Abstract

Discoloration of sliced packaged salami is contributing to rejection of the product, food waste and economical loss. A combination of residual O₂ in the headspace of packages and light is causing photooxidation and deterioration of colour. The aim of this study was to establish maximum tolerable concentrations of residual O₂ in packages of salami slices with 100 % N₂ under light display at 4 and 20 °C. Salami sausages had variable inherent O₂ consumption rate. Storage of salami in 1 % O₂ in darkness did not induce discoloration. The upper limits for O₂ for avoiding discoloration under light were variable in the range 0.1 – 1.0 %, depending on temperature and type of salami. Display at 20 °C increased the rate of O₂ depletion compared to 4 °C. To minimize discoloration, sliced and packaged salami should be stored in darkness at approximately 20 °C until the level of residual O₂ is reduced below a critical limit.
1. Introduction

Dry-cured fermented sausages of salami type are widely produced in Europe and other parts of the world. Salami usually contains pork and occasionally other meats, as well as pork fat, sodium chloride, curing agents like nitrite and nitrate, reducing agents like ascorbic acid and ascorbate, spices, carbohydrates and starter cultures (Toldrà, 2002). The process of fermentation, ripening and drying of the sausages results in a weight loss of more than 30% (Cevoli, Fabbri, Tabanelli, Montanari, Gardini, Lanciotti, & Guarnieri, 2014). The red to pink colour of salami is produced by nitric monoxide binding to the muscle pigment myoglobin, forming nitrosylmyoglobin, and is stabilized by reduction of the pH in the sausage (Møller & Skibsted, 2002). Sliced, packaged salami is prone to discoloration. Discolored salami and other meat products are contributing to undesirable food waste and lower commercial value at retail.

The discoloration of sliced, packaged salami is usually caused by the combination of residual O₂ in the packages and light exposure at display. Photooxidation of nitrosylmyoglobin comes from harmful light both in the ultraviolet and visible areas (Møller & Skibsted, 2002). Sliced, dry-cured Milano-type sausages were packaged in vacuum or 100 % N₂ and exposed to light and temperatures typical for retail display for 60 days (Zanardi, Dorigoni, Badiani, & Chizzolini, 2002). The vacuum packaged sausages were less red than those in N₂ at the end of display, probably due to higher residual O₂ levels in the vacuum packages, but specific O₂ concentrations were not established. Furthermore, Spanish dry fermented sausages of Salchichón type were slightly less red in vacuum than 20 % CO₂/ 80 % N₂, but only by a 0.5 a* redness value (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008). It seems like the level of residual O₂ in the headspace of the packages was not clarified.

The O₂ level in the headspace of the packages is a combination of several factors: residual O₂ level at time of packaging influenced by packaging machinery and operation, O₂ barrier properties of the packaging materials and oxygen consumption due to microbiological growth (Møller, Jakobsen, Weber, Martinussen, & Bertelsen, 2003). The O₂ barrier property of the packaging film is crucial for the access of O₂ to the product. Sliced, vacuum packaged salami was analysed for colour changes in films with O₂ transmission rates of 1, 11, 30, 72 and 90 ml/ m²/ 24 hrs at 23 °C and 0 % RH, respectively (Yen, Brown, Dick, & Acton, 1988). Films with 30 ml or higher O₂ transmission rates resulted in less redness and more discoloration at light display, and the discoloration increased with longer light display, up to 8 weeks. In this study, no change in redness occurred with any of these films under dark storage. The level of residual O₂ in the headspace of salami packaged in modified atmospheres is reduced due to
$O_2$ consumption by added and inherent bacteria in the product, mainly lactic acid bacteria. A study of $O_2$ levels of sliced, dry-cured sausages during storage in $N_2$ showed that the reduction of detrimental $O_2$ was faster at 22 than 4 °C, with initial $O_2$ concentrations of 8 – 14 % over 120 days storage (Scetar, Kovacic, Kurek, & Galic, 2013). In Norway, sliced salami is mostly packaged in modified atmospheres and displayed under light either in chill cabinets or at room temperatures. Cooked ham differs from dry cured sausages by having much lower total bacterial counts, at least early after packaging. Previously, the $C_2$ headspace levels for initiating discoloration of sliced, cooked ham with nitrite under light display have been set to 0.1 - 0.2 % $O_2$, partly depending on the headspace volume. (Møller, Jensen, Olsen, Skibsted, & Bertelsen, 2000; Larsen, Westad, Sørheim, & Nilsen, 2006). To our knowledge, such a critical level for residual $O_2$ in the headspace for modified atmosphere packaged sliced salami has not yet been established.

