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Colour formation in fermented sausages by meat-associated staphylococci with different nitrite- and nitrate-reductase activities

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Abstract

Three *Staphylococcus* strains, *S. carnosus*, *S. simulans* and *S. saprophyticus*, selected due to their varying nitrite and/or nitrate-reductase activities, were used to initiate colour formation during sausage fermentation. During fermentation of sausages with either nitrite or nitrate added, colour was followed by L^*a^*b measurements and the content of nitrosylmyoglobin ($MbFe^{II}NO$) quantified by electron spin resonance (ESR). $MbFe^{II}NO$ was rapidly formed in sausages with added nitrite independent of the presence of nitrite reducing bacteria, whereas the rate of $MbFe^{II}NO$ formation in sausages with added nitrate depended on the specific *Staphylococcus* strain. Strains with high nitrate-reductase activity showed a significantly faster rate of pigment formation, but other factors were of influence as well. Product stability for the sliced, packaged sausage was evaluated as surface colour and oxidation by autofluorescence and hexanal content, respectively. No significant direct effect of the *Staphylococcus* addition was observed, however, there was a clear correspondence between high initial amount of $MbFe^{II}NO$ in the different sausages and the colour stability during storage. Autofluorescence data correlated well with hexanal content, and may be used as predictive tools. Overall, nitrite- and nitrate-reductase activities of *Staphylococcus* strains in nitrite-cured sausages were of limited importance regarding colour development, while in nitrate-cured sausages strains with higher nitrate reductase activity were crucial for ensuring optimal colour formation during initial fermentation stages.

Keywords: Fermented sausage; *Staphylococcus*; Nitrate-reductase; Nitrite-reductase; Nitrosylmyoglobin; Cured colour formation

1. Introduction

Colour formation and colour stability are very important quality attributes of sliced fermented meat products. Such products are often marketed in convenient packages, thereby being more susceptible to oxidation, because of their large surface area exposed to oxygen and light under normal retail display conditions. In fermented sausages, the pigment responsible for the characteristic cured colour is the bright red nitrosylmyoglobin ($MbFe^{II}NO$), in which an axial ligand nitric oxide (NO) is coordinated to central Fe^{II} in heme (Møller & Skibsted, 2002). The chemical reactions leading to the cured meat pigment are a complex series of processes involving microbially, enzymatically and/or chemically catalysed steps, which depend on pH, pigment concentration, redox potential, curing agent distribution, temperature and relative humidity (Chasco, Lizaso, & Beriain, 1996). Especially, regarding the pathway for pigment formation in fermented sausage, little concrete knowledge is available with respect to the role of microbially derived enzymatic activities accelerating the overall process.

Addition of nitrite is traditionally justified by its inhibition of undesirable bacteria such as *Clostridium botulinum* (Cassens, 1997), but nitrite and derived compounds also act as effective antioxidants inhibiting lipid oxidation or development of rancid off-flavours (Cammack et al.,

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1999). However, the most important reason for adding nitrite and/or nitrate to fermented sausages is the formation of red colour, which is particularly enhanced when these curing agents are employed in combination with reducing agents (e.g. ascorbate/ascorbic acid) that accelerate the reduction of nitrite into NO. Alternative strategies for colour formation in sausages without using nitrate or nitrite have been attempted, i.e. via NO formation from L-arginine as a result of nitric oxide synthase (NOS) enzyme activity in either staphylococci (Morita, Sakata, & Nagata, 1998) or lactobacilli (Morita, Yoshikawa, Sakata, Nagata, & Tanaka, 1997), but since NOS is oxygen dependent along with other specific co-factors, it is considered unlikely to ensure a uniform colour formation within a whole sausage (Møller, Jensen, Skibsted, & Knøchel, 2003).

Oxidative discolouration of fermented sausages is characterized by conversion of $MbFe^{II}NO$ to nitrate and the brown derivative metmyoglobin ($MbFe^{III}$), and this quality deteriorating process is known to depend on partial oxygen pressure and myoglobin reducing systems in general and most likely related to subsequent lipid oxidation (Zanardi, Novelli, Ghiretti, Dorigoni, & Chizzolini, 1999). Fermented sausage is a very complex product in which the study of pigment oxidation is quite difficult due to the many factors affecting the redox potential within the products, and currently nothing is known concerning the influence of starter culture on colour stability during storage and retail display.

