Reducing the amount of nitrites in the production of pasteurized organic meat products

Summary of the project and implications

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Colophon

TNO Quality of Life

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Abstract

In the production of organic meat products like cold meats, nitrites and nitrates are used for several reasons: for the antimicrobial and anti-oxidative properties, forming and stabilizing the red, cured meat colour, and for forming a cured flavour. From literature, it is concluded that it is not possible to substitute nitrite in the production of organic cooked cured meat products with a single additive or combination of additives without changing the quality of the meat products. Nitrite is responsible for the distinctive cured colour and aroma and microbiological and chemical stability of the meat product. Nitrite is unique in its capability to prevent *Clostridium botulinum* spores to germinate and form poisonous toxins. In organic production, it is not desirable to use nitrite, mainly because of probable health risks associated with it; according to several publications, nitrite-derived products are supposedly to be carcinogenic. For food safety (mainly by preventing botulism), having small amounts of nitrites in the meat products is still desirable. The organic sector expects that a complete ban on nitrite use will only lead to uncontrolled application of (organic) alternatives still leading to nitrites in the final product; alternatives that may be used without registration Therefore, the present work focuses on a reduction of nitrites, to minimize microbiological risk, while reducing the risks of producing possible carcinogens.

From practical studies with organic Bologna type sausage and organic cooked ham products, it is concluded that the level of nitrite used in the process may be reduced to maximally 80 mg $NaNO_2$ per kg meat product without negatively affecting the quality of the two product categories. A reduction to 40 mg/kg also seems possible; however, in that case the products are more likely to be susceptible to external factors, like light and temperature abuse during storage. After cooking, the (residual) nitrite level in the produced products reduced to about 10-25% of the originally (ingoing) amount of nitrite. Completely excluding nitrite in the production of organic meat products like Bologna type sausage and cooked ham is not desirable; the typical cured colour will not turn up. Next to that, as a safety measure, small amounts are advisable. The efficiency of nitrite is affected by several factors. In particular, factors like water activity, pH, intensity of the heat treatment, initial load of spores, amount of iron, process and storage temperature, and presence of other ingredients like ascorbates. The study has only been performed on two types of organic cooked cured meat products. Although some implications of the present research for other products is hypothesized in this report, it is suggested to practically verify the results for other types of products before lowering the ordinarily used amount of nitrites.

Content

1 Introduction

In the production of organic meat products like cold meats, nitrites and nitrates are used for their antimicrobial and anti-oxidative properties, for forming and stabilizing the red, cured meat colour, and for forming a cured flavour. The use of nitrite in organic products, however, is very much subject for debate. Being an additional non-organic component, nitrite, in principle, is a non-desired component. In addition, possible reaction products of nitrites (N-Nitroso compounds) may be carcinogenic.
For safety reasons, restricted use of nitrites is allowed. In accordance with to the latest European legislation, a residual nitrite concentration of 50 mg per kg organic meat product is allowed, while no more than 80 mg nitrite per kg meat product may be added initially during the production process (EU, 2007). These figures still may change, since the current discussion in the EU on the use of nitrites in organic meat products is not finished yet.

To give input to the present discussion, a program on the reduction of the use of nitrite in the preparation of two types of organic meat products has been carried out. Although the effects of leaving out nitrite also have been studied, focus in this program has been on reducing the amounts of nitrites in organic meat products compared to regular meat products, while still guaranteeing food safety. The reasoning for this was that the organic meat sector assumes that a total ban probably will increase the risk of using nitrites uncontrolled. A reduction, however, might be possible, and seems to be the best option.

In this framework, a literature review and two experimental studies on this subject have been performed in 2005 and 2006 (Stegeman *et al.*, 2005, 2006, and 2007). During the first experimental study, cured ham and Bologna type sausages were produced on a pilot scale without using nitrite and with two reduced levels of nitrites. The effects of the nitrite level on colour and colour stability of the sliced products were investigated. In the same study, products have been inoculated with *Clostridium botulinum* spores to study the activity of nitrites in inhibiting the germination of Clostridium spores and successive toxin formation. As is mentioned in a previous study of TNO, *theoretically* it may be possible to produce meat products under specific conditions without nitrite, see Stekelenburg and Hoornstra, 2002. From the literature survey, it was concluded that *under the normal practical* conditions, nitrite should not be left out completely. If so, colour and taste will deviate largely from regular products. It also would introduce very severe and impracticable requirements on the complete supply chain (e.g., on raw material requirements, and water activity, pH, etc. in the final product, to strict guarantees on temperature (abuse) in the entire chain). Nevertheless, a reduction in the amount of (residual) nitrite should be strived for, because of the suggested carcinogenic properties of N-nitroso compounds. In the second experimental study, meat colour formation and stability of the same product categories manufactured industrially were studied. Next to that, the effect of lowering the amount of nitrites on the growth microorganisms, and more directly *Listeria monocytogenes* was studied.

The results of the literature study are presented in chapter 2, whereas the experimental studies are discussed in chapter 3. In chapter 4 general conclusions and implications of the studies are given.

2 Summary of the literature study

In the literature survey (Stegeman *et al.*, 2005), an overview of the functions of nitrite is given. Next to that, possible organic (and conventional), alternatives to reduce or replace nitrite are summarized.
The main functions of nitrite are food safety, colour formation and stability, inhibition of lipid oxidation and flavour formation. In the following paragraphs, these functions are discussed successively.

2.1 Food Safety

Since ages, nitrite is used in the production of meat products as a preservative. The actual mechanism behind this function of nitrites has been studied intensively in the previous century. However, this mechanism is still not unravelled completely. In general, it is agreed that the antibotulinum properties of nitrite is affected by multiple factors. In particular, factors like salt level, pH, intensity of the heat treatment, initial load of spores and presence of other ingredients like phosphates and ascorbates determine the activity of nitrite against *C. botulinum*. The most accepted theory is that nitrite (NO) forms a complex with the iron in the heam group of myoglobin. This iron is also required for spores to germinate. Thus, outgrow of *C. botulinum* now is prevented since all iron is captured in the complex. This also explains why nitrites are less effective in products containing high levels of heam-iron like products prepared with much liver, spleen, kidneys and/or blood.
The required level of nitrites depends on several parameters: product composition, processing (including ingredients, handling, packaging, etc.), and storage conditions. Meat products that have been sterilised at 121°C during at least 3 minutes (botulinum cook) do not require nitrites for safety reason. Based on literature data, for meat products subjected to less severe heat treatments (F_0[1] = 0.1 – 1.5 minute) and stored at ambient temperature minimum ingoing nitrite amounts of 100 mg $NaNO_2$/kg meat are advised. Product which are pasteurised and stored at ambient temperatures, a minimum ingoing nitrite amount of 100 mg $NaNO_2$/kg meat is also advised. For pasteurised meat products stored under refrigerated conditions, the need for nitrite addition for its microbiological purposes is less unambiguously. The required amount depends on several factors, particularly the water activity and pH of the final product. For products with relative high water activity values (>0.970), the presence of nitrite may give extra safeguard, especially for longer storage periods (3 weeks to 3 month). A minimal ingoing nitrite contents of 60 mg/kg meat expressed as $NaNO_2$ is advised. Again, this amount may be changed depending on the final pH of the product. More generally, ingoing amounts of 100 – 150 ppm were given by many authors as a rule of thumb to create safe products to be stored at ambient temperature. This amount may be reduced if nitrite is applied in combination with other conservation ingredients. Examples of ingredients with an antibacterial function that may be applied in organic products are lactic acid and their sodium, potassium- or calcium salts, malic acid, ascorbic acid, citric acid

