Effects of High Pressure and Sodium Nitrite Levels on Cured Color Development and Residual Nitrite Concentration in Pork Homogenates

Geun-Pyo Hong, Ji-Sook Kim, Ji-Yeon Chun, and Sang-Gi Min*

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea

Abstract

This study investigated the effects of high pressure with or without thermal treatment on the cured color development and residual nitrite contents of model meat systems (pork, NaCl and sodium nitrite). At low nitrite levels (≤ 50 $\mu\text{g/g}$), 200 MPa of pressure alone (P) did not develop the cured meat color ($p > 0.05$). Thermal treatment (T) showed curing pigmentation (higher CIE L^* and CIE a^*), and the impacts were more effective when pressure was combined with thermal treatment (PT). In contrast, nitrite levels did not contribute to the cured meat color when ≥ 200 $\mu\text{g/g}$ of nitrite was added to the meat. At high nitrite levels, although the typical cured color that is induced by thermal treatment did not present by pressure alone, the PT treatment still showed a pinker color with low residual nitrite content compared to the T treatment. The higher the pressure level (300 MPa), the greater the cured meat pigmentation with lower residual nitrite. Therefore, the present study demonstrates the possible application of high pressure, both for cured pigmentation and reducing residual nitrite, respective to typical thermal treatments.

Key words: pressure, reducing activity, cured pigment, residual nitrite, oxidation

Introduction

It has been widely recognized that pressurization elongated the shelf-life of foods without sensorial and nutritional losses (Zhou *et al.*, 2010). For protein-based food products, particularly meat and meat products, high pressure processing modifies protein functionality thereby providing unique textural properties in foods distinguishable from typical thermal treatment (Ma and Ledward, 2004). The main advantages of high pressure treatment of products include better water-binding and rheological properties (Cheftel and Culioli, 1997). These benefits of high pressure treatment are especially identified in pressure treated meat products. According to available literature, high pressure can enable the reproduction and restructuring of value-added meat and meat products without thermal treatment (Ma and Ledward, 2004). Contrarily, meat discoloration induced by high pressure limits the potential application of high pressure techniques on fresh meat (Hong *et al.*, 2007). Pressure-induced meat discoloration

has been reported in numerous studies (Carlez *et al.*, 1995; Hong *et al.*, 2007). As reported by Carlez *et al.* (1995), denaturation of myoglobin (Mb), displacement or release of heme, and oxidation of ferric metMb were major attributions involved in the mechanisms for meat discoloration under high pressure. In addition, these changes under high pressure have a strong relationship with lipid oxidation in pressure treated meat (Carlez *et al.*, 1995; Ma *et al.*, 2007). The formation of cured pigments would be one possible way to prevent pressure-induced meat discoloration (Carlez *et al.*, 1995).

Sodium nitrite (NaNO_2) is a key ingredient in the meat curing process. It has various functions in meat systems such as antimicrobial, antibotulinal, as well as antioxidation properties (Reddy *et al.*, 1983). It is believed that the metabolites of nitrites (HNO_2 and NO) react with Mb rather than nitrites themselves (Honikel, 2008). Generally, the formation of cured meat pigments can be simplified into a three stage reaction, i.e., nitric oxide (NO) formation, binding NO to Mb, and denaturation of the protein portion of Mb; although various branched metabolisms also present (Fujimaki *et al.*, 1975). Exogenous or endogenous reductants play roles of importance and are essential to reduce nitrous acid anhydride (N_2O_3) to NO, which is the final step in nitrite degradation (Honikel,

*Corresponding author: Sang-Gi Min, Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-450-3680, Fax: 82-2-455-1044, E-mail: foodeng301@paran.com

2008; Pegg and Shahidi, 2000). Therefore, to accelerate the curing process, reductants, especially ascorbate, are used in meat product formulations. In addition, reductants are also responsible for nitrate formation, which is a crucial factor to reduce residual nitrite (Lee *et al.*, 1978). Consequently, Mb, nitrite, and reductants (mainly ascorbate) are the essential ingredients for the meat curing process.

