*(t-M))] \quad (1)$$

The logistic model was also fitted, according to equation (2) proposed by Gibson et al. (1987).

$$Nt = A + C/[1+\exp(-B*(t-M))] \quad (2)$$

In equations (1) and (2), Nt indicates the cell number (log CFU mL^{-1}) at any given time (t). Results were fitted to the equations by nonlinear regression using a Marquardt algorithm running on the software Statgraphics Centurion XVI and obtaining the values of A, C, and M for each equation. Then, μ and λ were calculated according to the equations for each model as described by Gupta et al. (2012).

The standardized residuals of each model were evaluated, in order to test their suitability. The homoscedasticity and independence assumptions were checked by plotting the standardized residues against the fitted values and the independent variable. Moreover, the normal distribution of the standardized residues was checked with Chi-Square, Shapiro-Wilk W, Skewness Z-score and Kurtosis Z-score tests. The root mean square errors (RMSE) were calculated and used to compare the suitability of each model.

2.6. Statistical analysis

Multifactor analysis of variance was performed to determine those factors with statistically significant effect (p-value < 0.05).