

Élelmiszertudományi Kar

# **Reducing of microbiological**

## contamination of meats

## Thesis

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The Applicant met the requirements of the Ph.D. regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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#### AIMS

Foods of animal origin, among them meat as well, easily spoil by virtue of their chemical composition, thus their shelf-life is relatively short and as a consequence of this significant nutrient losses occur that have serious economic consequences, too. At the same time one can not neglect the fact that among spoilage-causing micro-organisms there are pathogens, too, that pose health concerns.

My aim was to examine what possibilities are there in the physical and chemical preservation methods or their combinations to extend the keeping quality of meat.

Out of the physical processes I wanted to determine the inhibitory and destructive effect of ionizing radiation on micro-organisms, and the combined effect of aerobic and vacuum packaging and cold storage in chicken meat model. I wanted to determine the effect of treatment as a function of irradiation dose and storage time by the changes in the counts of different micro-organism groups.

I chose high hydrostatic pressure as another physical treatment. In this case beside the natural microflora of chicken meat and beef I investigated the survival of *Listeria monocytogenes* and *Bacillus cereus*, as health hazardous micro-organisms as a function of high hydrostatic pressure and nisin, used as preservative.

From the chemical preservatives I wanted to study the effect of trisodium-phosphate dipping solution on the microbial contamination of chicken meat, and examine the possibility of decreasing the presently used chemical concentration so that at the same time shelf-life of cold-stored meat is increased.

#### **MATERIALS AND METHODS**

Chicken wing, chicken breast and thick flank of beef were used in the experiments. One, two, three and four kGy doses were used for irradiation, aerobic and vacuum packaging were applied and samples were stored at 8-10 and 3-4 °C, respectively. I studied the growth of mesophilic aerobic bacteria, pseudomonads and enterobacteria as a function of treatment, on the basis of which the time needed to reach the critical contamination level determines the shelf-life that indicates the efficiency of the treatment.

Chopped chicken breast and thick flank of beef were examined for their natural microflora, and after inoculation with *Listeria monocytogenes* and *Bacillus cereus* in the pressure range of 0-800 MPa. Count of mesophilic aerobic cells and pseudomonads were tested in chicken meat and changes in the count of *Listeria monocytogenes* and *Bacillus cereus* spores in beef. Effect of pressure on dormant and heat-activated spores was examined with and without nisin addition in order to get information about the inhibitory effect of nisin. I could determine the threshold pressure values for the various micro-organisms thus exact description of the eventual applications becomes possible.

Trisodium-phosphate dipping solution was used to investigate the keeping quality of chicken wing to determine a dipping solution concentration lower than the presently used one but still effective. I wanted to study the effect of the treatment on the decrease in the initial contamination of meat by mesophilic aerobic cells, pseudomonads and enterobacteria and the growth rate of surviving micro-organisms to find out the delay in reaching the critical contamination limit that unambiguously determines the shelf-life of meat.

Mathematical statistical methods were used to evaluate the results.

#### **NEW SCIENTIFIC RESULTS**

1. Ionizing radiation by 2 kGy dose increased the relative shelf-life of chicken wings packed in polyethylene foil bag and stored at 8-10 °C by 5-6-fold, in vacuum packaging and stored at 2-3 °C by 12-fold. Count of enterobacteria was decreased by 4,5-5 orders of magnitude, and it reached  $10^5 \text{g}^{-1}$  value only after 15 days.

2. Cell count of mesophilic bacteria in chopped chicken breast meat was decreased already in the pressure range of 120-150 MPa, treatment at 300 MPa caused a 3 orders of magnitude decrease. HHP treatment combined with nisin (670 IUg<sup>-1</sup>) reduced the cell count by 5 log cycles. Pseudomonads were more sensitive (75 MPa), pressurization by 200 MPa resulted in 4 orders of magnitude decrease. Count of colony forming units in chopped beef inoculated by *Listeria monocytogenes* decreased already around 250 MPa, at 450 MPa CFU showed 6 log cycles decrease. Influence of nisin on increasing this effect was hardly measurable.

3. Dormant spores of non-heat activated psychrotrophic *Bacillus cereus* were very resistant ( $D_{10} = 769,2$  MPa) in chopped beef as an effect of high hydrostatic pressure. Resistance of *Bacillus cereus* decreased ( $D_{10} = 294,1$  MPa) in samples stored for 2 weeks at 4 °C after high pressure treatment according to the survival curve referring to the death of injured cells.

 $D_{10}$  value of heat treated spores (80 °C, 10 min) was 588,0 MPa, that increased to  $D_{10}$  = 1000 MPa after a 16-day storage at 4 °C calculated from the regression line. This indicates that in the heat treated and subsequently pressurized population the presumably sub-lethally damaged cells repaired their injuries during the storage period, and as a consequence of this a similar or, resulting from the error of the test method, higher colony count could be determined.

4. Dipping of chicken wings in 7,6% trisodium phosphate solution for 1 minute decreased the cell count of mesophilic aerobic bacteria, enterobacteria, and pseudomonads by 2,5 orders of magnitude, relative shelf-life increased by 5,4-fold compared to the untreated samples stored at 3-4 °C temperature. As a consequence, various microbe groups reached the critical contamination level in different times, thus the shelf-life was extended.

5. Regression data calculated from the equations of growth curves fitted on the colony counts of mesophilic aerobic bacteria, enterobacteria, and pseudomonads showed that growth rate of mesophilic aerobic bacteria and enterobacteria decreased as a function of trisodium phosphate concentration, while that of pseudomonads practically didn't change. No significant differences in the maximum cell count yield were observed between groups.

