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

Experiments on industrial scale

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Abstract

In this study, cooked organic cured ham products and Bologna type sausages have been produced in an industrial setting with regular and two reduced amounts of nitrite. Nitrite levels in the recipe have been reduced by a factor two and four. The residual nitrite analysis showed that nitrite concentrations declined sharply after production for both type of products to about 10 – 15% of the ingoing level and further declined during storage time of about 7 weeks (of which 3-4 weeks as sliced packaged product) to a final amount in the range of the detection limit of 2 ppm. No significant difference is found between the different ingoing levels.

Applying the two reduced levels of ingoing nitrite still gave the desired cured colour for Bologna type sausage as well as for ham after cooking. During 30 days storage at 7 °C of the sliced and packaged product and 26 hours illumination at retail condition, the colour of all ham samples did not fade. For the Bologna type sausage no colour fading occurred during in the slices products during a 25 day period storage and 22 hour illumination in the products prepared with 158 and 79 ppm ingoing nitrite, while a small colour change occurred for the samples prepared with 40 ppm nitrite after being exposed to light for more than 8 hours.

Challenge tests with *L. monocytogenes* were carried out on the prepared products. Both recipes were inoculated with a cocktail of three types of *L. monocytogenes* at a dosage of 1000 bacteria per gram product. The tests showed no increase in growth of the Listeria bacteria on the Bologna type sausage products prepared with 178 and 79 ppm nitrite during the storage period of 32 days at 7 °C, while a rather small increase by a factor 5 was seen during mentioned storage period in the Bologna type sausage prepared with 40 ppm nitrite. In all ham products the number of *L. monocytogenes* bacteria increased by 2 log units in circa 1.5 weeks storage at 7 °C. This dissimilarity is probably due to a difference in water activity between the ham and the bologna type sausage products, i.e. about 0.965 vs. 0.973.

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 can be reduced to 40 ppm ingoing amount. Some product can lose their cured colour somewhat earlier when exposed to an extended light source. Good logistics and handling practice can prevent this discoloration. According to the latest developments a maximum value of 80 ppm ingoing nitrite will be set by EU legislation for organic meat products. Based on the present study a stable colour is expected for ham and Bologna type of sausage produced in compliance with the latest EU regulation EC 2092/91 according the recipes used in this study.

Content

1 Introduction

In the production of (organic) meat products like cold meats, nitrites (and nitrates) are used for several reasons: for forming and stabilizing the red, cured meat colour, and for the antimicrobial and anti-oxidative properties. The use of nitrite in organic products, however, is very much subject for debate, as is subjected to legislation on this subject. Being an additional non organic component, nitrite, in principle, is a non desired component. However, for safety reasons, the use of nitrites is (temporarily) allowed. According to the latest European legislation (annex to regulation 2092/91), a residual nitrite concentration of 50 mg per kg meat product is allowed, while no more than 80 mg nitrite per kg meat product may be added initially during the production process. A final regulation will be set up after reconsideration of the present regulation at the end of 2007.

To give input to the present discussion, a program on the reduction of the use of nitrite in the preparation of organic meat products has been set up. In this framework, a literature review and a pilot study on this subject already have been performed in 2005 (see A&F report 560, 2005 and A&F report 658, 2006).

In the present study the effects of lowering the amount of nitrites in sliced Bologna type sausages and in cured ham, both manufactured in an industrial setting, have been studied. The effects of a reduced amount of nitrite during preparation on the colour and colour stability as well as on the growth of *Listeria monocytogenes* have been investigated.

2 Materials and methods

2.1 Manufacture of the meat products

Organic Bologna type sausages[1] and cured cooked hams were produced in standard industrial setting. The manufacturing took place at the production plants of two Dutch commercial meat processing companies, applying their conventional processes as much as possible. After production, products were conditioned for a given period, successively hygienically sliced and packed under modified atmosphere at the industrial locations, transported under refrigerated conditions either to TNO in Zeist for challenge tests with *Listeria monocytogenes* or to A&F for the shelf life experiments. Schematically, these different processing and handling steps are given in Table 1 and 2.

Table 1 Schematic representation of the preparation and logistics of the Bologna type sausage.

Production of Bologna type sausage
Raw materials are chopped and mixed using a vacuum cutter and successively a colloid mill
Vacuum stuffing of the sausages
Cooking/smoking to internal temperature of 72 °C (pasteurization value Vp_{70}^{10} of 110 min.)
↓
Chilling to 0-2 °C and storage for 7 days at -2 °C
↓
Storage for 3 weeks at 0-2 °C
↓
Slicing and packaging under modified atmosphere
↓
Transport (< 2 °C) to A&F and TNO

Table 2 Schematic representation of manufacture and the logistics of the ham products.

Production of the ham
brine injection of ham pieces to 15% green weight with curing brine
tumbling, adding lost brine to achieve 115% retention
Vacuum filling in cooking bags
Cooking to internal temperature of 66 °C
↓
Chilling to 0-2 °C and storage for 5 days at 0-2 °C
↓
Removal of bags and smoking in a smoker at 60 °C for 1 hr.
↓
(Unpacked) Chilling to 0-2 °C and storage at 0-2 °C for 11 days
↓
Slicing and packaging under modified atmosphere
↓
Transport (< 2 °C) to A&F and TNO

2.1.1 Bologna type sausage

Bologna type sausages were produced batch wise starting with a commercially used organic recipe, see Table 3, varying the amounts of sodium nitrite in the seasoning/salt mixture, giving

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

recipes for doughs containing 40, 80, and 160 ppm (mg per kg meat) – ingoing – sodium nitrite and 1.88% sodium chloride.

Table 3 Recipe of the organic Bologna type sausages.

Ingredient	Content
Pig meat	77.5%
Water	12.9%
Seasoning /salt	9.6%

The production was started the 24th of August. The different products were prepared sequentially in batches of 175 kg, starting with the lowest amount of nitrite and ending with the highest (conventional) amount of nitrite. About 5 kilograms of dough were left in the colloid mill after unloading it. This amount was returned to the bowl cutter again to be mixed with the next batch. Therefore, the final nitrite concentrations were about 40 ppm, 79, and 158 ppm, respectively. After stuffing, the first and last pair of sausages of each batch were removed from the test, to be sure that all sausages produced had the desired amount of nitrites.
All sausages were cooked[2] and smoked the same morning in one batch and successively cooled as soon as possible. The first 5 days of storage were carried out at -2 °C to enable an end of shelf life comparable to the ham products. After 5 days, the temperature of the cold stores was set at the ordinarily used temperature of 0-2 °C. After another 7 days, the sausages were sliced, packaged and transported, see paragraph 2.2.

2.1.2 Cured Ham

Cured ham products were produced starting from a commercially used organic recipe, according to Table 4, varying the amount of nitrite salt in relation to the amount of sodium chloride in the seasoning/salt mixture of the brine, giving recipes for ham products containing 157, 80 and 40 ppm sodium nitrite and 1.93% sodium chloride.

Table 4 Recipes of the organic cooked cured ham.

Ingredient		Content
Organic uncured ham		87%
Brine (injected)		13%
Composition		
Water	75%	
Seasoning / Salt	25%	

In the ham production process, the standard organic hams (containing 157 ppm of nitrite) were manufactured completely at the standard industrial process line (injecting, tenderizing, tumbling, and stuffing), while the hams with reduced amounts of nitrite were prepared partly at a pilot setup and partly at the industrial line. The production of the organic cured ham products started the 31st of August with the preparation of the 40 and 80 ppm nitrite containing products. The raw meat was injected at the pilot line with the given brines. Successively, the hams were

[2] *Introducing a cooking loss of about 3%*

tenderized at the industrial line. The brine that was released during this process step was collected and added to the meat again. Next, the batches were tumbled during 3 hours in a small vacuum tumbler. The batches were left overnight at refrigerated conditions (0-2 °C) and tumbled for another hour the next day. After tumbling, the hams were stuffed in bags under vacuum again at the industrial line. The standard organic hams were manufactured and stuffed the same day that the other two types of ham products were stuffed.
All hams were collected and cooked in the bag in one batch to a core temperature of 66 °C, and held for 10 minutes at 66 °C. Successively, the hams were cooled down to 0-2 °C and stored at this temperature for 5 days. After removal of the vacuum bags, the hams were smoked at 60 °C for 1 hour and stored without any casing for another 11 days before slicing.

2.2 Slicing and packaging

Slicing and packaging was carried out at the premises of the industrial partners, using their standard lines to perform the operations. For ham, the products were refrigerated to -2 °C the night before slicing, while the Bologna type sausage was kept at 2 °C until slicing. After slicing and packaging under modified atmosphere (30% CO_2 / 70%N_2 and 70% CO_2 / 30% N_2 for respectively the sliced ham and the sliced Bologna type sausage), the packaged products were transported to the research institutes by cold storage trucks (T< 2 °C), and stored in cold rooms immediately after arriving at the institutes.

2.3 Conditions before and during shelf life experiments

The shelf life experiments were performed at A&F. After receipt at the institute, the meat products were stored again at refrigerated conditions according to the time schedule given in Table 5.

Table 5 Conditions during storage for the two types of packed sliced products.

Ham		**Bologna**	
1	Storage at 7 °C in the dark during 21 days	1a	Storage at 2 °C in the dark during 5 days
		1b	Storage at 7 °C in the dark during 12 days
2	Storage during 7 days at 7 °C; illumination (± 450) according a set time schedule	2	Storage during 7 days at 7 °C; illumination (± 450) according set time schedule

During the last week, the products were subjected to light for given periods of time and the colour (development) of the products was determined as a quality parameter. During this illumination phase, storage in retail display was simulated. Therefore, in the storage room (7 °C) the packaged meats were spread at a table surface and illumination took place during pre-defined periods according to Table 6, by means of standard fluorescent tubes, type Philips TLD 58W/33, colour CoolWhite – 4200 K. All illuminated samples were exposed to lighting of about 450 lux at the surface, as determined by a lux meter, during time of display. The samples were rotated between the periods that illumination took place to minimize the light intensity differences within the storage room. At the beginning of the illumination phase, packaged meats were exposed to light for only a short period, while later on, the duration of the illumination steps were increased

since initially no effect of light on the colour was observed, see Table 6. In that table, the exact conditions are given for the two different products.
N.B. The day numbers that are given in the schedule and figures represent the number of days after the production of the Bologna type sausage. The ham products were manufactured 1 week after the Bologna type sausages and, thus, were cooked at day 8; slicing of the Bologna type sausage and the ham products took place respectively at 28 days (day 29) and 17 days (day 25) after production (cooking).

