Inhibition of *Listeria monocytogenes* in minced beef by combined effect of oregano essential oil and caprylic acid

Radka Hulánková, Gabriela Bořilová

Department of Meat Hygiene and Technology
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
Brno, Czech Republic

Abstract

Both oregano essential oil (OEO) and caprylic acid (CA) are highly aromatic natural antimicrobials; their individual application in food is therefore limited. Their combined additive effect we proved previously *in vitro*. Following mixtures (v/w) were tested in minced lean beef inoculated with 6 strains of *L. monocytogenes* at concentration of 5 log cells/g: 0.25% of CA (MIC *in vitro*) + 0.1% of OEO (2×MIC); 0.5% CA (2×MIC) + 0.05% OEO (MIC); and both mixtures with 0.1% of citric acid. The treated meat was vacuum packed and stored at 3 ± 1 °C for 15 days. Approximately a 0.5 log reduction was observed at all experimental groups in comparison with the control samples (with distilled water) from day 6 to the end of storage. No differences in *L. monocytogenes* counts were found between the experimental groups. In sensory evaluation (odour, colour, overall acceptability) the mixtures with 0.1% of OEO showed during the whole experiment better scores in odour and overall acceptability than the control group.

Natural antimicrobials, Origanum vulgare, octanoic acid

Introduction

Over the past years, research in the area of microbiological safety and shelf-life of food has focused on natural antimicrobials as a result of concern of consumers regarding synthetic additives.

Caprylic acid (octanoic; CA) is a saturated medium-chain fatty acid, occurring naturally in milk, coconut oil or palm kernel oil in the form of triacylglycerol. According to the Joint FAO/WHO Expert Committee on Food Additives caprylic acid is considered as safe (EFSA, 2009). In the USA, caprylic acid is approved for surface application on RTE meat and meat products (USDA-FSIS, 2011).

Oregano essential oil (OEO) obtained from *Origanum vulgare* L. was successfully used many times for inhibition of microorganisms in meat including *L. monocytogenes* (Tsigarida et al. 2000; Skandamis and Nychas 2001).

The antimicrobial effect of these agents is aimed at cell membrane, where caprylic acid probably disrupts the electron transport chain and oxidative phosphorylation (Desbois and Smith 2010), whereas the major antimicrobial components of OEO, carvacrol and thymol, increase cell membrane permeability (Burt 2004).

The aim of this study on combined effect of CA and OEO was to evaluate the possibility of lowering the individual concentrations of these compounds on sensory acceptable level while maintaining the overall antimicrobial effect. Since these compounds are highly aromatic, their individual application in food is therefore limited, especially as higher concentrations of essential oils are usually needed in food (including meat) to obtain the same antimicrobial effect as *in vitro* (Barbosa et al. 2009).

Materials and Methods

The antimicrobials used in this study were oregano essential oil (Nobilis Tilia, Czech Republic), originating from Spain and containing mainly carvacrol (72%), *p*-cymene (7.6%) and γ-terpinene (5.7%), caprylic acid

Address for correspondence:
Mgr. Radka Hulánková, Ph.D.
Department of Meat Hygiene and Technology
University of Veterinary and Pharmaceutical Sciences Brno
Palackého tř. 1/3, 612 42 Brno, Czech Republic

Phone: +420 541 562 750
Fax: +420 541 321 230
E-mail: hulankovar@vfu.cz
http://www.vfu.cz/acta-vet/actavet.htm

(≥ 98%, Sigma-Aldrich, USA) and citric acid (anhydrous, p.a., Lach-Ner, Czech Republic). Concentrations of OEO and CA in the mixtures were based on their minimum inhibitory concentrations (MIC) determined for *L. monocytogenes* strains *in vitro* (Hulánková and Bořilová, In Press).

Fresh beef inside rounds were purchased from a local meat producer, grinded in a meat grinder (grinder plate hole size 3 mm) and inoculated with a mixture of six *L. monocytogenes* strains (serotypes 1/2a, 1/2b and 1/2c) isolated from minced meat, heat-treated and non-heat treated meat products. The suspension was prepared using the McFarland turbidity scale and further diluted to obtain the concentration of 5 log cells/g. After inoculation and homogenisation in a laboratory knife mill (3.000 rpm, 10 s), sterile distilled water (control) or the antimicrobials were added (v/w) and the mixture was homogenised again (3.000 rpm, 20 s). Aliquots of 50 g of the mixture were vacuum packed (99.0% of vacuum) in AMILEN foil (Verpackungen GmbH, Germany) (PA/PE 20 μm/60 μm, O_2 permeability 50 $cm^3 \cdot m^{-2}$ at 24 h 23 °C 75% r.h., CO_2 permeability 150 $cm^3 \cdot m^{-2}$ at 24 h 23 °C 0% r.h., water vapour permeability 3.0 $g \cdot m^{-2}$ at 24 h 23 °C 85% r.h.) and stored at 3 ± 1 °C for 15 days.

The analysis was performed after 0, 3, 6, 9, 12 and 15 days of storage. Numbers of *L. monocytogenes* were determined according to EN ISO 11290-2 using buffered peptone water (Oxoid, UK) and ALOA (Merck, Germany). Odour, colour and overall acceptability were evaluated by three trained assessors using a nine-point hedonic scale where 1-totally unacceptable, 5-neutral, 9-excellent. The results were evaluated using ANOVA (StatPlus 2008 Professional v. 5.2.5.0) and level of significance 0.05.

