The effects of starter culture on the biogenic amine accumulation in traditional Portuguese dry-sausages

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INTRODUCTION

Linguiça is a highly popular and appreciated traditional Portuguese dry-fermented sausage. Its production involves a ripening step, which provides favorable conditions for biogenic amines (BAs) formation due to microbial growth, acidification and proteolysis. The levels of biogenic amines in dry-fermented sausages are highly dependent on the type of product, producer and could even vary from batch to batch. The microbiological quality of raw materials, technological process and growth/type of microbial flora are some factors that may explain this variability. To date, only few studies focused the BAs level in Portuguese traditional sausages, showing variable levels of accumulation, being the tyramine (9.71 to 1289 mg kg^{-1}) the most abundant, followed by putrescine (7.28 to 2720 mg kg^{-1}) and cadaverine (4.91 to 1237 mg kg^{-1}) [1,2,3]. With the aim to prevent or reduce the biogenic amines formation during the manufacture of dry-fermented sausages, several studies have evaluated commercial and experimental starter cultures. Although in some studies the use of starter cultures have successfully reduce the BAs accumulation during the fermentation of sausages, [4] others studies were ineffective [5].

OBJECTIVES

This work aimed to evaluated the influence of one commercial starter culture composed by *Pediococcus pentosaceus, Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus* and *Debaryomyces hansenii* (Texel®ELCE Br, Danisco) on biogenic amine accumulation during manufacture process and storage of *Linguiça.*

MATERIALS AND METHODS

1. Dry sausage manufacturing process:

Raw pork meat was mixed with water (410mL/kg), salt (20g/kg), dry garlic (4.5g/kg), sweet pepper (12.5g/kg), laurel (0.5g/kg), dextrose (10g/kg), red wine (410mL/kg) and divided in two portions, of which one was inoculated with commercial starter cultures (Texel®ELCE Br, Danisco), and both portions macerated at 4°C for 3 days and then stuffed in the natural casings. The process was carried out in duplicate (two independent batches A and B). Sausages were ripen in a refrigerated chamber at 15°C with 77% relative humidity during 8 days. The final products were stored at 4°C for 21 days. Two sausage were analyzed at each sampling time (0, 3, 6 12 and 32 days).

2. Physicochemical analysis:

The pH was measured directly in the center of the samples with a pH-meter HI8424 183 (Hanna Instruments, Portugal), while water activity (a_w) was measured using a water activity meter Aqualab 4TE (Decagon, USA).

3. Microbiological analysis:

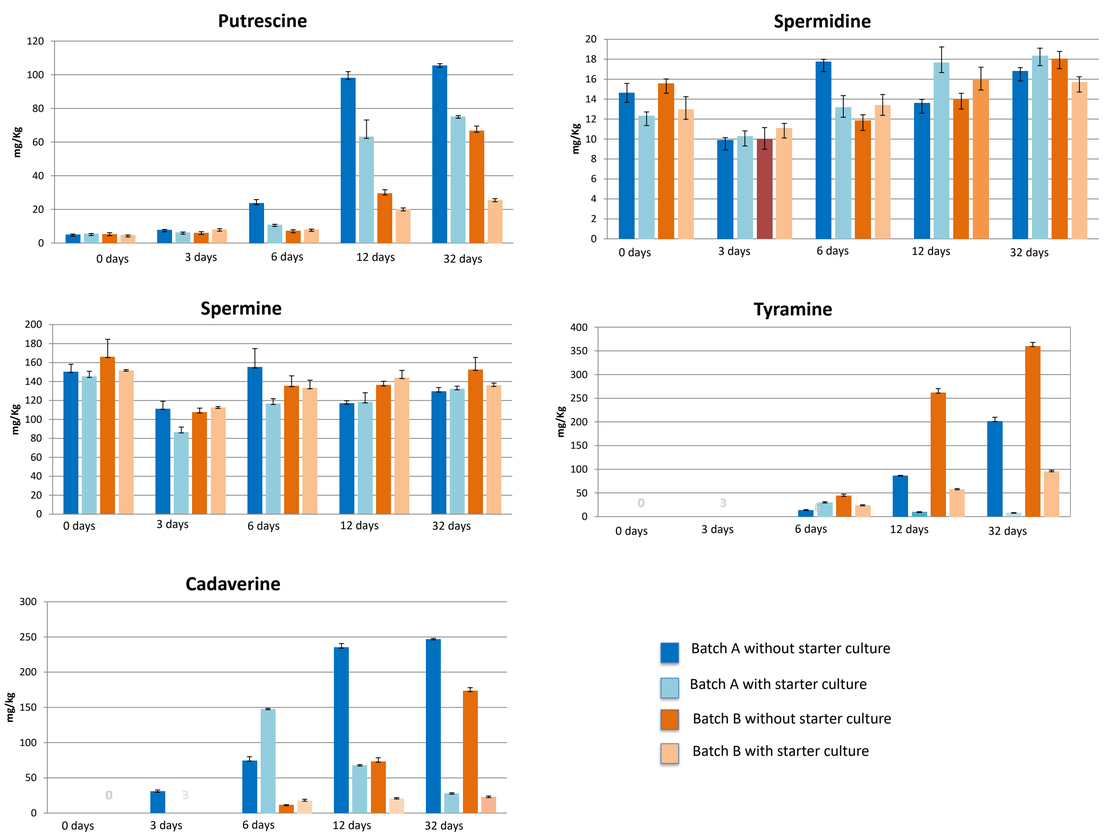
10 g of the sample was taken aseptically and homogenized for 2 min in 90 mL of sterile buffered peptone water. Lactic acid bacteria (LAB) counting was performed on Man, Rogosa and Sharpe (MRS) agar overlaid with 5 mL of agar (0.8 %) incubated at 30 °C for 48-72h. Yeast count was determined on Dichloran Glycerol agar (DG-18) and incubated at 25 °C for 4-6 days.