The purpose of this study was to determine the maximum levels of residual $O_2$ in the headspace of $N_2$ atmospheres to avoid discoloration of sliced Norwegian type salami displayed under light at 4 and 20 °C. The study comprised two types of commercial salami sausages, however, without investigating processing factors of the salamis in this study.

### 2. Materials and methods

#### 2.1. Products for the experiment

Sausages from two batches of dry fermented salami were supplied by two Norwegian meat companies, and called A and B for this study. The recipes for both sausage types contain meat of pork and beef, pork fat, sodium chloride, spices, garlic, sodium nitrite, sodium ascorbate (A only), rosmarin extract (B only), carbohydrates and starter cultures. The sausages were stuffed in 85 wide mm synthetic casings, and weighed approximately 4 kg each after drying. Salami A consisted of 35 % fat, 21 % protein, 6 % sodium chloride and 38 % water. Salami B consisted of 35 % fat, 18 % protein, 5 % sodium chloride and 41 % water. The sausages A and B were sliced and packaged approximately 1 and 2 months after completed production, respectively.

#### 2.2. Experimental set-up

In a first small experiment, packages with sliced salami were injected with air to obtain 1.0 % $O_2$ in the headspace, and then the reduction of residual $O_2$ was followed for 7 days. The
experiment included salami sausages A and B, and was performed under light display and in
darkness, both at 4 and 20 °C, amounting to 8 x 4 replicates = 32 samples in total.

The second main experiment was a full factorial setup of the experimental factors, including
type of salami (A and B), O₂ level (0, 0.10, 0.25, 0.40, 0.75, 1.0 and 20.95 %) and
temperature (4 and 20 °C). The concentrations of O₂ were chosen to mimic common levels at
packaging (0.1 – 0.4 %), elevated levels by malfunction of packaging (0.75 and 1.0 %) and
complete leakage (21 %). All samples were subjected to light, and in addition, the 1 %
samples were stored in darkness for comparison. Three replicates were made of each
experimental condition, amounting to 32 x 3 = 96 samples in total. Instrumental colour
analysis was performed on all samples at day 1, 2, 4 and 7, and visual colour evaluation on
one of the replicates at days 1, 2 and 4.

2.3. Slicing, packaging and light display

All these operations took place at the Nofima pilot plant. The sausages were sliced on a
Bizerba VS12D machine (Bizerba, Balingen, Germany). The slices were 1.0 mm thick and
weighed ca. 5 g. Two stacks with 14 slices each were used for one package, yielding ca. 140
g of sausage per package. The holding time for the stacks from slicing to completed
packaging was approximately 45 minutes at 18 – 20 °C.

Packaging was performed on a Multivac R145 thermoforming machine (Multivac,
Wolferschwenden, Germany). The black base film was of type Multipet 450 and the
transparent top film of type Biaxer 65 XX HFP AFM (both Wipak, Nastola Finland) with O₂
transmission rates of 10 and 5 ml/m²· day, 1 atm at 23 °C and 50 % RH, respectively. The
base film was formed into trays 21.5 cm long, 10.8 cm wide and 1.8 cm high. The slices were
packaged in 100 % N₂ (AGA, Oslo, Norway). The gas to product or sausage volume ratio
(G:P) was approximately 2 to 1. After packaging, the levels of residual O₂ in headspaces
were 0.1 – 0.4 %. All packages with salami slices were first stored in darkness at 4 °C for 14
days to allow for complete removal of residual O₂ from the headspaces. To obtain packages
with elevated levels of O₂ in the headspace, variable volumes of air were then injected into
the packages using syringes with needles through self-sealing septas of type 644209
(Dansensor, Ringsted, Denmark). Packages with air or 20.95 % O₂ were punctured once with
a needle. The light display trial started within one hour after supply of air. Storage time for
sufficient removal of O₂ and level of injection of air for suitable O₂ concentrations were
established through pre-trials.

The light display was standardised to approximately 930 lux continuously at the surface of
the salami slices for up to 7 days, both at 4 and 20 °C. The fluorescent lamps at 4 °C were
Natura de luxe L36W/76 (Osram, Munich, Germany) and at 20 °C Auralight T5 Supreme HO 49W/830 (Auralight International AB, Karlskrona, Sweden) both typically used for illumination of meat products in display cabinets and from ceilings in food shops in Norway. The light intensity simulating retail conditions was obtained by adjusting the distance between the light sources and the salami surfaces. The packages under light display were rotated on days 1, 3 and 5 to expose the different samples to nearly uniform levels of light.