[1] *F_0 is the total sterilization time at 121 °C. At F_0 = 3.0 minutes a reduction in C. botulinum with a factor of 10^{12} is achieved.*

and (extract of) organic herbs and spices, like oregano, cinnamon, garlic, clove, thyme. Of those additives, lactates are most frequently reported to be able to enhance shelf life. Although sometimes a positive effect is reported on *Clostridium* prevalence, it is concluded from literature that still a small amount of nitrite should be present to prevent outgrow of *C. botulinum* if the a_w is higher than 0,970 and if the products are stored at ambient temperature. Lactates are mainly used to decrease water activity, therewith reducing growth of microorganisms.
Many other preservatives have been studied to replace nitrites, like sorbic acids, sorbates, EDTA, and phosphates, with promising results (although at much higher concentrations than nitrite). These ingredients, though, are due to legislation, not allowed in organic meat products.

2.2 Colour formation and stability

The pigment responsible for the specific pink cured colour of meat products is a ferrous (Fe^{++}) complex of myoglobin containing nitric oxide (from nitrite); nitrosylmyoglobin (not heated) or the denatured stable form after cooking, nitrosylhemachrome. These pigments are formed by several reaction steps. When even a small amount of nitrite is available (1-2 ppm) a slight pink colour will appear after heating. However, nitrosylhemachrome may fade under the influence of UV light or bacterial spoilage, giving a gray-brown reaction product. Because residual nitrite contents is affected by different factors, like raw materials and additives, possible oxidation reactions, heating process, and storage conditions, in literature different amounts of ingoing nitrite are suggested, ranging from 50 to 100 mg/kg meat, to stabilize the nitrosylhemachrome complex, and thus the cured pink colour.

Next to nitrites, other additives can be used to obtain a pink colour in cooked meat products. These products must be divided in two categories:
- Vegetables, spices and herbs that contain nitrate, like leek and celery;
- Products that have a red colour or that enhance the red colour of meat.

In the first category, the nitrate level of the vegetables is the important factor. Nitrate must be reduced towards nitrite. This reduction step is mostly carried out by increased temperature and adding nitrate reducing microorganisms.
Moreover, nitrite still will be present in the product after stimulated reduction of nitrate by microorganisms.
The controllability of the process, however, becomes more precarious:
- Often, vegetables with high nitrate levels are added to the raw ingredients and in a successive step nitrate is reduced to nitrite. The extra reduction step requires a temperature increase. During this step, extra microorganism may grow. Although the meat product will be pasteurised after this, it is not mentioned in open literature whether or not possible toxin formation during the reduction step may taken place.
- An alternative procedure is performing the reduction step separately. The vegetables are reduced by microorganisms to form nitrite before they are added to the meat raw material.

During this extra step, which focuses on the conversion of nitrates in nitrites, possible other undesired reactions may take place.
- The concentration of nitrate in these additives may change over time, in particularly in organically grown ingredients. Therefore, too, the final amount of nitrite in the product may vary, and eventually it may exceed the amount of nitrite ordinarily used.

In the second category, fermented rice (Angak), beetroot juice, or other organically produced red colouring agents are suggested. Next to that, non-organic additives like commercial colouring additives and synthesized preformed cooked cured meat pigments have been reported in literature. The latter mentioned ingredients are not allowed in organic products. Furthermore, it is questionable whether the organic additives may be applied, since they merely have a cosmetic function and are no real functional additive or processing aid.
In addition, vitamin E (α-tocopherol) is mentioned either as a feed ingredient in animal production or as an additive in meat products to slow down oxidation and enhance redness, enabling the use of a reduced amount of nitrite in the final product. Whereas the addition of vitamin E to organic meat products is not allowed, it is also debatable whether it is allowed as a feed additive to organically grown animals if they are merely added to stabilize the colour of the final meat product.

2.3 Inhibition of lipid oxidation and flavour formation

Nitrite holds great anti-oxidative properties, inhibiting lipid oxidation. In the form of nitric oxide, transition metals (e.g., iron in myoglobin) are bound. This way, nitrite prevents these metals from their pro-oxidative function and auto-oxidation of lipids in meat is hindered. Next to this, nitrite may react with the double bounds in fatty acids, and thus stabilising these fatty acids. A third theory states that not the nitrite ion, but the nitroso and nitrosyl compounds formed in meat are responsible for the anti-oxidative function.
Though nitrite is a very strong antioxidant, alternative ingredients, organically grown, like rosemary, green tea, sage, and grapes may be used to take care of the anti-oxidative function.

The reason for the presence of the cured flavour is not clear at the moment. Often it is stated that the well-known flavour of cured products in fact is the base flavour of fresh, non-oxidized meat. Inhibition of lipid oxidation, for example, prevents the occurrence of off flavours like rancidity and warmed over flavour. From many studies, however, it is shown that alternative additives may be used to inhibit lipid oxidation. However, the produced meat products are lacking the characteristic cured flavour.
The amounts of nitrites, though, may be reduced severely, still ending up with the cured flavour. If the meat product is kept in an oxygen free environment (oxygen free packaging), probably 10 ppm nitrite will be sufficient to obtain the cured flavour. Under practical conditions, it is advised to use at least 40 mg $NaNO_2$ per kg meat in the production of meat products for obtaining the characteristic flavour.

2.4 Conclusion

From the literature survey, it is concluded that no single ingredient[2] is known to date that can replace nitrite in realizing all of its functions (antimicrobial, anti-oxidative, colour and flavour formation and stabilisation). Nitrite is a very effective preservative and anti-botulinal agent. For several products, good manufacturing practice and introducing extra hurdles like low water activity and low pH may be sufficient to end up with a safe product without nitrite that can be stored at low temperature for 2 or 3 month as has been shown by Stekelenburg and Hoornstra (2002) in their microbiological risk analysis. Compared to products made with nitrites, this, however, may have a great effect on product quality and supply chain requirements[3]. Some other products (e.g., pasteurised cold meats with a_w>0.97, or sausages with low a_w and pH, but stored at ambient temperatures for a longer period) probably still require nitrite, although in smaller amounts than presently used.

