Food Research International 43 (2010) 1719–1724

Contents lists available at ScienceDirect

Food Research International

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

Inactivation of food spoilage bacteria by high pressure processing: Evaluation with conventional media and PCR–DGGE analysis

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ARTICLE INFO

Article history:
Received 16 March 2010
Accepted 15 May 2010

Keywords:
High pressure processing
RNA extraction
DGGE
Active species
Sliced cooked ham

ABSTRACT

Microbial diversity and dynamic changes of sliced vacuum-packed cooked ham during refrigerated storage (0–90 days) after high pressure processing (400 MPa at 22 °C for 10 min) was investigated by using culture-dependent and culture-independent approaches. Isolation of genome DNA and total RNA directly from meat samples, followed by PCR–denaturing gradient gel electrophoresis (DGGE) and RT-PCR–DGGE on 16S rDNA V3 region, was performed to describe the structure of the bacterial community and active species in pressurized sliced cooked ham. The DGGE profile showed that most spoilage bacteria including *Lactococcus garvieae*, *Weissella cibaria*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Weissella paramesenteroides*, *Leuconostoc carnosum* and *Lactococcus lactis* subsp. *lactis* were completely inactivated after high pressure processing (HPP), whereas *Weissella viridescens* and *Weissella minor* survived HPP and induced the final spoilage. The microbial diversity of HPP samples during the whole refrigerated storage period was extremely simple. Our results clearly indicated that HPP was an efficient method for avoiding the growth of the major spoilage bacteria and could be used to prolong the shelf-life of sliced vacuum-packed cooked ham.

1. Introduction

High pressure processing (HPP) is a very promising non-thermal technology for the preservation of sliced cooked and cured meat products and it shows a big potential for the innovative development of new products with a relatively low energy consumption (Hugas, Garriga, & Monfort, 2002). By choosing the appropriate process conditions, it could give a great inactivation for yeasts, moulds and most vegetative bacteria, including most spoilage and pathogenic bacteria, but vitamins, color and flavor remain unaffected largely (Cheftel, 1995; Cheftel & Culioli, 1997; Matser, Krebbers, van den Berg, & Bartels, 2004; Morris, Brody, & Wicker, 2007; Smelt, 1998).

After high pressure treatment, one population of microorganisms may be killed, another population may survive (non-injured), and a third population maybe sublethally injured (Wu, 2008). The injured organisms are potentially as important as their normal counterparts because they can resuscitate and become fully functional in a favorable environment. Many researchers have investigated the recovery phenomenon of injured bacteria after HPP (Bozoglu, Alpas, & Kaletunç, 2004; Jofré et al., 2007; Koseki, Mizuno, & Yamamoto, 2007, 2008). However, to characterize and monitor the community diversity of active spoilage bacteria, including normal and injured microorganisms, in pressurized complex ecosystems is difficult. It is important to detect both injured and non-injured microorganisms, and to distinguish between live and dead cells to prevent false-positive or false-negative results (Wu, 2008). It is well documented for example, that stressed or injured cells are not recovered in selective media and that cells present in low numbers are very often inhibited by microbial populations numerically more abundantly (Hugenholtz, Goebel, & Pace, 1998). For these reasons, it is crucial to have tools that allow monitoring of the microbial populations without cultivation (Cocolin et al., 2007). This goal can be achieved by culture-independent methods such as PCR–DGGE.

However, when culture-independent methods were used to study the microbial ecology, RNA-based methods are likely to yield more useful information on viable and metabolically active microbial communities *in situ* than DNA-based methods, due to the fact that rRNA synthesis and bacterial cell growth are closely related (Wagner, 1994). By using DNA as a molecular marker, it is possible to determine the presence or absence of a particular bacterial species in the sample, but it is impossible to assure that whether these bacteria are alive or dead. However, the use of RNA-based methods can help in the understanding of the alive and active population within a microbial ecosystem (Diez et al., 2008).

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doi:10.1016/j.foodres.2010.05.012

The aim of this study was to investigate the microbial diversity and predominant spoilage bacteria in pressurized sliced vacuum-packed cooked ham by using culture-dependent approaches and RNA-based DGGE methods.

2. Materials and methods

2.1. Preparation of cooked ham

Sliced cooked hams were prepared in a local meat factory according to the conventional techniques without the addition of any preservatives, except for nitrite. The ham was made with pork meats, sodium chloride, pentasodium tripolyphosphate, sodium ascorbate, sodium glutamate, sucrose, flavoring additives, soya isolate protein, potato starch, nitrite and water. The raw materials were mixed, cured for 16 h at 4 °C under vacuum, and packed into an artificial casing and cooked until the core temperature reached 72 °C and then smoked for 2 h. After cooling by immersion in cold water, the products were maintained at 4 °C overnight, the hams were then aseptically sliced into 0.5 cm slices (Hu, Zhou, Xu, Li, & Han, 2009). After vacuum packaging with polyamide (PA)/polyethylene (PE) membrane (oxygen permeability <24 cm^3/m^2/day at 20 °C, 120 μm thickness with PA/PE ratio 20/100), 100 packages (100 g sliced hams, per package) were stored at 4 °C for sampling use.

