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# "Blown pack" probabilistic modeling for *C.algidicarnis* and *C.estertheticum* under the effects of storage temperature, vacuum level and package shrink temperature

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**Abstract**

A model for 'blown pack' probability (BPP) caused by spores of *C.estertheticum* DSM8809 and *C.algidicarnis,* was developed as a function of vacuum packaging variables: storage temperature(ST:-2, 2, 4 and 15°C), vacuum level(VL:6 and 9mBar) and heat shrink temperature(HST:83 and 87°C). Beef meat pieces, were inoculated with spore suspensions individually at $10^2$spores/cm$^2$, packed and daily monitored up to 90 days. The lower BPP, estimated by the log-logistic model, for *C.algidicarnis* was 0.8% at:-1.5°C/6mBar/87°C while for *C.estertheticum* was 99.13% at the same conditions. For both organisms, tested variables were unable to eliminate the risk of blown packaged spoilage, at $10^2$spores/cm$^2$ contamination level.



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*Keywords:* Psychrotrophic clostridia; 'blown pack' probability model, vacuum-packed meat, storage temperature, vacuum level

## 1. Introduction

Microbial spoilage of red meat is a complex event to which many different bacterial populations can contribute depending on the storage temperature and packaging conditions. Concerning the 'blown pack' spoilage, it is

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generally accepted that psychrotoletant/psychrophic Clostridial species are the main agents, including *Clostridium estertheticum, Clostridium gasigenes* and *Clostridium algidicarnis* (Broda et al. [1], Adam et al. [2]; Silva et al. [3]). It is associated with the outgrowth of *Clostridia* in packed fresh meat stored under refrigeration. The presence of both *C.estertheticum* and *C.algidicarnis,* has been reported in Brazil (Silva et al. [3]). This spoilage typically occurs in vacuum packaged meat, in the absence of temperature abuse or packing failure, after 4-6 weeks. This spoilage is responsible for significant economic losses to the meat industry (Bolton et al. [4]).

Several intervention strategies have been proposed to control the occurrence of 'blown pack' spoilage (Adam et al.[2]). Control measures basically rely upon the adoption of good manufacturing practices that should be strictly used and improved in abattoirs, such as keeping low storage temperatures (<−1.5 °C), avoiding the contact of hide with carcasses and recontamination during slaughtering (Moschonas et al. [5]). Besides, modifications in the operational patterns during vacuum packaging such as shrinking temperatures (Bell et al., [6]) along with vacuum level, type and integrity of the packaging system influence the onset of 'blown pack' spoilage. However, it has not been investigated, the risk of meat with high spore contamination, influenced by vacuum level, storage temperature and heat shrinking temperature. Research conducted by Silva et al. [7] showed that the combination of vacuum pressure (9mbar) combined with shrinking temperature (87°C) retarded the spoilage by vegetative psychrotrophic clostridia cells. This research aimed to model the 'blown pack' probability (BPP) caused by spores of *C.estertheticum* DSM8809 and *C.algidicarnis,* as a function of vacuum packaging control variables: storage temperature(ST), vacuum level(VL) and packaging heat shrink temperature(HST).

## 2. Material and Methods

### *2.1. Microorganisms and preparation of spores suspensions*

Spores suspensions were produced as Adam et al. [2] and Bell et al.[7], for *Clostridium estertheticum* DSM8809 and *Clostridium algidicarnis,* isolated from Brazilian spoiled red meat samples (Silva et al.[3]). Spore suspensions were standardized ($10^5$spores/mL) and storage at 4°C.

### *2.2. Preparation of beef steaks and study of growth / no growth interface*

Beefs of strip loin (*longissimus dorsi*), were purchased aseptically opened in a laminar flow cabinet, trimmed with a sterile knife and the steaks (10×5×2 cm) were aseptically cut. A reduction of surface contamination was performed by heating both surfaces of meat in a disinfected grill, until 100 °C was reached (in the center of steak), as monitored with a thermocouple inserted. After cooling, the steaks were packed in shrinkable multilayer ethylene–vinyl acetate (EVA) that was previously disinfected by UV radiation for 1 h/side.

For gas/no gas assessment, it was inoculated 0.5mL of spore suspension of each psychrotrophic *Clostridia* on the surface of each beefsteak. An inoculum of $10^2$spores/$cm^2$ was the highest level of spore contamination for fresh red meat samples found by Silva et al. [3]. The inoculum was uniformly distributed at the surface of steaks and the packages were sealed in a vacuum sealer (Minivac, Selovac) previously calibrated to 6 or 9mbar of vacuum pressure, monitored using a portable digital Vacuum Meter (GMH3160-12, Henkelman). Then, packages were heat shrieked in a water bath set at 83 and 87°C±0.2C /3s. The inoculated population of each *Clostridia* was confirmed by swabbing the steak surface (50 $cm^2$) as outlined by Silva et al. [7]. Finally, all the packages were identified and stored at -2, 2, 4 and 15°C (temperatures ranged from microorganism no growth (Broda et al. [1]) to abuse storage condition) until 90 days or 'blown pack'. For each storage conditions, one negative control (non-inoculated sample) was also included in the experiment and three packages were prepared. Packages were visually assessed daily for the presence of gas, causing package distension and code 0 was adopted for positive event and 1 for negative. Cases where gas was not observed, at 90 days were considered censured to the right and code 1 was used.