Nitrite probably is the only (legal accepted) component that brings about the typical pink colour and cured flavour in pasteurised meat products. Alternatives - like fermented red rice - are known that may also induce a red colour of the meat product. However, these products only may be added if they introduce extra functionality in the organic product. They should be abandoned if they are only used to change product's appearance. Several anti-oxidants are known, that probably may be used to substitute nitrite in realizing nitrite's anti-oxidant function. According to some literature references, the desired flavour will also be present if other anti-oxidants are used, since the flavour of cured meat products, stems from the flavour of meat that is not oxidized. Other researchers, however, state that although the mechanism behind the creation of the cured flavour is not known yet, it only will be present if nitrite is used.

In general, it can be concluded that simply abandoning nitrite in the production of organic meat products is not possible without changes in appearance and controlling product safety. Several alternative ingredients may be used to take care of part of the functions of nitrite. Depending on product characteristics (especially shelf life and product-colour), attention must be paid to processing conditions and temperature conditions in the supply chain, before lowering the amount of nitrite to a certain extend. The exact extent should be determined case by case.

[2] Nitrate and its reduced form left out of consideration

[3] Stekelenburg and Hoornstra have performed a microbiological risk analysis; effects of abandoning nitrites on colour and taste as well as on practical applicability were not included in the study

3 Summary of the experimental studies

In the present programme, experimental studies have been performed on two scales. The first experimental study, see Stegeman (2006), has been performed at a pilot scale. In this study the effects of lowering the amount of nitrites (to about 80-90 ppm, 40-50 ppm and 0 ppm) on the colour (stability), on the micro-organisms growth by bacterial plate counts, and on the growth of *Clostridium botulinum* and toxin formation by means of challenge tests has been studied in organic Bologna type sausage[4] and organic cured ham.

In the second experimental study, Bologna type sausage and cured ham products, both organic, with regular and reduced nitrite content (about 160, 80, and 40 ppm) have been produced by industrial partners and successively their colour stability has been followed. Next to that, challenge test with *Listeria monocytogenes* have been carried out on these products.

3.1 Experiments on pilot scale

Organic cured ham and Bologna type sausages were prepared with three levels of ingoing sodium nitrite, respectively 0, 41, and 82 mg/kg, and 0, 59, and 95 mg/kg, for the Bologna type sausage and the ham product. A generally applied product flow of 5 weeks at 4 °C before slicing, modified atmosphere packaging of the sliced product, and retail storage for 5 weeks at 7 °C with an excessive light source of about 880 lux (12 hours per day; to simulate a worse case scenario[5]) only the last week was simulated. At several moments in time analyses of colour, residual nitrite content, and microbiological condition were performed.

The results of the residual nitrite analyses (see Table 1), showed that nitrite concentrations declined sharply after production for both type of products to about 15 – 25% of the ingoing level and steadily declined further during storage time to a final amount of 2.5 – 6 ppm. In many presently produced organic meat products with ingoing nitrite amounts of about 120 -160 ppm these values range from 40 to 80 ppm. No significant difference is found between the two different ingoing levels.

Table 1 Analyses of nitrite content in organic Bologna type sausage and cured ham during storage.

Nitrite content [mg/kg]	Bologna type sausage			Cured ham		
Ingoing nitrite	0	41	82	0	59	94
After 1 week storage at 4 °C	0	6.1	6.4	0	16.7	24.7
At slicing, after 5 weeks storage at 4 °C	0	4.3	3.5	0	12.1	16.3
4 Weeks after slicing and MAP packaging, storage at 7 °C	0	2	2.3	0	4.9	7.1
5th Week after slicing, last week at 880 lux, storage at 7 °C	0	2.5	5.6	0	5.7	4

Analyses of the plate counts showed that nitrite can be left out without a significant growth of mesophylic bacteria if products are handled hygienically during processing.

[4] *the Dutch type of sausage called "Ardenner Boterhamworst"*

[5] *Ordinarily, light sources used in retail cabinets are 400 – 500 lux, where the top package blocks the light for the other packages.*

The visual evaluations of the colour of both products with nitrite were acceptable compared to the one without nitrite. Nevertheless, the Bologna type sausage prepared with the lowest amount of nitrite already showed severe fading at the end of the storage period (see next figures). In the picture of the product at the end, the top slice is partly folded back, to show the difference in decolourization of top slice compared to the interior that has been covered with other slices.

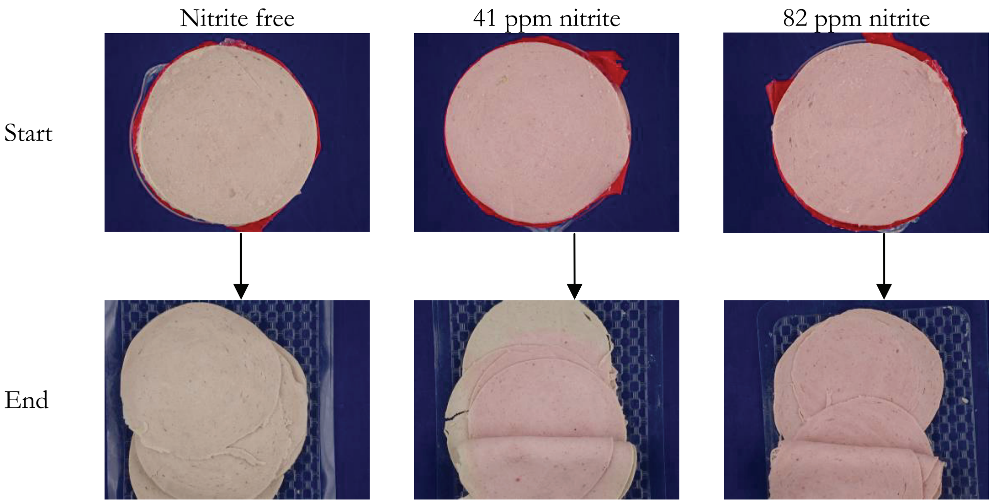

Figure 1 Photographic presentation of colour development and change in Bologna type sausage during storage

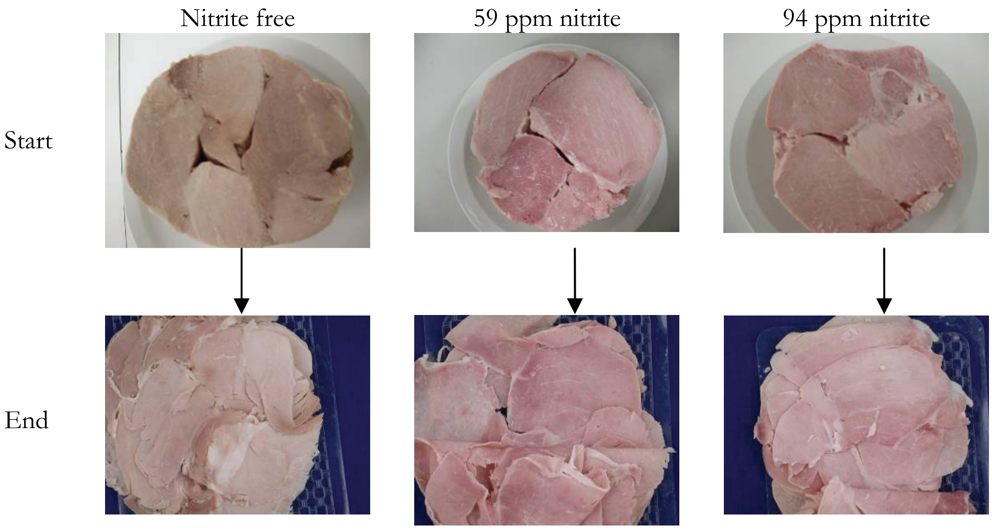

Figure 2 Photographic presentation of colour development and change in ham during storage.

