P45

## Cadaverine and putrescine contents in traditional Portuguese pork sausages linked to the addition of starter cultures

Dias R.[1], Pinto R.[1], Barros M.[1], Vaz Velho M.[1,2], Barros D.[1], Pires P.[1,2]

[1]Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal, [2]CISAS-Centre for Research and Development in Agrifood Systems and Sustainability, Viana do Castelo, Portugal

### Introduction

Starter cultures are used in meat sausages production mainly for technological reasons but, depending on the species, an added effect against other bacteria, including pathogens, can occur. Lactic acid bacteria (LAB), usually considered non-pathogenic and non-toxic, are the main fermenting microorganisms in starter cultures, but some LAB species can produce biogenic amines (BAs). The biogenic amines content in meat, although not regulated by law, is a meat freshness indicator. The formation and increase of the content of BAs is related to food degradation processes, reason why it is important to control the contents of these amines over product lifetime. Currently, European Commission Regulations (2073/2005, 144/2007, 365/2010) set food safety criteria for histamine in fishery products and no criteria have been established for other BAs or other food products, such as meat, dairy, or other products, despite the presence of important levels of BAs in all types of food and the potential public health concern due to their physiological and toxicological effects, when these products are consumed.

This study aims to ascertain the effect of the addition of a LAB strain, known to be active against some pathogens that can be found in cured meat products, on cadaverine and putrescine production in cured-smoked sausage-like pork products during storage.

### Material and Methods

The LAB strain *Lactobacillus plantarum* ST153ch was applied to three cured-smoked products. In "Alheira" it was added together with meat and other ingredients before the curing process and in "Salpicão" and "Lombo" it was included in finished products after slicing. Similar products were produced without the addition of starter culture, here called control samples.

