

## Research Article

### Effect of Glycosylated Nitrosohemoglobin on Quality of Cooked Meat Batters during Chill Storage

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**Abstract:** Sodium nitrite is a key traditional meat-curing agent in meat industry. However, because of its carcinogenicity, the studies about nitrite substitutes have been focused on for many years. In this study, Glycosylated Nitrosohemoglobin (G-NO-Hb) solution synthesized by porcine blood, nitrite and sugar through maillard reaction was applied in cooked meat batters to replace for nitrite. Color difference, Thiobarbituric Acid-Reactive Substances (TBARS) and total Aerobic Plate Count (APC) were determined in order to evaluate the quality of meat batters. UV-Vis spectra analysis showed that the produced pigment through maillard reaction was G-NO-Hb. The  $a^*$ -values of batters treated with G-NO-Hb showed a significant increase ( $p < 0.05$ ) compared to control sample and had no significant difference compared to that of nitrite treated samples ( $p > 0.05$ ), which indicated that the addition of G-NO-Hb contributed to the formation of red pigment in cooked meat batters. Also, both TBARS values and APC of meat batters treated with G-NO-Hb, especially those added with G-NO-Hb solution (6 g nitrite/kg reaction system) were significantly lower than the control samples ( $p < 0.05$ ). It revealed that G-NO-Hb was a potential nitrite substitute for coloring, antioxidation and antisepticise during meat curing.

**Keywords:** Glycosylated nitrosohemoglobin, meat batters, sodium nitrite, thiobarbituric acid-reactive substances

## INTRODUCTION

Nitrite, an conventional meat-curing agent, has been extensively used in meat and meat products for hundreds of years by virtue of its multi-functionality in meat curing, containing formation of the characteristic pink color of cured-meats (Mancini and Hunt, 2005), production of the specific cured flavor (Cho and Bratzler, 1970), preventing the formation of warmed-over flavor in cured products and inhibiting the germination of spores and toxin formation by *Clostridium botulinum* (Al-Shuibi and Al-Abdullah, 2002). Despite all of the desired properties, nitrite can react with secondary amines and amino acids in muscle proteins to form carcinogenic and teratogenic N-nitrosamines (Honikel, 2008; Stuff *et al.*, 2009), therefore, the meat industry have been searching for alternatives of nitrite in cured meats, especially for its color formation, antioxidative and antimicrobial effect.

Over the past five decades there has been increasing research interest in the development of nitrite-free meat-curing systems. This has resulted in alternatives to the use of nitrite in cured meats for color, antioxidative or antimicrobial effects. Therefore, a large number of colorants to replace nitrite have been studied. Shahidi and Pegg (1990) reported that a novel

cooked cured-meat pigment synthesized by the reaction of beef red blood cells with nitrite directly or indirectly through a hemin intermediate was responsible for the characteristic pink color of cured meats. Frankfurters with lower levels of sodium nitrite (50 mg/kg) and 12% tomato paste had the highest redness values ( $p < 0.05$ ) among all the experimental samples (Deda *et al.*, 2007), while annatto powder, as a natural colorant, was found to replace for 60% of the nitrite addition in two different formulations of sausage (Zarringhalami *et al.*, 2009). More recently, *Lactobacillus fermentum* was found to produce cured pink color in a Chinese-style sausage to replace for nitrite (Zhang *et al.*, 2007). Additionally, more other substitutes for color formation, such as histidine and monascus pigment have been also developed.

Although these substitutes have been studied and various patents have been filed on them, none had reached the commercial stage for some reasons (Shahidi and Pegg, 1992). The aim of this study was to evaluate the effect of Glycosylated Nitrosohemoglobin (G-NO-Hb) synthesized from porcine blood cells, nitrite and sugar on the color, antioxidative and antimicrobial attributes of cooked meat batters during chill storage.