2.2. High pressure treatment

Before high pressure processing, samples were vacuum packaged with another polyethylene membrane layer (Beijing Huadun Xuehua Plastic Group Co., Ltd., China) to prevent contamination from the high pressure transmission fluid (bis (2-ethylhexyl) sebacate, Li-Dong Precision Machinery Company, Shenzhen, China). Sliced cooked hams were subjected to high pressure in a 2 L vessel (52 Institute, Baotou, Neimeng, China) at 400 MPa for 10 min at room temperature (22 °C). The pressure level and time of pressurization were controlled by a computer program (BTNMC for HPP Control 1.0). Pressure holding time reported in this study does not include pressure come-up or release times. The pressure come-up rate was 350 MPa/min and the pressure-release time was almost immediate. The temperature increase due to adiabatic heating in the HPP chamber was less than 2 °C/200 MPa.

2.3. Sample storage and examination

Immediately after high pressure processing, the outer package was removed, and then the HPP samples were stored at 4 °C along with controls (not treated samples, NT). Duplicate samples of hams collected at time 0 (before HPP), 1 (after HPP), 15, 30, 90 days were used for microbiological and molecular biology analysis.

2.4. Microbiological analysis

Twenty-five grams of each sample (two replicates) was aseptically taken and homogenized in 225 mL of sterile peptone saline (1 g of peptone and 8.5 g of NaCl per liter). After shaking at 200 rpm for 10 min with a stomacher, the suspension was serially diluted in triplicate (1:10) in peptone saline. Serial dilutions were plated onto appropriate culture media and the following microbiological analyses were carried out on HPP and NT samples (Table 1).

2.5. pH measurements

Ten gram portions of each ham sample (two replicates) was homogenized in 10 mL distilled water for 2 min, and used for pH determination by Microprocessor pH meter (Hanna HI9025c, Portugal).

Table 1
Media and incubation conditions used in this study.

Microbe species	Plate count media	Purchase Co.	Incubation
Aerobic plate count	PCA[a]	Land Bridge (Beijing)	37 °C/48 h
Psychrotrophs count	PCA	Land Bridge (Beijing)	7 °C/10 d
Lactic acid bacteria	MRS[b]	Oxoid (England)	Double layer, 30 °C/48 h
Enterobacteriaceae	VRBDA[c]	Land Bridge (Beijing)	Double layer, 37 °C/24 h
Staphylococci	BP[d]	Land Bridge (Beijing)	37 °C/48 h
Brochothrix thermosphacta	STAA[e]	Oxoid (England)	25 °C/48 h
Pseudomonads	CFC[f]	Oxoid (England)	25 °C/48 h
Yeasts and moulds	PDA[g]	Land Bridge (Beijing)	25 °C/5 d

[a] Plate count agar.
[b] de Man, Rogosa, sharpe agar.
[c] Violet red bile dextrose agar.
[d] Baird–Parker agar.
[e] Streptomycin thallus acetate agar (STA selective supplement without actidione).
[f] Cetrimide–Fucidin–Cephaloridine agar.
[g] Potato dextrose agar.

2.6. Nucleic acids extraction

Direct extraction of total bacterial nucleic acids from samples at each sampling point was undertaken as follows: 20 g each sample, in duplicate was homogenized in a stomacher tube with 80 mL of saline peptone water and shaken for 30 min at 4 °C. The sediment was allowed to settle for 5 min, and then two 35 mL aliquots were transferred into two 50 mL sterile tubes, one for DNA and another for RNA extraction. Each of the two tubes was centrifuged (Avanti J-E, Beckman Coulter, American) for 10 min at 4000*g* (4 °C). The supernatant (20 mL), was aseptically transferred into a 50 mL sterile centrifuge tube, and re-centrifugation was performed at 10,000*g* for 20 min (4 °C). The pellet was transferred to a sterile 2 mL tube and then stored at -80 °C for nucleic acids extraction.

2.6.1. DNA extraction

Bacterial DNA was extracted using the TIANamp Bacteria DNA Kit (Tiangen Biotech Beijing Co., Ltd., China) according the manufacturer's protocol for the purification of genomic DNA. Finally, DNA was eluted with TE buffer (Tiangen) and stored at −20 °C.

2.6.2. RNA extraction

For RNA extraction, 1 mL RNAiso™ Plus reagent (TaKaRa Biotechnology Dalian Co., Ltd., China) was added into the tubes containing the bacterial cells and mixed immediately by vortexing for 2 min, and then incubated for 5 min at room temperature (15–25 °C). Then 200 μL of chloroform was added and mixed well. After extraction for 5 min, tubes were centrifuged at 12,000*g* for 15 min (4 °C). The supernatant was transferred into a new sterilized 1.5 mL tube and 400 μL of isopropanol was added, incubated at room temperature for 10 min, and then centrifuged at 12,000*g* for 10 min (4 °C). The supernatant was removed and1 mL 75% ethanol was added and the tube inverted several times and centrifuged at 12,000*g* for 5 min (4 °C). The supernatant was decanted and residual supernatant was removed by gently dabbing the inverted tube once onto a paper towel. RNAs were suspended with RNase Free dH_2O (TaKaRa), and 1 μL RNase Free DNase I