

# Effect of garlic, oregano and thyme essential oils on the *Clostridium sporogenes* behaviour in nitrite-free cured pork sausage

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**Objectives:** To evaluate the effect of essential oils from garlic, oregano and thyme on the behaviour of *Clostridium sporogenes* in a cured pork sausage made without nitrite.

**Materials and Methods:** *Clostridium sporogenes* (DSM 767) was used as a surrogate for *Cl. botulinum*. This surrogate shares many common characteristics with *Cl. botulinum*, namely the mechanisms of nitrite inhibition and other parameters used in food preservation. Therefore non-pathogenic species is commonly used to estimate the pathogen behaviour. The suspension of *Cl. sporogenes* used for inoculation was prepared from a fresh culture in Reinforced Clostridium Medium (RCM). The third generation of culture was poured into a Roux flask containing about 150 ml of modified PA3679 culture medium. The culture was incubated for 72h at 30°C in anaerobiosis. Culture maturity was determined by phase-contrast microscopy to detect the presence of spores in most of the observed cells. The culture was collected from the Roux flask, centrifuged and washed twice with a sterile water solution of 0.85% NaCl. The cell suspension was pasteurized at 80°C for 10 min. Before inoculation, the spore suspension was diluted to obtain a level of inoculation of 3-4 log CFU/g. Contaminated meat rested for 16 h at 4°C. The experiment was prepared using meat and fat from pork belly without skin. The meat was ground (15 mm) (Mainca, Barcelona). The preparation of the five formulations was adding 7.5% of red wine (pH 3.5, 12% of ethanol.) and 1.5% salt. The negative control did not have any other ingredient. The positive control had 150 mg/kg of sodium nitrite. The samples with EO were added with 50 mg/kg of the respective EO (Ventos, Barcelona). The mixture rested overnight (16 to 18h) at 4°C and was then filled into a natural thin casing (40-50 mm diameter), tied in a horseshoe shape (each sausage weighed ca.200g). *Chouriços* were smoked for 3 h in a smoking chamber (Begarar, Thermaxs 100EC, Berlin, Germany), burning beech wood scraps to produce smoke. The maximum temperature was never higher than 30°C and dried at 15°C, 85% RH (Aralab Fitoclima, Rio de Mouro, Portugal) until 30 days. Three samples were collected for analysis 4 h after the preparation of the batter, after smoking and at 7, 14 and 30 days of drying.

**Results and Discussion:** The initial count of *C. sporogenes* was  $3.52 \pm 0.16$  log ufc/g. After the smoking phase, the counts were reduced in more than one logarithmic unit, still with no detectable differences ( $p > 0.05$ ) between treatments. After seven days of drying, the counts were below 2 log ufc/g for all the experimental conditions. On the 15<sup>th</sup> drying day, *Cl. sporogenes* were still detectable in the negative control, with low counts ( $0.31 \pm 0.12$  log ufc/g) and not detectable in the sausages made with nitrite or with EOs. After 30 days, the presence of *Cl. sporogenes* was undetectable in all the sausages. The reduction on the bacteria population indicates that the effect of essential oils on clostridia is quite evident and that it is eliminated from the chorizo microbiota after 15 days, as observed with nitrite. In previous studies, we observed that the addition of EO at low levels is tolerated by the consumers, not having a serious impact on the flavour of the sausage. The observed *Cl. sporogenes* non-survival during the processing is mainly due to the drying and respective activity of water reduction, that was below 0.90 at the end of the processing. The results of the present study open exciting perspectives to eliminate nitrite from this cured sausage manufacturing.

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