STABILITY OF MYOGLOBIN DERIVATIVES UNDER FREEZING CONDITIONS

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Background and Objectives

Frozen beef has some problems, especially in discolouration during storage. In frozen beef, metmyoglobin(MetMb) production is accelerated by freezing-thawing treatment. Discoloured meat loses its value in the market place and is not highly regarded by customers. Cured meat pigment appeared essentially to be stable during freezing(Sakata et al.,1995a). The physico-chemical characteristics of cured meat pigment was compared with those of myoglobin(Mb) derivatives in a solution. The nitroso-Mb(NOMb) formed easily and was also stable towards an oxidizing agent(Sakata et al.).
As one of the objectives, the mechanism on incidence of discolouration was investigated, for that reason, an experiment to simulate discolouration was carried out in meat. A model solution was also prepared to study the effect of freezing on the stability of Mb derivatives, NOMb and oxy-Mb(MbO_2).

Experimental Methods

Frozen beef: A commercial vacuum-packed beef was obtained as an example of discoloured frozen meat. The beef was tranported from the USA in frozen cuts. The correct freezing period was unknown, but normally more than 3 months should have passed until the processing was done in Japan. At the meat packer, the cut was thawed, trimmed, sliced, vacuum-packed and frozen again. To bring about an artifical discolouration, fresh beef round and sirloin slices were purchased, vacuum-packed in barrier multilayer films and then frozen at -20 to -30℃. A part of the sirloin was minced, vacuum-packed, and then frozen.

Analysis of frozen beef: MetMb formation ratio(%) was measured with a surface reflectance(Sakata and Nagata,1992) and also with the buffer extraction of Mb from the sample(Trout,1990). Hunter-a value was estimated by a colour difference meter. TBA value was determined by the distillation method.
Model system: An experiment was prepared with a model solution using commercial Mb preparation. As a model, the pH5.5 reaction mixture was prepared containing Mb(1%) and ascorbic acid(AsA,0.5%), and then evacuated until bubbles in the sample solution could not be seen, to prepare MbO_2 as much as possible with a following oxygenation(SAKATA et al.). The solution was stored at 20℃ for 3 days, and then diluted to 1:10 with the medium(acetate buffer,pH5.5), evacuated and frozen. A Thunberg-type test tube and cuvette were used to evacuate and to mix the model solution. After 1 week of freezing, the sample was thawed and visible absorption spectra and MbO_2 %(against total Mb level) was measured. The second freezing was done with the thawed Mb solution by the same method as with the first freezing, and it was then frozen for over 1 month. NOMb was prepared with nitrite addition(0.1%) in the same system as for MbO_2 formation. The absorption spectra, NOMb% and residual nitrite content were determined with our method(Sakata and Nagata,1991; Sakata et al.,1995b).

Principal Results

Experiment with beef: The relative concentration of MetMb in the discoloured beef sample was 43% measured by both the surface reflectance and extraction method. As reported by Renerre(1990), sales decrease by a factor of 2 which is an important industrial problem, when the pigment on the meat surface comprises about 20% of MetMb. The discolouration could be seen optically. There were no significant differences found between all the samples of each of the analysed characteristics(meat colour and TBA value) for beef round slice in the case of being frozen repeatedly(3 times) during a 1 month period($p<0.05$). In this experiment apparent discolouration could not be noted even with repeated freezing and thawing. During the storage of the beef sirloin samples frozen for 5 months, MetMb formation proceeded and in particular the minced meat discoloured easily. MetMb formation showed 18.5% of the total Mb in the steak, while it showed more than half of the pigments in the minced meat(56%), which discolouration could be observed visually. During mincing of the meat, blooming occurred swiftly and then discolouration could be seen when the minced

red meat was vacuum-packed.

