Monascus-Extract a Possible Alternative to Nitrite in Meats L. LEISTNER, J. FINK-GREMMELS and J. DRESEL Federal Centre for Meat Research, D-8650 Kulmbach, Germany

<u>SUMMARY:</u> The use of nitrate or nitrite for the curing of meat products has a long tradition and provokes little health risk in the amounts legal in Germany. Nevertheless, alternatives are of interest. In Asia the mould *Monascus purpureus* WENT is traditionally used to colour and flavour different foods, and in addition this mould is believed to be beneficial for the health of consumers. Our investigations with *M. purpureus* (DSM strain 1379) revealed that the extract of this mould grown on rice is non-toxic, improves the formation and stability of colour in cooked and raw meat products, has a bacteriostatic effect on some undesirable bacteria, and influences the lipids in the blood favourably. Thus, Monascus-extract may be regarded as a possible alternative to nitrite. However, it probably will not replace in general the use of nitrite for meats, but could provide for health conscious consumers attractive meat products without curing agents.

INTRODUCTION: The addition of nitrite (nitrite curing salt) or nitrate (salpetre) should improve the colour, flavour and preservation of meats, and because nitrite is an antioxidant the rancidity of the products is delayed. However, nitrite is a substance with considerable toxicity, and therefore it should be used in amounts as little as possible, but as much as necessary. Following this rule, in Germany since 1981 the addition of nitrite has been reduced by 20 %, and the use of nitrate for meat products was restricted too (LEISTNER, 1981). Nevertheless, some consumers of meats object to nitrite even present in small amounts. About 5 % of the German meat products (e.g. Gelbwurst, Weißwurst, Pfälzer Leberwurst) are traditionally manufactured without nitrite or nitrate and such products are an alternative, however, they appear grey, lack the cured meat flavour and are easily perishable. Therefore, further alternative meat products are desirable.

In the Far East, especially in China, moulds of the genus *Monascus* are used to ferment rice, which acquires a pleasant red colour. After drying and grinding this "red-mould-rice" (also called Angkak) is added to pork, poultry, fish, tofu, bananasauce, rice wine, brandy, etc. to improve the colour, flavour and preservation of these products (HESSELTINE, 1983; LEISTNER, 1986; MEYER, 1990). In China "red-mould-rice" is used since more than 1500 years. In a book on Chinese medicine, LI SHIH-CHUN (1590) pointed out that "red-mould-rice" is a cure for various diseases, including indigestion, diarrhea and even anthrax. The "red-mould-rice" has also been recommended to extent the shelf-life of meat.

Since "red-mould-rice" could be an alternative for the addition of nitrite or nitrate to meat products, we have investigated the production as well as the toxicological, dietetic, colouring and preservative effects of Monascus-extract. In these investigations we used Monascus-extract instead of "red-mould-rice", because the extract has a more uniform composition, and thus the dosage is more exact.

PRODUCTION OF MONASCUS-EXTRACT: Moulds of the genus *Monascus* produce at least six secondary metabolites which are coloured (yellow, orange, pink or red). These pigments are structurally related, and the qualitative and quantitative pigment production depends primarily on the nutrients and the pH of the substrate as well as the incubation temperature (WONG et al., 1981; KUNZ and OBER, 1987; MEYER, 1990). In our investigation we utilized the *Monascus purpureus* strain 1379 from the German Culture Collection (DSM), which produces ample red pigments, especially if grown on a rice medium of the following composition: 20 % pulverized rice, 8 % sucrose, 2 % yeast extract, 0.5 % magnesium sulfate, 0.1 % potassium hydrogen phosphate, 0.1 % calcium chloride, dissolved in water. The autoclaved medium we inoculated with *M. purpureus* DSM 1379 and incubated for three weeks at 30°C. Thereafter the mycelium was dryed at 40°C, homogenized and several times extracted with methanol (analytical grade). Under nitrogen, the methanol was completedly evaporated and the