2.4. Analyses

The concentration of O$_2$ in the headspace of the packages was obtained with a Dansensor Checkmate 3 instrument (Dansensor, Ringsted, Denmark) by the use of a small vacuum pump and a needle inserted through self-sealing septas (Dansensor), withdrawing 7 ml of gas. All packages were analyzed at days 0, 4 and 7 of display, while spot tests were performed on days 1 and 2.

Instrumental values (L* - lightness, a* - redness and b* - yellowness) were obtained with a Minolta Chroma Meter CR-400 (Konica Minolta, Inc., Tokyo, Japan) with a 8 mm viewing port, 2° viewer angle and illuminant D$_{65}$. The instrument was calibrated against a white tile (L* = 97.16, a* = 0.25 and b* = 2.09). The samples were measured in intact packages at the product surface through the transparent film. The instrumental colour measurements were performed in four replicates on all samples.

Visual colour evaluation was performed by a 6 member trained panel. The colour of the salami slice surfaces was assessed on a scale of 1 = very red, 2 = slightly red, 3 = slightly brown, 4 = moderately brown and 5 = very brown, adapted from AMSA (2012). Additional standard samples exhibiting scores of 1 and 5 were used at all sampling days as examples for the assessors. During the evaluation, the samples were randomly displayed under PlusLux 3000 warm white light (Thorn, Durham, England) with a light intensity of 1600 lux at the salami surfaces.

Fading of the sausages was analyzed on a Foss XDS Opti Probe Analyzer Reflection and Immersion instrument (Foss NIRSystems Inc., Laurel, Maryland, USA). Spectra of 400 – 700 nm with 10 nm intervals were measured in samples from one replicate at day 4 of display directly through the top film of intact packages. The ratio 650/570 nm expressed the degree of fading of cured meat products with scale ca. 1.1 = no cured colour, ca. 1.6 = moderate fading, 1.7 - 2.0 = noticeable cured colour and 2.2 - 2.6 = excellent cured colour (AMSA, 2012).
pH was measured directly in the sausages with an Ingold Xerolyte electrode (Mettler-Toledo, Greifensee, Switzerland). Water activity of the sausages was analysed at 25 °C with an AquaLab CX-2 instrument (Decagon Devices Inc., Pullman, Washington, USA).

2.5. Statistics

The colour parameters were evaluated by a fixed-effects ANOVA model with main effects type of salami, temperature, O_2 level and days of storage. All two-factor interactions were also included in the ANOVA model. The samples with 21 % O_2 and the samples stored in darkness were kept out of the ANOVA analysis, but included in the figures for comparison. The analysis was performed in MATLAB (R2014b, The Mathworks, Inc., Natick. MA, USA, at www.mathworks.com).

3. Results and discussion

3.1. Consumption rate for residual O_2

A fast consumption of initial residual O_2 by the salami sausages is of importance for avoiding later light induced discoloration. As shown for both salamis type A and B, the reduction in concentration of headspace O_2 from the initial 1.0 % was faster at 20 than 4 °C, and faster in light than in darkness at the lowest temperature (Fig. 1 i and ii). These findings are in agreement with a study of dry cured sausage packaged in N2 and which had a faster removal of residual O_2 at 22 than 4 °C (Scetar et al., 2013). A higher rate of O_2 consumption under light than in darkness is consistent with results from a similar study of cooked cured sausage (Böhner, Hösl, Riebling, & Danzl, 2014), demonstrating O_2 consumption by photooxidative reactions.

The rate of O_2 depletion was higher for salami A than B at 4 °C, meaning that salami A needed shorter time to reach a certain level of residual O_2 than salami B at this low temperature. The specific cause or causes for this difference in O_2 consumption rate between the two salamis are not known, because there are a number of differences in recipes and processes. Salamis A and B had a pH of 5.02 and 4.88 (p < 0.05), and an a_w of 0.875 and 0.897 (p < 0.05), respectively. Bacterial O_2 consumption is likely to contribute considerably to removal of O_2 in this system (Toldrá, 2002). Starter cultures added to Scandinavian-type fermented sausages varied in metabolic activity (Klingberg, Axelsson, Naterstad, Elsser, & Budde, 2005). In the present study, salamis A and B were used for the packaging test approximately 1 and 2 months after production, which could contribute to reduced bacterial activity and O_2 consumption for the latter salami.
The O₂ consumption in packages of salami is depending on the gas to meat ratio. In this experiment, the G:P ratio was relatively high at 2:1, where O₂ concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 % corresponded to O₂ headspace volumes of 0.56, 1.12, 1.68, 2.24 and 2.80 ml, respectively. With a low volume of O₂ the gas is removed faster, and the packages with salami can sooner be ready for light exposure. In a modelling of five factors contributing to the discoloration of cooked ham, the headspace to product volume was one of the crucial factors (Møller et al., 2003). Even with low percentages of residual O₂ in the headspace, but combined with high G:P ratios, there will be sufficient O₂ for colour deteriorating processes to take place. For the preservation of the preferred red colour of sliced cooked or dry cured meat products, it is beneficial to try to obtain low G:P ratios at the time of packaging.