Table 6 Applied illumination schedule

Day number	**Illumination time (hours per day)**		**Illumination cumulative (total hours)**	
	Bologna	ham	Bologna	ham
46	1	1	1	1
47	1	1	2	2
48	1	1	3	3
49	2.5	2.5	5.5	5.5
50	2.5	2.5	8	8
51	0	0	8	8
52	4	4	12	12
53	4	4	16	16
54	4	4	20	20
55	2	6	22	26

The imposed temperature profile and different process steps are schematically summarized in Figure 1 and Figure 2, respectively for the Bologna type sausage and the cured ham products.

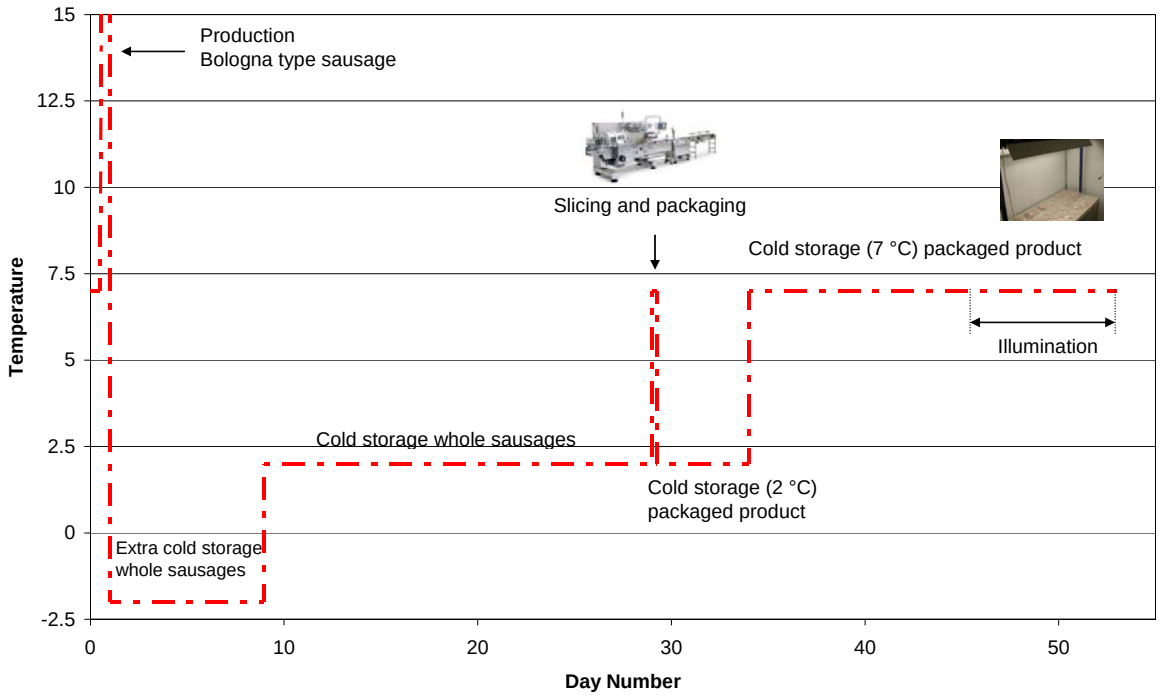

Figure 1 Schematic representation of the imposed temperature profile and logistics for the produced Bologna type sausage.

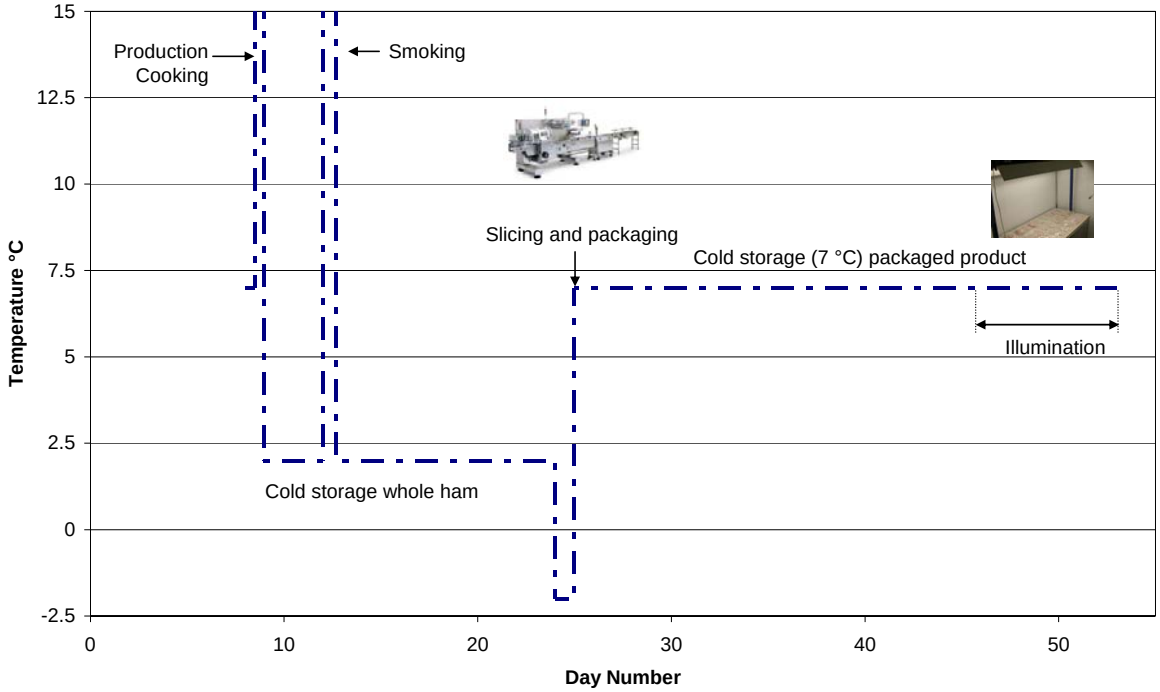

Figure 2 Schematic representation of the imposed temperature profile and logistics for the produced ham products.

2.3.1 Determination of the colour

The colour was measured by a Datacolor reflectance spectrophotometer, type S/N 1204, with a diaphragm of 6.5 mm, according to the CIE-lab colour space. The three parameters in the system represent the lightness of the color (L^*, L^*=0 yields black and L^*=100 indicates white), its position between magenta and green (a^*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow). The meter was calibrated against a white tile supplied by the manufacture before every session. The meter was placed on top of the surface of the meat products immediately after opening the packages. Each value was the mean of 10 determinations per package (of the top slice) and 3 packages per sample.
Next to the colour measurements, also pictures were taken in a light cabinet to give visual information on the colour development. The white balance of the camera was set each time by means of a white surface before taking the pictures.

2.3.2 Determination of pH

The pH values were measured for each type of product directly after receiving the samples, during the storage period as well as at the end of the storage period.
The values were determined by means of a Sentron Argus hand held pH meter equipped with a Hot-Line SurFET probe.

2.3.3 Determination of the amount of residual nitrites and microbiological analysis

During the shelf life experiments samples were taken randomly and were handed over to Silliker in Ede for the determination of the anaerobic and aerobic plate count and the amount of residual nitrite. The aerobic plate counts were determined by the standard method ISO 4833, for the anaerobic plate count a modified method using Schaedler Agar and incubated in the absence of oxygen at 30 °C for 3 days.
The residual amount of nitrite was determined by a spectrophotometer method.

2.4 Conditions and methods before and during the challenge test

The challenge tests were performed at TNO. After receipt at the institute, the products were stored for 1 day at 0 °C until further examination.

2.4.1 Challenge test

Each meat product with different nitrite content was inoculated with a cocktail of three types of *Listeria monocytogenes*: type 1/2a (ATCC 35152), type 4a (ATCC 19114) and type 4b (ATCC13932). Before inoculation the cultures, kept on slants in refrigerator, were pre-cultivated twice in Brain Heart Infusion broth (BHI, Oxoid CM225) for 24 hours at 30°C. The full grown cultures were diluted in physiological peptone saline (PPS) to obtain a mixture at the desired level. A quantity of the meat product (ca. 1000 g) of each composition was placed in the bowl of a disinfected laboratory cutter and inoculated with 10 ml of a suspension of mentioned bacteria to a final level of about 10^3 per g product. After inoculation, the meat products were minced and homogenised for 2 minutes. Subsequently, the minced product was divided into 20 portions of 40 g and vacuum packaged in plastic pouches with an oxygen permeability of less than 5.0×10^{-11} $m^3 \cdot m^{-2} \cdot Pa^{-1} \cdot day^{-1}$ at 20°C. The packages obtained were stored at 7°C for up to 32 to 35 days. During the experiment the temperatures were registered.

2.4.2 Microbiological analysis

At appropriate time intervals, samples of the minced meat product of each batch were taken in duplicate for microbiological analyses. From each single package a sample of 20 g was taken aseptically, diluted 10-fold in PPS and homogenized in a stomacher for 1 minute. Additional serial dilutions were made in PPS. Numbers of *L. monocytogenes* were determined using Palcam agar (Oxoid CM877 and SR150) as mentioned in ISO 11290-2: 1998. Plates were incubated at 37°C for 2 days. Aerobic colony counts were performed according to ISO 4833:1991 on Tryptone Soya Agar (TSA, Oxoid CM131) incubated at 30°C for 3 days. At the start of the challenge test pH values were measured of each type of cooked cured pork hams as well as at the end of the storage period.

3 Results

3.1 Shelf life experiments

3.1.1 Colour development and stability

At the following pages, the colour development and stability of the products are illustrated by means of pictures taken of the products directly after opening the packages. These pictures are presented together with the measured L* and a* values.

In Figure 3, 5, and 7, page 13, 14, and 15, respectively, the pictures of the Bologna type sausage products are presented for the three different ingoing amounts of nitrite, respectively the normal amount of nitrite (158 ppm), the reduced amount (79 ppm) and minimal amount of nitrite (40 ppm). For ham, overviews are given in Figure 9, 11, and 13 (page 16 and further) for respectively the regular organic ham (157 ppm), the product with a reduced amount of nitrite (80 ppm), and the product with the minimal amount of nitrite (40 ppm ingoing). In the figures, the number of days after production as well as the total amount of light (in hours exposed) is given.
At day 50 to day 55, the top four slices were taken of the stack of the Bologna type sausages and laid down at the bottom side to show the difference in colour between the top slices and the slices inside the stack that were not subjected to light. The same was done for ham, where the top two slices were partly moved at day 53 and day 55.

