

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 antibacterial drugs in meat (1-7). As far as known, only one author (2) investigated 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 ($57.5 \times 76 \text{ mm}$) and sterilized at 110°C for 80 min ($F_0 = 1$).

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; approx. 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 $25-27^\circ\text{C}$ 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 mg 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 $0.88 \pm 0.03 \text{ mg kg}^{-1}$ and $0.82 \pm 0.03 \text{ mg kg}^{-1}$, 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.

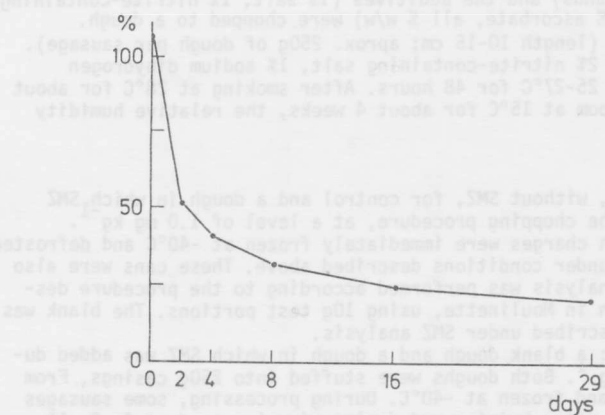
The HPLC method, originally developed for swine meat and kidney tissue, was found to be also suitable for SMZ analysis in the raw dough and in the final product. The chromatograms of the blanks, in these cases, were very clean either. Peaks of endogenous compounds appear only during the first 25 min, 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 sausages 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 approx. 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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Materials and methods:

Species-specific antisera: Antisera against bovine, porcine and equine species were raised in sheep with Freund's adjuvants. Each primary dose comprised 100 µg of antigen (10 mg fraction V products) emulsified in Freund's complete adjuvant (10 µl) and sterile saline (10 µl). Booster injections of 50 µg antigen (50 µl saline) were administered in the same manner and volume, except using Freund's incomplete adjuvant, were given at 28-42 day intervals. Antisera against ovine species albumin was raised in a 12-month old steer by a similar programme. Blood samples (10-20 ml) were taken 7-10 days after the second boost and antisera assessed for specificity by microdiffusion precipitin tests. In these, samples of each antiserum were reacted with normal species sera (diluted 1:2 in 0.2M) in 500 µl of 0.2M phosphate buffered saline (pH 7.0). A white precipitate had formed at the junction of the two liquids during 1-3 hr incubation at room temperature (RT) and

Preparation of meat extracts: Authentic species meat extracts were prepared from fresh, whole muscle tissue as follows: (1) Approximately 200g tissue was thoroughly comminuted in a fine strainer with 400ml of chilled water (in a stainless steel processor) allowed to stand for 1h at RT and centrifuged at 1500g for 10 min. The supernatant was filtered through Whatman No. 1 paper, to avoid clumping, and used directly. (2) Other meat species tissues (liver, kidney, heart, muscle) were prepared in the same manner and volume, allowed to stand for 1h at RT and centrifuged as above. (3) Both types of extract contained very soluble meat proteins, as well as the typical lipid components, such as cholesterol, which were removed by conventional biochemical double diffusion tests (applying fluids direct to wells) to ensure that the extracts were free of these components. (4) The respective supernatants were extracted with overnight extraction at RT.

Preparation of sterilized reagent discs: The procedure of Regard et al. (1985) was modified as follows: Glass reagent discs (20 mm diameter) were punched from Whatman No. 3 paper and stored in a desiccator until needed. The discs were sterilized by autoclaving at 121°C for 15 min. The discs were then stored in a desiccator until needed. The discs were then stored in a desiccator until needed.

Preparation of agar plates: Petri dishes (as above) were filled with 10-15 ml of agar, substitution water was prepared from the following: 20 ml distilled water, 1.5% NaCl, 1.5% calcium chloride and 0.05M water. The agar plates were prepared, were autoclaved at 121°C for 15 min and stored until needed.

Procedure for infect test samples: The test procedure for verification of infect test samples followed the same steps as the original OEBIT system as follows: The test plates were primed with reference meat discs (50 µg and 100 µg) of one of the species under test, the agar plates were covered carefully by each disc on the agar surface, so that all the agar surface was fully covered when viewed directly from above. A small incision was then made in the agar surface (thrust at previously marked) and the blank disc placed inside to secure the tissue flaps. In general, sufficient agar seeded from

during ripening 4, 8, 16 and 28 days after ripening, respectively. The sausages were defrosted just before analysis. The casing was removed and the whole contents were homogenized in a Bausch & Lomb SMZ analyzer. The casing was removed and the whole contents were homogenized in a Bausch & Lomb SMZ analyzer. The casing was removed and the whole contents were homogenized in a Bausch & Lomb SMZ analyzer. The casing was removed and the whole contents were homogenized in a Bausch & Lomb SMZ analyzer.

Results and discussion

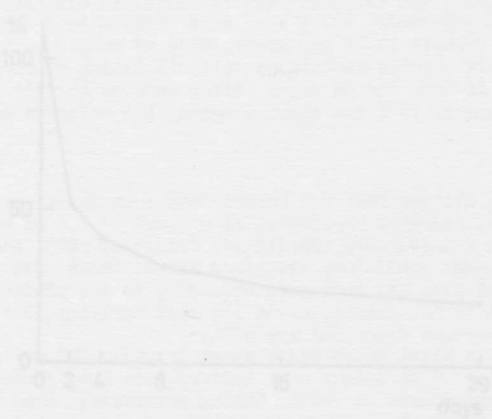
Losses of SMZ

No decrease of SMZ was observed during the ripening process. The final product contained 0.25 ± 0.05 mg SMZ per 100 g sausage. The loss of SMZ during ripening was 0.05 mg per 100 g sausage. The loss of SMZ during ripening was 0.05 mg per 100 g sausage. The loss of SMZ during ripening was 0.05 mg per 100 g sausage.

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After one month not more than approx. 25% of the original amount of SMZ was still detectable in the sausage. It was established that this decrease was not caused by diffusion of SMZ into the brine (as far as detectable) as 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 sausage during the different stages of preparation. Analytical recoveries of spiked SMZ amounted to 100% in the raw dough, 71% just after brining and 71, 70, 6, 79 and 100% during different stages of ripening. It seems legitimate to relate this decrease to the sulfonamide penetration into the bacteria, where SMZ competes with p-aminobenzoic acid - to which the sulfonamide has a close similarity - for dihydropteroate synthetase. In this way the formation of tetrahydropteridine acids, the immediate precursor of folic acid, is inhibited. During the processing of the raw fermented sausage SMZ might act in the same way. Particularly during the brining process a sharp rise in growth of some specific bacteria occurs. If SMZ is also incorporated in these bacteria it might be possible that this SMZ is not longer detectable. However, it is not clear if this is the case and not necessarily true that SMZ is not available after consumption of the sausage. Much more information is needed about what really happens in the product before the total impact of this effect can be considered. This is currently under