Despite endogenous reducing systems in meat losing their reducing activity post-mortem, Lee *et al.* (1975) reported that nitrate formation by endogenous reductants accounted for half of that formed by ascorbate. For pressurized meat, it is believed that endogenous reducing systems cannot maintain their activity after high pressure, and oxidative reactions in pressurized meat generally dominate (Carlez *et al.*, 1995). However, these oxidative reactions have mainly been shown when meat is treated with high pressure at greater than 350 MPa (Carlez *et al.*, 1995) or at sub-zero temperatures for a long time (Hong *et al.*, 2007). Interestingly, Cheah and Ledward (1997) observed better reducing activity in moderately pressure-treated meat (80 MPa). These observations were also supported by Jung *et al.* (2003) where the metMb content of pressurized beef decreased to around 350 MPa and then increased. Cheah and Ledward (1997) suggested that oxygen consuming systems, which are responsible for the oxidative reactions of meat, are pressure-sensitive compared to reducing systems. It is likely that the pressure tolerance of reducing systems is maximal at about 350 MPa, although the maximum tolerance should vary depending on various factors such as pH, temperature, and holding time (Jung *et al.*, 2003). In addition, heme displacement at extremely high pressure (>400 MPa) seems to facilitate oxidative reactions in pressure treated meat (Carlez *et al.*, 1995; Ma *et al.*, 2007). From the above points of view, it is suggested that high pressure can enhance the meat curing process and reduce residual nitrite levels due to better reducing activity in meat.

Among the research on meat curing, although various approaches have been made, including low or nitrite-free curing systems or substitutions of nitrite for functional ingredients (Heaton *et al.*, 2000; MacDougall and Hetherington, 1992; Shahidi and Pegg, 1992), little information on high pressure during the meat curing process is available. Therefore, this study aimed to investigate the effects of high pressure combined with thermal treatment on cured meat pigmentation and on residual nitrite levels using model meat systems.

Materials and Methods

Sample preparation and treatment

Pork loins (*M. longissimus dorsi*) were obtained randomly from six carcasses at 24 h post-mortem. All fat and connective tissue were trimmed, and the meat was ground twice using a 2 mm plate. For the experiment of nitrite levels, the minced meat was mixed with 1.5% NaCl with desired amounts of sodium nitrite. For the low nitrite treatments, 0-50 µg/g of sodium nitrite was added to samples while 200-300 µg/g was used in the case of the high nitrite treatments. Approximately 20 g of aliquotted sample was stuffed into a cellulose casing and vacuum packaged in a polyethylene pouch. The samples were stored at 4°C for 24 h, and divided into 4 treatments: non-treated control (C), pressure treatment (P), thermal treatment (T), and pressure followed by thermal treatment (PT). To pressurize the samples, lab scale high pressure equipment was applied as described in Hong *et al.* (2007). The temperature inside the vessel was maintained at 4°C throughout the pressure treatment, and ethanol was used as the pressure transmitting medium. The compression and depression rates were 2.7 and 20 MPa/s, respectively, and pressurization was conducted at 200 MPa for 15 min. The thermal treatment was carried out in a 75°C water bath for 15 min, and cooled in an ice slurry for 15 min. All samples were analyzed after treatment without further storage. To evaluate the effects of pressure variations, minced pork was mixed with 1.5% NaCl and 200 µg/g of sodium nitrite and packaged using the same method described above. The samples were pressurized up to 300 MPa (maximum pressure of the device) for 15 min at 4°C and used as the pressure treatment (P) group. After this pressure treatment, half of the samples were heated at 75°C for 15 min and cooled in an ice slurry for 15 min, and were designated as the pressure followed by thermal treatment (PT) group. For replications, all procedures were repeated on three different days.

Color

Immediately after the treatments, CIE Lab color values were measured from the surface of samples with a color reader (CR-10, Konica Minolta Sensing Inc., Osaka, Japan) calibrated with a white standard plate ($L^* = +97.83$, $a^* = -0.43$, $b^* = +1.98$). CIE L^* , a^* , and b^* values were determined as indicators of lightness, redness, and yellowness, respectively. Six replicate determinations were made from each sample surface. The means of the CIE L^* , CIE a^* , CIE b^* color parameters were visualized

using Adobe Photoshop CS5 software (Adobe Systems Inc., USA) and compared to the instrumental color. The total color difference was calculated from the instrumental color as follows:

$$\text{Total color difference} = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*}$$

where the color values of the thermal treated sample (0.1 MPa) were used as references.

Cured pigment content

Nitrosylhemochrome (NHc) concentration was measured by the method of Heaton *et al.* (2000). A sample was diced into 3 mm cubes and 1 g of sample was immersed into 9 mL of 90% acetone. The sample solution was stirred gently for 10 min, and then filtered using a Whatman 42 filter paper. Absorbance was measured in duplicate at 540 nm and cured pigment content (ppm) was calculated by multiplying by a factor of 290.