The corresponding charts for the L* and a* values are given below the pictures, thus for the Bologna type sausage, these are given in Figure 4, 6, and 8. While for the ham products, these corresponding charts are given in Figure 10, 12, and 14.

3.1.2 Microbiological and chemical analyses and pH values

In Appendix 1, the pH values for the different products and storage times are given in Table 9, the results of the microbiological and chemical analyses of the Bologna type sausage and the ham products are given in Table 10 and Table 11 respectively.

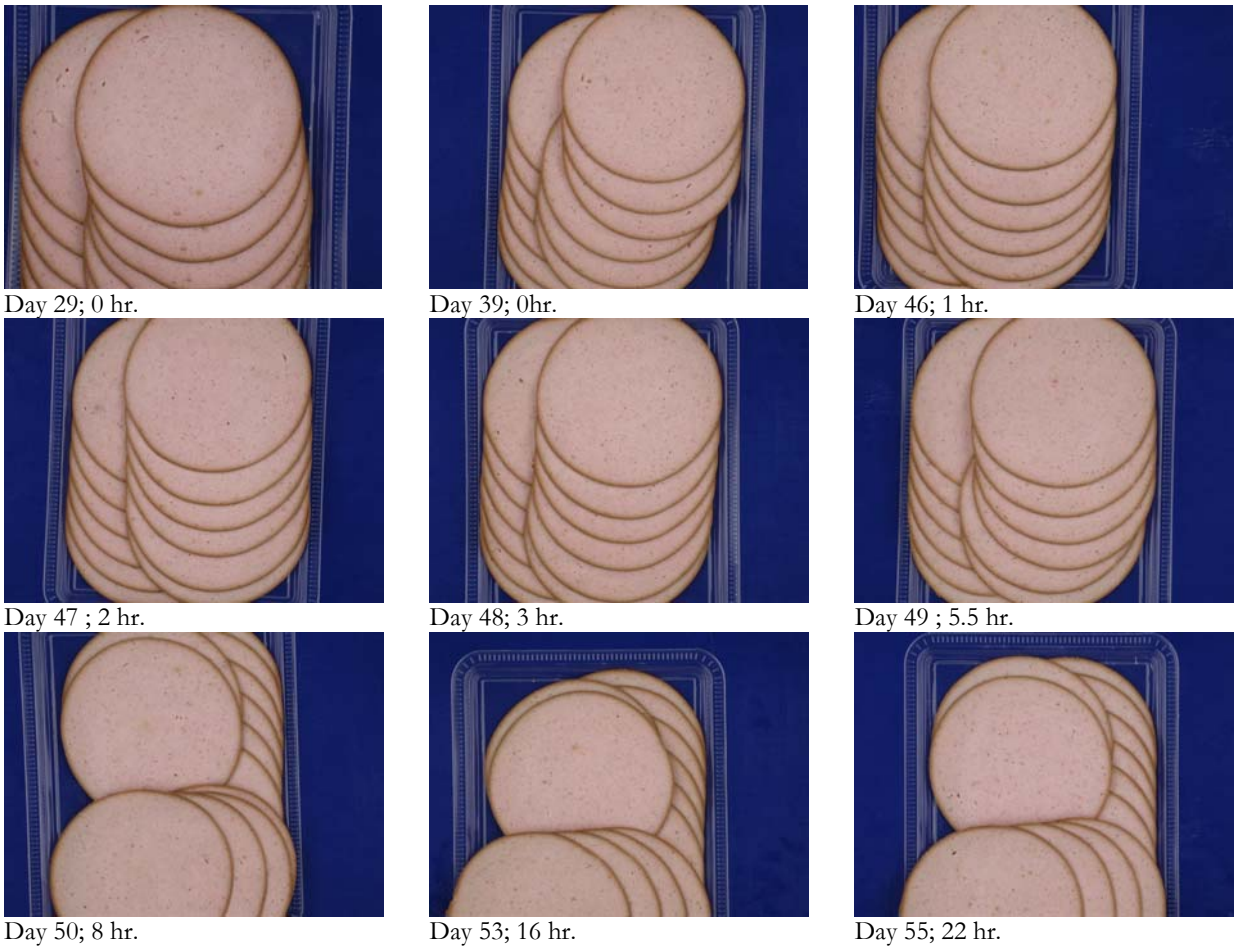

Figure 3 Representation of the colour and colour development of Bologna type sausage produced with normal amounts of nitrites (158 ppm ingoing) as a function of days after production (=day number) and the hours exposed to light.

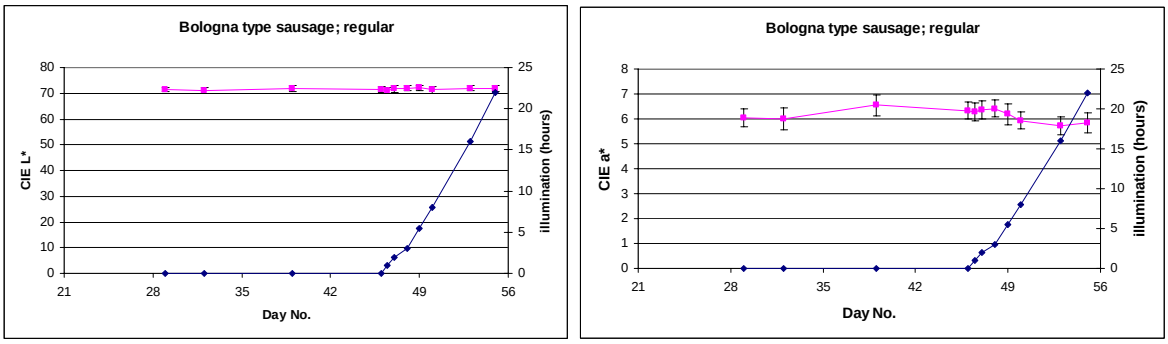

Figure 4 Values for L* (left) and a* (right) of Bologna type sausage produced with standard amounts of nitrites (158 ppm) as a function of shelf life given at the primary y-axis. At the secondary y-axis, the corresponding illumination time is given.

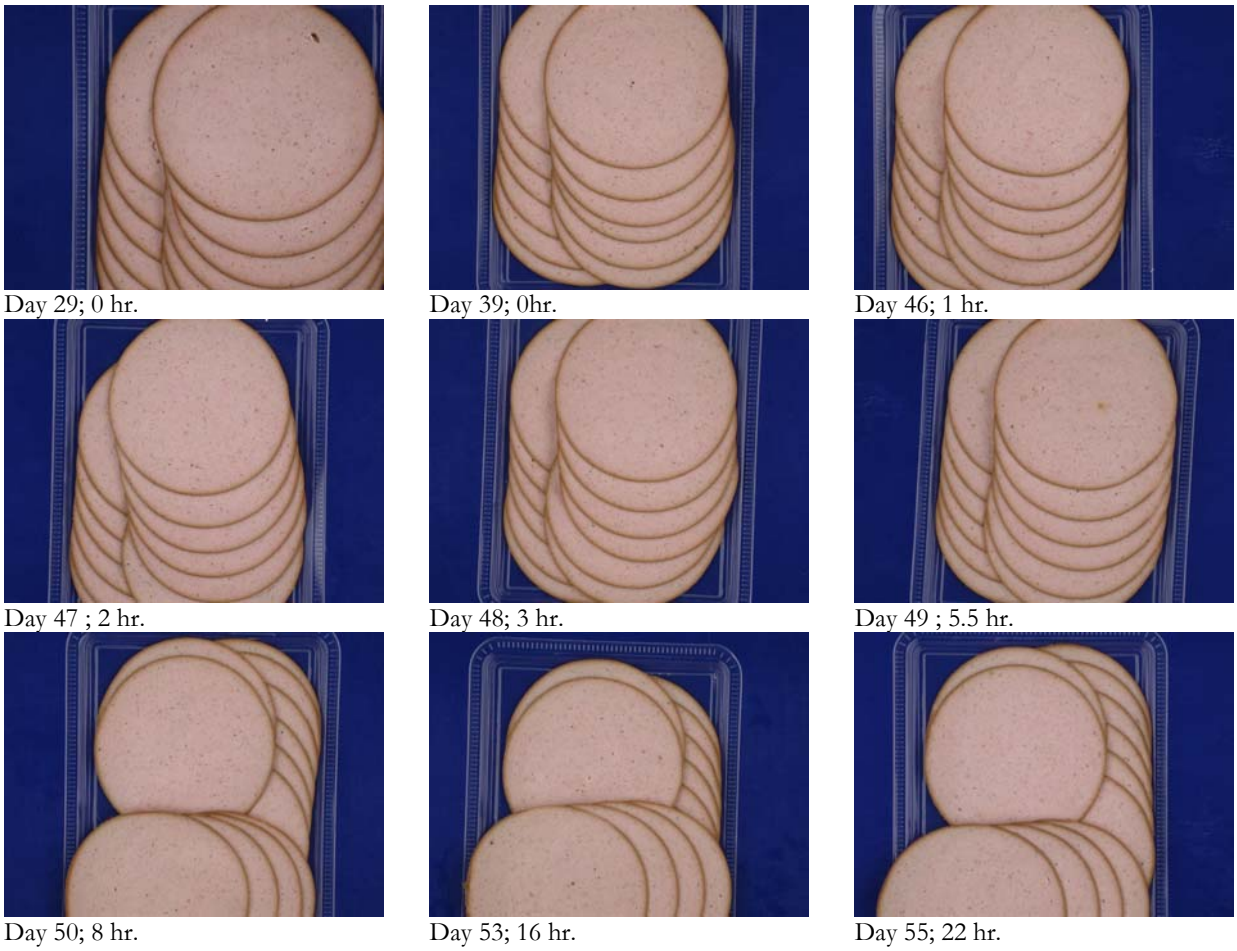

Figure 5 Representation of the colour and colour development of Bologna type sausage produced with reduced amounts of nitrites (79 ppm) as a function of days after production (=day number) and the hours exposed to light.