3.2. Concentrations of initial residual O₂

The main experiment consisted of 7 concentrations of O₂ that were obtained by injecting various volumes of air, except the samples with pure air, which were made by puncturing the packages. The average initial levels of 0, 0.10, 0.25, 0.40, 0.75, 1.0 and 20.95 % O₂ were reached with only minor deviations from targets, although with some variations within each O₂ group (Table 1).

3.3. Effects of residual O₂ on visual and instrumental colour

The ANOVA analysis of the main experiment demonstrated that the type of salami, A or B, explained much of L* lightness (Table 2). Storage temperatures of 4 or 20 °C influenced the colour attributes, and most for a* redness. Initial level of residual O₂ in the headspace explained a high degree of the variation in visual colour, a* values and ratio 650/570 nm for fading. b* yellowness was not influenced by level of residual O₂ or type of salami. The day of display, 1, 2, 4 and 7, had a bearing on b*, but had almost no effect on visual colour, L* and a*.

The correlation between visual redness score and a* instrumental redness values was high, with R² of 0.97 (Fig. 2). The panel for visual colour evaluation established a maximum score of 2, corresponding to an a* value of 13.5, of which a noticeable difference in colour or slight discoloration was observed for both sausages, A and B.

The visual colour evaluation at day 4 of the experiment showed that in order to keep the salami at or below an acceptable score of 2, a higher level of initial residual O₂ under light could be tolerated at 20 than 4 °C (Fig. 3). In addition, salami A was more robust and could maintain an acceptable colour at higher levels of O₂ than salami B. Therefore, the specific requirements for maximum residual O₂ were variable, depending on the type salami and storage temperature. Approximate maximum O₂ level for salami A at 4 °C was 0.30 % and at...
20 °C 1.0 %, while for salami B at 4 °C it was 0.15 % and at 20 °C 0.35 %. Samples of salami with 1.0 % O₂ stored in darkness were all red and had visual colour scores of approximately 1.

The a* redness colour values at day 4 of display of the salamis A and B stored at 4 and 20 °C are shown in Fig. 4 i. Data for a* values at 1 and 2 days of light display showed a similar discoloration as for 4 days, but to a less degree, and slightly more at 7 days (results not shown). Increasing level of initial O₂ in the headspace of the packages reduced a* values for both salamis and temperatures. The sausage controls with 0 % O₂ in light, as well as those with 1 % O₂ in darkness, both had high a* values of approximately 16. Decreasing a* values were measured for O₂ levels from 0.1 to 21 %, with ultimate a* values as low as approximately 4. The most rapid decline in a* was observed at 4 °C and for salami B. This high degree of discoloration can be linked to a slower reduction in residual O₂ levels in this type of samples (Fig. 1). To keep the sliced salami sausages above a critical a* value of 13.5 after 4 days storage, salami A at 4 °C needed to be below approximately 0.35 %, salami A at 20 °C below 1.0 %, salami B at 4 °C below 0.10 % and salami B at 20 °C below 0.25 % at this sampling time. The limits for maximum acceptable residual O₂ were almost similar for the a* values and visual colour evaluation (Fig. 3).

L* lightness values were consistently 3 - 4 units higher for salami B than A (p < 0.01) and 1 - 2 units higher at 4 than 20 °C (p < 0.01) (Fig. 4 ii). The lighter colour for sausage B could be due to fat properties or a higher fraction of pork versus beef in the recipe. The L* values were not affected by level of initial O₂, except increased values for samples in 21 % O₂.

b* yellowness values did not vary between the different treatments or types of salami in this experiment (results not shown).

The results for fading of the salami are shown in Fig. 5. The ratio 650/570 nm is expressing the degree of cured colour, with samples with high numbers having a red, typical cured colour (AMSA, 2012). Again, the samples stored at 20 °C and from sausage A were higher in 650/570 nm values than those at 4 °C and from sausage B. Values at or above 2.2 with excellent cured colour were noted for salami A at both temperatures and salami B at 20 °C with 0 and 0.1 % O₂ in light and 1 % O₂ in darkness. Samples of salami B at 4 °C were considerably lower than salami A in 650/570 ratios at all O₂ levels with light, as well at 1.0 % O₂ in darkness (p < 0.05). The results on fading for these salami B samples differed slightly from a* values and visual colour scores, and indicates that changes in the pigment may have occurred, although not evident to the human eye.