TOXICOLOGICACAL EVALUATION OF MONASCUS-EXTRACT: We detected no mycotoxins by chemical analysis in our Monascus-extract, however, only 40 mycotoxins were assayed and more than 300 are known. Therefore, further investigations were carried out with biological assays. The acute toxicity of the Monascus-extract was evaluated with mice and rats and here again, contrary to many other moulds, the strain *M. purpureus* DSM 1379 proved non-toxic (FINK-GREMMELS and LEISTNER, 1989). In addition, the cytotoxicity and genotoxicity of the Monascus-extract were studied *in vitro*, and it was concluded that the genotoxic potential of Monascus-extract is much lower than of nitrosamines which possibly occur in cured meats. Therefore, from the public health point of view Monascus-extract would be a desirable alternative to nitrite (FINK-GREMMELS et al., 1991b).

extract residue we kept in a dessicator. Before use measured amounts of the extract are dissolved in demineralized water

and suspended by ultrasonic treatment (FINK-GREMMELS et al., 1991a).

DIETETIC EFFECTS OF MONASCUS-EXTRACT: Monascus fermentation products are recommended in Asia also for dietary reasons, e.g. to protect against hyperlipoproteinemia. ENDO (1980) isolated from *M. ruber* a metabolite named ^{monacolin} K, which normalized induced hyperlipoproteinemia in rats. Later the reduced form of monacolin K was introduced ^{as} a drug, which proved successful in lowering the cholesterol level in the blood of patients (TOBERT, 1987). Our modell ^{experiments} demonstrated that pigments of *Monascus* influence the lipiprotein metabolism, which was estimated by de-^{creased} cholesterol, HDL-cholesterol and triglyceride levels in the blood of rats with dietary induced hyperlipoproteinemia (FINK-GREMMELS and LEISTER, 1989). The efficiency of the Monascus-extract was lower than that of the therapeutically ^{used} substances. Therefore, the observed dietetic effects of Monascus-extract correspond more to the effect of certain ^{spices}, such as garlic (HÄNSEL and HAAS, 1984).

MONASCUS-EXTRACT USED AS COLOURANT FOR MEATS: The red *Monascus* pigments are stable toward heat (120°C), and this is an important feature for a food colourant. In our experiments the Monascus-extract improved the colour of fermented sausages, without influencing the desired white colour of the fat. In cooked sausages (Bologna-type sausage, frankfurters) the Monascus-extract caused in products manufactured with salt an attractive reddish colour which, however, did not really correspond to the cured meat colour. On the other hand, the colour stability of such products under the in-fluence of light was much better than in nitrite-cured meats. The combined use of nitrite curing salt and Monascus-extract re-^{sulted} in a typical cured meat colour and especially in a much better colour stability under the influence of light, as was pro-^{Ven} by objective colour measurements. If the cooked meat products contained 4000 ppm Monascus-extract (recommended level) the nitrite addition could be reduced to 70 % or even 90 % and still the cured meat colour was satisfactory and the ^{Colour} stability impressive (FINK-GREMMELS et al., 1991a).

MONASCUS-EXTRACT AS A PRESERVATIVE: One of the reasons for using nitrite for meats is its preservative effect, and therefore for any alternatives to nitrite an inhibitory effect toward undesirable microorganisms would be an asset. WONG