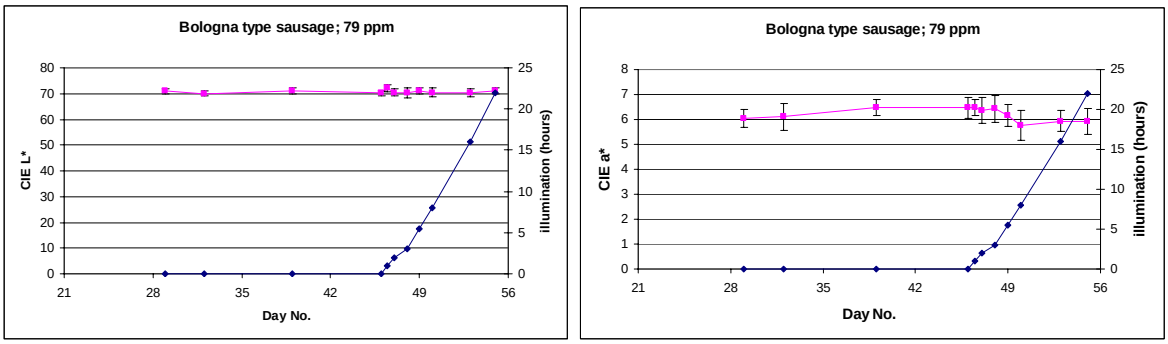

Figure 6 Values for L* (left) and a* (right) of Bologna type sausage produced with reduced amounts of nitrites (79 ppm) as a function of shelf life given at the primary y-axis. At the secondary y-axis, the corresponding illumination time is given.

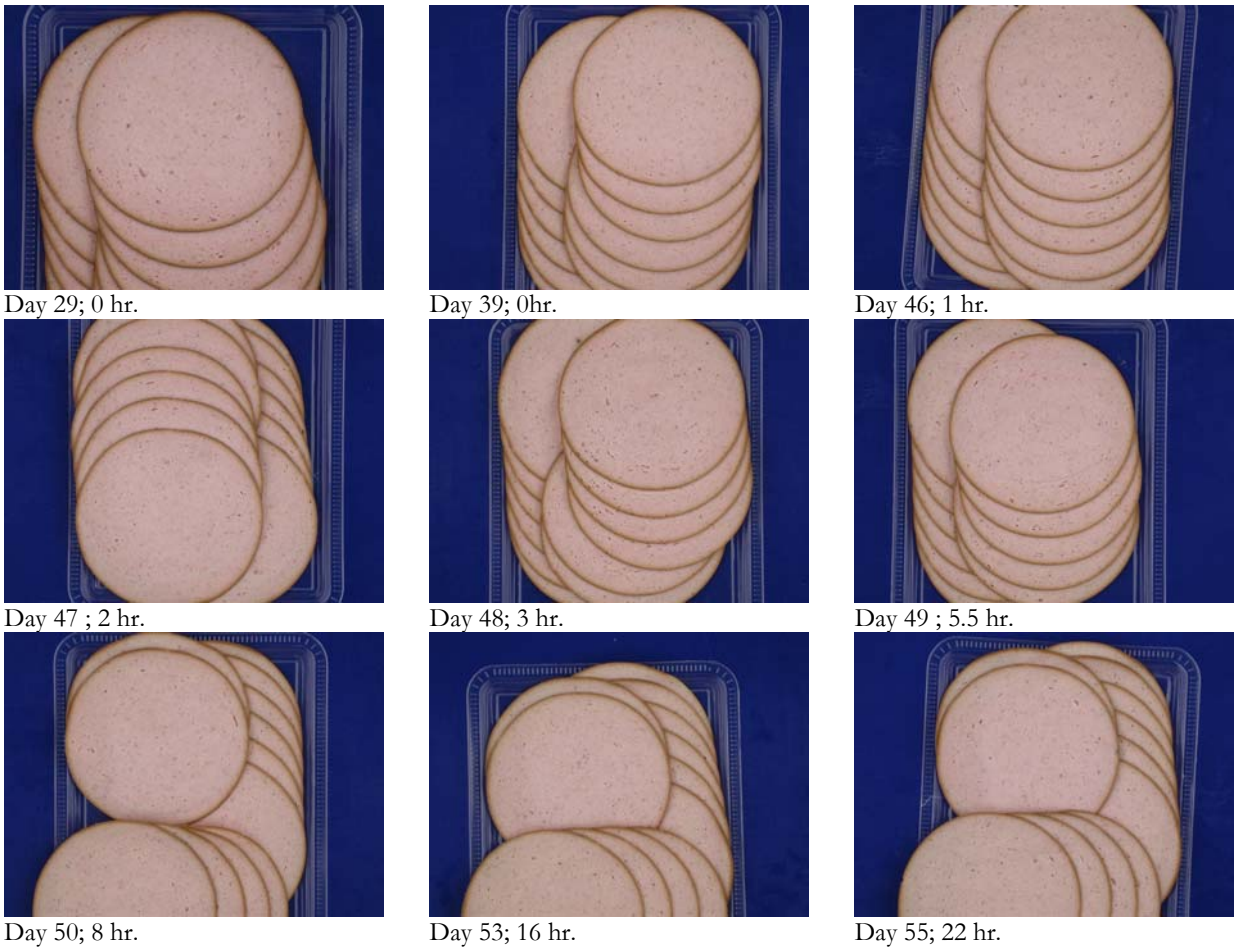

Figure 7 Representation of the colour and colour development of Bologna type sausage produced with minimal amounts of nitrites (40 ppm) as a function of days after production (=day number) and the hours exposed to light.

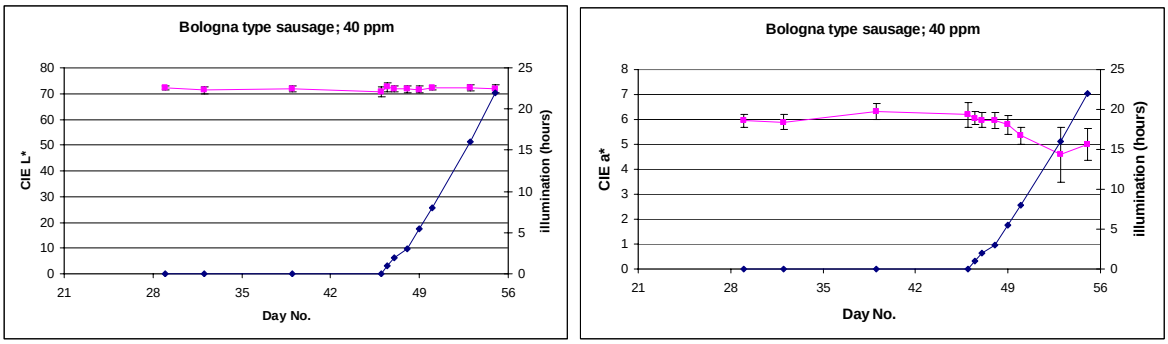

Figure 8 Values for L* (left) and a* (right) of Bologna type sausage produced with minimal amounts of nitrites (40 ppm) as a function of shelf life given at the primary y-axis. At the secondary y-axis, the corresponding illumination time is given.

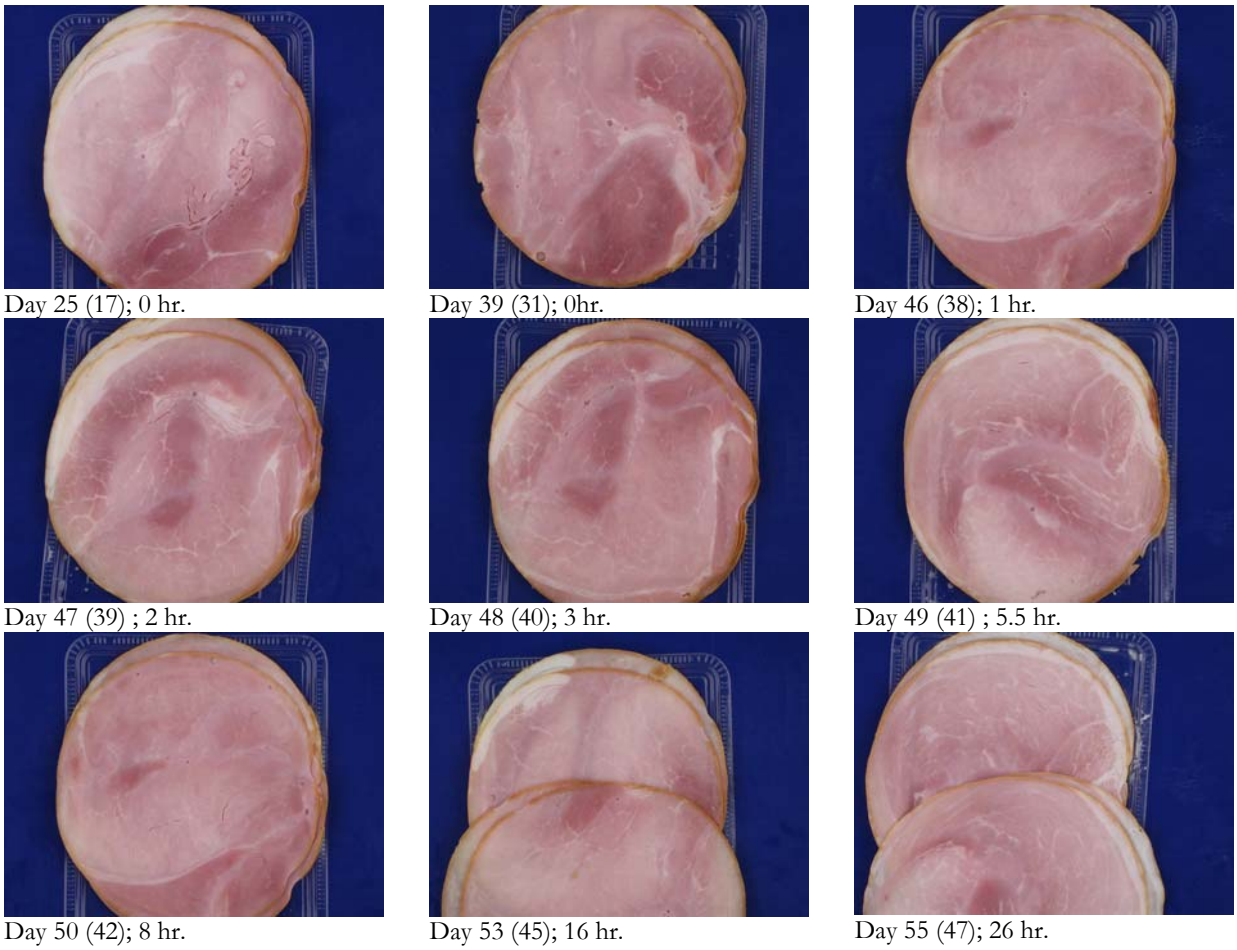

Figure 9 Representation of the colour and colour development in Ham produced with regular amounts of nitrites (157 ppm). Below the pictures day numbers are given and the hours the products are subjected to light. Day numbers correspond to the ones given for Bologna type sausage; in brackets the number of days after cooking is given.

