Effect on some processing methods on sulphamethazine residues in meat products.

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Summary

A model study is described concerning the effect of two different types of processing procedures, i.e., sterilization and fermentation, on sulphamethazine (SMZ) residues in the final product. Both luncheon meat and raw fermented sausages were prepared from dough to which SMZ was added, during the chopping procedure, at a level of 1.0 mg kg^{-1}. The SMZ content was determined at different stages of processing by means of a high performance liquid chromatographic technique and UV detection.
No decrease of SMZ was observed during the preparation of luncheon meat. In the raw fermented product, after a ripening period of one month, only 20% of the original amount of SMZ was still present. Most of the SMZ disappeared during brining. This decrease was not due to diffusion of SMZ into the brine.

Introduction

Sulphonamides, in particular sulphamethazine (SMZ), are widely used in veterinary practice. This implies the possible presence of residues in tissues of treated animals used for human consumption.
Though meat is, in general, heat-treated before consumption, there are some products which are consumed unheated, either fermented or not.
Several studies have been published concerning the effect of processing (e.g., cooking) on residues of anti-bacterial drugs in meat (1-7). As far as known, only one author (2) invetigated the fate of SMZ residues. It was observed that roasting and grilling of meat from treated bovine animals affected the biological activity of SMZ residues either minimally or not at all.
In the present model study a sterilization and a fermentation process was applied to assess the effect of these processing procedures to the SMZ content. Analysis of SMZ was performed by means of a high performance liquid chromatographic procedure (8) with UV detection after SMZ extraction from the matrix followed by solid phase extraction from the extract. This procedure was earlier developed for swine meat and kidney tissue.

Experimental

Preparations of products.

The luncheon meat and the raw fermented sausages were made in the experimental butchery of the Department according to a procedure usually applied in the Netherlands.
Luncheon meat : The basic materials (27% beef, 5% pork rind powder, 18% pork, 33,5% pork back fat and 10% snow-ice, all % w/w) were chopped to a dough together with the additives (4.0% flour, 1.8% nitrite-containing salt, 0.33% spices, 0.05% glutamate, 0.02% ascorbate and 0.3% phosphate mixture, all % w/w). The doughs were stuffed in 200g cans (57.5 x 76 mm) and sterilized at 110°C for 80 min (F_o = 1).
Raw fermented sausages :
The basic materials (45.8% beef, 10% pork, 30% back fat and 10% pork rind powder, all % w/w), a starter culture (special starter sausage from CIVO-TNO, Zeist the Netherlands) and the additives (1% salt, 1% nitrite-containing salt, 0.7% glucose, 0.44% spices, 0.2% glutamate and 0.05% ascorbate, all % w/w) were chopped to a dough.
The doughs were stuffed into permeable artificial casings (length 10-15 cm; aprox. 250g of dough per sausage).
The sausages were left in a brine (composition : 7% NaCL, 2% nitrite-containing salt, 1% sodium dihydrogen phosphate and 90% water, all % w/w; pH = approx. 4.2.) at 25-27°C for 48 hours. After smoking at 28°C for about 30 min, the sausages were allowed to ripen in a climate room at 15°C for about 4 weeks, the relative humidity being 80%.

Experimental design

Luncheon meat : Two batches were prepared : a blank dough, without SMZ, for control and a dough in which SMZ (Sigma Chemicals) was added to the raw material, during the chopping procedure, at a level of 1.0 mg kg^{-1}.
Both doughs were stuffed in 200g cans. Some cans from both charges were immediately frozen at -40°C and defrosted just before analysis. The remaining cans were sterilized under conditions described above. These cans were also frozen at -40°C and defrosted just before analysis. SMZ analysis was performed according to the procedure described below after homogenation of the contents of the can in Moulinette, using 10g test portions. The blank was also used for establishing the analytical recovery, as described under SMZ analysis.
Raw fermented sausages : Again two batches were prepared : a blank dough and a dough in which SMZ was added during chopping to the raw material, at a level of 1.0 mg kg^{-1}. Both doughs were stuffed into 250g casings. From each charge some sausages were immediately vacuum packed and frozen at -40°C. During processing, some sausages from each batch were taken for each examination (directly after brining and during ripening on days 4, 8, 16 and 29 after preparation). These sausages were also vacuum packed and frozen at -40°C.
The sausages were defrosted just before analysis. The casings were removed and the whole contents were homogenized in a Moulinette. SMZ analysis was performed using 10g test portions. The blank was also used for establishing the analytical recovery as described under SMZ analysis.

SMZ analysis

The SMZ content was determined as described by Haagsma and van de Water (8). This method comprises sonication-aided extraction of SMZ from the ground sample with chloroform/acetone 1 : 1 v/v. After filtration and acidification the extract was cleaned up and concentrated on a solid-phase extraction column packed with a silica-based aromatic sulphonic acid cation exchanger. Analysis was performed by high performance liquid chromatography on a C_8 reversed-phase column using acetonitrile/sodium acetate (0.01 mol l^{-1}, pH = 4.6) 30 : 70 v/v as an eluent. Detection was performed at 254 nm and the flow rate was 1.5 ml min^{-1}.
Analytical recovery experiments were carried out in different stages of processing. For luncheon meat, SMZ was spiked both to the fresh-prepared dough and to the final product at levels of 0.25, 0.50, 0.75 and 1.0 mg kg^{-1}, at least 15 min before extraction by the procedure described above. For the raw fermented sausages SMZ was successively spiked to the fresh-prepared dough, to the sausages directly after brining and to the sausages

during ripening 4, 8, 16 and 29 days after ripening, respectively, at levels also used for the luncheon meat. The sausages were defrosted just before analysis. The casing was removed and the whole contents were homogenized in a Moulinette. SMZ analysis was performed using 10g test portions. The blank charge was also used for establishing the analytical recovery as described under SMZ analysis.