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Table 1: Formulations of raw pork batters different treated

Sample	NaNO <sub>2</sub>	G-NO-Hb-1	G-NO-Hb-2	G-NO-Hb-3	G-NOHb-4
Control	-	-	-	-	-
NaNO <sub>2</sub>	100 mg/kg	-	-	-	-
G-NO-Hb-1	-	2 mL	-	-	-
G-NO-Hb-2	-	-	2 mL	-	-
G-NO-Hb-3	-	-	-	2 mL	-
G-NO-Hb-4	-	-	-	-	2 mL

## MATERIALS AND METHODS

**Materials:** Fresh porcine red blood cells were collected from porcine blood supplied by Heilongjiang Baodi Agri and Tech Co., LTD and then immediately frozen at -20°C. Porcine rump was purchased from a local retail meat market. Sodium nitrite, glucose and sodium ascorbate are of food grade. All other chemicals and solvents are of analytical grade.

### Methods:

**Preparation of G-NO-Hb:** NO-Hb was prepared according to the method of Shahidi and Pegg (1991) with slight modifications. Red blood cells were thawed overnight at 4°C before use. One kg Porcine red blood cells were added into 2 kg distilled water and then stored at 4°C for 3 h with intermittent stirring. Reducing agent (sodium ascorbate), glucose and different levels of sodium nitrite (9, 12, 15, 18 g, respectively) were then added to formulate different glycosylated reaction systems which were named G-NO-Hb-1, G-NO-Hb-2, G-NO-Hb-3, G-NO-Hb-4, respectively. The systems were heated at 60°C in water bath for 15 min with intermittent stirring with a glass rod and then cooled in ice water to room temperature. After cooling to room temperature, the systems were stored in the dark at 4°C. All these operations were carried out in a darkened condition.

**UV-Vis spectral analysis:** UV-Vis spectral analysis was used to identify the final product, as different heme derivatives have different characteristic absorption bands (Millar *et al.*, 1996). Spectral analysis was performed according to the method of Arihara *et al.* (1993) and Xu and Verstraete (2001). The absorption scans were made from 350 to 700 nm at 1 nm increments using a UV-Vis spectrophotometer (UV-6000PC; Shanghai Metash Instruments, Co., Ltd., China).

**Preparation of cooked pork batters:** Under aseptic condition, the fresh porcine rumps were trimmed off connective tissues and ground through 4 mm plates in a cold room. Six batches of raw pork batters (100 g for each) were formulated as described in Table 1. The batters were thoroughly mixed, vacuum packaged in sterilized plastic bags, heated at 90°C for 20 min, cooled to the room temperature and then taken out of the bags and immediately placed onto Styrofoam trays. Oxygen permeable polyethylene film (Miaojie Co Ltd., China) was over-wrapped on the surface of the trays. The samples were stored at 4°C for 15 days. Color

difference, Thiobarbituric Acid-Reactive Substances (TBARS) and total Aerobic Plate Count (APC) were determined at day 0, 3, 6, 9, 12 and 15 during storage.

**Color evaluation:** Color difference was measured using a ZE-6000 colorimeter (Nippon Denshoku, Kogyo Co., Tokyo, Japan). The color differences of all samples were performed at day 1 after the cooked batters were prepared. The results were shown as *L\**-value (lightness), *a\**-value (redness) and *b\**-value (yellowness). The instrument was calibrated using a white standard plate (*L\** = 95.26, *a\** = -0.89, *b\** = 1.18). All the treatments were analyzed using a 3 cm port size sample-plate, as described by McClure *et al.* (2001). The values were gained from three different parts of pork batters, each with three different sample-plate directions.

**Thiobarbituric Acid-Reactive Substances (TBARS):** The TBARS values were determined according to the method of Sinnhuber and Yu (1958) with slight modifications as described by Kong and Xiong (2006).

**Aerobic plate count:** The batters (25 g) was removed from each treatment under aseptic conditions and homogenized in a sterile Waring blender containing 225 mL 0.9% normal saline. After shaking at 230 rpm for 10 min, 1 mL of this suspension was serially diluted in triplicate (1:10) using 0.9% normal saline. For the enumeration of total APC, 0.1 mL volumes of appropriate serial dilutions of homogenized samples were spread on the surface of Plate Count Agar and incubated at 37°C for 48 h. Colonies developing on plates were counted. Results were expressed as log<sub>10</sub> numbers of colony forming units/grams (cfu/g).

**Statistical analysis:** All specific experiments were repeated at three times. Statistical analysis was performed using Statistix 8.1. Analysis of Variance (AOV) was done to determine the significance of the color difference (values of *L\**, *a\** and *b\**) between different samples. Significant differences (p<0.05) between means were determined using Least Significant Difference procedures.