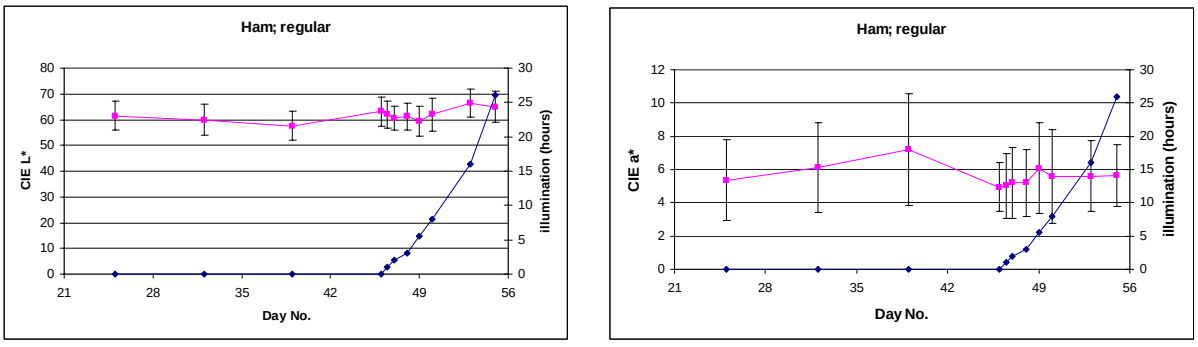

Figure 10 Values for L* (left) and a* (right) of ham with regular amounts of nitrites (157 ppm) as a function of day number (=shelf life + 8 days). At the secondary y-axis, the corresponding illumination time is given.

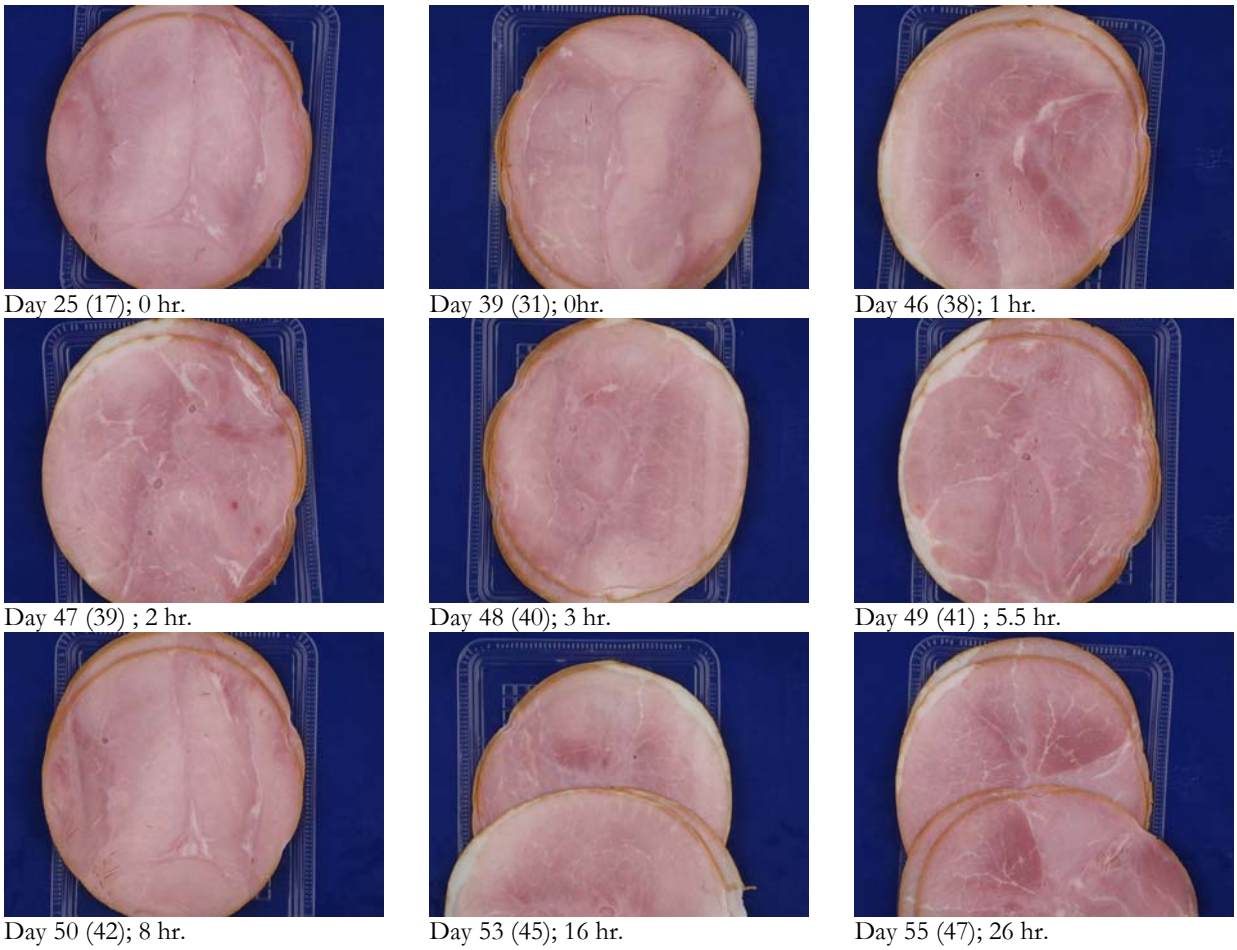

Figure 11 Representation of the colour and colour development in Ham produced with reduced amounts of nitrites (80 ppm). Below the pictures day numbers are given and the hours the products are subjected to light. Day numbers correspond to the ones given for Bologna type sausage; in brackets the number of days after cooking is given.

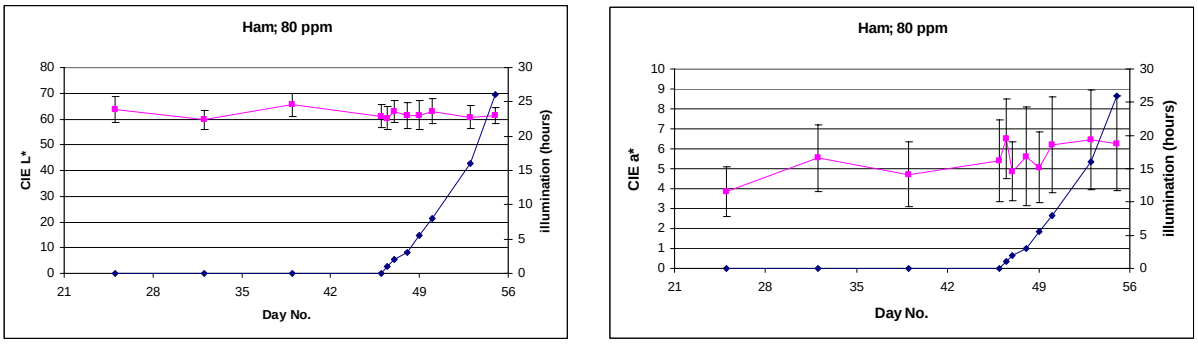

Figure 12 Values for L* (left) and a* (right) of ham with reduced amounts of nitrites (80 ppm) as a function of day number (=shelf life + 8 days). At the secondary y-axis, the corresponding illumination time is given.

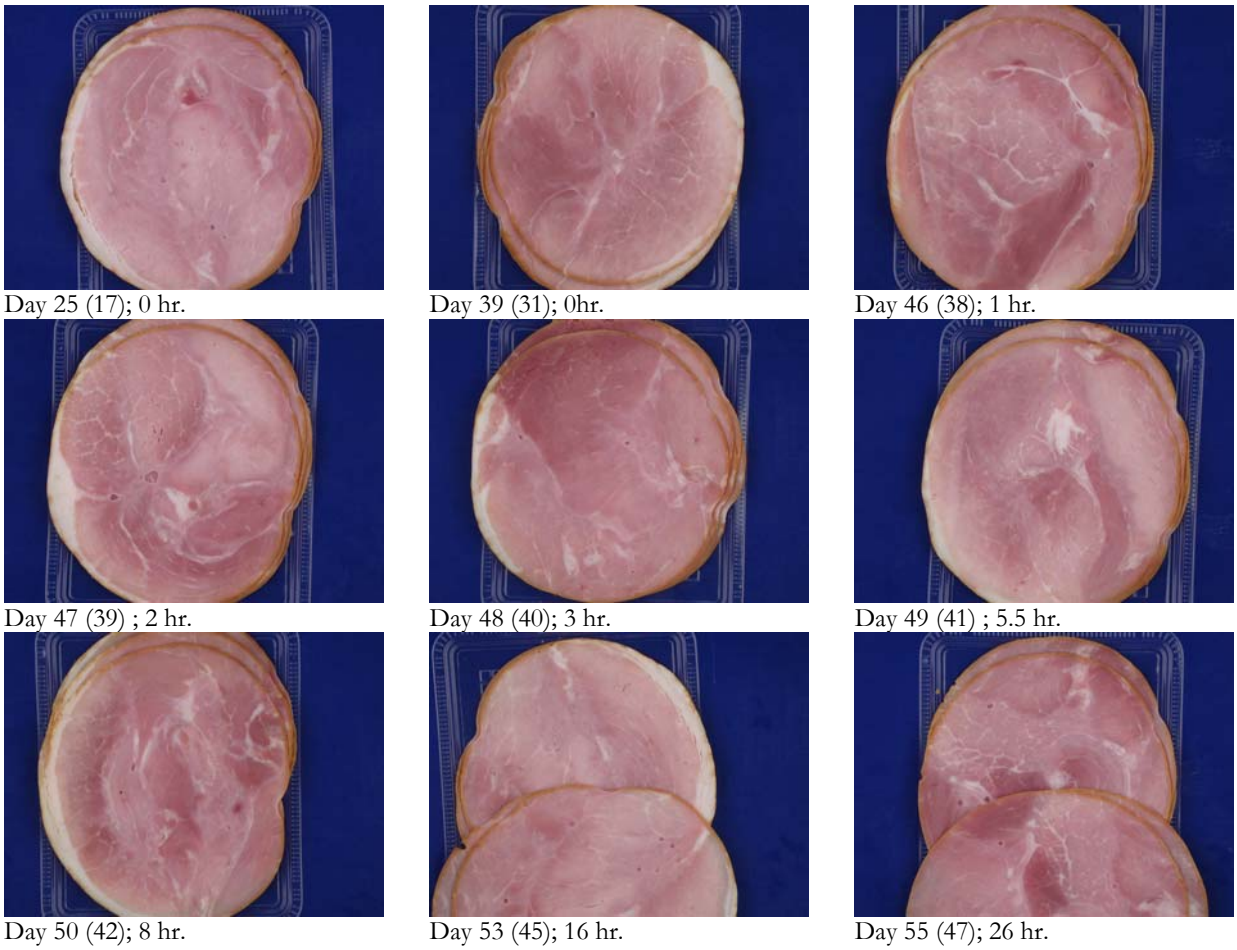

Figure 13 Representation of the colour and colour development in Ham produced with minimal amounts of nitrites (40 ppm). Below the pictures day numbers are given and the hours the products are subjected to light. Day numbers correspond to the ones given for Bologna type sausage; in brackets the number of days after cooking is given.