Results and discussion

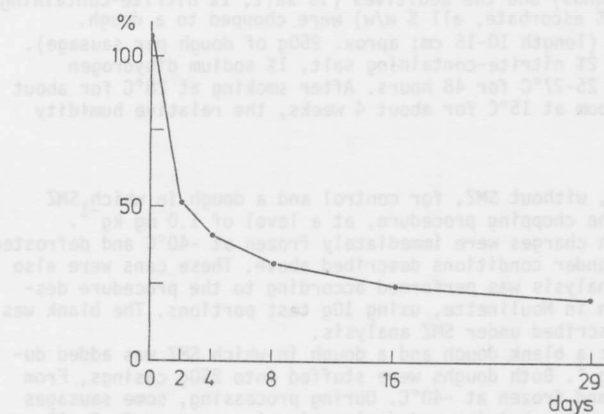
Luncheon meat

No decrease of SMZ was observed during the preparation of luncheon meat. The SMZ content of the raw dough and of the final product amounted 0.88 $\pm$ 0.03 mg kg^{-1} and 0.82 $\pm$ 0.03 mg kg^{-1}, respectively. These results are not significantly different (t test; P = 99%). No explanation could be given for the somewhat lower SMZ content in the dough as related to the added amount of SMZ.
As the product was subjected to a rather strong heat treatment in comparison to a pasteurized product, it seems unlikely that during preparation of other types of products under the same or less intense heating conditions any decrease in SMZ content, as a result of heat treatment, will occur.
The HPLC method, originally developed for swine meat and kidney tissue, was found to be also suitable for SMZ analysis in the raw dough and in th final product. The chromatograms of the blanks, in these cases, were very clean either. Peaks of endogenous compounds appear only during the first 25 min, while SMZ elutes at a retention time of about 5.9 min.
Analytical recoveries were 82% for SMZ spiked to the dough and 86% for SMZ spiked to the luncheon meat, both with good reproducibility.

Raw fermented sausage

Fig.1 relates the SMZ content, in different stages of storage, to the original amount of this compound (= 100%). As during ripening the suasages lost some weight due to moisture loss, the absolute amount of the remaining SMZ is given.

Fig. 1 : Relation between storage time after preparation of raw fermented sausage (at 15°C) and SMZ content (in percentages of the original amount). Percentages are calculated on absolute amounts.



After one month not more than aprox. 20% of the original amount of SMZ was still detectable in the sausages. It was established that this decrease was not caused by diffusion of SMZ into the brine (as far as detectable), as was demonstrated by HPLC analysis of the brine.
The HPLC procedure proved to be also suitable for the determination of SMZ in raw fermented sausages and in the dough and sausages during the different stages of preparation. Analytical recoveries of spiked SMZ amounted 75% in the raw dough, 71 just after brining and 71, 78.5, 79 and 79% during different stages of the ripening period.
It seems legitimate to relate this decrease to the sulfonamide penetration into the bacteria, where SMZ competes with p-aminobenzoic acid - to which the sulphonamides bear a close similarity - for dihydropteroate synthetase. In this way the formation of tetrahydropteroic acid, the immediate precursor of folic acid, is inhibited. During the processing of the raw fermented sausages SMZ might act in the same way. Particularly during the brining step a sharp rise in growth of some specific bacteria occur. If SMZ is also incorporated in these bacteria it might be possible that this SMZ is not longer detectable. However, it is needless to say that this does not necessarily mean that SMZ is not available after consumption of the sausages. Much more information is needed about what does really happen in the product before the total impact of this effect can be considered. This is currently under

investigation.

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Results and discussion

Luncheon meat

No decrease of SMZ was observed during the preparation of the luncheon meat. The SMZ [illegible] the final product amounted 0.88 ± 0.03 mg kg^{-1} and 0.82 ± 0.03 mg kg^{-1}, respectively. These results are not significantly different (t test; [illegible]) [illegible] for the somewhat lower SMZ content in dough as related to the added amount of SMZ.
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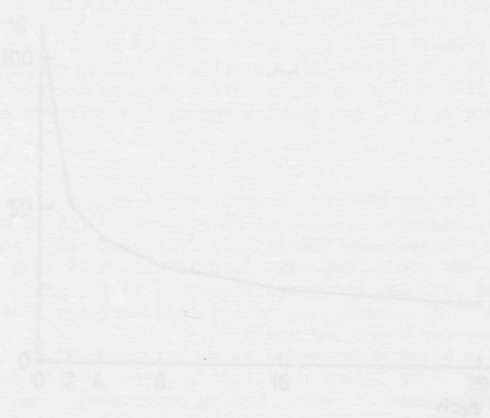

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