



10º Encontro Nacional de Cromatografia

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COM O ALTO PATROCÍNIO DE SUA EXCELÊNCIA



O Presidente da República

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10º Encontro de Cromatografia

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PC

Panel Communications

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PC-56

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

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Linguíça is a highly popular and appreciated traditional Portuguese dry fermented sausage. Its production involves a ripening step, which provides favorable conditions for biogenic amines 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 the authors' best knowledge, only few studies focused on the quantification of biogenic amines in Portuguese traditional sausages, reporting variable levels of accumulation, being the tyramine the most abundant followed by putrescine and cadaverine. Starter cultures have been used aiming to prevent or reduce the formation of biogenic amines during the manufacture of dry-fermented sausages. Based on the results reported in the literature, the use of starter cultures may reduce or not the biogenic amines accumulation during the fermentation of sausages.

In this work, it was evaluated the influence of one commercial starter culture (Texel®ELCE Br, Danisco) on biogenic amine accumulation during manufacture process and storage. Parameters such as pH value, water activity and microbial counts were also assessed. In general the results pointed out that the starter culture inhibited the accumulation of biogenic amines (putrescine, cadaverine and tyramine) as well as the growth of *S. aureus* and Enterobacterias. This inhibitory effect was clear during ripening and storage periods. On the other hand the starter culture did not have a significant effect on spermidine and spermine concentrations.

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

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INTRODUCTION

Linguíça 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 ineffective⁵.

OBJECTIVES

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

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 (Textel®ELCE Br, Danisco), and both portions macerated at 4°C for 3 days and then stuffed in the natural casings. 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 links were analyzed at each sampling time (0, 3, 6, 12 and 32 days) and two independent batches (A and B), with and without starter cultures were performed.

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:

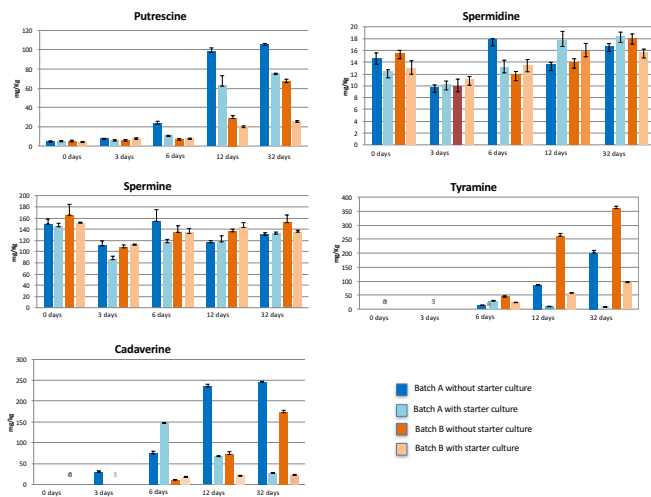
10 g of the sample was taken aseptically and homogenized for 2 min in 90 mL of sterile buffered peptone water. Lactic acid bacteria (LAB) counting was performed on Man, Rogosa and Sharpe (MRS) agar overlaid with 5 mL of agar (0.8%) incubated at 30 °C for 48-72h. Yeast count was determined on Dichloran 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 (2002) and quantified by HPLC with a chromatograph (Thermo Finnigan, San Jose, CA, USA) equipped with a SCM 1000 degasser, a P4000 pump, an AS 3000 automatic injector and a photodiode array UV6000LP detector. Separation of the different biogenic amines was carried out on a reverse phase C18 mod Kromasil 100 column (15 cm, 4mm ID) (Teknokroma Analítica, S.A.). The column was thermostated at 40 ± 1 °C, flow rate was 1,2 mL/min and the wave-length of the detector 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 *Linguíça*:



- 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 *Linguíça* 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 ^a	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 ^a	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 ^{bc}	0.9327±0.0031 ^{cd}	7.24±1.24 ^{cd}	6.47±0.19 ^{cd}
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 ^{cd}

- 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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