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ScienceDirect

Procedia Food Science 7 (2016) 173 – 176

Procedia Food Science

9th International Conference on Predictive Modelling in Food

The Antimicrobial Effect of Rosemary and Thyme Essential Oils Against *Listeria monocytogenes* in *Sous Vide* Cook-Chill Beef During Storage

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Abstract

Sous vide cook-chill (SVCC) is a technology characterized by vacuum-packaging of raw or partially prepared foods before pasteurization, followed by rapid chilling and storage below 3ºC. The application of essential oils (EOs) is a strategy to control pathogens and to extend the shelf life of products by reducing microbial levels and oxidative processes. The aim of this study was to evaluate the efficacy of *Rosmarinus officinalis* L. (rosemary) *Thymus vulgaris* L. (thyme) EOs against *L. monocytogenes* ATCC 679, inoculated in beef processed by SVCC stored at 2ºC and 8ºC for 1, 2, 3, 7 and 14 days. Leaves were dried and hydrodistilled in a Clevenger. Determination of minimum inhibitory concentration (MIC) assay was performed. Beef samples of *m. longissimus thoracis et lumborum* were packaged in bags inoculated and added individually with one of each EO at MIC values. Bags were vacuum-sealed, and samples were processed at 55ºC/65 min (for 3-$\log_{10}$ reduction). *L. monocytogenes* enumeration was done according to ISO 11290-2. A reduction of the population of *L. monocytogenes* was observed in all samples at 2ºC. At day 14, the population of *L. monocytogenes* was similar in thyme and control at 2ºC and 8ºC. Inversely, rosemary at both temperatures show an added reduction of about 2-$\log_{10}$, comparatively to control. These results support the possibility of using rosemary as natural preservative to contribute in the reduction of *L. monocytogenes* and confirm the importance of using adequate chilling storage for maintain this pathogen at acceptable levels in view to prevent the risk for consumers.

Peer-review under responsibility of Department of Food Science, Faculty of Food Engineering, University of Campinas.

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doi:10.1016/j.profoo.2016.10.001

Keywords: Sous Vide cook-chill, Essential oils, *Listeria monocytogenes*

1. Introduction

Thermal processing is an important method of food preservation, especially for foods that required an extension of shelf-life. This process must be designed to destroy pathogenic microorganisms in their maximum heat resistant state [1,2]. *Sous vide* cook-chill (SVCC) is a technology characterized by vacuum-packaging of raw or partially prepared foods before pasteurization, followed by rapid chilling and chilled storage below 3ºC [3,4,5]. The risk associated to this technology is that heating treatment does not reduce pathogenic to a safe level [5]. *Listeria monocytogenes*, considered one of major cause of severe food-borne diseases worldwide, is one of main microbiological hazard associated with *sous vide* processing due to its ubiquitous nature, ability of growing at chilled temperatures and heat resistance [6]. Essential oils (EOs) of different plant species have become popular, as safe alternative instead chemical preservatives to control pathogens. *Thymus vulgaris* L. (thyme) is an aromatic plant and antibacterial activity of *T. vulgaris* EO has been previously found *in vitro* experiments [7,8,9]. Thymol and carvacol are the main antibacterial compounds of this EO [10]. *Rosmarinus officinalis* L. (rosemary) is recognized by their antimicrobial activities. The principal compound is 1.8-cineol and has a activity against bacterial membrane [11,12].The aim of this study was to evaluate the efficacy of these two EOs against *L. monocytogenes*, inoculated in beef processed by SVCC technique and stored at 2ºC and 8ºC.

2. Material and Methods

2.1. Essential oils

Fresh aerial parts of *Thymus vulgaris* and *Rosmarinus officinalis* were collected from Trás-os-Montes, Portugal. Plants were dried until stable weight and submitted to water distillation for 3 hours in a Clevenger-type apparatus.

2.2. Antimicrobial screening of EOs

L. monocytogenes ATCC 679 was cultured in tryptone soya broth (Oxoid, Hampshire, UK) at 30°C. After 14h, a single colony was transferred to 10 ml Brain Heart Infusion (BHI) broth (Biokar Diagnostics BK015HA) (37°C). A 12 hours culture was diluted with isotonic saline (0.9%) to give a final suspension of ~5 log CFU/ml. The microdilution test was conducted in 96-well plates. A dilution series of EOs was obtained with BHI. The positive control was obtained with 100 μL BHI and 20 μL of the bacterial suspension. The negative control was completed with 100 μL BHI. At the end, was added 20 μL of the aqueous solution resazurin. The microplates were incubated 24h at 37ºC. The MIC values were defined according the lowest concentration of the EO, which inhibited the bacteria growth.

2.3. Antimicrobial activity in beef

2.3.1. Inoculum preparation

L. monocytogenes strain ATCC 679 was grown in tryptone soya broth (30°C, 18h) to achieve a viable cell population of 8 log CFU/ml. The culture was centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was decanted and the pellet suspended in sterile 0.1% peptone solution by vortexing. The washing step was repeated twice. Bacterial cell count was determined by optical density (O.D) method at 600 nm, a population of 1×10^8 cfu/mL. Serial (10-fold) dilutions in 0.9% isotonic saline were performed in duplicate to yield approximately $1x10^6$ CFU/mL. To verify the number of viable *L. monocytogenes* in the suspension, dilutions were spread on Oxford Agar (Biokar Diagnostics BK110) supplemented with OXFORD (Biokar Diagnostics BS003) in duplicate at 37ºC for 48 h.

2.3.2. Sample preparation, inoculation and thermal inactivation

Longissimus thoracis et lumborum muscles were obtained from Portuguese bulls (9-11 months; 90-150 kg carcass weight) at 72h *post mortem*. The pH was measured directly in the muscle using a combined glass electrode with a pH-meter (Crison Instruments, Spain). Only muscles whose pH were below or equal to 5.8 were used. Muscles were cut into pieces of approximately 200 g and two samples of each piece were immediately (24 h *post mortem*) investigated for the presence of *L. monocytogenes* according to ISO 11290-1. If at least one positive-sample from each muscle were detected in one meat cut, all piece cuts were totally excluded from the inoculation experiments. Meat samples were prepared by removing a layer of ~1 cm from the meat surface, and aseptically cut in small meat pieces (0.5 cm thick, surface 2.5×2.5cm) of a weight of 10 g. Meat samples were inoculated by microsyringe with 0.1 mL aliquots