LWT - Food Science and Technology 72 (2016) 19–25

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Growth and toxigenic potential of *Bacillus cereus* during storage temperature abuse in cooked irradiated chicken rice in combination with nisin and carvacrol

Samia Ayari [a, b, c], Dominic Dussault [a], Moktar Hamdi [c], Monique Lacroix [a, *]

[a] Research Laboratory in Sciences Applied to Food, INRS-Institut Armand-Frappier, Canadian Irradiation Centre, 531 Boulevard des Prairies, Laval, Quebec, H7V 1B7, Canada
[b] National Center for Nuclear Sciences and Technologies (CNSTN), Tunis Cedex, 2020, Tunisia
[c] National Institute for Applied Sciences and Technology (INSAT), B.P. 676, Tunis Cedex, 1080, Tunisia

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
12 April 2016
Accepted 15 April 2016
Available online 19 April 2016

Keywords:
Bacillus cereus
Carvacrol
Nisin
Combined treatments
Chicken rice

ABSTRACT

This study investigated the effect of combined treatments involving low doses of gamma irradiation in combination with nisin and carvacrol as antimicrobial agents on the growth and potential toxicity of *Bacillus cereus*, during storage temperature abuse. The chicken rice was spiked with endospores and was incubated at 10 °C for 2 weeks. Microbial population was examined using plate counting on MYP agar. Toxigenic potential was measured through the record of enterotoxins and phosphatidylcholine-specific phospholipase C (PC-PLC) activity. The results showed that samples processed with a combination of low irradiation dose; (D_{10}, ½ D_{10}) and reduced concentration of antibacterial agents (MIC, ½ MIC), resulted in a significant decrease ($p \leq 0.05$) of *B. cereus* count compared to the samples treated only with higher amount of antimicrobial agents (2 × MIC). Toxin production was also delayed by irradiation and a total absence of enterotoxin was observed in irradiated rice at 1.8 kGy in the presence of nisin alone or in combination with carvacrol until the 12th day of storage at 10 °C. Reduced proliferation of *B. cereus* obtained by combined treatment was associated with a limitation of toxin production and led systematically to significant decrease ($p \leq 0.05$) of PC-PLC activity during storage.

1. Introduction

Consumer demands are more and more directed towards high-quality, minimally processed, high nutrients and fresh like foods. Therefore, ensuring microbiological safety of food products, while maintaining their nutritional and organoleptic properties, is still a priority nowadays (Pattanayaiying, Kittikun, & Cutter, 2015). The prepared dishes are generally subjected to a moderate heat treatment in order to limit alteration on desirable properties such as texture, flavor, color and nutrient value. If such treatment is generally effective in destroying vegetative bacterial forms present in the food, it is insufficient to eliminate heat resistant spores. Therefore, an appropriate disinfection treatment is needed to completely destroy contaminating spores.

Food scientists and the food industry are therefore searching for innovative and emerging methods that may destroy undesired microorganisms with less effect on food quality. For this reason, major efforts have been made to develop non-thermal technologies which can prevent adverse thermal effects and produce safe food products (Birmpa, Sfika, & Vantarakis, 2013; Severino et al., 2014). A growing interest in non-thermal preservation method, particularly the combination of non-thermal methods with antimicrobial treatments (Luu-Thi et al., 2015; Masana, Barrio, Palladino, Sancho, & Vaudagna, 2015) can enhance the lethal effects of non-thermal processing and preserve food physico-chemical properties without affecting the nutritional value (Raso & Barbosa-Canovas, 2003; Ross, Griffiths, Deeth, & Mittal, 2003).

Several studies highlighted that the combined use of antimicrobial compound incorporated or not in edible coating and γ-irradiation can increase the radiosensitivity of bacteria, resulting in lower radiation doses required for lethality (Caillet, Millette, Turgis, Salmieiri, & Lacroix, 2006; Huq, Vu, Riedl, Bouchard, & Lacroix, 2015; Severino et al., 2014; Takala, Salmieri, Vu, & Lacroix, 2011).

Rice-based products and farinaceous foods such as rice breads

* Corresponding author.
E-mail address: Monique.lacroix@iaf.inrs.ca (M. Lacroix).

http://dx.doi.org/10.1016/j.lwt.2016.04.025
0023-6438/

and noodles are frequently contaminated and have been found to contain *Bacillus cereus* spores (Kramer & Gilbert, 1989). Rice meals served in hotels, restaurants, oriental tacke-away restaurants, hospitals and food stalls are sometimes associated with cooked chicken. Many studies have demonstrated the presence of *B. cereus* in poultry products and cytotoxic *B. cereus* strains was recently isolated from cooked chicken (Lopez, Minnaard, Perez, & Alippi, 2015; Smith, Berrang, Feldner, Phillips, & Meinersmann, 2004). The manner in which chicken rice is prepared for human consumption can promote the survival and outgrowth of *B. cereus*. Toxin production by surviving *B. cereus* depends on the number of environmental factors including storage temperature, and then depth study of *B. cereus* behavior in this type of product is crucial. The objective of this study was to assess the effectiveness of combined treatment involving low doses of gamma radiation and/or antimicrobial agents (nisin and carvacrol) in reducing *B. cereus* spores and its toxin production in cooked chicken rice incubated at 10 °C (a common refrigeration abuse temperature) over 15 days of storage.