4. Biogenic amines evaluation:

Biogenic amines were extracted according Vinci and Antonelli[6] and quantified using a Varian HPLC system with a Prostar 220 pump, a Rheodyne 7725i manual injector with a loop of 20 µL and a Varian ProStar 330 Photodiode Array detector. The Software Star Chromatography Workstation, version 4.5, was used to control the solvent flow rate (1.2 mL/min), data acquisition, and data processing. A Jones Chromatography 7981 column oven and a reverse phase C18 mod Kromasil 100 column (15 cm, 4mm ID) (*Teknokroma Analitica*, S.A.) column were used. Separation of the different biogenic amines was carried out at 40 ± 1 °C and at 254 nm. The mobile phase was a gradient elution program with a binary mixture of 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B)[6].

RESULTS

1. Evaluation of biogenic amines during manufacture process and storage in *Linguiça:*



- There was a wide variation in the biogenic amines content among all the batches.
- Starter cultures reduced the production of putrescine, cadaverine and tyramine.
- Putrescine, cadaverine and tyramine increased, namely during ripening and storage.
- Spermidine and spermine were present in the raw material and during ripening and storage. There were no significant effects of starter cultures on their concentrations.

2. Physicochemical and Microbiological analyses

Physicochemical and Microbiological parameters during *Linguiça* manufacture and storage. A: Control batch; B: batch inoculated with starter culture. Different superscripts within a column represent significant differences ($P < 0.05$).

Batch	Days	Physicochemical parameters		Microbiological counts (log10CFU/g)	
		pH	a_w	LAB	Yeast
No starter	0	5.57±0.08^{ab}	0.9854±0.0013^{a}	4.27±0.85^{a}	3.03±0.00^{a}
	3	5.50±0.08^{ab}	0.9858±0.0016^{a}	4.67±0.73^{ab}	4.39±0.34^{b}
	6	5.46±0.02^{ab}	0.9575±0.0000^{b}	7.90±0.82^{d}	5.40±0.00^{c}
	11	5.28±0.08^{b}	0.9305±0.0096^{cd}	7.20±0.08^{cd}	5.56±0.10^{cd}
	32	5.37±0.06^{ab}	0.9327±0.0031^{cd}	7.24±1.24^{cd}	6.47±0.19^{d}
With starter	0	5.67±0.00^{a}	0.9865±0.0003^{a}	5.51±0.27^{ab}	3.15±0.78^{b}
	3	5.51±0.06^{ab}	0.9874±0.0044^{a}	6.12±0.13^{bc}	4.24±0.17^{b}
	6	5.37±0.32^{ab}	0.9633±0.0051^{b}	7.98±1.44^{d}	5.57±0.79^{cd}
	11	4.88±0.04^{c}	0.9143±0.0032^{d}	8.54±0.17^{d}	5.58±0.48^{cd}
	32	4.98±0.13^{c}	0.9151±0.0069^{cd}	8.75±0.02^{d}	6.54±0.50^{d}

- The pH values of batches inoculated with starter cultures during ripening and storage were lower compared to the other batches.
- a_w decreased in all samples, with no statistical differences between batches.
- Batches didn´t significantly affected the LAB or Yeast counts.

CONCLUSIONS

- The use of starter culture is recommended for the production of safer dry-fermented sausages with low biogenic amines content.
- The starter culture used inhibited the accumulation of biogenic amines (i.e., putrescine, cadaverine and tyramine).

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Acknowledgements:

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) for financial support to: CIMO (strategic project PEst-OE/AGR/UI0690/2014); *Associate Laboratory LSRE-LCM (Project POCI-01-0145-FEDER-006984); CQ-VR, Project UID/BIO/04469/2013 .*