STUDIES ON THE CONTENT OF CHOLESTEROL OXIDES IN HEATED MEAT PRODUCTS

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Background

Cholesterol can oxidise with air to a number of cholesterol oxides (CO) [1]. Oxidation could be started by heat, light or free radicals - for example from fat oxidation. The compounds shown in fig. 1 are preferred reaction products. Hazardous biological effects are attributed to several CO. They could act cyto- and angiotoxic [2] as well as cancerogenic [3]. A relationship is supposed between the absorption of CO with the food and cardiovascular disorders like atherosclerosis [4, 5]. It was proven in animal tests that CO could initiate atherosclerotic lesions of blood-vessels [5]. There is evidence, that CO act much more effective in this manner than cholesterol itself [6].

In recent, own studies with a method designed for CO-analysis in meat the CO-contents in raw, lean pork and beef meat were scarcely different from fried or cooked samples, if the analysis was taken directly after heating. One week of storage (8°C; samples were wrapped up in aluminium foil) significant changes were observed. Whereas the CO-contents in raw pork and beef changed only slightly, both considerable increased in the fried samples with 7β-Diol by about a factor of 30. The largest changes were observed after refrigerated storage of cooked meat. After seven days the concentration of 7β-Diol in pork and beef raised by about a factor of 100. Noticeable increases were also ascertained with all other cholesterol oxides. After a normal household refrigerator storage (one or two days) of cooked meat a considerable increase was found, the 1-mg/kg-level was crossed by three oxides (7α- and 7β-Diol, 7-Keto) already after one day of storage.

Methods

In our investigations ready-to-eat chilled meals and sausages were analysed. They were stored appropriate to the declarations of the manufacturers in the original packings and were repeatedly investigated until the recommended shelf life. The meat products (without trimmings) were twice analysed without separation of fat and without previously heating by a method developed for CO-analysis in meat.

The internal standard 19-Diol (an in meat not existing CO) was added to the samples and the fat was repeatedly extracted in the mixer with hexane/i-propanol in the cold. This fat was transesterified for one hour by 30 % sodium methylate with occasional shaking. The reaction was stopped by adding of citric acid and the products of transesterification were repeatedly extracted with ether in a separation funnel. The united ether fractions were dried over sodium sulphate and evaporated to dryness with a rotary evaporator and subsequent with nitrogen. A Solid-Phase-Extraction (SPE) on basis of aminopropyl was applied to the residue to get an enrichment of CO and to separate them from the surplus of cholesterol. The CO were eluted with acetone and evaporated to dryness with nitrogen. The sample was derivatisated with a reactive mixture of BSA/TMSI/TMCS for two hours at 70 °C. The separation was achieved on gas chromatograph with a apolar DB-5 similar column. The eight CO (7α- and 7β-Diol, α- and β-Epoxid, 20α-Diol, Triol, 25-Diol, 7-Keto) were identified by mass spectrometer.

Results and discussion

The amount and the development of CO-concentrations from four different meat products during refrigerator storage (6 °C) are described. The CO-contents in liver sausage (fig. 2) increased to a maximum, which was up to nine-fold compared to the amounts found in heated, non stored pork and beef in own recent studies ("values of comparison"). On the other hand the concentrations in cured loin (fig. 3) were constant relative to the values of comparison. There were only small changes of CO-contents during refrigerator storage till the end of recommended shelf life in liver sausage packed in a synthetic casing and in cured loin, too. The amounts of other, here not shown liver sausages packed in natural or synthetic casings were in good agreement with those of fig. 2, the material of casing seemed to be not crucial.

During storage of a heated and vacuum packed, non cured emulsion type sausage especially the concentrations of the compounds 7α-Diol, 7β-Diol and 7-Keto increased, which are all oxidised on position seven of the cholesterol molecule (fig. 4). The amounts at the end of recommended shelf life shown in fig. 4 were in good agreement with those of other non cured, emulsion type sausages. The concentrations increased up to a factor of four relative to the values of comparison.

Reformed turkey meat exhibited the highest CO-contents (fig. 5) of all poultry meat products analysed so far, up to a factor of twenty relative to the values of comparison (7β-Diol: > 1 ppm). This product showed additional particularities like non-uniform development of CO-concentrations during refrigerator storage. At "day 7" the highest, at "day 27" the lowest and at "day 42" intermediate contents with a high standard deviation were analysed. This was obviously caused by the fact, that in this series of analysis samples of two different batches were used ("day 7" = both samples from batch 1; "day 27" = both samples from batch 2; "day 42" = one sample from both batches respectively). As a result of this experiment, homogenous meat products from one manufacturer can show considerable differences from batch to batch. This may be caused by production or storage of the reformed turkey meat, less by raw meat or treatment of the raw meat.

The presented results only concern a small part of the samples, which are analysed in course of the research project. In particular frozen meat products and preserves with long shelf life are not analysed yet, but also additional chilled products. Moreover, uncommon meat products like the reformed turkey meat shown in fig. 5 need further investigation.

Conclusions

Cholesterol oxides are attributed to hazardous biological effects. The studies are concerned with the concentration of CO in several heated meat products. These are ready-to-eat chilled meals, prepared and chilled stored meat and preserves of meat and meat products, storable at ambient temperatures for a long time. The influence of ingredients (rosemary, ascorbate and nitrite curing salt) with antioxidative behaviour is also investigated. First results are presented exemplary from four meat products (liver sausage, cured loin, non cured emulsion type sausage, reformed turkey meat). As a result, the amount and the development of the CO-content during storage differs considerable depending on meat product.

