

# Multilayer furcellaran/chitosan nano/microemulsions with the addition of bioactive peptides and their effect on microbiological quality of cold stored pork loin

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**Objectives:** The objective of this work was to analyze the antimicrobial properties of newly developed multilayer furcellaran/chitosan nano/microemulsions with oregano essential oil and antimicrobial peptides on a model meat product - fresh pork loin, stored for 11 days at 4 °C.

**Materials and Methods:** The chitosan/furcellaran emulsions without peptides were prepared by mixing 400 mL of furcellaran or chitosan solutions (1000 ppm in 0.005 M NaCl) with 0.24 mL of oregano essential oil (Etja, Elbląg, Poland) and 0.24 mL of TWEEN 80 (Sigma-Aldrich, St. Louis, USA). In case of furcellaran and chitosan emulsions with the addition of peptides, 1.22 mL of RW4 or LL37 peptide was additionally added to the emulsion system. In this way 3 types of furcellaran nanoemulsions and 3 types of chitosan microemulsions were obtained: without peptide, with RW4 peptide and with LL37 peptide. Fresh pork loin slices were obtained from local retail chain located in Kraków, Poland. The slices were hermetically sealed by the processors and used in the experiment exactly 7 days before their due date. Each emulsion layer was applied on the surface of the pork loin by the use of electro spraying technique using Fluidnatek FL-10 (Bioinica, Valencia, Spain) equipped with scanning emitter motion and top- down vertical electro spraying configuration. To ensure appropriate spraying angle an industry spray nozzle was used (Euspray M-C2, Barcelona, Spain) with spraying angle of 95°. The spraying was performed with the maximum flow size of 6000 mL/h and the emitter collector voltage drop of 20 kV. The spraying of each layer lasted exactly 5 ± 1 s. Before placing each slice on the horizontal collector, the collector surface was also sprayed to provide a layer of the emulsion also at the bottom side of the meat slice. After each spraying the slice was left for 15 minutes for drying before another layer of the emulsions was applied. Each sample was coated in 3 layers of emulsions with the following order: (I) chitosan microemulsion layer, (II) furcellaran nanoemulsion layer, (III) chitosan microemulsion layer. After last layer of emulsions was applied the samples were hermetically packed in PP trays using Oceania Mini Tray Sealer machine (Italian Pack, Como, Italy) and stored at 4 °C for 11 days. Afterwards the samples were analyzed for their total aerobic mesophilic bacteria (TAM) counts, with incubation done on PCA agar (Oxoid, Basingstoke, UK) at 30 °C for 72h. The experiment was conducted using three independent replications.

**Results and Discussion:** The initial TAM of the pork loins used was 3.08 ± 0.90 log cfu/g and increased after 11 days to 5.79 ± 1.49 log cfu/g. All applied multilayer emulsions exhibited strong antimicrobial properties and successfully inhibited bacterial growth. The contamination of samples coated in nano/microemulsions was in the range of 2.36 (emulsions with RW4 peptide) - 3.80 (emulsions with LL37 peptide) log cfu/g for lemon grass essential oil, 3.24 (emulsions with RW4 peptide) - 3.72 (emulsions without peptides) log cfu/g for rosemary essential oil, 2.58 (emulsion with RW4 peptide) - 3.74 (emulsion with LL37 peptide) log cfu/g for thyme essential oil and 1.71 (emulsions without peptides) - 2.58 (emulsion with LL37 peptide) log cfu/g for oregano essential oil. The emulsions with oregano essential oil proved to have the most potent antimicrobial properties with all samples exhibiting TAM contamination of below the initial contamination after 11 days of storage. Also in case of most essential oils analyzed, the addition of RW4 resulted in the highest microbial inhibition, aside from emulsions with oregano essential oil.

**Conclusions:** The newly developed 3-layer nano/microemulsions exhibit high antimicrobial potential and successfully inhibited the microbiological spoilage of fresh pork loin slices for at least 11 days. There is a need to perform further, more robust research, which will include longer storage period and more types of microorganisms tested along with the sensory, oxidation rate and physicochemical analyses to provide a detailed information on the effectiveness of the developed coatings.

**Key words:** Antimicrobial peptides, Multilayer nanoemulsions, Meat, Electro spraying