### LIST OF PUBLICATIONS RELATED TO THE THESIS

#### Articles in journals IF articles

- 1. **Castillo** A., L.A., Mészáros, L., Kiss, I:F: (2005): Effect of trisodium-phosphate on the microbiological contamination of chicken meat Acta Alim. 34 (1), pp.5-11.
- 2. **Castillo** A., L.A., Mészáros, L., Kiss, I:F: (2004): Effect of high hydrostatic pressure and nisin on microorganisms in minced meat Acta Alim. 33 (2) p.183-190.

#### Articles without IF

In foreign language

- 1. **Castillo** A., L.A., Mészáros, L., Kiss, I:F: (2003): Efecto de las altas presiones en varios microorganismos de la carne Revista Tecnología en Marcha Ed.Technol. de Costa Rica 16 (3) p.7-11.
- 2. **Castillo** A., L.A., Mészáros, L., Kiss, I:F: (2003): Efecto de las altas presiones en esporas de *Bacillus cereus* en carne de vacuno Revista Tecnología en Marcha, Ed.Technol. de Costa Rica 16 (4) p.3-6.

#### **Conference proceedings**

Hungarian (full paper)

 Kiss, I.F., Castillo A., L.A., Mészáros, L., Mohácsiné-Farkas, Cs., Reichart, O. (2013): Csomagolás és besugárzás kombinált hatása húsok eltarthatóságára, XLX. Konzervipari Napok, 2013. május 6-7. Nagykőrös, MÉTE Konzervipari Szakosztály, 3-4.

Hungarian (abstract)

- Castillo A., L.A., Kiss, I.F., Mészáros, L. (2003): Effect of trisodium-phosphate on microorganisms on chicken meat Acta Microbiol. et Immunol. Hung. 50 (2) p.304. Abstr.
- 2. **Castillo** A., L.A., Kiss, I.F., Mészáros, L. (2001): A trinátriumfoszfát hatása a baromfihús mikroorganizmusaira in: MMT 2001. évi Jubileumi Nagygyűlés Előadásainak és Posztereinek Összefoglalója, p.24. 2001. október 10-12.
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- 4. **Castillo** A., L.A., Mészáros, L., Kiss, I., Kiskó, G.(2000): A nagy hidrosztatikus nyomás és a nizin hatása a hús mikroorganizmusaira in: MMT 2000. évi Nagygyűlés Előadásainak és Posztereinek Összefoglalói, p.74. Keszthely, 2000. augusztus 24-26.
- 5. **Castillo** A., L.A., Al-Gamodi, F., Kiss, I.(1998): Besugárzás hatása aerob és vákuumcsomagolt csirkehús eltarthatóságára in: MMT 1998. Évi Nagygyűlés Előadásainak és Posztereinek Összefoglalói p.16. 1998. augusztus 24-26. Miskolc.
- 6. **Castillo** A., L.A., Al-Gamodi, F., Kiss, I. (1999): Effect of irradiation on shelf life of aerobic- and vacuum-packed chicken meat Acta Microbiol. et Immunol. Hung. 46 Abstr. p.70-71.

#### International Conferences (abstract)

- 1. Mészáros, L., **Castillo**, L., Kiss, I.F.(1999): Effect of High Hydrostatic Pressure and Nisin on *Listeria monocytogenes* in Minced Beef, p.78. in: Abstracts of Papers and Posters of the European Conference on Emerging Food Science and Technology, 22-24. November, 1999. Tampere, Finland.
- Kiss, I.F., Castillo A., L.A., Al-Gamodi, F.(1998): Shelf-life Extension of Chicken Meat by Irradiation in: Abstracts of 3rd Karlsruhe Nutrition Symposium (Eds.: Gaukel V., Spiess, W.E.L.) p.59. Karlsruhe, 18-20 October 1998.

### **R+D** project

- Kiss, I., Farkas, J., Mészáros, L., Polyák, K., Zukál, E., Andrássy, É., Castillo A., L.A., Márialigeti, K., Krommer, J., Szabó, G.(1999): Nisin Plus in Hungary, Interim Progress Report #5/6 in:Development and Practical Implementations of Nisin with other Biopreservatives and Mild Processes that Expand the Range of Application of the Bactericin in Assuring Food Safety and Quality (FAIR-CT96-1148) Period 30-09-1998 to 31-3-1999. pp.1-8.
- 2. Kiss, I.F., J. Farkas, L. Mészáros, K. Polyák, E.Zukál, É. Andrássy, L. Castillo, K. Márialigeti, J. Krommer, G. Szabó (2000): Nisin Plus-Final Research Report (Overwiev and Conclusion from University of Horticulture and Food Industry, Budapest), Time period: February 1.-December 31. 1999. in: Development and Practical Implementations of Nisin with other Biopreservatives and Mild Processes that Expand the Range of Application of the Bactericin in Assuring Food Safety and Quality (FAIR-CT96-1148) 3rd annual progress report pp.1-35.
- Kiss,I.F.(coord.): Kiss,I.F., Farkas,J., Mészáros,L., Polyák, K., Zukál,E., Andrássy,É., Castillo A., L.A., Márialigeti,K., Krommer,J.,Szabó,G.(2000): NISINPLUS, Time period 1 November 1998-31 December 1999. in: Individual Annual Progress Reports Period 1-10-1998. to 31-12-1999., Development and Practical Implementation of Novel Combinations of Nisin with Other Biopreservatives and Mild Processes that Expand the Range of Application of the Bacteriocin in Assuring Food Safety and Quality,pp.113-148. (FAIR-CT-1148), Co-ordinator: Smid, E.J., (DLO)Wageningen, Holland