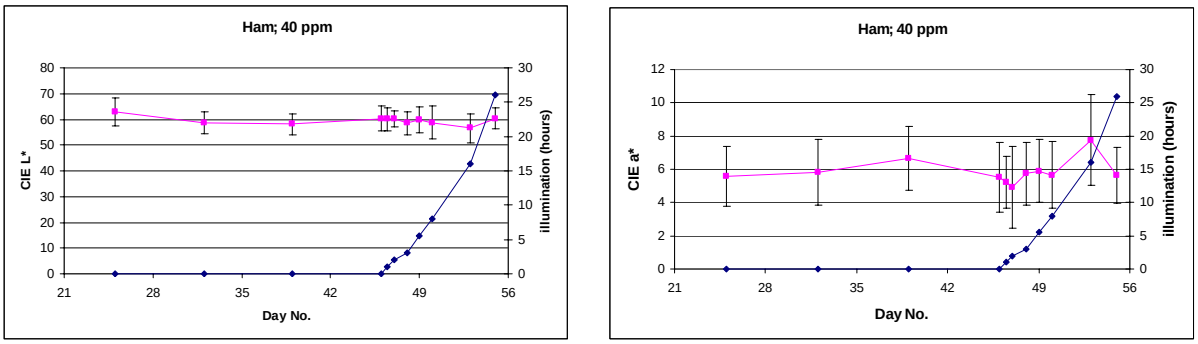

Figure 14 Values for L* (left) and a* (right) of ham with minimal amounts of nitrites (40 ppm) as a function of day number (=shelf life + 8 days). At the secondary y-axis, the corresponding illumination time is given.

3.2 Challenge test

The a_w values and pH values at the start and the end of the test are given Table 7 for the Bologna type sausage and in Table 8 for the ham products. In Appendix 2, the results of the microbiological analyses during storage at 7°C for the Bologna type sausage and the ham products are given in Table 12 and 13, and Table 14 and 15, respectively. Graphically, the mean counts of *L. monocytogenes* and aerobic bacteria in the Bologna type sausages are displayed in Figure 15 and 16, while for the ham products these mean counts are displayed in Figure 17 and 18.

Table 7 a_w and pH values of Bologna type sausage with different nitrite contents.

Product	aw value	pH value At the start	pH value After 32 days at 7°C
Bologna type sausage with 158 mg/kg nitrite (standard product)	0.965	6.05 6.04	6.05 5.98
Bologna type sausage with 79 mg/kg nitrite	0.965	6.09 6.09	6.03 6.00
Bologna type sausage with 40 mg/kg nitrite	0.966	6.10 6.10	6.05 6.03

Table 8 a_w and pH values of ham with different nitrite contents.

Product	aw value	pH value At the start	pH value After 35 days at 7°C
Ham with 157 mg/kg nitrite (standard product)	0.974	6.24 6.26	5.96 5.91
Ham with 80 mg/kg nitrite	0.972	6.25 6.26	5.82 5.84
Ham with 40 mg/kg nitrite	0.972	6.26 6.27	5.89 5.88

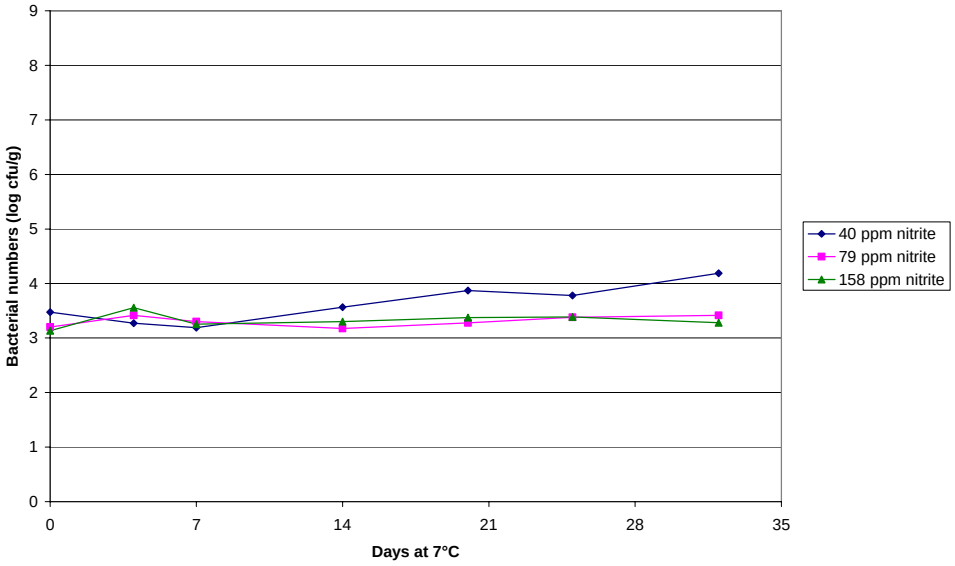

Figure 15 Development of *L. monocytogenes* on vacuum packed Bologna type sausage with different nitrite contents.

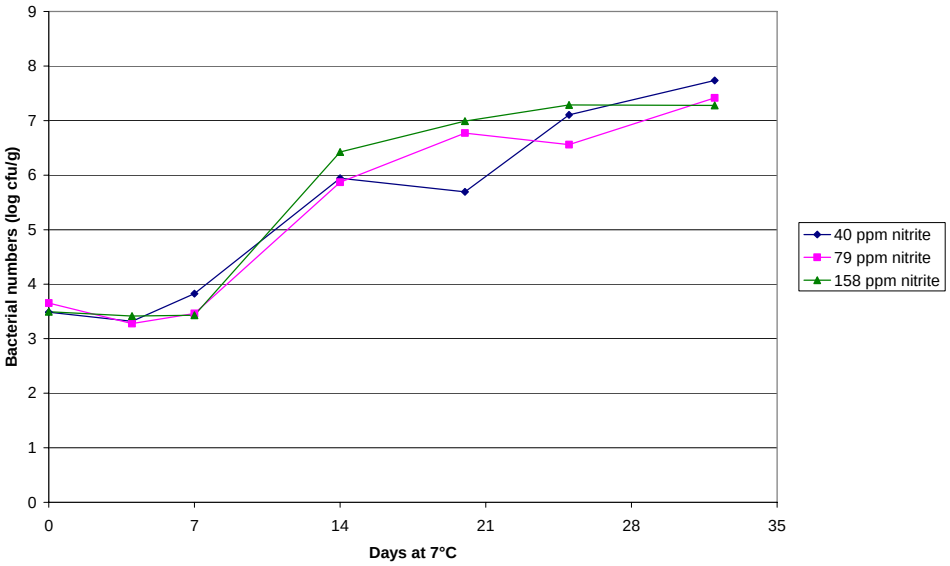

Figure 16 Development of aerobic bacteria on vacuum packed Bologna type sausage with different nitrite contents.

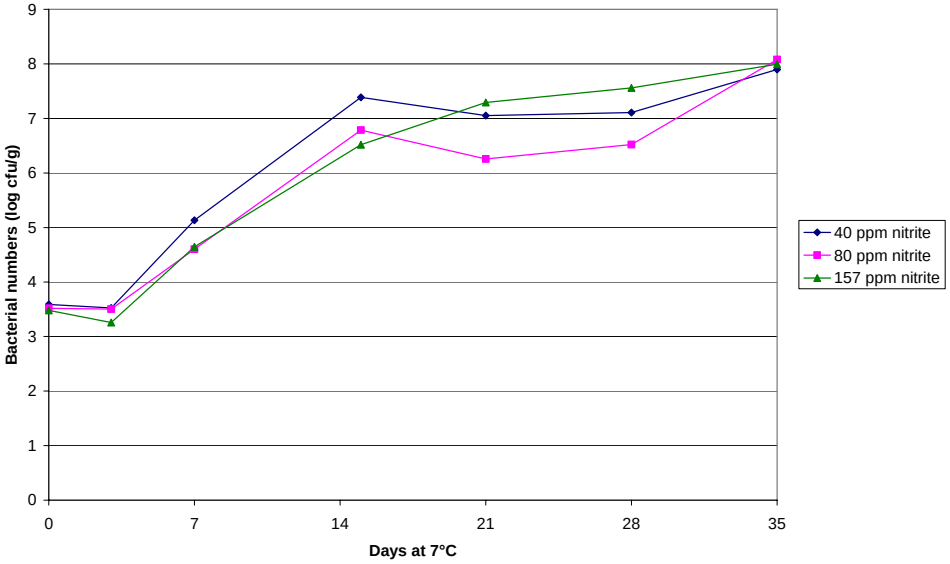

Figure 17 Development of *L. monocytogenes* on vacuum packed ham with different nitrite contents.