References

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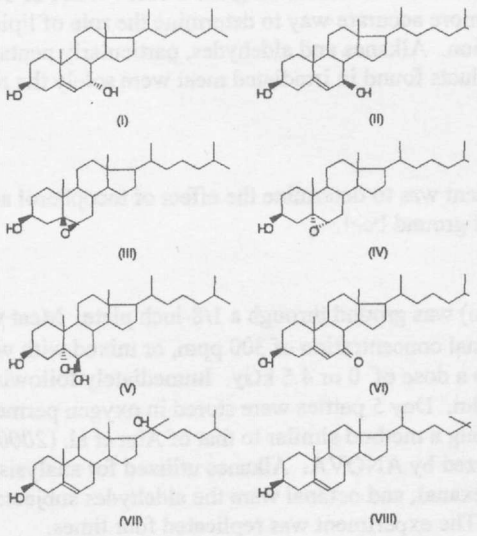

fig. 1: Autoxidation of cholesterol – structures of the main products:
Cholest-5-en-3b,7a-diol (I)
Cholest-5-en-3b,7b-diol (II)
Cholestan-5b,6b-epoxy-3b-ol (III)
Cholestan-5a,6a-epoxy-3b-ol (IV)
Cholestan-3b,5a,6b-triol (V)
Cholest-5-en-3b-ol-7-on (VI)
Cholest-5-en-3b,20a-diol (VII)
Cholest-5-en-3b,25-diol (VIII)

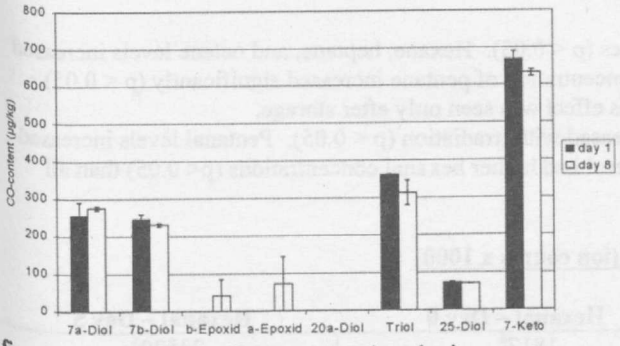

fig. 2: CO-contents in liver sausage (synthetic gut; storage: 6 °C); Means and standard deviations from two determinations of one sample.

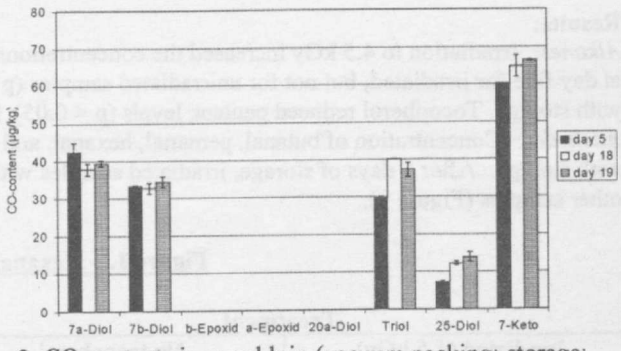

fig. 3: CO-contents in cured loin (vacuum packing; storage: 6 °C); Means and standard deviations from two determinations of one sample.

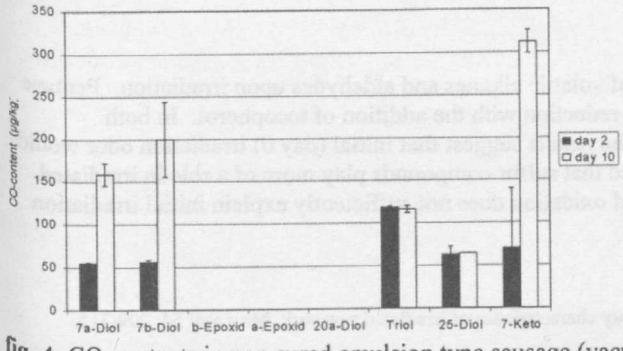

fig. 4: CO-contents in non cured emulsion type sausage (vacuum packing; storage: 6°C); Means and standard deviations from two determinations of one sample.

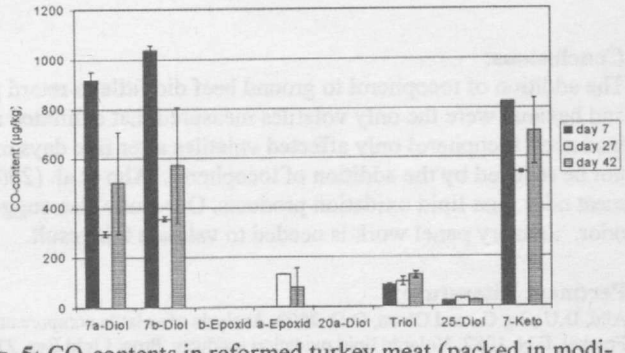

fig. 5: CO-contents in reformed turkey meat (packed in modified atmosphere; storage: 6 °C); Means and standard deviation at „day 7" and „day 27" from 2 determinations of 1 sample, at „day 42" from single determinations of two different batches.