## RESULTS AND DISCUSSION

**Identification of G-NO-Hb:** Absorbance at 540 nm was regarded as an indicator to quantify the

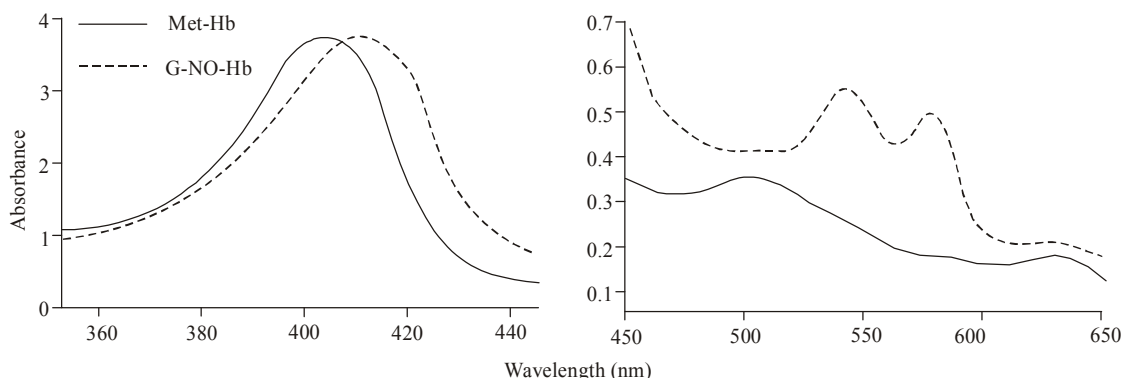


Fig. 1: Absorption spectra of Hb and G-NO-Hb from 350 to 700 nm

Table 2: Maximal absorbance maxima of aqua heme derivatives<sup>a</sup>

Heme derivatives	Absorbable maxima (nm)	
Reduced-heme	434	557
Oxy-heme	418	544
Nitroso-heme	421	548
Met-heme	410	505

<sup>a</sup>: Cited data are from Millar *et al.* (1996)

Table 3: Influence of different G-NO-Hb solutions on the color of cooked meat batters

Sample	L*-value	a*-value	b*-value
Control	65.24±1.22a	8.23±0.25b	13.54±0.4b
NaNO <sub>2</sub>	66.79±0.84a	14.17±0.64a	14.31±0.30ab
G-NO-Hb-1	66.43±0.64a	14.59±0.91a	14.54±0.44ab
G-NO-Hb-2	66.21±0.41a	15.06±0.42a	14.91±0.58ab
G-NO-Hb-3	65.82±0.93a	15.68±0.78a	15.21±0.50ab
G-NO-Hb-4	65.43±1.15a	16.23±0.57a	15.39±0.52a

<sup>a,b</sup>: Different letters in the same column indicate significant differences (p<0.05)

development of G-NO-Hb, while UV-Vis spectral scanning was used here to identify its generation. The absorption spectra of appropriate dilutions of the fresh prepared G-NO-Hb solution and Hemoglobin (Hb) solution held at room temperature for 7 days were determined to identify the formation of G-NO-Hb, as different heme derivatives have characteristic bands depending primarily on the bound state of the central iron and the molecule (Millar *et al.*, 1996). The control sample showed distinctive bands at around 410, 505 and 635 nm (Fig. 1), which were the characteristic peaks of Met-Hb in aqueous solution (Table 2). In contrast, the synthesized G-NO-Hb solution had absorption peaks at 420, 542 and 578 nm after maillard reaction, which demonstrated the formation of G-NO-Hb.

**Influence of different G-NO-Hb on the color of meat batters:** The a\*-values of cooked meat batters added with G-NO-Hb showed a significant increase (p<0.05) compared to that of control and had no significant difference compared to nitrite treated samples (p>0.05) (Table 3), which indicated that the addition of G-NO-Hb had a positive influence on the red color formation of cured meat batters. a\*-values of the meat batters added with different G-NO-Hb solutions had no significant difference (p>0.05). Furthermore, there was