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and BAU (1977) isolated from cultures of M. purpureus an antibiotic substance, named monascidin A, which proved inhibitory toward bacteria of the genera Bacillus, Streptococcus and Pseudomonas. WONG and KOEHLER (1981) isolated from certain strains of M. purpureus two yellow pigments which occured outside of the mycelium and were in rather low concentrations bacteriostatic for Bacillus subtilis. On the other hand, OBER and KUNZ (1989) were not able to demonstrated any bacteriostatic effects of M. purpureus DSM 1379 toward three tested strains of bacteria. CHEN and TSENG (1989) observed bacteriostatic effects in eight of ten investigated Monascus strains especially against Staphylococcus aureus. The bacteriostatic fractions were not pigmented and stable toward heat (120°C for 20 min.) as well as pH (5.5). Unpurified extracts delayed the microbiological spoilage of pork. FINK-GREMMELS et al. (1989) demonstrated that even M. purpureus DSM 1379 is bacteriostatic for several species of bacteria, however, only the extract and not the intact mycelium of the mould. LEISTNER and DRESEL (1991) investigated the bacteriostatic effects of this Monascus-extract in modell systems as well as in meat products. They demonstrated that the inhibitory effect depends on the species of the bacteria, the inoculation size and the time of incubation. Quite sensitive proved several Bacillus species as well as Listeria monocytogenes, after longer incubation this was true for S. aureus too. Generally grampositive bacteria were more inhibited than gramnegative organisms. In vacuum packaged sliced Bologna-type sausage, stored at 7°C, the growth of lactobacilli was delayed more by the addition of 4000 ppm Monascus-extract to the product, than by the normal nitrite addition (80 ppm). L. monocytogenes was inhibited in this product by 4000 ppm Monascus-extract similar as with 80 ppm nitrite, but definitely more than in products containing sodium chloride only. In fermented sausages the usual nitrite addition (120 ppm) inhibited Salmonella ssp. definitely more than 4000 ppm Monascus-extract, and the results were similar for L. monocytogenes. The desirable lactobacilli in fermented sausages were inhibited neither by the extract nor by the nitrite addition. Therefore, Monascus-extract definitely could have bacteriostatic effects on undesirable bacteria not only in modell systems but in meat products too. These effects are qualitatively and quantitatively different from nitrite in meats (LEISTNER and DRESEL, 1991).

DISCUSSION AND CONCLUSIONS: In our experiments it was demonstrated that the extract of the mould *Monascus purpureus* DSM 1379 is toxicological unobjectionable, and favourably influences the colour formation and especially the colour stability of cooked and raw meat products. Furthermore, this mould influences positively the lipid metabolism in laboratory animals, and the Monascus-extract inhibits several undesirable bacteria in model systems as well as in meat products. However, the bacteriostatic effect of Monascus-extract differs in some respects from that of nitrite curing salt.

A feature which until now has not been investigated thoroughly is the impact of Monascus-extract on the flavour of meat products. The concentrated Monascus-extract has a slightly bitter taste, however, the addition of 4000 ppm Monascu⁵⁷ extract to cooked or raw meats has no adverse influence on the flavour or aroma of these products. In comparison to the meat products only manufactured with sodium chloride, the cooked and raw meats with the addition of Monascus-extract were judged to be of better taste (FINK-GREMMELS et al., 1991a; LEISTNER and DRESEL, 1991). However, a systematic investigation of the flavour of meat products which contain "red-mould-rice" or Monascus-extract is needed.

In Switzerland (according to a tentative regulation - vorläufige Weisung - of the Federal Health Office - Bundesamt für Gesundheitswesen - of July 25, 1989) the addition of rice fermented with *Monascus purpureus* to Bologna-type sausage has been allowed. However, products with "red-mould-rice" must not contain nitrite and the addition has to be labeled. In Germany the colouration of meat products is not legal. One of the reasons is that a "freshness" of spoiled products could be simulated, and therefore the consumer could be deceived. Comparable to nitrite, the Monascus-extract is not just a colourant

but has other properties too, and this could be an asset. Before the legalization of "red-mould-rice" or Monascus-extract for meat products the advantages (colour, flavour, preservation, dietary effect) and the disadvatages (the consumer may be deceived) should be considered carefully. In general, the legalization of "red-mould-rice" poses less problems, since it may be considered to be a food (as in Switzerland), whereas the approval of Monascus-extract would be more difficult, since it has to be treated as an additive.

In Canada recently as an alternative to nitrite in Bologna-type sausage a multifactoral system has been recommended, which consists of a preformed curing colour (dinitrosyl-ferro-hemochrome-complex), sodium ascorbat in combination with sodium tripolyphosphate as antioxidants, and sodium hydrophosphite or sodium lactate as preservatives (O'BOYLE et al., 1990). This approach is promissing, however, the legalization of several substances as replacement for nitrite would cause in Germany even more difficulties than the approval of the Monascus-extract.

In conclusion it could be stated that efforts to replace nitrite and nitrate in meat products are worthwhile. "Red-mouldrice" or Monascus-extract may be regarded as possible alternatives for the addition of nitrite or nitrate to meats. However, they probably would not replace in general the use of nitrite for meats, but could provide for health conscious consumers attractive meat products without curing agents.

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