A novel method to stabilize meat colour: ligand coordinating with hemin

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Abstract Carbon monoxide (CO), L-cysteine and L-histidine were tested to coordinate with hemin chloride (pigment containing haem iron). In the presence of sodium dithionite, both CO and L-cysteine could react with hemin to afford respectively the corresponding complexes: CO-hemin and L-cysteine hemin; while L-histidine could not react with hemin. Both CO-hemin and L-cysteine hemin could decompose and release the corresponding ligand to generate hemin. Both light and temperature had an obvious effect on stabilization of these complexes. By sensory evaluation, both CO-hemin and L-cysteine hemin have bright red colour and show a potential as cured cooked-meat pigments (CCMP) in the manufacture of meat product.

Keywords Hemin · Coordination complex · Cured cooked-meat pigments (CCMP) · Ligand · Stabilization

Introduction

Colour that affects consumer decisions on the purchase of meat product is one of the most important sensory qualities (Mancini and Hunt 2005). Myoglobin (Mb) plays an important role in meat colour variation (Honikel 2008). However, Mb is not stable. Ferrous ion (Fe^{2+}) locating centrally in porphyrin ring of Mb is easily transformed to iron (Fe^{3+}), even porphyrin ring

of Mb is deteriorated when meat is exposed to oxygen atmosphere for a long period (Kerry et al. 2006). Originally, nitrates and/or nitrites are added to give the product its characteristic colour (Ordonez et al. 1999). Nitrosomyoglobin (NO-Mb) is formed by a reaction of Mb with nitric oxide during this process (Chasco et al. 1996). Modified atmosphere packaging (MAP) system is an effective method for fresh meat to keep pleasant colour (Behrends et al. 2003). In this case, Mb coordinates with oxygen to form stable oxymyoglobin ($\text{O}_2\text{-Mb}$) (Brody 1997). More recently, carbon monoxide (CO) acting as one of important ingredients for MAP has attracted a considerable interest (Søheim et al. 1999). It is considered to form carboxymyoglobin (CO-Mb) by a reaction of Mb with CO during this process (El-Badawi et al. 1964). In essence, stabilization of meat colour is realized by various ligands coordinating with Mb in all cases. Hemin is the important structural unit which locates centrally in Mb. Usually, ligand coordinating with Mb occurs in hemin moiety (Bonnett et al. 1978, 1980a, b).

Ligand field theory (LFT) shows that some low weight molecular containing S, O and N can bind Mb and stabilize meat color (Shi et al. 1990). So, nitric oxide (NO), carbon monoxide (CO), L-cysteine and L-histidine are promising ligands that can bind hemin to afford the corresponding complexes. Shahidi and his group (1996) prepared the CCMP-dinitrosylferrohemeochrome from hemin and nitric oxide. However, to our best knowledge, no report was found that CO, L-cysteine or L-histidine was applied to reacting with hemin for preparation of cured cooked-meat pigments (CCMP). The present paper aimed to develop an effective method that keep hemin an attractive and stable colour by a coordination of hemin with CO, L-cysteine, and L-histidine, respectively.

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Fig. 1 Absorption spectra of CO-hemin and hemine

Materials and methods

Materials and chemicals

Hemin chloride (pigment containing haem iron), L-cysteine and L-histidine were purchased from Sinopharm Chemical Reagent Co., Ltd. Other chemical reagents were purchased from Shanghai Boer Chemical Reagent Co., Ltd.

Reaction of hemin with carbon monoxide, L-cysteine and L-histidine

The hemin derivatives were prepared in the dimethyl sulfoxide (DMSO) model system similar to that described by Jankiewicz et al. (1994), but with modifications. The specific details were described as follows.

With carbon monoxide

Carbon monoxide (CO) was prepared by reaction of concentrated vitriol with oxalic acid under heating condition (60–70 °C). The resulting CO was filtered with solution of sodium hydroxide.