3.4. Practical consequences for packaging of salami
One of the main experiences from this study is that for each sliced salami-type product in modified atmosphere packages it is important to learn the rate of reduction in residual O₂ in the headspace. The packages with salami should be stored in darkness until all residual O₂ is consumed, and then the packages can safely be displayed under light. The present study only addressed packaging and display factors, for example level of initial O₂ and storage temperature on the rate of O₂ consumption. To obtain a more complete understanding of the system, future studies should include processing factors like the type and activity of starter cultures, influence of raw materials and ingredients, and fermentation and drying processes.

For this shelf stable dry salami, a display temperature of approximately 20 °C favours a rapid O₂ consumption. In situations where the level of initial residual O₂ is too high, it is advisable to store the sliced salami packages at 20 °C in darkness, to facilitate a fast decline in O₂ levels before exposure to light takes place. The holding time to reach acceptable concentrations of residual O₂ depends on factors like initial O₂ level, G:P ratio and O₂ depletion rate, and should be determined by gas measurements. However, the temperature should not be increased much over 20 °C, where partial melting of fat from the salami can cause consumer rejection of the product, depending on the content of various fatty acids.

4. Conclusions

The strategies for maintaining a red colour of sliced, packaged salami are two-fold: either shielding the sausage from light or ensuring that the concentration of residual O₂ comes below a critical level at the time the light exposure is starting. The present experiment demonstrates that the limit for maximum residual O₂ in the headspace of salami packages at the start of illumination should be in the range 0.1 to 1.0 % O₂, and depending on the O₂ consuming activity of the sausages and the display temperature. The two batches of salami in this experiment were different in their ability to consume O₂ and hence the extent of discoloration. Possible causes for this difference have been indicated above, but more research is required on the mechanisms of O₂ consumption in a product like salami.

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References


Scetar, M., Kovacic, E., Kurek, M., & Galic, K. (2013). Shelf life of packaged sliced dry 


packaging films and light exposure effects on the color stability of vacuum-packaged dry 


Text to figures

Fig. 1.
The reduction in concentration of residual O₂ (%) in the headspace of packages with sliced 
salami over 7 days storage in darkness (i) and light (ii). All packages were injected with air 
volumes to 1.0 % O₂. Symbols: x = 4 °C, 0 = 20 °C, unbroken line = salami type A, dotted line 
= salami type B.

Fig. 2.
Correlation between visual colour score and a* redness values for sliced packaged salami. 
R² was 0.97. Scale for visual colour: 1 = very red, 2 = slightly red, 3 = slightly brown, 4 = 
moderately brown and 5 = very brown. Acceptable colour above a* of 13.5 and below visual 
score of 2.
Visual colour score for sliced packaged salami at day 4 as affected by initial O₂ concentration in the headspace of packages. Scale for visual colour: 1 = very red, 2 = slightly red, 3 = slightly brown, 4 = moderately brown and 5 = very brown. Acceptable colour below a score of 2. For symbols; see Fig. 1.

Instrumental colour for sliced packaged salami at day 4 as affected by initial O₂ concentration in the headspace of packages, and illustrated for a* redness values (i) and L* lightness values (ii). Acceptable colour above an a* value of 13.5. For symbols; see Fig. 1.

Fading by the ratio 650/570 nm for sliced packaged salami at day 4 as affected by initial O₂ concentration in the headspace of packages. Scale ca. 1.1 = no cured colour, ca. 1.6 = moderate fading, 1.7 - 2.0 = noticeable cured colour and 2.2 - 2.6 = excellent cured colour (AMSA, 2012). For symbols; see Fig. 1.
Highlights

- Discoloration of salami occurs by a combination of residual $O_2$ and light
- Maximum $O_2$ headspace concentrations to avoid discoloration in light were 0.1-1.0 %
- Depletion of residual $O_2$ from headspace was faster at 20 than 4 °C
- Keep sliced packaged salami in darkness until residual $O_2$ has passed a critical level
Table 1
Concentrations of $O_2$ (%) at 45 minutes after injection of air in packages of sliced salami.

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Table 2

ANOVA analysis for visual evaluation and instrumental color measurements. The numbers are explained variances (%), and bold numbers are statistically significant at a 5 % level. The visual evaluation is based on day 1, 2 and 4, the instrumental colour values are based on day 1, 2, 4 and 7, and fading is based on day 4 only.

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