Model system: Fig.1(A) shows the absorption spectra of the Mb model solution over a 3 day period of reaction. The typical spectral pattern of MbO_2 could be seen and the initial MbO_2 formation ratio attained 86% at 3 days of storage. However it did not increase to more than that value. Thus, the following experiment on freezing was conducted using this reaction mixture of MbO_2. MbO_2 formation was reported to be difficult in a solution. The oxygenation of reduced-Mb(RMb) has been generally conducted to prepare MbO_2 (Siedler and Schweigert,1959). Though we used a similar method in this experiment, 100% MbO_2 formation could not be obtained. RMb appears to be essentially unstable compared with MbO_2, because it tends to be oxidized to MetMb(Hamm,1975). Fig.1(B) shows the absorption spectral pattern of the diluted Mb reaction mixture after freezing and thawing. The MbO_2 % decreased to 53% after 1 week of freezing. On the other hand, a cooled sample without freezing(2～3℃) held the formed MbO_2 concentration. By the 2nd freezing(refreezing) for 1 month after thawing of the Mb solution, formed MbO_2 decreased to ca.32% as shown in the dotted line. Regardless of the aerobic conditions, addition of $NaNO_2$ (0.1%) to Mb model system formed NOMb to 100% within 24hrs. The typical absorption spectrum of NOMb solution is shown in Fig.2(－). From these results, Mb tends to bind NO easily, and a higher affinity with NO than O_2 may be the reason for the stability of NOMb. Fig.2(--) shows the spectral pattern of NOMb solution thawed after refreezing for 1 month. Though NOMb level decreased gradually, its characteristic spectral pattern with 2 peaks could be seen. Even after 6 months of storage, a high level of NOMb remained and the red colour could be observed. With the numbers of freezing and its duration, residual nitrite content reduced considerably. Residual nitrite could not be detected after storage for 6 months. The decrease phenomenon of nitrite could be observed in cured meat when it was frozen(Sakata et al.,1995a).

From these results, MbO_2 formation showed to be difficult compared with NOMb, and the formed MbO_2 was essentially unstable toward freezing. The reason why the oxidation of MbO_2 to MetMb proceeds during freezing is presently not clear. It may be the reduced pO_2 pressure during storage. The discolouration can be controlled partly using films with high-permeability for oxygen or gas-packaging with abundant oxygen. Further investigations are necessary to solve such a meat discolouration.

Conclusions

Freezing of beef under our experimental conditions did not cause a considerable MetMb formation. Even with repetitive freezing and thawing, no negative effect could be noted on the colour of the fresh beef slices. MbO_2 was rather stable in meat cut but unstable in a model solution compared to NOMb. The stability of MbO_2 changed under various freezing conditions, e.g. storage period.

Pertinent Literature

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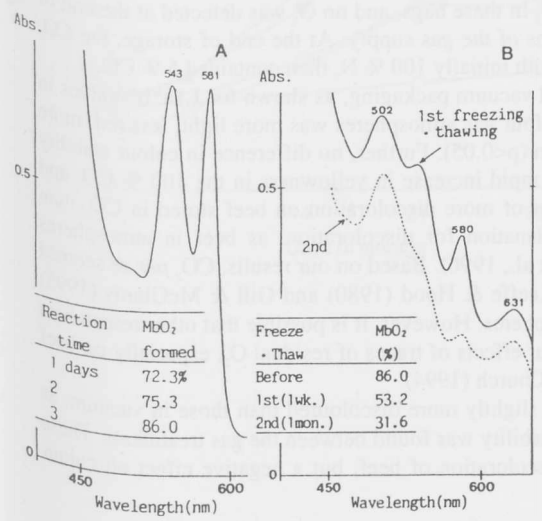

Fig.1. Absorption spectra of the Mb model solution after 3 days of reaction in the presence of AsA and oxygen(A) and after freezing and thawing treatment(B).

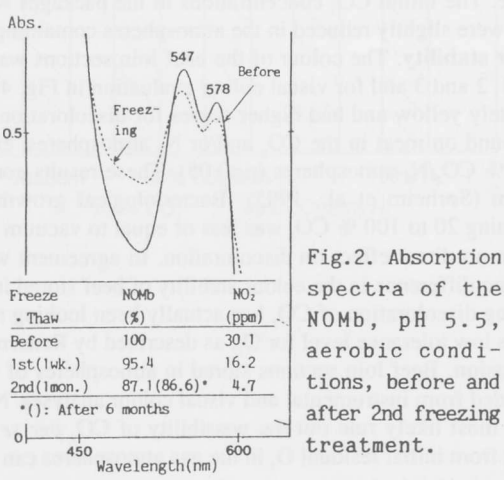

Fig.2. Absorption spectra of the NOMb, pH 5.5, aerobic conditions, before and after 2nd freezing treatment.