Results and Discussion

Figure 1 shows numbers of *L. monocytogenes* during storage. There were no statistically significant differences between the experimental groups ($P = 0.205$), but the numbers of *L. monocytogenes* were considerably lower in comparison with the control group ($P < 0.001$). Since the sixth day of storage there was a decrease of about 0.5 log $CFU \cdot g^{-1}$.

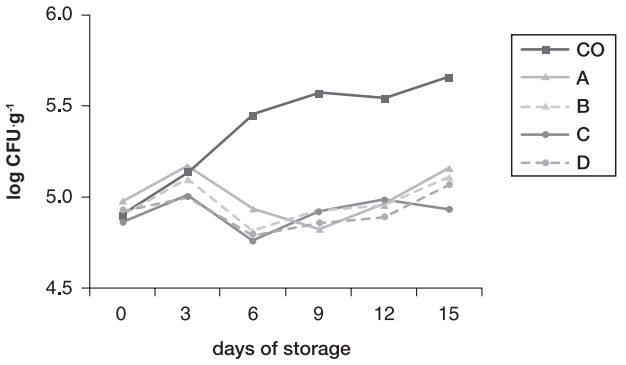

Figure 1. Number of *L. monocytogenes* during storage. **CO**: control; **A**: 0.25% CA + 0.1% OEO; **B**: A + 0.1% citric acid; **C**: 0.5% CA + 0.05% OEO; **D**: C + 0.1% citric acid

Although the reduction of *L. monocytogenes* numbers was relatively low it should be noted that the effect of natural antimicrobials in foodstuffs is influenced by many intrinsic and extrinsic factors and to obtain the same inhibition in food as *in vitro* it is often necessary to use concentrations that are several times higher (Burt 2004; Barbosa et al. 2009). Lower inhibition corresponds to lower addition of the antimicrobials (OEO in maximal concentration of 0.1%) compared with e.g. the study of Tsigarida et al. (2000) where a decrease of *L. monocytogenes* in vacuum packed beef of about 2-3 logs was observed after addition of 0.8% OEO (Tsigarida et al. 2000).

The sensory evaluation revealed that all the groups including the control one showed similar scores in colour (5.2 – 5.4). Odour and overall acceptability of experimental groups with higher ratio of CA (C, D) were comparable with the control group (mean scores 4.8, 5.0 and 4.2 in colour and 4.8, 4.9 and 4.5 in overall acceptability for control, C and

D, respectively). The groups with high ratio of OEO (A, B) showed better results than the control group achieving the mean score of 5.5 and 5.9 in odour and 5.3 in overall acceptability. Better sensory properties of minced beef with OEO were also reported by Tsigarida et al. (2000) or Skandamis and Nychas (2001), who used a 0.8% and 1% addition of OEO. However, in several other studies concentrations of essential oils in meat including OEO approaching to 1% were considered as unacceptable (Chouliara et al. 2007; Solomakos et al. 2008).

Many authors reported a synergy between essential oils and organic acids used as common food additives (acetic, lactic, citric). For example Dimitrijevic et al. (2007) observed *in vitro* increased antibacterial activity of OEO against *L. monocytogenes* after addition of lactic acid in concentration as low as 50 ppm. In our study, addition of citric acid had no effect on antimicrobial activity of the mixtures of CA and OEO, although during tests *in vitro* it significantly reduced MIC of CA and to lesser extent MIC of OEO of type strains (Hulánková and Bořilová, In Press).

Conclusions

The mixtures in both ratios (MIC CA + 2 × MIC OEO; 2 × MIC CA + MIC OEO) inhibited the growth of *L. monocytogenes* in beef, but the decrease was only around 0.5 log CFU/g in comparison with the control group. Addition of citric acid did not lead to significantly increased inhibitory effect of the mixtures. In sensory analysis the mixtures were comparable to control and the mixtures with higher amount of OEO showed even better sensory attributes than control. Further research is needed to evaluate the effect of higher concentrations of CA and OEO on sensory and microbiological parameters of meat.

Acknowledgement

This study was funded by Project No. MSM 6215712402 of the Ministry of Education, Youth and Sports of the Czech Republic.

References

Barbosa LN, Rall VLM, Fernades AAH, Ushimaru PLI, Da Silva Probst I, Fernandes A 2009: Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease **6**: 725 – 728

Burt S 2004: Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology **94**: 223 – 253

Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG 2007: Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. Food Microbiology **24**: 607 – 617

Desbois AP, Smith VJ 2010: Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology **85**: 1629 – 1642

Dimitrijevic SI, Mihajlovski KR, Antonovic DG, Milanovic-Stevanovic MR, Mijin DŽ 2007: A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L. Food Chemistry **104**: 774 – 782

EFSA 2009: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on calcium caprylate and magnesium caprylate as a source of calcium and magnesium added for nutritional purposes to food supplements, following a request from the European Commission. The EFSA Journal **7**: no.6, 20 pp.

Hulánková R, Bořilová G: Combined Effect of Oregano Essential Oil and Caprylic Acid against *Salmonella* Serovars, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes*. Acta Veterinaria Brno. In Press

Skandamis PN, Nychas G-JE 2001: Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. Journal of Applied Microbiology **91**: 1011 – 1022

Solomakos N, Govaris A, Koidis P, Botsoglou N 2008: The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. Food Microbiology **25**: 120 – 127

Tsigarida E, Skandamis P, Nychas G-JE 2000: Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. Journal of Applied Microbiology **89**: 901 – 909

USDA-FSIS 2011: Safe and suitable ingredients used in the production of meat and poultry products. FSIS Directive 7120.1, Rev. 7, 48 p. Last modified July 7, 2011. Accessed July 20, 2011. Available at: <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf>