INFLUENCE OF THE STARTER CULTURE ON THE BIOGENIC AMINE FORMATION DURING THE RIPENING OF PORK SAUSAGES

Sara Bover-Cid; Maria Izquierdo-Pulido and M. Carmen Vidal-Carou*
Departament de Nutrició i Bromatologia-CeRTA, Facultat de Farmàcia, Universitat de Barcelona. Avinguda Joan XXIII, s/n. 08028 Barcelona. Spain.

INTRODUCTION

Biogenic amines (BA) are basic nitrogenous compounds common to most plants, animals and microorganisms. The main origin of BA in foods is believed to be the result of microbial activity due to the enzimatic decarboxylation of the precursors amino acids (1). Some of the BA usually found in foods are the monoamines histamine, tyramine, tryptamine, and phenylethylamine wich may cause toxicological effects when ingested at relatively large amounts. Thus, these monoamines can be related to histaminic intoxication, hipertensive crisis in patients under mono-amine-oxidase inhibitor (MAOI) treatment, and food-induced migraines (1, 2), especially when the detoxification mechanisms are compromised by individual sensitivity, gastrointestinal diseases, alcohol ingestion or treatments with MAOI drugs. The diamines putrescine and cadaverine may potenciate the toxic effects of monoamines. On the other hand, polyamines such as putrescine, spermine, and spermidine, are known to play an important role in physiological processes of plants and animals, and they are also commonly found in foods. Formation of potential carcinogenic nitrosamines, especially in meat products that contain nitrite and nitrate curing salts, constitutes an additional toxicological risk associated to polyamines (3). In addition, BA are of concern in relation to food spoilage. Since they can be the result of the proteolytic and amino acid decarboxylase activity of undesired contaminating microbial flora., some BA have been suggested as indicators of the spoilage extent of some food (4, 5).

During the ripening of dry fermented sausages, there is a growth of microorganisms and also a certain degree of proteolysis which yields the amine precursor amino acids. These events offer favourable conditions for the BA formation. Production of amines in meat and meat products has been often related to *Enterobacteriaceae*, *Pseudomonas* and lactic acid bacteria (LAB) (1). However, the addition of an adequate starter culture to fulfil the fermentation could be an advisable practice to prevent the amine accumulation (6-8). Starter cultures usually consist of one or several strains of lactic acid bacteria (LAB), *Micrococcaceae* or a combination of both (9). On the other hand, the processing conditions (e.g. temperature and humidity) can affect the growth and activity of microbial flora, either initially present in the raw material or added as starter culture. The amounts of precursor amino acids resulting from proteolysis, and the capability of BA formation by bacterial population might also be affected by such processing conditions.

In the present work, the influence of single as well as mixed starter cultures on the BA production during the ripening of dry fermented sausages was examined in comparision with sausages obtained from the same raw materials and the same processing conditions but without addition of starters. Moreover, the effect of an inadequate sausage drying on the formation of BA was also studied.

MATERIAL AND METHODS

Samples. The sausage elaboration was carried out at pilot-scale. Sausage mixture (10 kg per batch) was made from shoulder pork meat and lard in a proportion of 80:20, and (all per kg) 25.0 g of sodium chloride, 0.1 g of sodium nitrite, 0.2 g of sodium nitrate, 0.5 g of sodium ascorbate, 4.0 g of dextrose and 3.0 g of pepper. Meat and fat pork were ground to a 6 mm particle in a meat cutter and divided into four fractions for the different batches. The control batch (I) was spontaneously fermented without addition of any starter culture while batch II was manufactured with the addition of *Lactobacillus curvatus*. Batches III and IV were made with a mixed culture of *Lactobacillus curvatus* and *Staphylococcus xylosus*. Starter cultures, dissolved in 500 ml of water, were added to achieve a final concentration of 10^6 colony forming units (CFU)/g of sausage mixture. Each mixture batch was homogenised with the other ingredients and stuffed into artificial casings (approx. 250g per sausage). Sausage surfaces were imbibed with the mould *Penicillum candidum* by immersing them into a spore suspension.

The ripening and drying process was conducted at 15 °C and 70 to 80% relative humidity (RH) for a period of 21 days. All the samples were placed in the same room at the same conditions. However, in order to obtain an inadequate sausage drying in batch IV, the casings were not well conditioned before stuffing. Thus, casing were not enough humit to allow the usual water evaporation during the ripening.

Duplicate samples for raw meat mixture (zero time) and three sausages from each batch were sampled on the 2nd, 3rd, 7th, 14th and 21st days of ripening.

Analysis. BA were determined by a high performance liquid chromatography (HPLC) method described by Hernández-Jover *et al.* (10). The pH was measured directly from samples using a microcomputerized pH meter (Crison 507, Spain) inserting the electrode into the middle of the sausage. Water content was determined by drying the sample at 100 to 104 °C until constant weight. Due to a large decrease in relative humidity during ripening process, biogenic amine contents are referred as dry matter.

Statistical analysis. Statistical calculations were performed by using the SPSS 6.0.1. software for Windows (SPSS Inc., Chicago, IL, USA). All the values shown in the tables are the mean of three determinations together with their standard deviation (*SD*).

