# CHANGES ON BIOGENIC AMINE PROFILES IN "PAINHO DE PORTALEGRE" DRY FERMENTED SAUSAGE FROM STARTER CULTURES ADDITION

Santos, C.[1]*; Roseiro, C.[1]; Silva, L.[1]; Elias, M.[2]

[1]*Instituto Nacional de Engenharia, Tecnologia e Inovação, DTIA, Estrada do Paço do Lumiar, 1649-038 Lisboa. Portugal.*
[2]*Universidade de Évora, Departamento de Fitotecnia, Apartado 94, 7002-554 Évora*

**Key Words:** Dry fermented sausage, Biogenic amines, Microbiological counts, Starter culture.

## Introduction

Protein hydrolysis carried on during the processing stages and along the storage period of dry fermented sausages is the source of non-proteic material production, including small peptides (<1000 Da m.w.) and free amino acids (Toldrá *et al*., 1992; Ordóñez *et al*., 1999). These compounds contribute to the taste and, as precursor agents, to the formation of relevant volatile and non volatile flavouring components in the final product. Within the last group, biogenic amines may appear with increased concentrations in traditional dry fermented sausages (Roseiro *et al*., 2005) since, among other reasons, the curing salts and starter cultures are usually absent in the formulation. Then, a greater variety of bacteria species and strains find out environmental conditions to grow up longer, namely those $GRAM^-$ having pronounced carboxilase activity. Biogenic amines are considered undesirable components due to their possible toxic and allergic impact in consumers. For histamine, the most studied amine, limits of 8-40mg $kg^{-1}$, 40-100 mg $kg^{-1}$ and over 100mg $kg^{-1}$ have been associated to slight, intermediate and intensive poisoning outbreaks (Maijala & Eerola, 1993), while the toxicity threshold for tyramine is not so precise, ranging from 100 to 800 mg $kg^{-1}$. In relation to putrescine and cadaverine, both known as not producing "per se" adverse health effects but increasing the effect of others by inhibiting the amino oxidase enzymes (Bardócz, 1995; Eerola *et al*., 1997), no limits were established so far.

## Objectives

The present study aimed to clear out in what extent starter cultures addition, from the natural flora, may influence quantitative and qualitatively the biogenic amines contents and profiles in "Painho de Portalegre" a Portuguese traditional IGP dry fermented sausage.

## Methodology

*Preparation of sausages* – Meat from the shoulder and ham and belly pieces, obtained from 24h *post-mortem* carcasses of "Alentejano" pigs, raised according the handling system described in "IGP" production requirements, were used on formulation. Minced raw materials (plate holes diameter-3 cm) and seasoning ingredients were mixed together (atmospheric pressure during about 5 minutes) to give the final proximate composition: lean pork (73%); fatty belly (8%); NaCl (2%); paprika paste (5%); garlic paste (0.5%); tap water (11.5%). Batches with approximately 10 kg were prepared as follows: Batch C (control with no starter inoculation); Batch Ls1, same as batch C but inoculated with *Lactobacillus sakei*-strain 1; Batch Ls2, inoculated with *Lactobacillus sakei* - strain 2; batch Sx1, inoculated with *Staphylococcus xylosus*-strain 1; batch Sx2 inoculated with *Staphylococcus xylosus* - strain 2; batch LS1; batch LS2, followed of two minutes of additional mixing. Between batches preparation, the mixer bowl was cleaned up, sanitized and washed with abundant water flow. Batches were held afterwards in a chilling room at $+2^{o}$ C for two days (seasoning up-take purposes) before stuffing into natural casings (pig rectum) and put at the drying/smoking house. Environmental conditions observed during the early phase of this processing stage reflected the typical traditional manufacturing process. Sampling included raw meat/fat mixture before salt/seasoning and starters addition, the end of the seasoning uptake period and stuffed product ripened up to day 15, 30, 40 (finished product) and 70 (corresponding to 30 days of storage). At storage, sausages were packaged under vacuum and held at room temperature, which was not controlled but ranged between 12-17°C.

