Formation of cured meat colour in meat model systems and fermented sausages by *Lactobacillus fermentum* strains (JCM1173 & IFO3956)

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Background

Cured meat products have an oxidative stability, which is markedly enhanced due to the addition of nitrite. However, the mechanism by which nitrite acts as an antioxidant is not yet fully understood. In this context, it is uncertain whether nitrite or the major pigment nitrosylmyoglobin (MbFe(II)NO) provides cured meat with its stability towards development of oxidative rancidity. A number of studies prove nitrite to posses antioxidative properties both in meat (1) and in meat model systems (2). The antioxidative mechanism of nitrite is ascribed to its ability to chelate free metal ions. However, other studies show that low molecular nitric oxide complexes and MbNO itself will act as an antioxidant even in very low quantities (3). Oxidative discoloration of cured meats converts the nitrosylated myoglobin to metmyoglobin (MbFe(III)), which is not only detrimental for appearance but may in addition have serious consequences for the oxidative status. The possible formation of carcinogenic nitrosamines from nitrite and secondary amines has recieved much attention over the years. The meat industry has shown interest in avoiding the application of nitrite and instead using other alternatives.

Microbial conversion of myoglobin has been investigated previously and several bacteria have been found capable of reducing MbFe(III) to MbFe(II), thereby making the coordination of molecular oxygen possible (4), thus changing the colour from brown to bright red. Others have reported certain strains capable of forming nitric oxide (NO), which in presence of MbFe(III) or MbFe(II) yields the cured meat pigment, MbFe(II)NO (5).

Objectives

The objective of the present study was to evaluate the ability of two *Lactobacillus fermentum* strains (IFO3956 and JCM1173) to convert MbFe(II)/MbFe(III) to MbFe(II)NO in either 1) model systems and 2) fermented sausages thereby providing the characteristic cured meat colour without addition of nitrite.

Methods

Bacteria cultures, *L. fermentum* IFO3956 and JCM1173, were kept at 4°C for three weeks and thereafter incubated for 18 hours at 37°C before being used for inoculation of MRS broth or sausages.

Man-Rogosa-Sharp (MRS) broth: Bacteria were incubated anaerobic in MRS broth (0.2% glucose) with or without MbFe(III) (2.0 mg/ml). Broth cultures with or without addition of manganese (Mn) were included. After 18 hours incubation bacteria cells were removed by centrifugation and absorption spectra were recorded. Samples without Mn were submitted to electron spin resonance spectroscopy.

Fermented sausages: A receipt for a salami type of sausage (3% salt and 30% fat) was used with the following four types of additions: 1) a commercial, acidifying *Pediococcus pentosaceus* (Chr. Hansen A/S), 2) 60 ppm nitrite, 3) *L. fermentum* IFO3956 and 4) *L.fermentum* JCM1173. Inoculation levels were between $10^4 - 10^6$ pr. gram sausage for the three starter cultures. Sausages were dried for 16 days during which they were submitted to tristimulus colourimetry, ESR spectroscopy, chemical and microbiological analyses.

Results and discussion

Figure 1 shows various absorption spectra obtained from MRS broth added MbFe(III). All samples inoculated with one of the two strains show spectral patterns characteristics of bright red colour. However, it is not possible solely based on spectral data to distinguish between the two complexes; MbFe(II)O_2 and MbFe(II)NO. Figure 2 shows ESR spectra of MRS broth without Mn and with added MbFe(III). These spectra indicate that paramagnetic myoglobin species, most likely MbFe(II)NO, have been formed in the samples, with the strain *L. fermentum* JCM1173 having a much higher content compared to *L. fermentum* IFO3956. The g-factor obtained for the two spectra is similar to previous reported values by (6), whereas the lack of hyperfine splitting can be explained by the presence of penta-coordinated complex of MbFe(II) and NO.

The sausages fermented with one of the three starter cultures all showed an increase in redness evaluated by the change in a-values from about 4-5 at day 2 to 8-10 at the end of the drying at day 18. The sausages added nitrite exhibited a-values around 12 from day 2 to 18. In addition, samples added starter culture all have a zone towards the outer edge, which is much more red compared to the centre of sausage. This is illustrated by a-values between 11-13 measured at the edge of sausages inoculated with starter culture, which is comparable the a-values obtained for sausages added nitrite.