Colour measurements during bulk storage, slicing, and presentation in a chilled cabinet were performed by using the CIElab method. Measured a*- values (red values), including the error bars of the two product types are given in the following figures. Error bars for the ham products are obviously rather high as they are attributed to the inherent heterogeneity of the product.

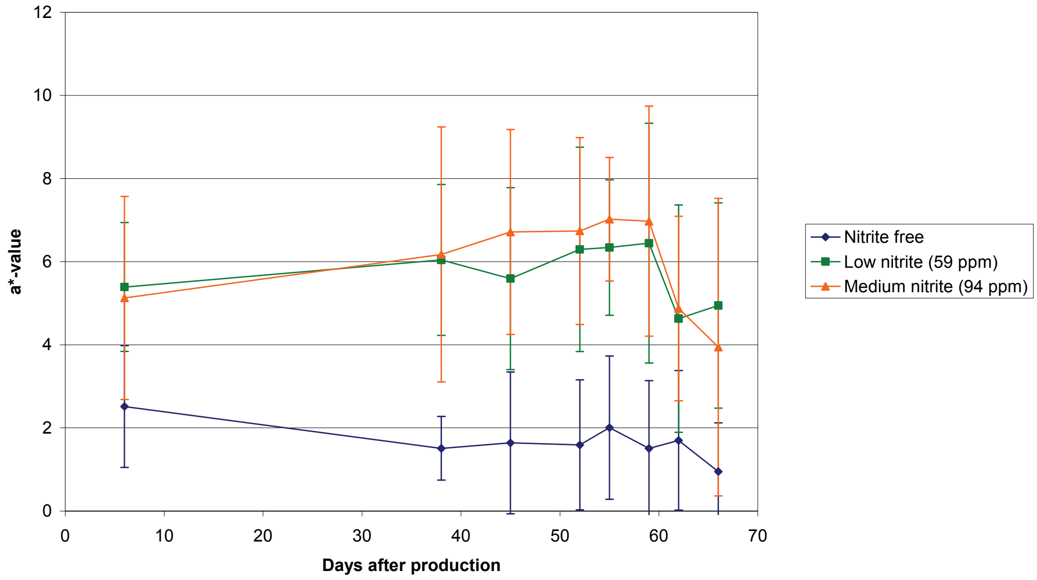

Figure 3 Red colour change during storage for the ham product.

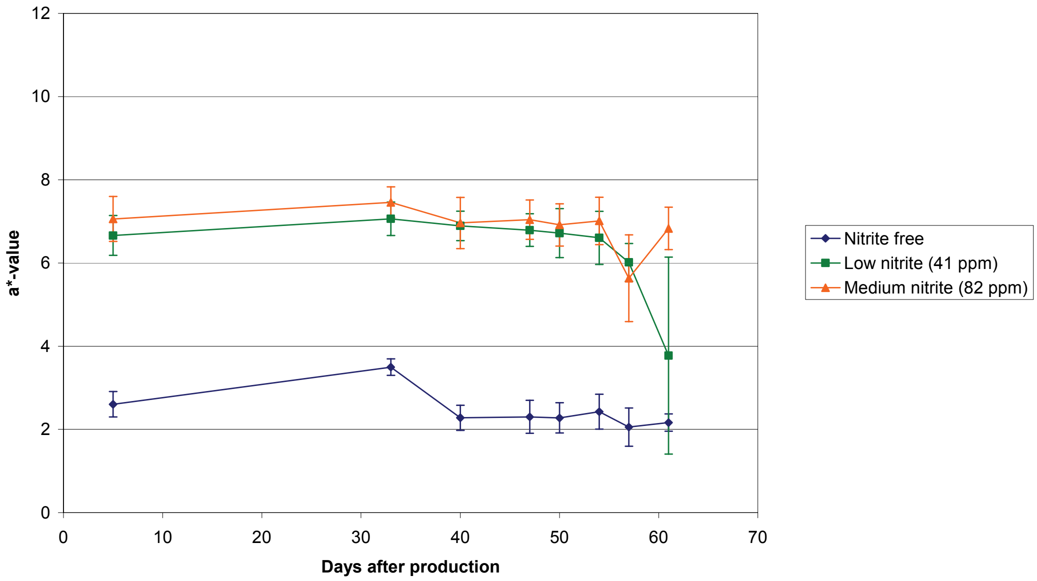

Figure 4 Red colour change during storage for the Bologna type sausage.

The experiments showed that the colour of products produced without nitrite already were grey immediately after production, and even became greyer during storage. Applying the two reduced levels of ingoing nitrite led to the desired cured colour for the Bologna type sausage as well as for the ham product. During storage and illumination, the samples' red colour decreased gradually. For part of the samples, colour faded more rapidly, attributed to a not optimally packaged product, enabling oxygen to enter the package. In general, the colour stability of most samples was judged as satisfactory, in particular if one takes into account that the applied illumination was about twice at high and over a much longer period than sliced meat products normally are subjected to in a retail display. No significant difference between the two levels of nitrite was observed.

For the purpose of the challenge tests with *C. botulinum* spores, two organic Bologna type sausage versions were made and pasteurized (P_{70} = 55 min), each with three different levels of ingoing nitrite: 0, 54, and 108 mg/kg respectively. One recipe was a lean version, 10% fat and an a_w value of 0.971 - 0.975, the other recipe was a higher fat version, 33% fat and an a_w value of 0.968 - 0.973. Both recipes were inoculated with *C. botulinum* at a dosage of 100 spores per gram product. The tests showed growth after 4 weeks in the lean Bologna type sausage with a higher a_w (0.975), no nitrite added and stored at 15 °C, see Figure 5. After 6 weeks also toxin was formed. After 12 weeks of storage at 15 °C, in the other products also growth of *C. botulinum* was established. When stored at 7 °C and 10 °C no growth of *C. botulinum* was measured during the 12 weeks of storage. Therefore it can be concluded that under the practical conditions as been carried out in this study (cold storage at temperatures below 7 °C, low a_w- values, production and handling under hygienic conditions), the risk of development of *C. botulinum* in pasteurised meat products produced without nitrites is very small.