Residual nitrite content

Residual nitrite was determined according to the AOAC method (1990). Five grams of each ground sample was placed in an individual 500 mL volumetric flask to which approximately 300 mL of preheated distilled water was added. The mixtures were heated for 2 h at 80°C and then cooled at room temperature. The meat solution was diluted up to 500 mL, and 10 mL aliquots were mixed with 2.5 mL *N*-(1-naphthyl)ethylenediamine dihydrochloride reagent and massed up to 50 mL. After standing at ambient conditions for 15 min, the absorbance was measured at 540 nm. The residual nitrite content was calculated by a standard curve using nitrite solution. The measurements were repeated three times.

Statistical analysis

A completely randomized design was adopted in this study. All treatment effects (nitrite levels, treatment methods, and pressure levels) were tested three times on different days using a new batch of pork loins. The data obtained from triplicate determinations were analyzed by the general linear model procedure using SAS (ver. 9.1). Two-way analysis of variance was performed, and the means were separated by Duncan's multiple range test when the main effects were significant ($p < 0.05$).

Results and Discussion

Low nitrite levels

The changes in cured meat color were dependent on

both the processing conditions and added nitrite levels (Fig. 1A). The addition of sodium nitrite up to 50 µg/g did not attribute to the lightness of the cured pork. The processing conditions, however, affected the lightness of the cured pork, and the highest L^* values were shown after thermal processing. No differences in L^* values were found between T and PT. Although high pressure treatment increased L^* values in the pork compared to the control, the increase in lightness was lesser than by thermal treatment ($p < 0.05$). Alternately, a^* and b^* values of the pork showed different patterns depending on the processing conditions. For the control and pressure treatment, a^* values sharply decreased at 10 µg/g of added nitrite and slightly increased with increasing nitrite levels ($p < 0.05$). Unlike the non-thermal treatments, the redness of the thermal treated pork was proportional to the added nitrite levels. The addition of nitrite tended to decrease the b^* values of the pork with increasing nitrite levels.

MacDougall and Hetherington (1992) reported that pink color in cured and cooked pork homogenate was detected with 1-2 mg/kg of added nitrite. Their observation is in partial agreement with this study. Although 10 µg/g of added nitrite initiated pink coloration (characterized by high CIE L^* and CIE a^* and low CIE b^* values) of the thermal treated cured pork (T and PT), the visual color of these products was still not distinguishable to their respective nitrite-free counterparts (Fig. 1B). Major differences in the results between MacDougall and Hetherington (1992) and the present study might be attributed to the presence of NaCl, exogenous reductants, and storage before physical treatment. The development of cured meat color likely occurs by the formation of nitric oxide Mb (NO-Mb) and thereafter the denaturation of globin (Aberle *et al.*, 2001). Considering the nitrosation of Mb, HNO_2 must be reduced to NO via N_2O_3 , whereas the reduction of NO from N_2O_3 requires reductants (Honikel, 2008; Pegg and Shahidi, 2000). Although meat contains various endogenous reducing systems in which reducing activity requires NADH, low NADH in post-mortem meat manifests as a loss of reducing ability in these systems (Faustman and Cassens, 1990). In addition, there is a loss of nitrite since a portion is oxidized to nitric acid (HNO_3) or transformed to gaseous forms (N_2O and N_2) during storage (Pegg and Shahidi, 2000). For pink coloration, NO-Mb must be turned into NHc, which is a characteristic pigment of cured meat. The transformation of NHc implies globin denaturation and detachment from the heme complex. For protein unfolding, compression energy at 200 MPa provides less unfolding energy com-

Fig. 1. Effects of low levels of added nitrite and processing conditions on the (A) instrumental color, (B) visual color, and (C) cured pigment content of model cured pork. Arabic numbers indicate added nitrite concentrations (µg/g). C, control; P, pressure treatment (200 MPa for 15 min); T, thermal treatment (75°C for 15 min); PT, pressure followed by thermal treatment

pared to thermal treatment at 75°C (Cheftel and Culioli, 1997). Smeller *et al.* (1999) reported that Mb unfolded at >600 MPa, indicating that pressure treatment at 200 MPa in the present study offered a reversible molten globule state for Mb. Therefore, pressure treatment did not prevail to form cured pigmentation compared to thermal treatment.