To a mixture of 3.0 mg of hemin in 18 ml of DMSO, 30 mg of sodium dithionite, vitamin C or sodium sulfite dissolved in

2 ml of distilled water was added. pH of the mixture was adjusted to 8.0. Subsequently, the resulting CO was introduced to this reaction mixture for 30 min. 1.0 ml of the reaction mixture was transferred to a 100 ml volumetric flask, and then diluted to the scale with dimethylsulfoxide and analyzed directly by a UV-1600 spectrophotometer.

With L-cysteine

To a mixture of 3.0 mg of hemin in 18 ml of dimethyl sulfoxide, 30 mg of sodium dithionite vitamin C or sodium sulfite dissolved in 2.0 ml of distilled water and 3.0 mg of L-cysteine dissolved in 5.0 ml of dilute hydrochloric acid (0.025%) were added in turn with a stirring at room temperature. The reaction was carried out for 30 min. 1.0 ml of the reaction mixture was transferred to a 100 ml volumetric flask, and then diluted to with dimethyl sulfoxide and analyzed directly by a UV-1600 spectrophotometer.

With L-histidine

To a mixture of 3.0 mg of hemin in 18 ml of dimethyl sulfoxide, 30 mg of sodium dithionite vitamin C or sodium sulfite dissolved in 2.0 ml of distilled water and 3.0 mg of L-histidine dissolved in 5.0 ml of dilute hydrochloric acid (0.025%) were added in turn with a stirring at room temperature. The reaction was carried out for 30 min. 1.0 ml of the reaction mixture was transferred to a 100 ml volumetric flask, and then diluted to the scale with dimethyl sulfoxide and analyzed directly by a UV-1600 spectrophotometer.

Stability of CO-heme and cys-hemin

CO-heme and cys-hemin were stored in the sealed tube under two different conditions: (1) exposed to natural light at 25 °C for 0, 2, 4 and 6 h; (2) exposed to natural light at 4, 25 and 65 °C for 3 h; and (3) placed in the darkness at 25 °C for 5 h. The mixture was monitored by a UV-1600 spectrophotometer.

Fig. 2 Absorption spectrum of cys-hemin

Spectroscopy

Vis absorption spectra are good to show what are hemin and its complexes. Therefore, vis absorption spectra of the hemin and its complexes were measured with a UV-1600 spectrophotometer (Rayleigh Analytical Instrument Corp.). The spectra were recorded from 300 to 700 nm at the scanning rate of 100 nm/min. An 80% (v/v) dimethyl sulfoxide-water mixture was used as the blank.

Results and discussion

As shown in Fig. 1, two new absorption peaks locating at wavelength of 527 nm and 571 nm respectively appeared in the spectrum of CO-treated hemin. The result showed that hemin reacted with carbon monoxide to generate a new compound, carbonyl-heme (CO-heme). However, the reaction could not process in the absence of sodium dithionite. When sodium dithionite was replaced by other reducing agents such as vitamin C or sodium sulfite, the reaction could not process smoothly. Therefore, sodium dithionite was necessary for this reaction. The role of sodium dithionite is to reduce iron in hemin into ferrous ion. Usually, ferrous ion in hemin is easily oxidized to into iron (Pegg and Shahidi 1996), and the colour varied correspondingly from bright red to dark black. The stability of ferrous ion in hemin increase by a coordination with CO (Shi et al. 1990), and thus resulting in the increase of colour stability. CO-heme shows a potential as CCMP in view of its attractive colour. The prior reports showed that the reaction of NaNO_2 with haemoproteins under mildly acidic conditions could occur at the ferrous ion to give the nitrosylhaem pigment (Bonnett et al. 1980b), in the porphyrin ring (Bonnett et al. 1978, 1980a) or in the protein (Bonnett and Nicolaidou 1979). In 1985, Shahidi and his cooperators (1996) prepared CCMP from hemin and nitric oxide. Later, Shahidi and his cooperators (1996) revealed that only one molecule of NO ligated itself to the iron of the porphyrin

Fig. 4 Absorption spectra of CO-hemin stored in the darkness or exposed to natural light at 25 °C

molecule. Similarly, CO-heme in present study was formed possibly either by coordination of CO ferrous ion of hemin or by a reaction of CO and the porphyrin ring of hemin. More experimental details are required to elucidate its chemical structure.