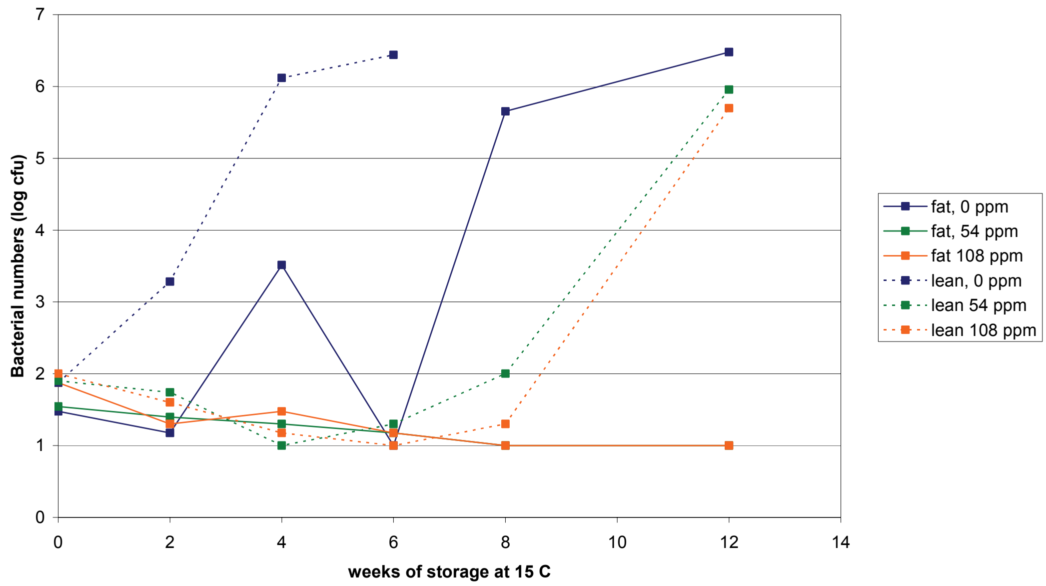

Figure 5 Growth of *C. botulinum* in Bologna type sausage, stored at 15°C

3.1.1 Conclusion of the experiments on pilot scale

Reducing the amount of ingoing nitrite at the production of organic ham and Bologna type sausage is possible. It may result in a very small effect on colour stability. By a reduction of the amount of nitrite to an extend of approximately 80 mg/kg ingoing, the colour forming and stability will be sufficient to reach a shelf life of 65 days. During production, heating, cooling, and

storage, the amount of residual level nitrite will be far less than the 50 mg/kg as mentioned in EU Directive 2092/91.
A further reduction of the ingoing amount of nitrite to 59 ppm in the ham product also showed to be feasible without causing a negative effect on the product quality.
The reduction of the ingoing amount of nitrite in the Bologna type sausage to a level of 40 ppm leads to a more pronounced effect on the colour stability. However, under practical conditions, it is expected that the effect will be less pronounced and the product quality still may be judged as acceptable.
Completely abandoning nitrite in the processing of organic meat products is not advisable. This will result in serious colour instability of the product compared to conventional products and for this point of view, the product does not look as consumers still expect. Furthermore, the risk of botulinum toxin formation will increase at higher storage temperatures (> 10 °C).

From the pilot experiments, it was concluded that the amount of nitrite in organic ham and Bologna type sausages could be reduced to 80 ppm, and probably to 40-50 ppm without negatively affecting the colour development and stability, as long as the meat products are manufactured and handled hygienically and stored under refrigerated conditions. To underpin the conclusions of the pilot experiments, successive experiments have been performed in standard industrial setting.

3.2 Industrial test

The organic Bologna type sausages and cured cooked hams were manufactured at the production plants of two Dutch commercial meat processing companies, applying their conventional organic production processes as much as possible, only changing the amounts of nitrite used in the process. The ingoing amounts of nitrite were 158, 79 and 40 mg/kg for the Bologna type of sausage, and 157, 80 and 40 mg/kg for the cured cooked ham. After production, the Bologna type sausages were conditioned for 5 days at -2 °C, followed by a 7-day period at 0-2 °C, whereas the cooked hams were conditioned for 5 days at 0-2 °C after production. After conditioning, all products were hygienically sliced and packaged under modified atmosphere. Part of the sliced products was subjected to challenge tests with *Listeria monocytogenes*, while shelf life experiments were carried out with the rest.
For the shelf life test of the sliced Bologna type sausage a generally applied product flow of 5 days at 2 °C and 19 days at 7 °C, retail illuminated with a light source of 450 lux was simulated according to a given time schedule only the last week. Sliced ham products have been stored for 28 days at 7 °C, also applying the same illumination during the last week according to a given time schedule. For the challenge tests, all meat products were inoculated with a cocktail of three types of *Listeria monocytogenes*: type 1/2a (ATCC 35152), type 4a (ATCC 19114) and type 4b (ATCC13932) to a final level of about 1000 bacteria per g product.

Analyses of the residual nitrite content showed that after slicing about 10 to 15% of the ingoing nitrite was still present, while only a very small amount of the ingoing nitrite was still present at the end of the storage period.
Pictures from the two types of products made at the start and end of the experiment are given in Figures 6 and 7 (at the end of the storage period, the top slices have been shifted to compare the colour of slices, subjected to light with the colour of covered slices). Visual observations as illustrated by the pictures show no colour change during the entire storage time in all ham products and in the Bologna type sausage produced with the regular and partly reduced nitrite concentration. Only the colour of the Bologna type sausage produced with 40 ppm nitrite has faded slightly. That product started to become brownish after being exposed to light for about 8 hours. The colour change was more pronounced after 16 hours. After that time, the colour is still acceptable, but the colour change may be noticed by consumers, if they compare the product with products that are not or only for a shorter time being exposed to light.

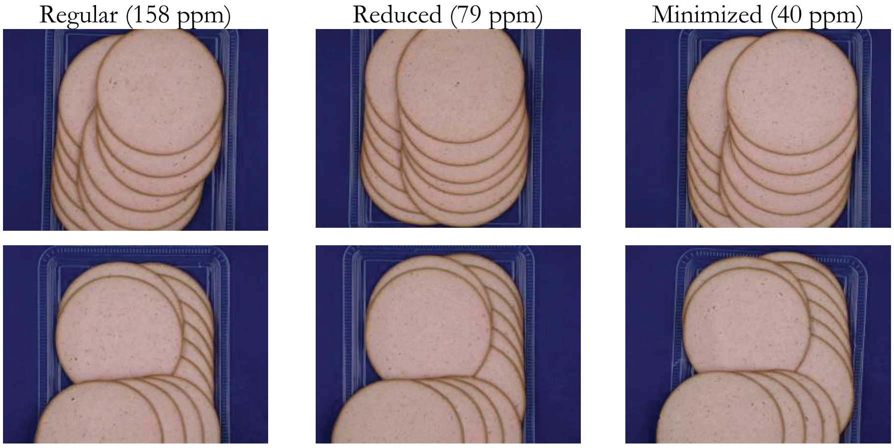

Figure 6 Representation of the colour of Bologna type sausage produced with different amounts of nitrites directly after slicing and at the end of the storage period.

