CONVERSION OF METHMYOGLOBIN INTO RED MYOGLOBIN DERIVATIVE BY *PSEUDOMONAS FRAGI*

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Keywords: *Pseudomonas fragi*, *Brochothrix thermosphacta*, lipid oxidation, colour, myoglobin

Introduction

Meat colour largely results from the chemical state of myoglobin. Undesirable discolouration of meat during preservation is due to the accumulation of a myoglobin derivative, metmyoglobin (Renerre, 1990). Generally, bacteria increase metmyoglobin ratio. An increase of metmyoglobin occurs concomitantly with an undesirable increase in lipid oxidation. A common meat bacterium, *Pseudomonas* sp., decreased a red myoglobin derivative, oxymyoglobin (Bala *et al.*, 1977), via increased oxygen consumption (Chan *et al.*, 1998). On the other hand, Faustman *et al.* (1990) reported that ground beef homogenates inoculated with *Pseudomonas* sp. showed a decrease in metmyoglobin and a colour reversion from brown to red. In this study, we isolated *Pseudomonas fragi* from beef, and investigated these bacterial effects on meat colour and lipid oxidation.

Materials and Methods

Bacterial Preparation

The bacteria used in this study were obtained from slimy beef preserved at 4°C for 2 weeks. Single colonies were purified on nutrient agar (1.5% w/v) plates after 2 days of incubation at 4°C under aerobic conditions. Isolates were identified by the following criteria: cell morphology, motility, growth temperature, and 16S rRNA sequences. Regarding *Pseudomonas* sp., further identification was performed by sequencing *rpoB* (Tayeb *et al.*, 2005). Pure culture were maintained at 4°C in nutrient broth and subcultured at a weekly interval.

Inoculation test on Sterile Meat

Semitendinosus and *Longissimus* muscles of Japanese Black beef steers were used. The outsides of the muscles were sterilized by dipping them into boiling water for 5 s. Each sample was formed into a circular disk 5 cm in diameter and 1 cm in thickness. The pure cultures were inoculated meat upper surface (20 cm^2) with 1.0×10^4 cfu/cm^2. Each sample was placed in an autoclaved 60 ml weighing boat, overwrapped with oxygen-permeable PVC film and stored for 10 days at 4°C under fluorescent lighting. The light intensity was 2000 lx. Lipid oxidation was evaluated in terms of 2-Thiobarbituric acid reactive substances (TBARS) values. TBARS were measured in samples displayed for 0 and 10 days by spectrophotometric analysis (Witte *et al.*, 1970, Mitsumoto *et al.*, 1993) using Shimadzu UV-2400PC spectrophotometer (SHIMADZU CORPORATION, Kyoto). The TBARS values were expressed as mg of malonaldehyde (MDA) equivalents per kg of muscle. Meat colour was measured by reflectance spectrometry using the Shimadzu UV-2400PC, and the redness was expressed as an a^* value. The values were analyzed by the general linear model procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC).

Inoculation Test with 0.05% Myoglobin Containing Nutrient Broth

0.05% horse skeletal myoglobin (Sigma Chemical Co., St Louis, Mo) containing nutrient broth was prepared. After filter-sterilization with a 0.45 μm pore-size filter (Dismic-25CS, Toyo Roshi Kaisha, Tokyo), the pure cultures were inoculated with 1.0×10^7 cfu/ml. After incubation for 92 h at 25°C, the cells were removed by centrifugation (10,000 rpm, 5 min) and absorption spectra of the supernatant were measured from 650 to 350 nm at room temperature (23-25°C) using the Shimadzu UV-2400PC spectrophotometer.

Results and Discussion

Seven different type colonies were identified, five were identified as *Pseudomonas* sp. (including *Pseudomonas fragi*; *P. fragi*) and two were identified *Brochothrix thermosphacta* (*B. thermosphacta*). Both bacteria are commonly observed in chilled meat (Dainty and Mackey, 1992). *B. thermosphacta* did not change meat colour and lipid oxidation (Table 1). *P. fragi* significantly improved redness ($P < 0.05$), and showed a tendency to lower the increase in TBARS. This observation agreed with Bala *et al.* (1977). Nutrient broth with the presence of *B. thermosphacta* as well as no-inoculated nutrient broth showed the metmyoglobin spectrum pattern, with characteristic peaks at 503 nm (Sakata *et al.*, 1996) (Figure 1). The α and β absorption bands were 580 and 543 nm, respectively. The myoglobin derivative(s) converted by *P. fragi* had a spectrum

Table 1: Effect of growth of *B. thermosphacta* and *P. fragi* on meat redness and TBARS

Treatment	Redness (a^*)		TBARS	
	Day0	Day10	Day0	Day10
Control	21.8	8.1	0.1	2.1
B. thermosphacta	19.3	6.1	0.1	2.0
P. fragi	18.7	12.4 †	0.1	0.8

† Significantly different from the control ($P < 0.05$). n=6.

that was very similar to that of oxymyoglobin (Sakata *et al.*, 1996). Therefore, oxymyoglobin was presumably responsible for high a^* value of the *P. fragi* inoculated beef at day10.

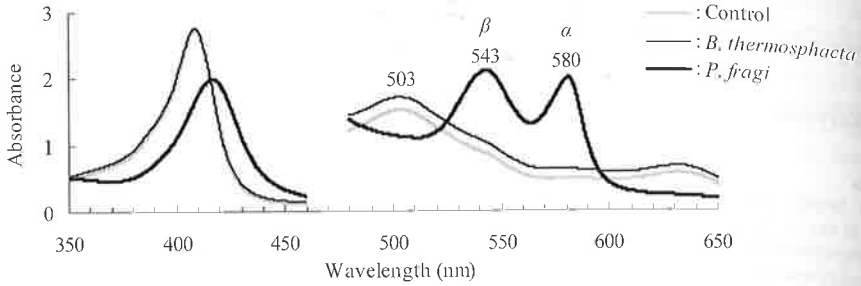

Figure 1: Spectra of bacteria in inoculated/non-inoculated 0.05%Mb-containing nutrient broth after 92h culture. The control contained sterile nutrient broth in place of cultures. The α and β absorption bands observed in *P. fragi*-inoculated-broth were 580 and 543 nm, respectively.

Conclusions

We isolated *P. fragi* from chilled beef. *P. fragi* improved meat colour during preservation. The improvement meat colour was probably due to the formation of oxymyoglobin. Today, a colouring agent for meat products, nitrite, is currently being questioned from the standpoint of human health. Nitrite may be a precursor of carcinogenic N-nitroso compounds (nitrosamines). Therefore, this bacterial metmyoglobin conversion system may be a key to a new technology for preserving and/or improving the appearance of meat and meat products.

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