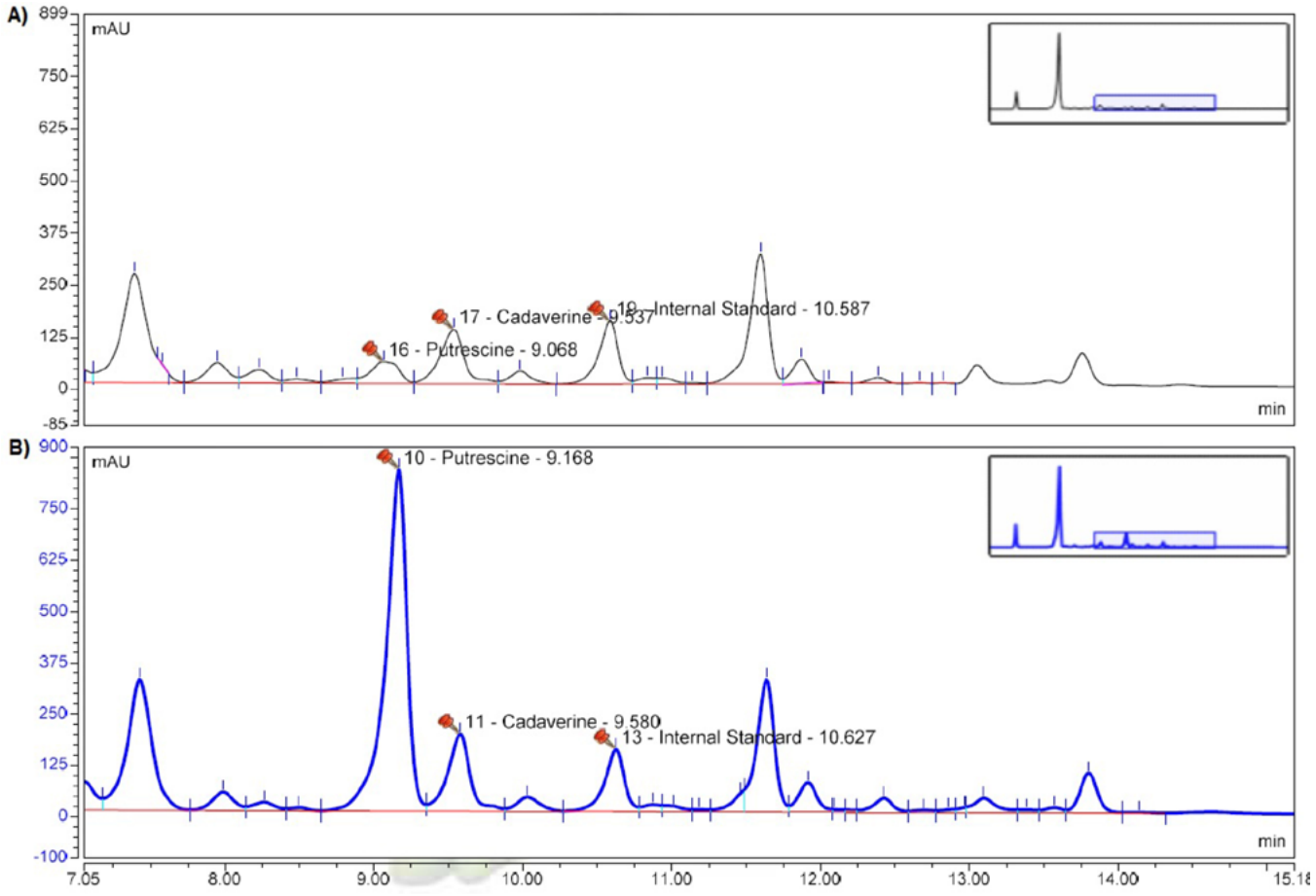


*Figure 1: HPLC chromatogram of biogenic amines in "Alheira" control sample (A: 0 days, B: 60 days).*

All samples were vacuum packed and stored at 4°C. Putrescine and cadaverine determinations were performed along a storage time of 0, 15, 30, 45, 60 e 90 days for "Alheira" product, and at 0, 15, 30, 45, 75, 90, 120, 165 e 180 days for "Salpicão" and "Lombo" sliced products.

BAs levels were determined by HPLC with an analytical Thermo Ultimate 3000 system and a Dionex Ultimate 3000 diode array detector. Amines were extracted with perchloride acid solution and derivatized with dansyl chloride following J. AOAC International method (AOAC, 1999) with some modifications: Hypersyl ODS C18 column (5mm, 250x4.6 mm I.D., Agilent) conditioned at 40ºC, injection volume of 20 mL, flow rate of 1mL/min, UV at 254nm and a mobile phase with a binary mixture of water and acetonitrile. A paired *t*-test was performed for each parameter (putrescine and cadaverine) and for each product ("Alheira", "Lombo" and "Salpicão") over the study time using Microsoft Excel 2016. In this analysis it was used the average of two measurements.

**Results**

A good separation of putrescine and cadaverine was obtained and two representative chromatograms of biogenic amines in samples with 0 and 60 days of storage for "Alheira" control sample are presented (Figure 1).

The level of putrescine in "Alheira" control product was between 11.4 and 161.3 mg/kg during the storage time, until 90 days. Similar results were observed for this product with added lactic acid bacteria, with a similar rate of increase (Figure 2), the concentration was between 11.9 and 161.2 mg/kg respectively at 0 and 90 days. Concerning the cadaverine content, it remained lower than 41.5 mg/kg (at day 90) and more constant for control and LAB samples (Figure 2).

During storage time there are no significant differences for putrescine concentration between control and LAB products, "Alheira", "Salpicão" and "Lombo" ($p$=0.068, $p$=0.976 and $p$=0,848 respectively using paired *t*-test). Concerning the other biogenic amine, there are no significant differences in cadaverine content for "Lombo" ($p$=0.759), but there are differences for the other two products, "Alheira" e "Salpicão" ($p$=0.019 and $p$=0.047 respectively).

The levels of biogenic amines with storage time are presented in Figure 2. In "Alheira" there is an increase in putrescine values with time, without significant differences between groups. However it is observed a constant level for cadaverine values in LAB samples with significant differences when compared to the control samples which amine concentration increases with time. In "Lombo" sausage there is no tendency for putrescine and cadaverine increase with time, both for LAB and control samples with no significant differences (Figure 2). For "Salpicão", the cadaverine concentration does not change significantly with time for both groups, however for putrescine there is a slightly increase with storage for the LAB group, without much change for the control group.