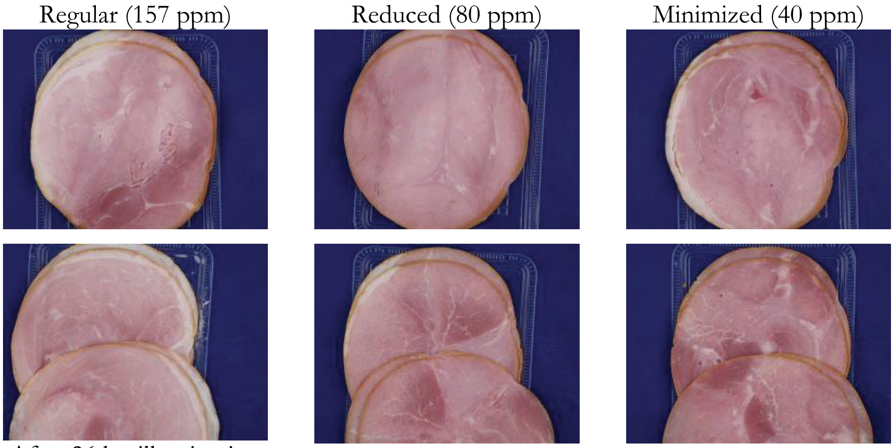

Figure 7 Representation of the colour of ham with different amounts of nitrites directly after slicing and at the end of the storage period.

More objectively, these results are shown by the changes in L*, a*, and b*-values over time. No significant changes were observed for all ham products and the Bologna type sausage prepared with the higher two nitrite concentrations. A decrease in a*-values (a reduction in magenta), and an increase in b*-values (an increase in yellow) was observed for the Bologna type sausage product produced with 40 ppm nitrite, see Figures 8 and 9 (L* and b*-values not shown). For the sliced ham, again, large error bars were given, caused by the natural heterogeneity of this product.

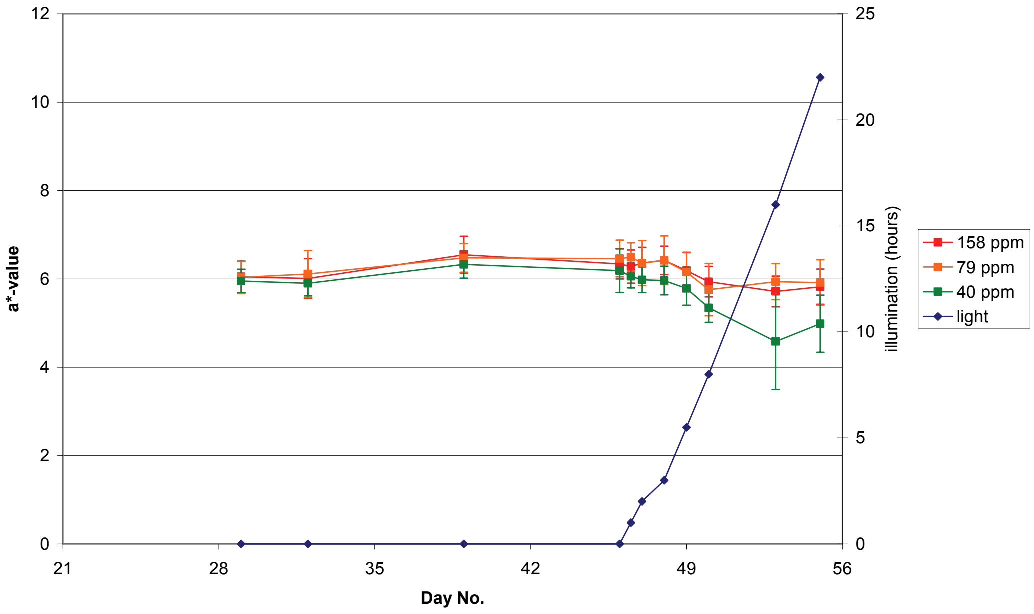

Figure 8 Red Colour change during storage for the Bologna type sausage.

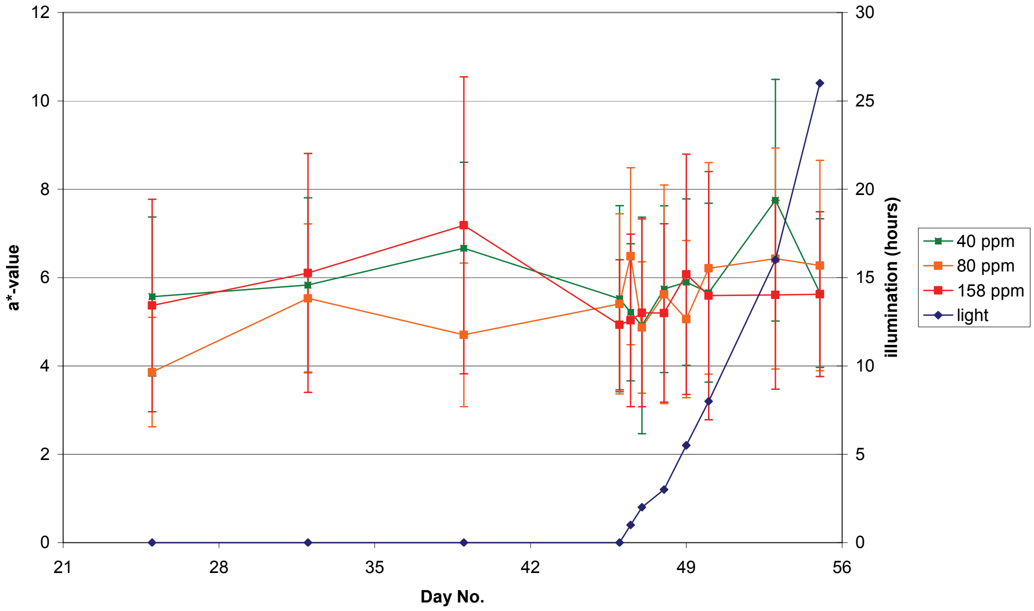

Figure 9 Red colour change during storage for the ham product.

L. monocytogenes inoculated in Bologna type sausage with 158 or 79 mg/kg nitrite did not increase during the entire storage period of 32 days at 7 °C, see Figure 10. In the Bologna type sausage with 40 mg/kg nitrite a rather small increase of *L. monocytogenes* by a factor 5 (0.7 log units) was seen during mentioned storage period. The aerobic plate counts in all sausages, mainly consisting of lactic acid bacteria, increased to a level of 10^7 cfu per g in 3 to 4 weeks. The pH of all Bologna type sausages was normal and remained constant during the storage period.