RESULTS AND DISCUSSION

Changes in water content and pH values of the four batches are presented in Figure 1. Water content decreased in all the batches, although the moisture of samples from batch IV was significanly higher ($p<0.0001$) than of the other batch samples, due to the defective drying process involved. Batches II and III attained faster and lower pH values than the sausages spontaneously fermented due to the higher lactic acid production by *L. curvatus* added as starter culture. Nevertheless, batch VI showed a slighly higher pH than batches II and III ($p<0.05$), probably because the higher water content allowed more the development of proteolytic

microorganisms rather than favouring the competitiveness of starter culture. Both parameters, water content and pH could contribute to explain the differences on the BA contents among the four batches.

In all the batches, physiological polyamines, spermine (SM) and spermidine (SD) contents did not differ. They kept relatively constant values during the whole process at levels of 51.3 mg/kg (*SD* = 2.4) for SM and 6.3 mg/kg (*SD* = 0.4) for SP, which are in accordance with the fact that the same raw materials were used as well as with the hypothesis of no polyamine formation during the fermentation step. Histamine (HI) was also found constant during the ripening with a mean value of 0.4 mg/kg (*SD* = 0.06). Tryptamine (TR) and phenylethylamine (PHE) were only detected in the last samples of the spontaneously fermented sausages at levels lower than 0.7 mg/kg.

Figure 2 shows the occurrence of the three BAs formed during the ripening of the four batches. The main amine was tyramine (TY), which is generally related to meat fermentation. The amounts of TY varied extensively among the batches. In comparison to the control (batch I), the addition of a mixed starter culture (batch III) could reduce the formation of TY more than a single culture (batch II) ($p<0.05$). The addition of only *L. curvatus* resulted in a reduction of 25%, whereas combined with *S. xylosus*, which contains double controlled microbial charge, the reduction was 65%. The defective dried batch (IV) showed equal contents of TY than the control sausages (batch I) despite of batch IV was also manufactured with a mixed starter culture. The higher water content of batch IV can be the reason for the higher TY formation. During the ripening, there is an increase of salt concentration due to the drying process which might be the responsible of the inhibition or reduction of decarboxylase activity.

Cadaverine (CA) was the second amine formed, showing the batches with starter cultures much lower amounts (less than 4 mg/kg) than the naturally fermented sausages (Figure 2). The lower pH attained in batches II, III and IV could efficiently reduce the growth and the decarboxylase activity of gram negative bacteria, which are the microorganisms mostly related to CA production (1). Little formation of putrescine (PU) was observed. Although PU amounts were also higher in control batch than in batches with starter culture, no statistical differences were found.

Conclusions

As a conclusion, the use of adequate microorganisms as starter culture and also the processing conditions, such as the drying step, may influence on the BA production by the microbial flora present in the sausage mixture. In addition to the presence of BA producer microorganisms, other factors such as favourable conditions for the growth and decarboxylase activity of these microorganisms are responsible for the amine formation.

Literature

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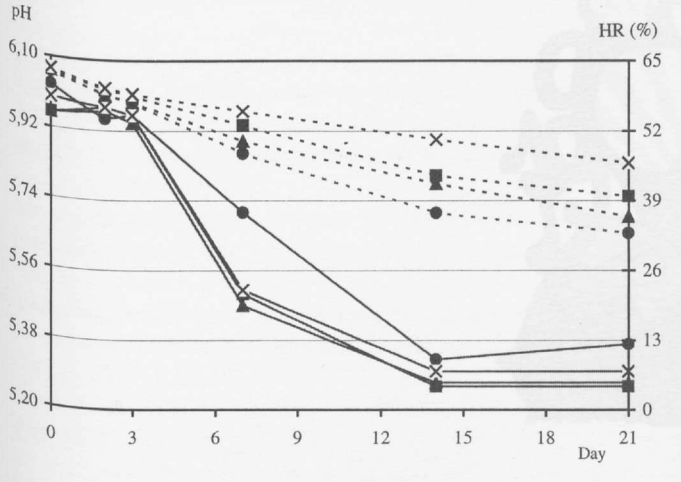

Figure 1. *Changes in pH values (——) and water content (- - - -) during the ripening of (●) control sausages of batch I, (■) sausages with single starter culture of batch II, (▲) with mixed starter culture batch III, and (x) with mixed starter culture and defective drying batch IV.*

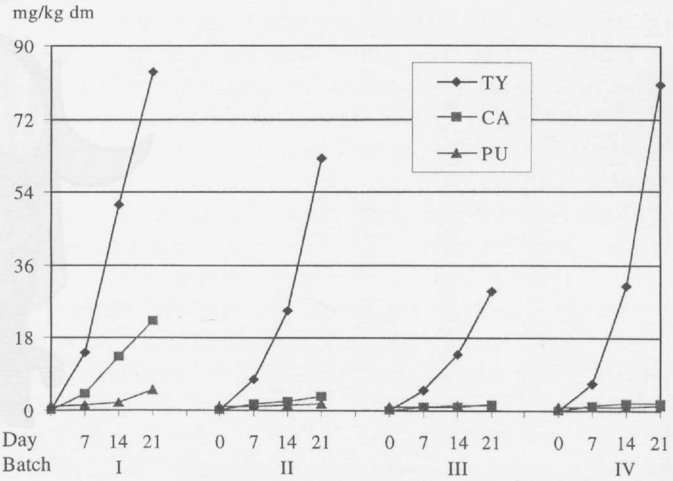

Figure 2. *Biogenic amines, tyramine (TY), cadaverine (CA) and putrescine (PU), formation (mg/kg of dry matter) during the ripening of four different dry fermented sausages: control (batch I), with a single starter culture (batch II), with a mixed starter culture (batch III) and with a mixed starter culture and defective drying (batch IV).*

NOTES