Biogenic amine analysis - Biogenic amines were determined by HPLC according to Eerola et al. (1993). Eight grams of the samples were homogenized in 40 mL 0.4M perchloric acid with a Polytrom homogenizer. The samples were centrifuged for 10 min. at 3000 r. p. m. and the supernatant rinsed into 25 mL bottle through filter paper. The extraction was repeated with 40 mL 0.4M perchloric acid. The supernatants were combined and adjust to 25 mL with 0.4M perchloric acid. A volume of 1 mL of the sample extract was derivatisated with dansyl chloride by incubation for 40 min. in alkaline media. The samples were finally dissolved in acetonitrile and filtrated through a membrane Acrodisc 25 mm GHP, GF 0.45  m (Gelman Sciences, Inc.). As internal standard, 1,7-diaminopentane was used.

Biogenic amines (tryptamine,  -phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) were separated using a HPLC system Waters consisted of the quaternary pump (Waters model 510 HPLC pump), automatic sampler (Waters 717Plus autosampler), diode array detector (Waters 2996). Separation was performed on the reverse phase Spherisorb ODS2 cartridge, 5  m, 4.0x125 mm. A gradient elution program with mixture of 0,1M ammonium acetate as solvent A and acetonitrile as solvent B was used. Samples were analysed in duplicate. The biogenic amines content was expressed as $mg.kg^{-1}$ dry matter of muscle.

Inoculum preparation – Strains used in this study were collected and selected from the natural microbiota of portuguese traditional dry fermented sausages produced in Portalegre district. Brain heart infusion (BHI) and deMan, Rogosa, Sharpe (MRS) broths were used for Micrococcaceae and Lactobacilli growth, respectively.

## Results & Discussion

Data obtained from starters addition on BA formation during "Painho de Portalegre" processing stages and a further short storage period is shown on Table 1. The observed trends varied with BA considered and depended on the starter composition. Regardless spermidine and spermine, which concentrations did not expressively differ from control batches, the other evaluated biogenic amines were, in general, strongly inhibited on their formation by both individual and combined starters. Exceptionally, batches inoculated with Sx1 presented significantly higher cadaverine and histamine contents than in controls, for samples picked up at day 30 and 40 of the drying stage and over the entire ripening phase, respectively. This specific ability for Histamine formation exhibited by certain microorganisms must be taken into account when selected active starter cultures are to be used instead of wild fermentations. In our case, in Sx1 inoculated samples at day 30 of the drying/smoking phase, which correspond in practice to a product ready for consumption, the histamine concentration reached a level 2 fold higher than that detected in the controls (95.79 vs 29.07 $mg.kg^{-1}$, on dry matter), a level close to the upper limit of acceptability recommended in food (TenBrink *et al.*, 1990). Among the microflora commonly found in meat products, different potentials for producing and degrading BA have been detected (Masson *et al*., 1996; Martuscelli *et al*., 2000).

The effect of storage time, run under vacuum at room temperature on the evolution of BA concentrations in "Paínho de Portalegre" depended on the microbial composition of the installed flora and, in agreement with this, it varied with the BA considered. Apart the phenylethylamine in Ls1 samples, the other BA mean concentrations decreased during the storage period in batches with strains 1. This main trend diverged in batches with strains 2 for putrescine, cadaverine and tyramine. In agreement with the explanation of Eerola *et al*., (1997), the anaerobic conditions associated to the vacuum packaging, by favouring the activity of amine producing bacteria, could effectively be the main reason involved in the increased levels detected.

Strains 2 were more effective in reducing BA contents all over the ripening periods than their counterparts, with differences being particularly relevant on cadaverine for both "genus" and on histamine in relation to *Staphylococcus*. Regardless the selected microrganism strains, *Lactobacillacae* showed, in most situations, superiority in controlling the BA level, mainly for those compounds detected in greater concentrations. However this condition was not confirmed for putrescine in batches inoculated with Sx2, which showed lower values than those with Ls2. These different bacterial potentials in influencing BA formation were confirmed when the impact of starters addition on the sum of vasoactive compounds (tryptamine+phenylethylamine +histamine+tyramine) and histamine potentiators (putrescine+cadaverine) (Chu and Bjeldanes, 1981) were considered. In fact, the level of both indexes in controls at day 30 of the drying/smoking phase (345.99 and 1287.02 $mg.kg^{-1}$ on dry matter, respectively) decreased about the double in batches with strains 2 (64.05 and 202.16 $mg.kg^{-1}$ – Ls2; 88.84 and 234.47 $mg.kg^{-1}$ – Sx2; 50.37 and 147.07 $mg.kg^{-1}$ – LS2) comparatively to those inoculated with strains 1 (153.5 and 562.91 $mg.kg^{-1}$ – Ls1; 269.26 and 917.8 $mg.kg^{-1}$ – Sx1; 105.98 and 223.15 $mg\text{-}kg^{-1}$ – LS1). Data also clearly shows that combined staters were more effective in their action than pure cultures and that, within those, Ls1 and Ls2 decreased more those indexes than Sx1 and Sx2.