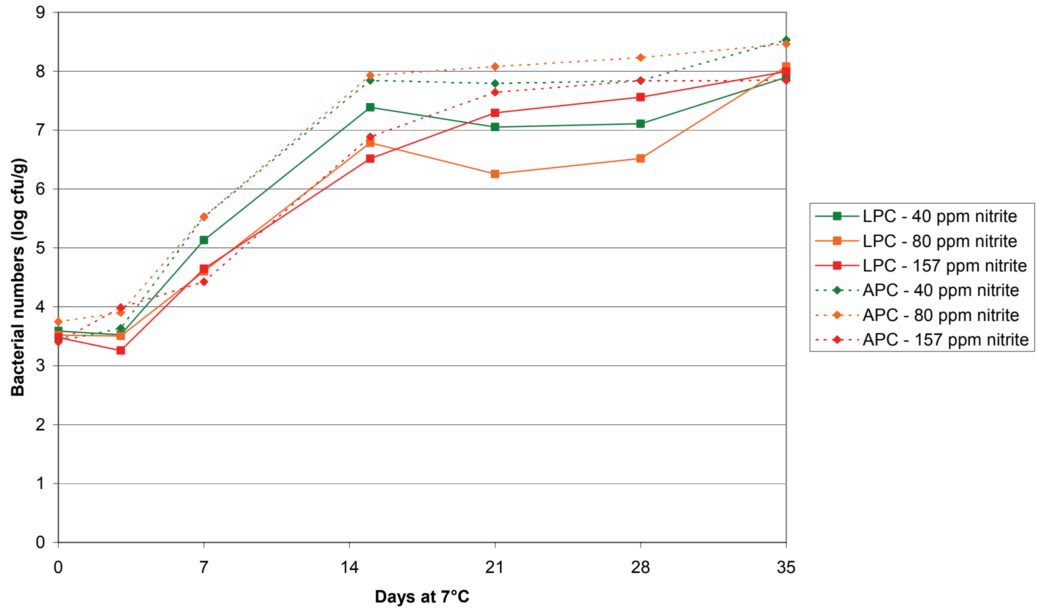

Figure 10 Development of *L. monocytogenes* (LPC) and aerobic bacteria (APC) on vacuum-packed Bologna type sausage with different nitrite contents, stored at 7 °C.

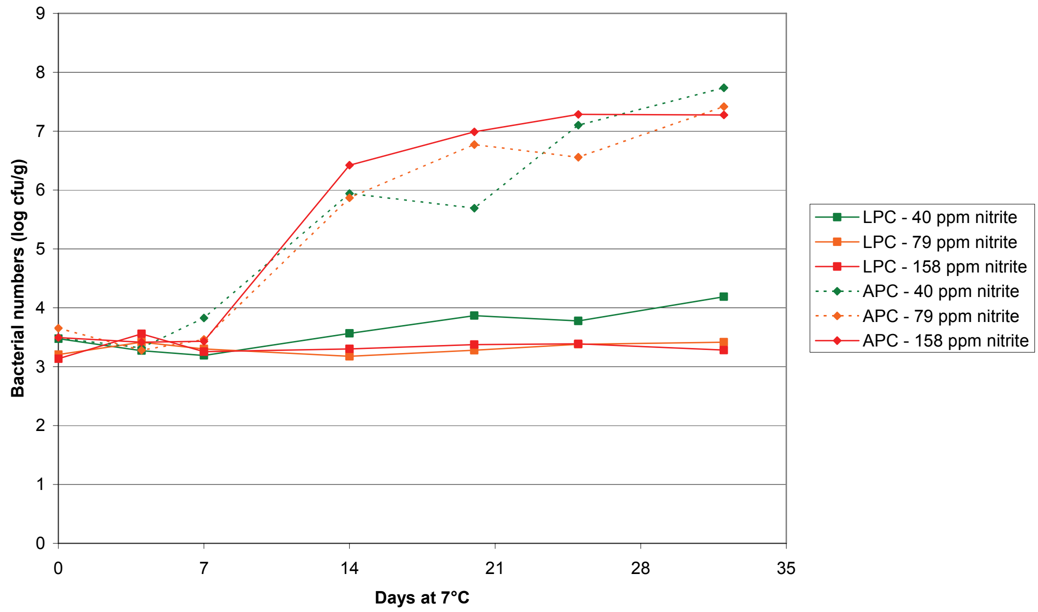

Figure 11 Development of *L. monocytogenes* (LPC) and aerobic bacteria (APC) on vacuum-packed ham with different nitrite contents, stored at 7 °C.

In all ham products with ingoing nitrite levels of 157, 80 or 40 mg/kg the inoculated *L. monocytogenes* bacteria increased by a factor 100 (2 log units) in circa 1.5 weeks storage at 7 °C, see Figure 11. Also, the aerobic plate counts, mainly consisting of lactic acid bacteria, did increase rather fast in all ham products, exceeding a generally accepted maximum level of 10^7 (log 7) cfu per g within 2 weeks 7°C. Differences in growth rates for both bacteria between hams with standard, medium or low nitrite contents were not significant. The relative rapid growth of both *L. monocytogenes* and lactic acid bacteria can be attributed to the rather high, but quite common, water activity (a_w value) of the ham products (i.e., about 0.973 in ham vs. 0.965 in the Bologna type sausage). Furthermore, the presence of lactate in the Bologna products is known to have anti-listerial activity. In addition, the pH of all ham products was quite common in the initial products and showed some decrease at the end of the storage period because of microbial metabolism.

3.2.1 Conclusions of the industrial scale experiments

From the colour experiments, it is concluded that the same colour is developed in the organic ham and Bologna type sausage products with a reduced ingoing nitrite content of 40 and about 80 ppm compared to products with an ingoing nitrite content of about 160 ppm. During the entire storage period of approximately 4 weeks at 7 °C the cured colour did not fade in the ham products prepared with 40 to 157 ppm nitrite, where the products were subjected to light of about 450 lux during 26 hours in the last period of the storage time.

For the Bologna type sausage products with 79 and 158 ppm ingoing nitrite, also colour was stable during the storage period of 22 days with illumination during 22 hours. The products prepared with 40 ppm nitrite, however, showed reduced colour stability, and started to become a bit brownish after about 8-16 hours illumination with 450 lux.

The duration of the illumination periods were rather extreme. In retail, packages ordinarily are covered by other packages, and only the top package is exposed to light. Therefore, it is concluded that during normal logistic and retail conditions the colour of organic Bologna type sausage and cooked ham will be stable if ingoing nitrite concentrations of 80 mg per kg product are applied.

From the challenge test with ham and Bologna type sausages with standard, medium and low nitrite levels it can be conclude that *L. monocytogenes* as well as lactic acid bacteria are not particularly sensitive to nitrite. Differences in growth rate of these bacteria in both products with different nitrite contents were small.