The main objective of the present study was to investigate the role of staphylococcal nitrite- and nitrate-reductases on the colour development of nitrite- or nitrate-cured sausages. This was studied by fermenting sausages with three different strains of *Staphylococcus* with varying nitrite- and nitrate-reductase activities. The strains of *S. carnosus*, *S. simulans* and *S. saprophyticus*, were selected in an earlier study (Table 1). At appropriate time intervals during fermentation, surface reflectance and electron spin resonance (ESR) spectroscopy were used to measure colour and the specific amount of cured pigment, $MbFe^{II}NO$, respectively. A retail display simulation was subsequently performed with the manufactured sausages evaluating the effects of the *Staphylococcus* strains on colour and oxidative stability of sliced products during illuminated storage. Novel methods for assessing chemical changes relating to both colour and oxidation were implemented, namely multispectral image analysis using VideometerLab equipment and furthermore application of surface autofluorescence spectroscopy for evaluation of lipid oxidation.

2. Materials and methods

2.1. Selection of bacterial strains

Three *Staphylococcus* strains, *S. carnosus*, *S. simulans* and *S. saprophyticus*, were selected from a pool of isolates originating from either fermented sausages, bacon brine or cured meats. The selection of these three strains was based on their previously demonstrated differences (Gøtterup et al., in press) in nitrate/nitrite reduction capacity (Table 1). Their biochemical characteristics were assessed according to the API Staph test (Biomerieux, Marcy-l'Etoile, France).

2.2. Preparation of fermented sausages

Eight different batches were produced according to a standard recipe used at the Chr. Hansen A/S pilot plant facilities in Holdorf, Germany. Batches were prepared with either 160 ppm of nitrate or nitrite as curing agent, and one of the three *Staphylococcus* strains (5×10^6 CFU/g) or no *Staphylococcus*. All batches had *Lactobacillus sakei* (10^7 CFU/g) added as standard acidifying culture. The other ingredients were: lean pork meat (85%), pork back fat (15%), sodium chloride (27 g/kg), dextrose (6 g/kg), sodium ascorbate (0.5 g/kg) and white pepper (2 g/kg). Frozen pork meat and back fat were chopped and mixed with salt and spices in a bowl chopper (KG Wetter, Biedenkopf–Breidenstein Germany) and stuffed in 60 mm cellulose casings. The sausages were fermented and ripened in climate chambers for 21 days during which the temperature declined from 24 °C to 16 °C and the relative humidity dropped from 96% to 86%. The products were mildly smoked after 3 and 6 days of processing. Water activity, pH and weight loss of every batch were examined during the fermentation/ripening period, and these data, together with the experimental design for the eight batches, are summarised in Table 2.

Table 1
Enzyme activities of nitrate-, nitrite-reductase and catalase for the three selected staphylococci strains determined for viable cells (data obtained from Gøtterup et al. (in press))

Isolate no.	Strain	Nitrate-reductase[a] ($nmol \times min^{-1} \times ml^{-1}$)	Nitrite-reductase [a] ($nmol \times min^{-1} \times ml^{-1}$)	Catalase[b] ($\mu mol \times min^{-1} \times ml^{-1}$)
389	*S. saprophyticus*	nd[c]	6 ± 1.0	2.0 ± 2.6
392	*S. simulans*	796 ± 15	42 ± 2.2	2.7 ± 0.7
506	*S. carnosus* subsp.*carnosus*	439 ± 9	4 ± 0.7	4.5 ± 1.9

[a] Early stationary cells ($n = 3$) were cultivated anaerobically in the presence of 20 mM KNO_3 and suspended in phosphate buffer (100 mM; pH 7.0) to a final OD_{600} of 2.0. Nitrite was measured by Griess reagent.

[b] Early stationary cells ($n = 2$) were cultivated aerobically and suspended in phosphate buffer (50 mM; pH 7.0) to a final OD_{600} of 0.5. Catalase activity was assayed as the declining absorbance at 240 nm.

[c] Not detectable.