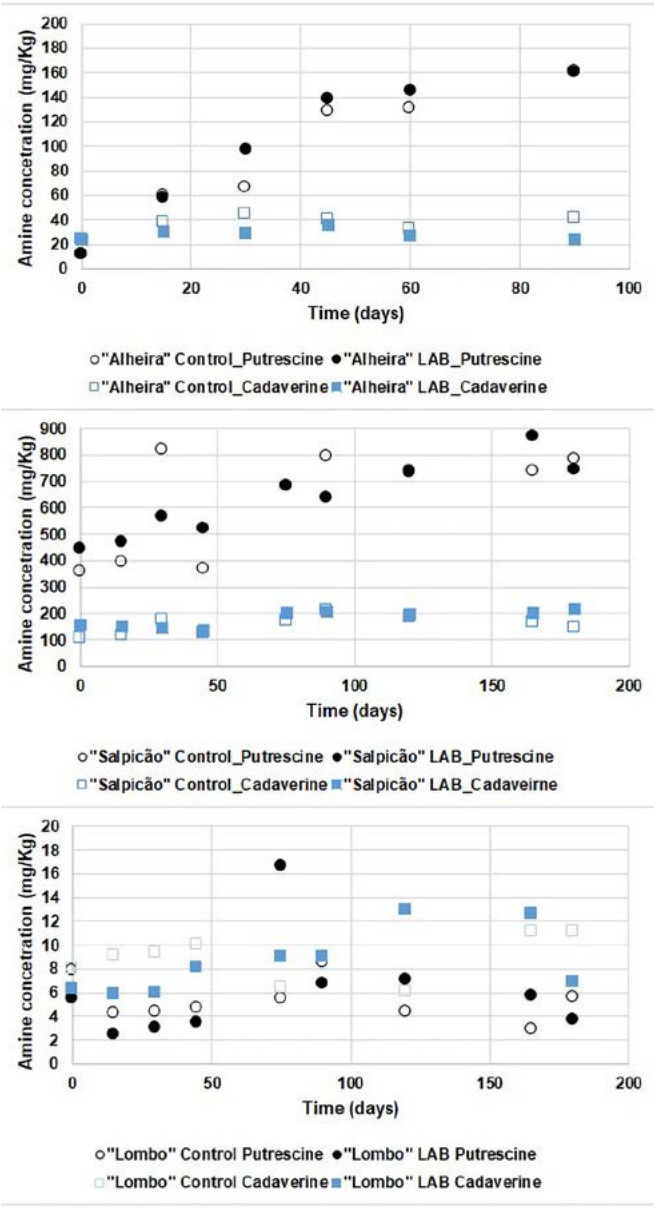


*Figure 2: Biogenic amines content for "Alheira", "Salpicão" and "Lombo" in LAB and Control groups*

**Discussion and conclusion**

The tendency observed is that the starter culture addition did not influence the putrescine contents in "Alheira", "Salpicão" and "Lombo" cured products, and did not also influence the level of cadaverine

in "Lombo" and in "Salpicão" it is in the limit with *p*=0.057. However in "Alheira" product the starter culture addition influences the concentration of cadaverine, with a lower concentration in the LAB group.

In this study it can be concluded that the inclusion of LAB starter cultures did not influence the putrescine and cadaverine contents along the storage of all the tested products, except for "Alheira" with a positive effect in cadaverine.

Usual contents of putrescine in fresh pork are < 2, < 5, and 20-40mg/kg, respectively (Kalac, 2006). The amount of cadaverine in fresh meat is at the level of 1 mg/kg but can raise even to 120 mg/kg (Bover-Cid *et al*., 2006).

**References**

AOAC Int. (1999): Liquid chromatographic method for determination of biogenic amines in fish and fish products, 82, 1097-1101.

Kalac, P. (2006): Biologically active polyamines in beef, pork and meat products: A review. Meat Science, 73, 1-11.

Bover-Cid, S., Miguelez-Arrizado, J., Latorre Moratalla, L. L., & Vidal Carou, M. C. (2006): Freezing of meat raw materials affects tyramine and diamine accumulation in spontaneously fermented sausages. Meat Science, 72, 62-68.