If the safety of the traditional production is clearly enhanced through starters addition, the influence of BA reduction may be adverse on the sensorial properties. Eerola *et al*., (1997), referred a positive relationship between sensorial evaluation and the putrescine concentration in samples stored up to 30 days in vacuum, but this results were not confirmed in all tested situations. Otherwise, this trend is contradictory to the undesirable sensorial properties of diamines.

## Conclusions

The addition of selected starter cultures in the manufacture of traditional dry fermented sausages may deeply promote the safety of the final products by decreasing the biogenic amines formation.

## References


Martuscelli, M.; Crudele, M.A.; Gardini, F.; Suzzi, G. 2000. Biogenic amine formation and oxidation by Staphylococcus xylosus strains from artisanal fermented sausages. Letters in Applied Microbiology, 31, 228–232.

Roseiro, C.; Santos, C.; Sol, M.; Silva, L.; Fernandes, I. 2005 Prevalence of biogenic amines in "Painho de Portalegre", a dry fermented PGI pork sausage at different ripening stages in relation to the amount of NaCl addition. Submitted to publication.

Maijala, R. & Eerola, S. (1993). Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat Science, 35(3), 387–395.

Bardócz, S. (1995). Polyamines in food and their consequences for food quality and human health. Trends in Food Science & Technology, October, 1995 (vol. 6), 341–346.

Eerola, S., Hinkkanen, R., Lindfors, E. & Hirvi, T. (1993). Liquid chromatographic determination of biogenic amines in dry sausages. Journal Association of Official Analytical Chemists, 76, 575–577.

Eerola, S.; Sagués, A.R.; Lilleberg, L.; Aalto, H. (1997). Biogenic amines in dry sausages during shelf-life storage. Z Lebensm Unters Forsch A, 205, 351–355.

TenBrink, B.; Damink, C. Joosten, H.M.I.J.; Huis in't Veld, J.H.J. (1990). Occurrence and formation of biologically amines in food. International Journal Food Microbiology 11:73–84.

Chu, C.H. & Bjeldanes, L.F. (1981). Effect of diamines, polyamines and tuna fish extracts on the binding of histamine to mucin in vitro. Journal of Food Science, 47:70–80, 88.

Toldrá, F.; Rico, E. & Flores, J. (1992). Activities of pork muscle proteases in model cured meat systems. Biochimie, 74, 291–296.

Ordóñez, J.A.; Hierro, E.M.; Bruna, J.M.; Lorenzo de la Hoz (1999). Changes in the components of dry-fermented sausages during ripening. Critical Reviews in Food Science and Nutrition, 39(4):329–367.

Masson, F.; Talon, R.; Montel, M.C. (1996). Histamine and tyramine production by bacteria from meat products. International Journal Food Microbiology, 32, 199–2087.