These observations are confirmed by the cured pigment content (Fig. 1c). Overall, the cured pigment contents of samples increased proportionally with increasing nitrite levels, and the increasing rates of cured pigments were about 2-fold higher in the thermal treatments (T and PT) than in the pressure treatment ($p < 0.05$). For the thermal treatments (T and PT), pressure in combination (PT) showed better efficiency for cured pigmentation than thermal treatment alone ($p < 0.05$). Some literature has suggested that the pressurization of meat (<200 MPa) enhanced reducing activity (Cheah and Ledward, 1997; Jung *et al.*,

2003). Cheah and Ledward (1997) postulated that pressure diminished the activity of oxygen utilizing systems thereby enhancing the reducing activity of pressurized meat. Consequently, the higher the reducing activity of pressurized meat, the more efficient the reduction from N_2O_3 to NO may be.

High nitrite levels

Under the presence of a high amount of sodium nitrite (≥ 200 µg/g), nitrite levels did not attribute to meat color, and the meat color was dependent on the processing conditions (Fig. 2a). Both pressure and thermal treatment increased CIE L^* and CIE a^* values in the pork, still pressure treatment resulted in lower CIE L^* and CIE a^* values compared to thermal treatment ($p < 0.05$). Although, CIE b^* values by the T treatment were significantly higher compared to the control ($p < 0.05$), the pressure

Fig. 2. Effects of high levels of added nitrite and processing conditions on the (A) instrumental color, (B) visual color, and (C) residual nitrite level of model cured pork. Arabic numbers indicate added nitrite concentrations (µg/g). C, control; P, pressure treatment (200 MPa for 15 min); T, thermal treatment (75°C for 15 min); PT, pressure followed by thermal treatment

treatments (P and PT) did not show different b^* values than the control. These results were also confirmed by the visual color (Fig. 2b). Although, pressure alone did not present a bright pink color, the PT treatment showed better cured coloration compared to the T treatment. Consequently, pressure treatment might provide more NO-Mb formation, hence more NHC pigmentation after heating under the absence of exogenous reductants.

For residual nitrite content, added nitrite levels showed a proportional relationship to residual content irrespective of the processing conditions (Fig. 2c). Before physical treatment (C), residual nitrite contents were about 60–70%. Pressurization reduced residual nitrite levels ($p < 0.05$) to about 80% from those of the control. As reported by Fujimaki *et al.* (1975), all added nitrite in cured meat products was transformed to the precursor of cured pigmentation (NO-Mb), gaseous forms (N_2O and N_2), its

oxidized form (HNO_3), or residuals (NO_2). Among the transformations, the majority of nitrite was oxidized to nitrate, which itself could not take part in cured pigmentation (Fujimaki *et al.*, 1975; Lee *et al.*, 1978). Lee *et al.* (1978) noted that a larger amount of nitrate was formed in the presence of ascorbate, and suggested that the ascorbate acted in the metMb reducing system, simultaneously nitrite was oxidized to nitrate as a reaction with ferrous Mb. Considering the better reducing activity of pressure treated meat (Cheah and Ledward, 1997), it is likely that more residual nitrite is oxidized to nitrate under pressure treatment. Nevertheless, pressure alone (200 MPa) had less impact on reducing residual nitrite levels compared to thermal treatment ($p < 0.05$). However, it is expected that this inefficacy of pressurization might be diminished by increasing pressure levels. Further investigations on this aspect are required. Alternately, the residual nitrite in

the PT treatment was significantly lower than that in the T treatment ($p<0.05$). Although, the mechanisms involved in the better reducing activity of pressure treated meat have not been clearly demonstrated, the present study implicates that changes in the reducing activity of pressurized meat are related to chemical reactions in the curing process.

High pressure levels

Under the presence of 200 $\mu\text{g/g}$ of nitrite, cured meat color values, particularly L^* and b^* values, were different according to the applied pressure levels (Fig. 3a). As shown earlier, 200 MPa of pressure alone did not generate a cured pink color as shown by the thermal treatment. Overall, pressure levels did not affect the a^* values of samples regardless of thermal treatment, although the thermal treatments showed higher a^* values than the pressure treatment ($p<0.05$). However, a significantly low b^* value was shown at 200 MPa of pressure ($p<0.05$), and then remained unchanged when the pressure increased up to 300 MPa. Furthermore, the L^* value was greatly increased at 300 MPa, where meat lightness approached that of the thermal treatment ($p<0.05$). These color changes in the P treatment were distinguishable from the PT treatment, i.e., increasing pressure levels did not cause changes in color in the PT treatments, and only minor changes in b^* values were observed at ≥ 200 MPa ($p<0.05$). For the total color difference, the treatment of 300 MPa of pressure showed similar values to that of the thermal treatment (Fig. 3b). However, it should be noted that these values were dependent on the lightness, and the pressure treatment still resulted in lower redness as compared to the

thermal treated samples ($p<0.05$).

