Global African Swine Fever Research Alliance

ASFree M.e.a.t. (Meet Export Agreement On Trading): Safeguarding The Italian Cured Pig Products From African Swine Fever

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INTRODUCTION

The Italian export of processed meats represents about 56% of all Italian pork meat exports. The marketing of safe pork products ("safe commodities") contributes to managing the risk of disease spread. The ASFV tenacity and the conditions under which it remains viable over time in aged pork products dated back to the late 80s.

Aim of the project

The ASFree M.e.a.t. (Meet Export Agreement On Trading) project aims at updating the existing knowledge on ASFV persistence in aged pork products and to investigate the efficacy of **High-Pressure Processing (HPP)** in ASFV inactivation to provide health guarantees to importing countries regarding the absence of ASFV in Italian aged products. The ASFree M.e.a.t. project consortium is lead by the National Reference Laboratory for ASFV in Italy (CEREP) and foresees the participation of four Italian Institutes and will produce cured pig products including ham either by artificial contamination and by in vivo experimental infections of pigs.

MATERIALS & METHODS

Six short-aged products were included in the study:
Felino salami types 1 and 2, Milano salami, Spianata, Cacciatore, and Napoli salami.

1. Isolation and culture of the ASFV

- Titration of the BA71/V viral strain, adapted to VERO cell lines, was conducted at the BSL-3 Laboratory of CEREP;
- Cytotoxicity tests were led on VERO cell cultures (ATCC-CL81) using seasoned pork meat paste intended for Milano salami production;
- Additional toxicity and sensitivity assays were conducted on Immortalized Porcine Kidney Macrophage (IPKM) cells.

2. Production and contamination of salami

- So far, three batches of Milano salami each (Fig. 1), 3 batches of Spianata and 3 batches of Cacciatore were experimentally contaminated, according to the FSIS guidelines, with 1% w/v with a BA71/V and cured for two months;
- Processing parameters were standardized according to the technical specifications provided by Salumificio Valtiberino;

Figure 1. Milano salami batch, ready for curing.

- Curing (drying) phase was monitored through a scheduled assessment of weight loss, water activity (Aw) and pH;
- Enumeration of mesophilic lactic acid bacteria was performed by counting colonies grown on selective solid media according to the ISO 15214:1998 standard method (Fig. 2);
- Control trials were conducted to assess the reproducibility of the curing process and to evaluate the potential influence of the addition of antibiotic-supplemented MEM medium on technological processing parameters and lactic acid bacterial behavior.

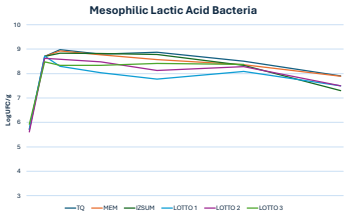


Figure 2. Comparison of lactic acid bacteria counts.
(TQ) salami produced and cured at *Salumificio Valtiberino*;
(IZSUM) salami produced in-house and cured in the climate-controlled chambers at CEREP;
(MEM) salami produced in-house and supplemented with antibiotic-enriched MEM;
(LOTTO1, LOTTO2, LOTTO3) salami produced and experimentally contaminated at CEREP.

3. ASFV detection and quantification

- At 1/3, 2/3 and the end of aging 3 salami were sampled for ASFV detection by Real-Time PCR;
- 1 g of homogenate was mixed with 10 mL of antibiotic-supplemented MEM and centrifuged at 2,000 rpm for 10 minutes;
- The supernatant was used for automated DNA extraction;
- DNA was amplified using the ID Gene™ ASF Duplex kit (IDASF-100) and two WOAH-recommended protocols (King *et al.*, 2003; Fernández-Pinero *et al.*, 2013);
- Real-Time PCR was performed on the CFX Opus 96 System;
- At the end of curing, positive ASFV salami were treated with HPP.

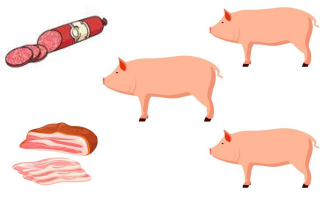
RESULTS AND DISCUSSION

- ✓ Preliminary data by Real-Time PCR showed that Milano salami sampled at 2/3 of aging are ASFV positive, with Ct values ranging between 30 and 35;
- ✓ The experimental production trials of Milano salami carried out at CEREP demonstrated the **reproducibility of the production process**, thus ensuring that the test was representative and reproducible compared to commercial production;
- ✓ Preliminary Real-Time PCR analysis performed on meat batter and Milano salami at the end of the ripening process, as well as before and after HPP treatment, highlighted the **need for viral isolation tests to confirm the presence of infectious ASFV;**
- ✓ The data generated by this project will be essential to support the export of Italian cured meats, providing scientific evidence on the safety of aged products with respect to the presence of ASFV.

FUTURE ACTIVITIES

In vivo experimental infection

- Animals of suitable size and will be infected with viral doses of circulating genotype II virus. Their anatomical parts will be used for the production of aged products. The virus will be quantified in the fresh product, that is, during the pre-production phase and at the end of the aging process;
- A selection of contaminated products will be used to feed experimentally housed pigs. The animals will be evaluated for the following parameters: clinical signs, viremia and viral shedding, seroconversion, presence of anatomical-pathological lesions, and presence of the virus in target organs.



References: McKercher *et al.* (1978; 1985; 1987); Mebus *et al.* (1997); Petrini *et al.* (2019).

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