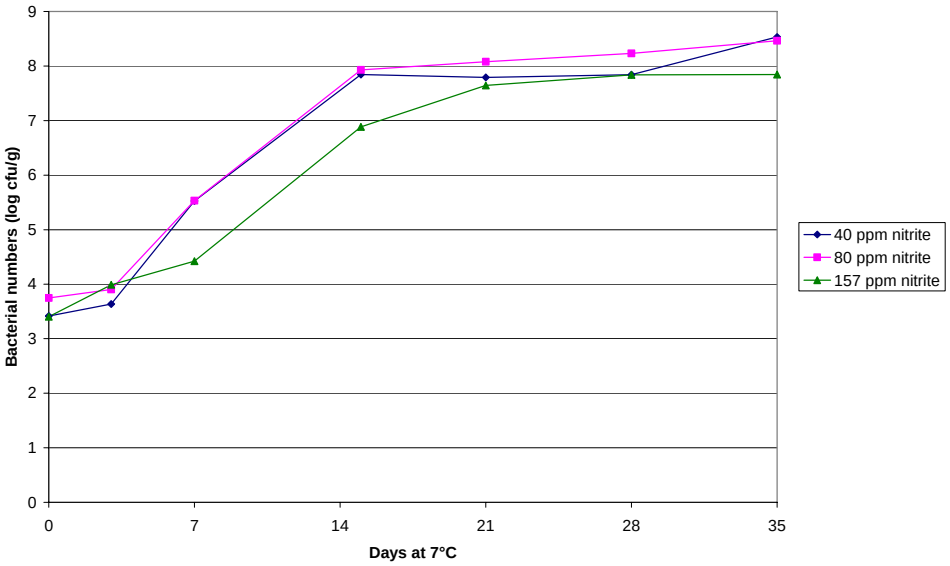

Figure 18 Development of aerobic bacteria on vacuum packed ham with different nitrite contents.

4 Discussion

4.1 Shelf life experiments

Bologna type sausage

Visual observations as indicated by the pictures in Figure 3, 5, and 7 show no colour change in the Bologna type sausage during the experiment for the regular and partly reduced nitrite concentration, while the sausages produced with only 40 ppm nitrite start becoming brownish after being exposed to light for about 8 hours. The colour change is 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 exposed to light. More objectively these results are also shown by the changes in L* and a*-values over time. No significant changes are observed for the products prepared with 158 and 79 ppm nitrite during the test (Figure 4 and 6), while a decrease in a*-value (a reduction in magenta) is observed for the product produced with 40 ppm nitrite (Figure 8).

Next to L*a*b*-values, colours can also be represented by hue values (with tan(hue) = b*/a*), that correspond more closely to human visual observations. In Appendix 3, graphs showing the development of the hue angle values are given, indicating that the colour change at the end of the storage time for the minimal nitrite containing product is less pronounced than one would expect from the specific L* and a* values. At the same time it is noticed that the difference in colour change between the standard product and the reduced nitrite products are only very small.

The pH-values of the Bologna type sausage did not change over the storage period of about 3 weeks at 7 °C after slicing and packaging. In the same period, the growth of anaerobic and aerobic bacteria in the products prepared with reduced amounts of nitrite was acceptable, although less retarded than in the standard product; maximum plate counts for the reduced nitrite containing product were in the range of 10^6 bacteria per gram versus 10^3 in the regular product at the end of the storage period.

Analyses of the residual nitrite content showed that after slicing about 10 to 15% of the ingoing nitrite is still present, while at the last week of the holding period the residual nitrite concentration ranged from 5 ppm for the high level ingoing nitrite products to less than 2 ppm (the detection limit) for the reduced nitrite products, indicating that only a small amount of the ingoing nitrite is still present at the end of the storage period.

Ham Products

Because of the natural inhomogeneity of ham, L*, a* and b* (not shown) as well as the hue angle (Appendix 3) values of the sliced ham products show a large scatter. However, during the entire shelf life no significant colour change is observed. Also, visual observations, as indicated by the different photographs (Figure 9, 11 and 13) do not show any fading during the experiment.

The pH-values of all ham products show a decrease of about 0.2 over the storage period. At the same time the total plate counts are rather high at the end of the storage period for all products. No significant effects of the nitrite concentration on plate count and pH is observed. The high

plate counts and drop in pH values are most likely caused by growth of lactic acid bacteria, due to a relatively high water activity.
Analyses of the residual nitrite content showed that after slicing about 10 to 15% of the ingoing nitrite is still present. At the end of the storage period, the residual nitrite concentration in the regular products was about 5 ppm, while the values were below the detection limit of 2 ppm for the other two products, prepared with lower amounts of nitrite. At the end of the storage period colours of all products have not faded. This may be an indication that a very low amount of nitrite is still present is the products. It may also be caused by the very small amount of celery that is used in the formulations and still may "supply" nitrites in the form of nitrates.

General remark both product types
The amount of light, the products were exposed to (22 or 26 hours), most likely are very extreme, and will be higher than under normal conditions. In retail, packages may be presented in displays for a longer period. However, they ordinarily are covered by other packages, and only the top package is exposed to light. Therefore, it is assumed that under normal conditions the desired cured colour will evolve in Bologna type sausage and cooked ham if nitrite concentrations of at least 40 mg per kg product are applied, and this colour will be stable during normal logistic and retail conditions.

4.2 Challenge tests

Bologna type sausages
L. monocytogenes inoculated in Bologna type sausage with 160 or 80 mg/kg nitrite did not increase during the entire storage period of 32 days at 7 °C (see Figure 15). 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 (see Figure 16). 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 reason for the better microbiological stability of the Bologna type sausage compared to the ham product is the relative low water activity (compare Table 7 and 8). Furthermore, the presence of lactate in the products is known to have anti-listerial activity. The pH of all Bologna type sausages was normal and remained constant during the storage period.

Ham products
In all ham products with nitrite contents 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 17). 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 (see Figure 18). 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 (see Table 8). Also the pH of all ham products was quite common in the initial products and showed some decrease at the end of the storage period as a result of microbial metabolism.

5 Conclusions

From the colour experiments it is concluded that the same colour is developed in the manufactured ham and Bologna type sausage products with a reduced ingoing nitrite content of 40 ppm and about 80 ppm compared to products with an ingoing nitrite content of about 160 ppm. During the entire storage period of about 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 manufactured 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 amount of light, the products was exposed to was rather extreme. In retail, packages ordinarily are covered by other packages, and only the top package is exposed to light. Therefore, it is assumed that the desired cured colour will be stable during normal logistic and retail conditions for Bologna type sausage and cooked ham if ingoing nitrite concentrations of 80 or 40 mg per kg product are applied.

From the challenge test with ham and Bologna type sausage 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.

Differences in water activity (a_w value) between the ham products and the Bologna type sausages appeared to have a more pronounced effect on inhibition of lactic acid bacteria, whereas the relative low water activity in combination with the presence of lactate in the Bologna type sausage almost completely inhibited the growth of *L. monocytogenes* during the storage period of 32 days at 7°C.

For meat products with higher water activity and/or absence of lactate *L. monocytogenes* can only be controlled by prevention of contamination due to stringent hygiene during production.

References

Stegeman, D., T.J. Verkleij, and F.K. Stekelenburg, 2005, Reducing the amount of nitrite in the preparation of organic meat products – a literature review (*in Dutch*). Wageningen: Agrotechnology & Food Innovations BV, Report A&F 560 – ISBN 9067549797.

Stegeman, D., W.W.J.T. Jansen, A.Z. Zegveld, T.J. Verkleij, and F.K. Stekelenburg (2006) *Reductie van nitrietgebruik bij de biologische vleeswarenbereiding*. Wageningen: Agrotechnology & Food Sciences Group, Report AFSG 658 - ISBN 9085850185.

Appendices

Appendix 1 pH value and results of the microbiological and chemical analyses of the sliced packed products used in the colour stability experiments

Table 9 Change of pH the products during storage.

Product	18 Sep. (day 25)	22 Sep. (day 29)	9 Oct. (day 46)	17 Oct. (day 54)
Bologna 158 ppm nitrite		6.06 ± 0.11	6.03± 0.06	6.03 ± 0.07
Bologna 79 ppm nitrite		6.00 ± 0.08	6.10± 0.07	5.99 ± 0.07
Bologna 40 ppm nitrite		5.98 ± 0.05	6.05 ± 0.05	6.05 ± 0.08
Ham 157 ppm	6.26 ± 0.16		6.12 ± 0.04	5.95 ± 0.10
Ham 80 ppm	6.20 ± 0.10		6.09 ± 0.14	6.02 ± 0.06
Ham 40 ppm	6.22 ±0.07		6.03 ± 0.14	6.03 ± 0.14

Table 10 Results of the microbiological analysis and amount of residual nitrite in the Bologna type sausage products during the experiments

	Day 29			Day 46			Day 53		
Ingoing nitrite (ppm)	40	79	158	40	79	158	40	79	158
Residual nitrite (ppm)	6.7	11	15	<2	<2	<2	2,2	3,5	5.7
Aerobic bacteria (cfu/g)	<100	500*	<100	$3{,}8 \times 10^4$	$1{,}9 \times 10^5$	300*	$2{,}7 \times 10^6$	$2{,}0 \times 10^6$	$6{,}2 \times 10^3$
Anaerobic bacteria (cfu/g)	<10	10*	<10	$3{,}2 \times 10^4$	1×10^5	340	$>1 \times 10^6$	$>1 \times 10^6$	$4{,}2 \times 10^3$

* indicative value

Table 11 Microbiological analysis and amount of residual nitrite in the ham products in time during the experiment.

	Day 25			Day 46			Day 53		
Ingoing nitrite (ppm)	40	80	157	40	80	157	40	80	157
Residual nitrite (ppm)	5.5	10	26	<2	<2	<2	<2	<2	5.3
Aerobic bacteria (cfu/g)	200*	3500	100*	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$	$2{,}9 \times 10^6$	$>1 \times 10^7$
Anaerobic bacteria (cfu/g)	<10	480	10*	1×10^5	$>1 \times 10^6$	$>1 \times 10^6$	$>1 \times 10^6$	$>1 \times 10^6$	$>1 \times 10^6$

* indicative value

Appendix 2 Results of microbiological analyses

Table 12 Results of *L. monocytogenes* counts of vacuum packed Bologna type sausage with different nitrite contents during storage at 7°C given in duplicate.

Product	Bacterial counts in log cfu per g Bologna type sausage after storage during						
	0 days	4 days	7 days	14 days	20 days	25 days	32 days
Bologna type with 158 mg/kg nitrite (standard product)	3.43	3.40	3.18	3.18	4.59	3.95	4.12
	3.52	3.15	3.20	3.95	3.15	3.60	4.26
Bologna type sausage with 79 mg/kg nitrite	3.34	3.40	3.18	3.26	3.20	3.40	3.32
	3.20	3.41	3.30	3.18	3.28	3.38	3.41
Bologna type sausage with 40 mg/kg nitrite	3.23	3.56	3.28	3.20	3.20	3.23	3.11
	3.04	n.d.	3.23	3.40	3.54	3.54	3.45

n.d. = not determined

Table 13 Results of aerobic colony counts of vacuum packed Bologna type sausage with different nitrite contents during storage at 7°C given in duplicate.