For meat products with high water activity ($a_w \geq 0.970$) and/or absence of lactate, *L. monocytogenes* can only be controlled by prevention of contamination due to stringent hygiene during production.

3.3 General conclusion of the experimental studies

It can be concluded that, under practical conditions (production and handling under hygienic conditions, cold storage at temperatures below 7°C and a_w in accordance to the shelf life) nitrite content in organic cooked cured ham and Bologna type of sausage might be reduced to 40 ppm ingoing amount. In all products produced with al least 40 ppm nitrite, the same colour was developed, regardless the amount of ingoing nitrite. Some products produced with only 40 ppm nitrite can lose their cured colour somewhat earlier when exposed to an extended light source. Good logistics and handling practice can prevent this discoloration.

A reduction of the amount of ingoing nitrite directly leads to a reduction of the residual nitrite content. Only a fraction (10-25%) of the ingoing nitrite will be still be present in the meat product after cooking and slicing. In the period between slicing and consumption, this level will decrease even further.

From the pilot study it can be concluded that under the practical conditions as been carried out in the pilot study (cold storage at temperatures below 7 °C, low a_w- values, production and handling under hygienic conditions), the risk of development of *C botulinum* in the concerning pasteurised meat products produced without nitrites is very small. However, if products, especially products with higher water activity values (e.g., 0.975), are stored at higher temperature (T> 10 °C) *C. botulinum* spores may germinate and toxins may be formed during storage periods of several weeks.

From the challenge test with ham and Bologna type sausages with standard, medium and low nitrite levels it can be conclude that *L. monocytogenes* as well as lactic acid bacteria are not particularly sensitive to nitrite.

According to the latest developments, a maximum value of 80 ppm ingoing nitrite will be set by EU legislation for organic meat products, while the residual nitrite content should be lower than 50 ppm. Based on the present study a stable colour is expected for ham and Bologna type sausages produced in compliance with the latest EU regulation EC 2092/91 according the recipes used in this study.

4 General conclusions and implications of the studies for organic meat products

From the literature and the experimental study, it is concluded that it is not possible to substitute nitrite in the production of organic cooked cured meat products with a single additive or combination of additives without changing the quality of the meat products. Nitrite is a typical substance responsible for the specific cured colour and aroma and for the microbiological and chemical stability. Nitrite is unique in its capability to prevent *C.botulinum* spores to germinate and form poisonous toxins, especially during storage of products at ambient temperatures.

From the studies with the Bologna type sausage and ham products, it nevertheless is concluded that the level of nitrite used in the process may be reduced to 80 mg $NaNO_2$ per kg meat product without negatively affecting the quality of the two product categories. In the model products, the (residual) nitrite level already shrinks to about 10-25% of the originally (ingoing) amount of nitrite during production of the meat product. A reduction of the level of nitrite used in the process towards 40 mg/kg also seems possible; however, with this amount the products are more likely to be susceptible to external factors, like light and temperature abuse during storage.

Completely excluding nitrite in the production of organic meat products like Bologna type sausage and cooked ham is not advisable for several reasons:

1. Nitrite serves as an extra safeguard to prevent *Clostridium* growth and toxin formation, in case of any fault that may occur in the complete production and supply chain, like contamination in the raw material or production process, deviations in used recipe, temperature abuse in the chain, temperature abuse at the consumer;
2. Lowering the ingoing amount of nitrites resulted in reduced residual nitrite levels, respectively to less than 20 mg/kg product just after slicing, and less than 10 mg/kg at the end of the storage period.
3. Food safety probably will not be guaranteed for products manufactured artisanal;
   Especially in artisanal manufacturing processes handling and production may give rise to higher risk of contamination or faults in the production process;
4. A ban on (sodium) nitrite will not lead to nitrite-free products in all instances.
   Some producers will still use (organic) nitrate containing additives that do not have to be declared as such (E250 or E252), although nitrite still will be present in the final product and the amount of "ingoing" nitrite is not known.
5. Organic meat products prepared without nitrite will not have the recognizable cured pink colour like the non-organic meat products. It is expected that this will lead to an extra marketing effort to convince consumers to buy these in colour aberrant grey products.

4.1 Implications of the study for other cold cuts

In the present framework only two types of cold cuts have been studied experimentally, i.e., organic Bologna type sausage and organic cured ham. Therefore, the given results are only verified for these two products. In this paragraph, implications for other products are hypothesised. Moreover, these implications should be handled with care, and the actual needs for nitrite should be studied case by case to be sure to end up with food safe products.

4.1.1 *Pasteurised cured organic meat products*

It is assumed that products made from other muscle tissue than pork, except beef, are comparable to the studied meat products. Therefore, generally 80 ppm ingoing nitrite may be sufficient as long as the water activity is lower than 0.970 and products are stored under 7 °C for probably not much longer than a three weeks period. Pasteurised cured meat products with a higher water activity or stored at higher temperatures will require higher amounts of nitrite.
To obtain colour stability comparable to the products experimentally studied, ingoing nitrite concentrations may have to be increased for products manufactured from raw material containing high levels of heam iron, like liver, blood and spleen. The amount of heam iron may play a role since iron may bind nitrite, disabling its functionality.
Also for beef products (having about twice as high heam iron than pork products), it might be advantageous to adapt the nitrite content to obtain the desired food safety.

4.1.2 *Sterilised meat products*

These products are heated for a prolonged period and stored at ambient temperature. For products that have undergone a complete botulinum cook (F_0 >3.0) no nitrite is required as an extra safeguard for preventing Clostridium botulinum growth. However, colour forming, oxidative stability, and flavour need to be taken care of if nitrite is left out.
For products heated less severe ($F_0 \leq 3.0$), extra safety risks are introduced if these products are stored at ambient temperature. These products still will require addition of a higher amount of nitrite; the more severe subjected to a heating process, the lower the amount of rest nitrite will become.

4.1.3 *Non heat treated cured dried products*

In products like dry fermented sausages and dry cured smoked meat products, nitrites[6] are required to obtain the pink colour; the pink colour is caused by a bound of NO to the porphyrin complex[7]. Nitrites, moreover, may also be required for safety reasons. For these products, the initial phase, when water activity and pH are still rather high, is the most critical phase in which pathogenic bacteria may develop. After this period, the low pH and/or low water activity in most of the products will prevent bacteria to develop. Experimental studies are necessary to determine what amount of nitrite is actually required for these product types and what the implications of a lower ingoing nitrite content will be. Also the effect of a high a_w and a higher pH on the development of groups of microorganism can be subject to this study.

[6] *Supplied in the form of nitrites or nitrates*

[7] *In some dry ham products that are aged for a very long period (18 – 30 month) like Parma and Iberian hams a rosy-red colour is also developed without making use of nitrites or nitrates. The colour is caused by a Zinc porphyrin complex. The development of this pigment is very slow and aging times over 12 month are required.*

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