NHc pigment content in the P treatment increased with increasing pressure levels (Fig. 4a) ($p<0.05$); however, the cured pigment content of the 300 MPa treatment did not exceed that of the 0.1 MPa PT treatment ($p<0.05$). As hypothesized, the greater the reducing activity of meat, the better the cured pigmentation may be. For the reducing activity of the pressurized meat, it was identified that 300 MPa was the maximum tolerance for activating reducing systems in the meat, and the meat lost its reducing ability with increasing pressure above 300 MPa (Jung *et al.*, 2003). One can propose that oxidative reactions in meat begin to prevail at higher pressure levels (>300 MPa) and auto-reducing metabolism from NO-metMb to NO-Mb may no longer occur (Walters *et al.*, 1967). Some literature has addressed the color changes of pressure treated meat products (>300 MPa) and supports this suggestion (Andres *et al.*, 2004; Campus *et al.*, 2008). Ultimately, this work represents that pressure alone is not appropriate to develop the typical cured color shown in thermal treated cured meat. However, pressure can reduce residual nitrite levels, and the effect was greater with increasing pressure levels ($p<0.05$) (Fig. 4b). This pressure effect on reducing residual nitrite was also maintained after simultaneous thermal treatment. Among the PT treatments, the sample pressurized at 300 MPa showed about 55% of residual nitrite compared to that at 0.1 MPa ($p<0.05$). Therefore, in application to meat products, this study demonstrates the superior effects of PT treatment both on cured color development and on reducing residual nitrite as compared to thermal treatment alone.

Fig. 3. Effects of pressure levels on the (A) instrumental color and (B) total color difference of model cured pork with the addition of 200 $\mu\text{g/g}$ of nitrite. Arabic numbers indicate pressure levels (MPa). P, pressure treatment; PT, pressure followed by thermal treatment

Fig. 4. Effects of pressure levels on (A) cured pigment content and (B) residual nitrite levels of model cured pork with the addition of 200 µg/g of nitrite. P, pressure treatment; PT, pressure followed by thermal treatment

Implications

This study has demonstrated the effects of high pressure in the meat curing process. To identify pressure enhanced reducing activity in meat, exogenous reductants were not added during meat curing. In general, the metabolism of nitrite in meat curing has been well reviewed by numerous researchers (Fujimaki *et al.*, 1995; Honikel, 2008; Lee *et al.*, 1975; Pegg and Shahidi, 2000), and is simply schematized in Fig. 5. Upon the given experimental conditions, most reactions would spontaneously occur, with the exception of catalyzing reactions of reducing systems. For NO-Mb formation, nitrite must be decomposed into NO and Mb must be in the ferrous form (Pegg and Shahidi, 2000). As mentioned above, pressure appeared to substitute the function of exogenous reductants and likely acted on two pathways of nitrite metabolism, i.e., the reduction of N_2O_3 and the formation of nitrate. The former would attribute to more NHC pigmentation after thermal treatment and the latter to reducing residual nitrite levels. Although it was found that the con-

version of nitrite to nitrate was accompanied with the oxidation of ferrous Mb to the ferric form, Lee *et al.* (1975) noted that nitrate formation did not occur when extracted metMb was reacted with nitrite under the absence of ascorbate. This would indicate that ascorbate reduced metMb to the ferrous form, and subsequently, the reduced Mb reacted with nitrite metabolites. In this aspect, pressure enhanced metMb reducing activity in the meat (Jung *et al.*, 2003), and hence preceded the conversion of nitrite to nitrate without the addition of ascorbate. This occurrence might result in low residual nitrite levels compared to respective non-pressure treated samples. Due to the high structural stability of Mb under high pressure, pressure alone does not form a stable NHC pigment compared to thermal processing. However, this work presents evidence that pressure-activated reducing systems provide potential benefits in cured meat coloration after thermal treatment, and pressure combined with thermal treatment is a promising technique to minimize residual nitrite content.

Fig. 5. Schematic representation of cured meat pigment formation and degradation of added nitrite.

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