The above-mentioned observation concerning measurements of colour is confirmed when submitting sausages to ESR spectroscopy. From Figure 3 and 4 it is evident that none of the two *L. fermentum* strains are capable of forming MbFe(II)NO in the centre of the sausage to any appreciable amount. However, when recording ESR spectra of sausage sample obtained from the edge of the three sausages fermented with starter culture, it is possible to obtain intense signals for MbFe(II)NO comparable to the amounts of cured pigment found in sausages added nitrite. In fact, the commercial *P. pentosaceus* starter yields signal intensities for MbFe(II)NO as high as *L. fermentum* JCM1173 does, whereas sausages added *L. fermentum* IFO3956 as observed in the MRS broth once again contains less MbFe(II)NO. It can be speculated that it is the availability of oxygen than determines the formation of MbFe(II)NO near

the surface of sausages without added nitrite, but still the microbial metabolism may affect the physico-chemical conditions, thereby either favouring or hindering formation of MbFe(II)NO.

Conclusions

Based on the present findings it seems that *L. fermentum* JCM1173 and IFO3956 to various degree can convert MbFe(III) to MbFe(II)NO in model system. In fermented sausages no formation of MbFe(II)NO are observed in the centre of the sausage, whereas high content of MbFe(II)NO is present in all fermented sausage in a 10 mm zone from the surface and inwards.

Pertinent literature

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Data

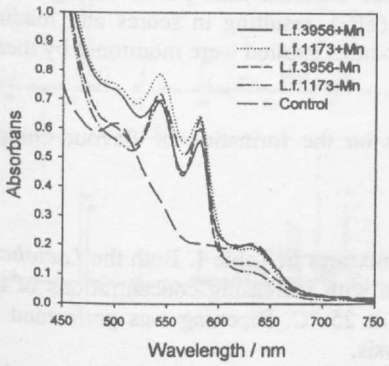

Figure 1. Representative absorption spectra of MRS broth added MbFe(III) with or without bacteria added.

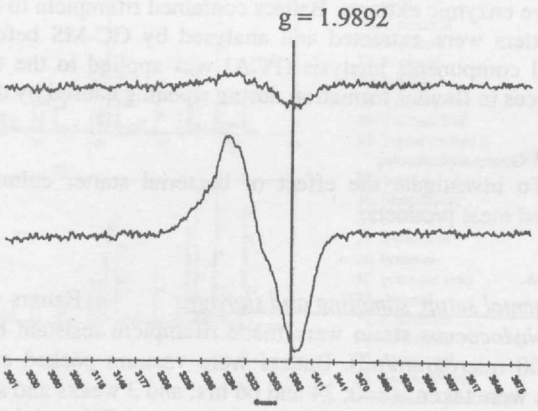

Figure 2. ESR spectra of MRS broth added MbFe(III) and *L. fermentum* IFO3956 (top) and JCM1173 (bottom). Spectra are recorded at 180°K

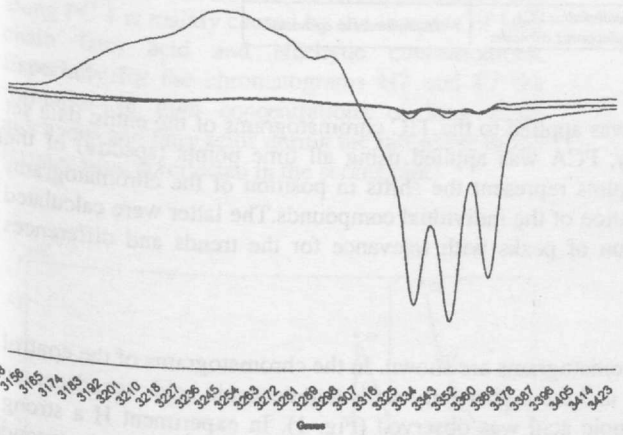

Figure 3. ESR spectra recorded at 140°K from the centre of salami sausages added nitrite (high intensity signal) and one of three starter cultures (weak signals).

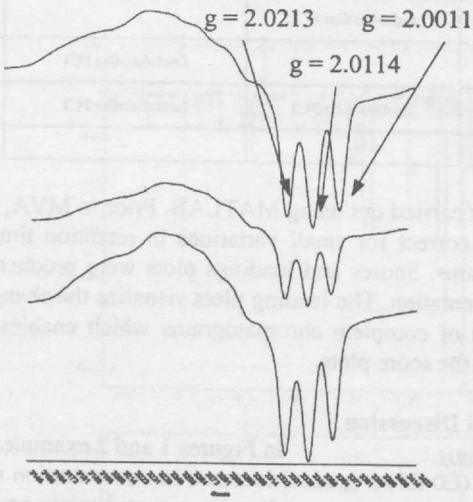

Figure 4. ESR spectra recorded at 140°K from the edge of salami sausages fermented with *L. fermentum* JCM1173 (top), *L. fermentum* IFO3956 (middle) and commercial *P. pentosaceus* (bottom).