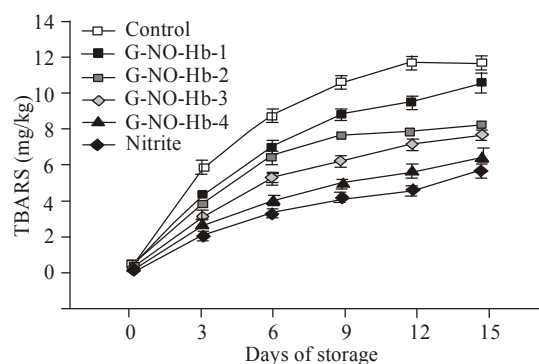


Fig. 2: TBARS values of cooked meat batters different treated  
Error bars refer to the standard deviation obtained from three analyses

no difference (p>0.05) on L\*-values among all samples. The colorimetric results were in excellent agreement with the observations of Chasco *et al.* (1996). Therefore, G-NO-Hb could be synthesized as a colorant to substitute for nitrite in meat curing.

**Thiobarbituric acid reactive substances:** TBARS were measured in meat products as indicators of lipid oxidation. The changes of TBARS values of different treatment samples during refrigerated storage were shown in Fig. 2. The TBARS values of all samples increased as refrigerated storage time prolonged, while the samples added with G-NO-Hb or nitrite had lower TBARS than control sample. However, all the samples treated with four different G-NO-Hb solutions had higher TBARS than nitrite-treated sample. At day 15, the TBARS value of sample treated with G-NO-Hb-4 had no difference compared with that of the nitrite-cured sample (p>0.05). This was probably due to the antioxidation of residual nitrite from the G-NO-Hb reaction system, as well as G-NO-Hb, the maillard reaction products (Chang *et al.*, 2011; Miranda *et al.*, 2011). Therefore, the G-NO-Hb-4 solution was chosen as the nitrite substitute for antioxidation in meat batters during chill storage.

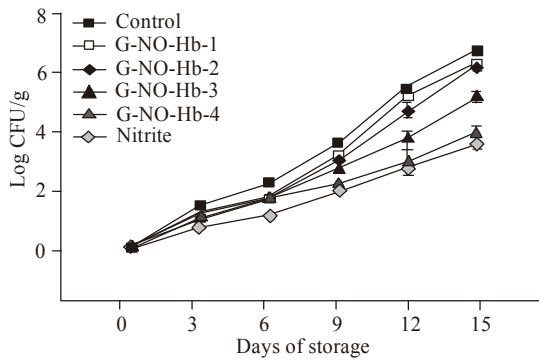


Fig. 3: APC of cooked meat batters different treated  
Error bars refer to the standard deviation obtained from three analyses

**Aerobic plate count:** The APC of all samples increased during chilled storage. After storage of 15 days, the APC of control and samples treated with G-NO-Hb-1 and G-NO-Hb-2 exceeded 6 Logcfu/g, which surpassed the standard level of the fresh meat, while the other treatments were above the level. Furthermore, the samples treated with G-NO-Hb-4 had slightly higher APC than those treated with sodium nitrite, but not significant ( $p>0.05$ ). It could be concluded that G-NO-Hb had a positive effect on inhibiting the microbial growth, which was in good agreement with the results that Maillard Reaction Products (MRP) had antimicrobial activity (Daglia *et al.*, 1994; Huang *et al.*, 2007). Another explanation was the iron chelation and residual nitrite from the G-NO-Hb solution (Rufián-Henares and De la Cueva, 2009). It has been reported that chitosan exhibits extensive spectrum antimicrobial activity against many bacteria (Dutta *et al.*, 2009), therefore the glucose could be replaced by chitosan as the saccharide source for synthesizing G-NO-Hb to reinforce the antibacterial effect.

### CONCLUSION

Glycosylated Nitrosohemoglobin (G-NO-Hb) was successfully prepared via maillard reaction and the synthesized G-NO-Hb pigment contributed to the formation of red color in cooked pork batters. It was also conformed four G-NO-Hb samples with different amounts of nitrite added had a positive influence on protecting the lipid oxidation and inhibiting the outgrowth of unwanted bacteria in cooked meat batters during chill storage. Moreover, the G-NO-Hb solution (6 g nitrite/kg reaction system) was found to exhibit the strongest antioxidative and antimicrobial activity. Additional research is required to evaluate the influence of G-NO-Hb on the flavor of meat products (Fig. 3).

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