## Tables and Figures

| Biogenic amine | Time | | Starter | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | Ls1 | Sx1 | LS1 | Ls2 | Sx2 | LS2 |
| **Tryptamine** | **0** | 1.59 | 1.59 | 1.59 | 1.59 | 1.59 | 1.59 | 1.59 |
| | **2** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | 13.39 | 12.06 | 11.30 | 7.71 | 3.90 | 0.00 | 4.16 |
| | | 0.00 | 4.45 | 0.00 | 0.00 | 0.00 | 0.16 | 0.20 |
| | | 27.07 | 0.00 | 2.40 | 0.70 | 1.42 | 2.55 | 2.25 |
| | | 7.86 | 1.49 | 1.36 | 0.35 | 0.00 | 1.36 | 0.00 |
| | | 3.96 | 3.96 | 3.96 | 3.96 | 3.96 | 3.96 | 3.96 |
| | **2** | 7.07 | 9.41 | 10.84 | 5.83 | 11.75 | 7.22 | 10.05 |
| | | 4.31 | 7.28 | 5.48 | 3.91 | 0.64 | 0.00 | 0.87 |
| | | 16.33 | 3.73 | 3.19 | 1.14 | 0.21 | 0.78 | 3.65 |
| | | 34.14 | 2.84 | 9.89 | 6.66 | 7.58 | 7.31 | 4.98 |
| | | 53.79 | 6.63 | 2.75 | 4.87 | 0.89 | 2.75 | 0.03 |
| | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| | **2** | 0.56 | 1.71 | 0.61 | 0.60 | 0.35 | 0.12 | 2.48 |
| | | 401.73 | 45.61 | 105.39 | 57.15 | 60.84 | 12.11 | 28.94 |
| | | 662.46 | 110.66 | 215.12 | 66.76 | 153.47 | 71.67 | 102.59 |
| | | 511.34 | 139.93 | 163.71 | 61.09 | 55.74 | 17.95 | 55.65 |
| | | 466.13 | 63.85 | 112.59 | 13.00 | 112.26 | n.d. | 72.98 |
| | | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| | **2** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | 509.59 | 250.16 | 492.48 | 132.12 | 104.89 | 63.06 | 110.63 |
| | | 959.33 | 540.71 | 1159.07 | 223.86 | 283.04 | 378.85 | 195.84 |
| | | 775.73 | 422.98 | 808.13 | 162.06 | 146.42 | 216.52 | 91.42 |
| | | 720.95 | 233.55 | 644.22 | 74.69 | 237.93 | n.d. | 146.06 |
| | | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 |
| | **2** | 1.11 | 1.87 | 1.58 | 2.88 | 0.71 | 0.33 | 0.64 |
| | | 14.15 | 5.20 | 23.78 | 6.48 | 2.40 | 3.08 | 2.18 |
| | | 29.07 | 7.18 | 95.79 | 12.63 | 4.62 | 9.38 | 2.70 |
| | | 18.97 | 4.26 | 61.56 | 6.54 | 1.49 | 7.53 | 1.78 |
| | | 24.48 | 0.87 | 39.08 | 1.88 | 0.32 | n.d. | 0.62 |
| | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | **2** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | 191.53 | 69.36 | 218.74 | 93.88 | 67.71 | 19.32 | 40.98 |
| | | 345.26 | 118.88 | 270.53 | 121.53 | 112.20 | 108.09 | 72.22 |
| | | 265.81 | 146.20 | 195.41 | 92.08 | 53.56 | 71.45 | 41.36 |
| | | 252.90 | 55.98 | 137.60 | 48.34 | 56.07 | n.d. | 42.99 |
| | | 3.49 | 3.49 | 3.49 | 3.49 | 3.49 | 3.49 | 3.49 |
| | **2** | 4.14 | 4.65 | 4.72 | 2.82 | 3.67 | 5.57 | 5.13 |
| | | 3.52 | 3.71 | 3.41 | 3.64 | 3.84 | 0.77 | 4.21 |
| | | 3.60 | 3.47 | 3.73 | 3.16 | 3.37 | 5.49 | 5.50 |
| | | 2.92 | 5.33 | 4.24 | 3.33 | 2.61 | 3.31 | 3.61 |
| | | 2.88 | 2.17 | 2.85 | 2.59 | 1.50 | n.d. | 2.65 |
| | | 38.04 | 38.04 | 38.04 | 38.04 | 38.04 | 38.04 | 38.04 |
| | **2** | 49.53 | 54.80 | 57.10 | 34.93 | 36.91 | 45.28 | 66.57 |
| | | 34.10 | 57.44 | 45.43 | 38.37 | 33.12 | 8.88 | 46.39 |
| | | 37.04 | 41.64 | 37.23 | 33.97 | 34.35 | 56.75 | 58.59 |
| | | 34.24 | 61.18 | 50.08 | 38.06 | 33.04 | 41.79 | 42.57 |
| | | 32.16 | 30.95 | 33.09 | 34.12 | 24.96 | n.d. | 29.92 |

Ls1 – Lactobacillus sakei (strain 1); Sx1 – Staphylococcus xilosus (strain 1); LS1 - Lactobacillus sakei (strain 1) e Staphylococcus xilosus (strain 1); Ls2 – Lactobacillus sakei (strain 2); Sx2 – Staphylococcus xilosus (strain 2); LS2 - Lactobacillus sakei (strain 2) e Staphylococcus xilosus (strain 2).