2. Materials and methods

2.1. Bacterial strain and preparation of spore suspension

B. cereus strain LSPQ 2872 (Laboratoire de Santé Publique du Québec, Canada) was used throughout the experiments. Cells were grown at 30 °C in brain heart infusion (BHI) medium (Difco Laboratories, Sparks, MD) supplemented with 0.5% (w/v) glucose. Before the experiment the bacterial strain was stored at −80 °C in BHI containing 25% glycerol. The spores were obtained, according to the modified method described by Finlay, Logan, and Sutherland (2002) and were then maintained at 4 °C until used (De Lara, Fernandez, Periago, & Palop, 2002).

2.2. Determination of minimal inhibitory concentration (MIC) value of carvacrol and nisin

The minimal inhibition concentration was determined using a modified critical dilution assay using a 96 well microtiter plates (Ayari, Dussault, Millette, Hamdi, & Lacroix, 2009; Gänzle, Hertel, & Hammes, 1996). Nisin and carvacrol were obtained from Sigma Aldrich Chemicals (St. Louis, MO). Carvacrol was emulsified in sterile, distilled water, and sterile Tween 80 (LaboratoireMat Inc., Quebec, Canada) was added (1% as highest final concentration) to stabilize the emulsion. A stock solution was stored at 4 °C and the range of the initial concentration was 5000 ppm. Nisin was diluted in Mueller Hinton broth (MH; Oxoid, England). The range of the initial concentration was 10,000 IU/ml of nisin.

2.3. Preparation of cooked chicken rice and inoculation

Commercial samples of long-grain white rice were purchased from a local supermarket and used for all experiments. For cooking, the rice was added to the boiled water with a ratio of 4:5 and the mixture was stirred continuously for 15 min. The chicken was cooked separately in the oven at 180 °C for 45 min. The mixture of 70% rice and 30% chicken was then divided aseptically in equal quantities and putted in sterile polyethylene stomacher bags. Suitable quantities of antimicrobial agents have been added on the surface of chicken rice to achieve the final concentrations required. Homogenization was carried out by manually mixing. Then, 500 µl of the suspension of endospores (preheated to 80 °C for 10 min) have been deposited on the surface of samples and mixed properly to have an approximate final concentration of 10^4 CFU/g.

2.4. Irradiation treatment

The aerobically packaged chicken rice samples were irradiated at the Canadian Irradiation Center (Laval, Quebec, Canada) at room temperature (20 °C). After irradiation, the irradiated and non-irradiated samples were immediately stored at 10 ± 1 °C and tested periodically during 15 days. The γ-irradiation treatment was performed using an underwater irradiator UC-15A (MDS Nordion International Inc., Kanata, Ontario, Canada) equipped with a ^{60}Co source at a dose rate of 15.6 kGy/h. This irradiator was certified by the National Institute of Standards and Technology (Gaithersburg, MD), and the dose rate was established using a correction for decay of the source. Amber Perpex 3042D (Atomic Energy Research Establishment, Harwell, Oxfordshire, United Kingdom) was used to validate the dose distributions.

2.5. Experimental approach

In this study, the basic experimental approach has been to generate survival curvesof *B. cereus* spores (LSPQ 2872) inoculated in chicken rice, in a variety of conditions (Table 1). Prepared chicken rice was separated into 3 groups depending on the treatment: (1) treated only with antimicrobial agents (2 times the MIC); (2) treated with antimicrobial components (1 time the MIC) followed by irradiation at 0.9 kGy (equal to half of the D_{10}); (3) treated with minimal inhibition concentration reduced to half (1/2 of MIC) followed by irradiation at 1.8 kGy (equal to the D_{10}). All groups were stored at 10 °C. Growth and toxigenic potential of *B. cereus* were then evaluated for 15 days (day 0 corresponded to the day of the treatment).

2.6. Microbial analysis

Following each treatment, the total spores count was determined successively each 3 days over 2 weeks. Serial dilutions of the homogenate were prepared and appropriate dilutions were spread plated onto the MYP (Mannitol-Egg-yolk-polymyxin) agar. Detection limit of the enumeration method was 50 CFU/ml. Growth rates were calculated to compare the groups. The slope function (Excel 2007, Microsoft) was used to do a linear regression of the linear part of the growth curve to estimate the growth rate of *B. cereus* µ in Ln CFU g^{-1} day^{-1}.

2.7. Determination of enterotoxin hemolysin BL (HBL)

The HBL toxin assay was performed using the "*B. cereus* enterotoxin reverse passive latex agglutination" kit BCET-RPLA TD950 (Oxoid, Dardilly, France) which detects the L2 component of the toxin. The protocol was used according to the supplier's manual. Dilutions of the food extracts or culture filtrate were carried out in microplate wells to which was added an appropriate amount of latex and followed agitation of the plate. If *B. cereus* enterotoxin was present, agglutination appeared as diffuse disorder downhole.

Table 1
Experimental approach.

	Experience 1	Experience 2	Experience 3
Antimicrobial components	2 × MIC[a]	MIC	½ MIC
Carvacrol(1)	625	312	156
Nisin(2)	1250	625	312
Carvacrol/Nisin	625/312	312/156	156/78
Gamma irradiation (kGy)	0	0.9 (1/2 D_{10})	1.8 (D_{10})

[a] MIC: Minimal inhibitory concentration, **(1)**: ppm, **(2)**: IU/g.