Product	Bacterial counts in log cfu per g Bologna type sausage after storage during						
	0 days	4 days	7 days	14 days	20 days	25 days	32 days
Bologna type with 158 mg/kg nitrite (standard product)	3.61	3.28	4.12	6.05	5.53	7.36	7.68
	3.36	3.36	3.53	5.84	5.85	6.85	7.79
Bologna type sausage with 79 mg/kg nitrite	3.53	3.38	3.49	6.21	6.73	7.41	7.32
	3.65	3.28	3.46	5.87	6.77	6.56	7.41
Bologna type sausage with 40 mg/kg nitrite	3.54	3.56	3.46	6.45	6.83	7.01	7.23
	3.45	3.28	3.40	6.40	7.15	7.56	7.32

Table 14 Results of *L. monocytogenes* counts of vacuum packed ham with different nitrite contents during storage at 7°C given in duplicate.

Product	Bacterial counts in log cfu per g ham after storage during						
	0 days	3 days	7 days	15 days	21 days	28 days	35 days
Ham with 157 mg/kg nitrite (standard product)	3.45	3.63	5.16	7.43	6.85	7.04	7.89
	3.73	3.41	5.11	7.34	7.26	7.18	7.90
Ham with 80 mg/kg nitrite	3.64	3.66	4.54	6.68	6.56	6.70	8.05
	3.52	3.51	4.60	6.79	6.26	6.52	8.08
Ham with 40 mg/kg nitrite	3.58	3.04	4.56	6.67	7.38	7.61	7.99
	3.38	3.48	4.73	6.36	7.20	7.51	8.00

Table 15 Results of aerobic colony counts of vacuum packed ham with different nitrite contents during storage at 7°C given in duplicate.

Product	Bacterial counts in log cfu per g ham after storage during						
	0 days	3 days	7 days	15 days	21 days	28 days	35 days
Ham with 157 mg/kg nitrite (standard product)	3.41	3.59	5.85	7.89	7.77	7.90	8.38
	3.41	3.68	5.20	7.80	7.81	7.78	8.68
Ham with 80 mg/kg nitrite	3.72	3.90	5.66	7.80	8.21	8.26	8.36
	3.75	n.d.	5.53	7.93	8.08	8.23	8.46
Ham with 40 mg/kg nitrite	3.43	3.53	4.20	6.95	7.54	7.94	7.90
	3.38	4.45	4.64	6.82	7.74	7.73	7.79

n.d. = not determined

Appendix 3 Colour stability of the different products represented by the hue angle values.

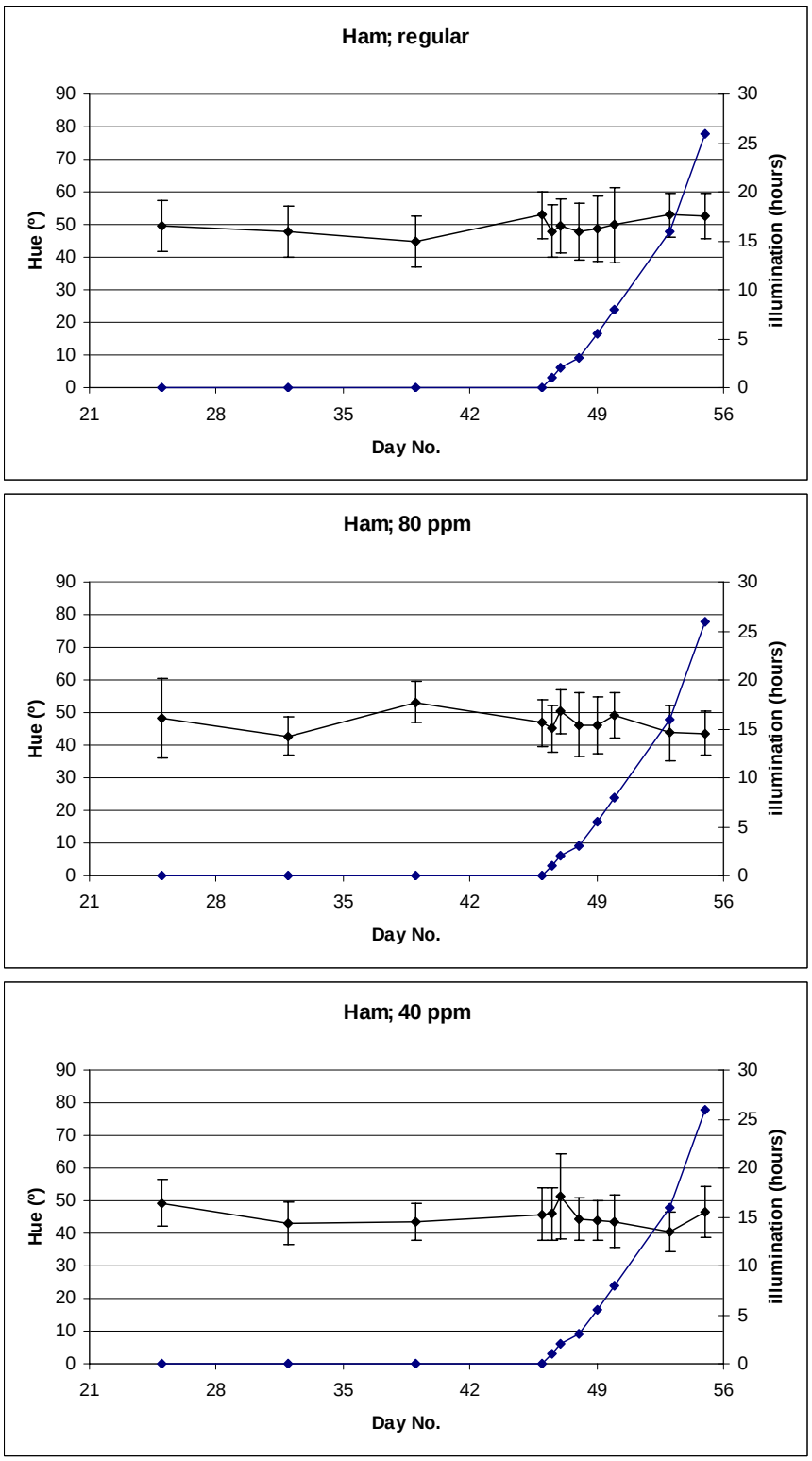

Figure 19 Hue - values for ham products as a function of shelf life given at the primary y-axis. At the secondary y-axis, the corresponding illumination time is given.

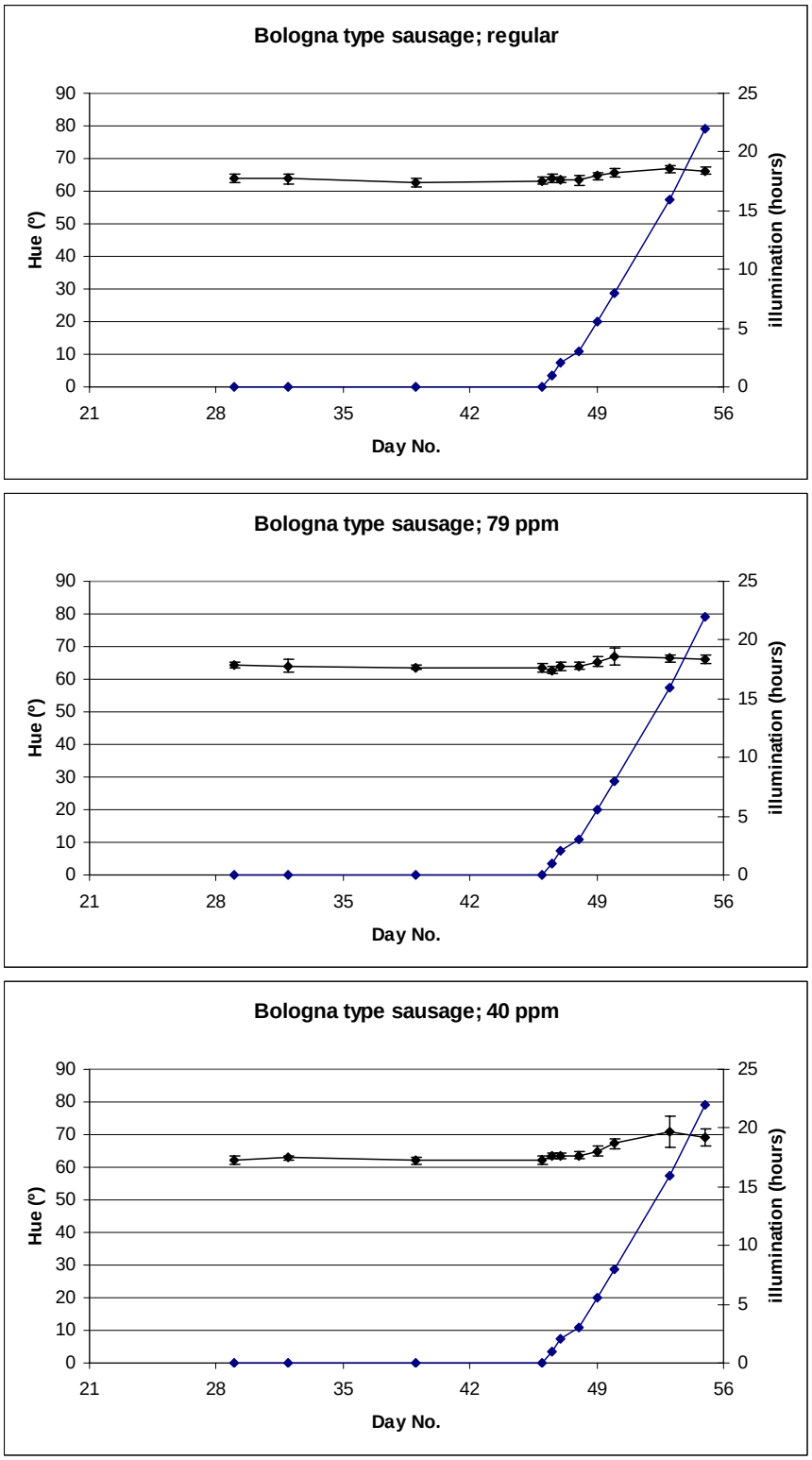

Figure 20 Hue - values for Bologna type sausage products as a function of shelf life given at the primary y-axis. At the secondary y-axis, the corresponding illumination time is given.