Subsequently, L-cysteine and L-histidine were examined under the same condition. The reactions were detected by a UV-1600 spectrophotometer. When L-cysteine was added to the mixture for 30 min, the characteristic peaks that located at wavelength of 418 nm, 515 nm, 549 nm and 616 nm respectively appeared (Fig. 2). These results showed that L-cysteine could react with hemin under the present condition. Also, sodium dithionite was necessary. However, no new peak appeared when L-histidine was added to the mixture. It showed that L-histidine could not react with hemin under the present condition. Similar to CO-hemin, cys-hemin exhibits bright red and has a potential as CCMP in the manufacture.

Stability is one of the most important qualities for CCMP. So, stability of CO-hemin and cys-hemin was also investigated.

The samples of CO-heme were exposed to natural light at 25 °C for 0, 2, 4 and 6 h and were detected by a UV-1600 spectrophotometer, respectively. The results were shown in Fig. 3. From Fig. 3, it was found that the two characteristic

Fig. 3 Absorption spectra of CO-hemin exposed to natural light at 25 °C for different time

Fig. 5 Absorption spectra of CO-hemin exposed to natural light at 4, 25 and 65 °C

Fig. 6 Absorption spectra of cys-hemin exposed to natural light at 25 °C for different time

peaks of CO-heme locating at wavelength of 527 nm and 571 nm were weakened gradually and disappeared basically for 6 h. The results showed that CO-heme was decomposed to generate another product during this process. By a careful analysis of these spectra, the resulting product was ascribed to hemin, indicating that the coordination of hemin with CO was reversible (Kakar et al. 2010) and CO-heme released free CO during this process. It was investigated on the effects of light affected CO-heme stability. As shown in Fig. 4, in addition to spectrum of CO-heme in the darkness had no obvious variance, the characteristic peak of CO-heme spectrum weakened significantly. Also, the effects of heat on CO-heme stability were investigated. Figure 5 showed the results that CO-heme was stored at different temperatures (4, 25 and 65 °C) for 3 h. From Fig. 5, it was found that the characteristic peaks of CO-heme disappeared completely at 65 °C, weakened significantly at 25 °C and had no obvious variance at 4 °C. These results showed that both light and heat fastened the decomposition of CO-hemin. It was observed that light and heat had the similar influences on carboxy-heme in carboxyl-hemoglobin (Seto et al. 2001; Rothberg et al. 1990).

Similarly, cys-hemin was dissociated to give hemin at room temperature (Fig. 6). In comparison to CO-heme, cys-hemin was relatively stable. In addition to 5 h for CO-heme decomposition, it took 26 h for cys-hemin to decompose itself completely under the same condition. The coordination theory notes that hemin coordinating with ligand can lead to crystal energy decrease (ΔE). ΔE reflects the complex's stability. It depends on the property and structure of ligand. The stability difference of these two complexes was possibly resulted from the diversity of ligand. Also, both light and heat could affect this process.

Conclusion

In conclusion, hemin reacted with CO and L-cysteine to afford the corresponding complexes in the presence of sodium dithionite. These two complexes can decompose to release the corresponding ligand and generate hemin. In addition to CO-hemin, cys-hemin is more stable. Both light and heat

fastened this process. CO-hemin and cys-hemin are bright red, and thus showing potentials as CCMP in the manufacture of meat product. The investigation about effects of CO-hemin and cys-hemin on the colour of meat product is in progress in our laboratory.

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