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Effect of high pressure treatment on the color of fresh and processed meats: A review

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ABSTRACT

High pressure (HP) treatment often results in discoloration of beef, lamb, pork, and poultry. The degree of color changes depends on the physical and chemical state of the meat, especially myoglobin, and the atmospheric conditions during and after pressurization. A decreased redness is attributed to a large degree to the oxidation of the bright red oxymyoglobin or the purplish deoxymyoglobin into the brownish metmyoglobin, as well as to the denaturation of myoglobin. Surely, the high myoglobin content makes beef more exposed to this discoloration compared to the white chicken meat. In addition, HP treatment causes denaturation of myofibrillar proteins followed by aggregation, consequently, changing the surface reflectance and increasing lightness. Other intrinsic and extrinsic factors may affect the pressure-induced color changes positively or negatively. In this review, the pressure-induced color changes in meat are discussed in relation to modification of the myoglobin molecule, changes in the meat microstructure, and the impact of the presence of different chemical compounds and physical conditions during processing.

KEYWORDS

High pressure; color; myoglobin; protein; beef; pork; poultry

Introduction

Myoglobin, the oxygen-binding protein found in vertebrae muscle tissue, is by far the most important meat pigment, usually making up 90–95% of the total pigment content. The color of fresh and processed meat depends on the concentration and the chemical and physical state of myoglobin, the attached ligand (e.g. O_2, CO, NO) and to some extent also on the structure of the meat surface. Meat color can vary dramatically depending on the chemical state of myoglobin and its relative proportions present: from the purple-red color of deoxymyoglobin (with the reduced form of iron), and the bright red color of the oxygenated oxymyoglobin form, to the oxidized brown color of the metmyoglobin form (with the oxidized form of iron) (Feiner, 2006), see Figure 1. Several abbreviations are found in literature for the different myoglobin species; deoxymyoglobin: MbFe(II), DeoxyMb, or DMb, oxymyoglobin: MbFe(II)O_2, OxyMb, O_2Mb, or OMb, and metmyoglobin: MbFe(III), MetMb, or MMb. In the present review, Mb is short for myoglobin irrespective of its oxidation state.

Meat is commonly categorized according to its color appearance into red and white meat (Table 1). Thus, beef is popularly known as "red meat" due to its marked red color followed by lamb, pork, and poultry, the latter known as "white meat". Meat color is one of the most important quality characteristics for the consumer in a purchase situation. For instance, a bright red color is desirable and perceived as a sign of freshness of beef meat, whereas a stable reddish/pink color is usually desired in cured pork products.

Myoglobin is a globular protein of in general 153 amino acids with a molecular weight of ∼16.7 kDa. Indeed, some amino acid differences exist in the primary structure of myoglobin between various animal species (Rousseaux, Dautrevaux, and Han, 1976), yet, the overall physiological O_2 storage function is considered to be similar. The protein part of myoglobin, globin, consists of eight α-helices (A-H) making up about 73% of the protein, and the rest of the amino acid chain is primarily turns and loops between the helices. The exterior of the protein is largely hydrophilic/polar, whereas the interior is hydrophobic/non-polar, except for two histidine residues, which are critical to the structure of myoglobin. Probably the most important part of Mb, and crucial for its function, is the hydrophobic pocket in which the heme is buried (Figure 2). The iron atom is covalently bound to the four porphyrin nitrogen atoms and a histidine residue in the heme group, whereas the sixth and final binding site is available for ligand binding. The bond between the iron and the proximal histidine imidazole nitrogen (F8) is the only covalent linkage between the heme group and the protein. However, other intramolecular, stabilizing forces are Van der Waals, hydrophobic, and electrostatic interactions. The heme-globin interactions influence the electronic configuration of the heme.

Pressure is a fundamental physical parameter that affects chemical reactions and physiological functions of biomolecules differently than temperature. High pressure (HP) treatment (100–800 MPa) has unique effects on biomolecules. Native proteins are in a stable conformational state. Because of changes in the balance of the molecular forces, HP drives the stable protein
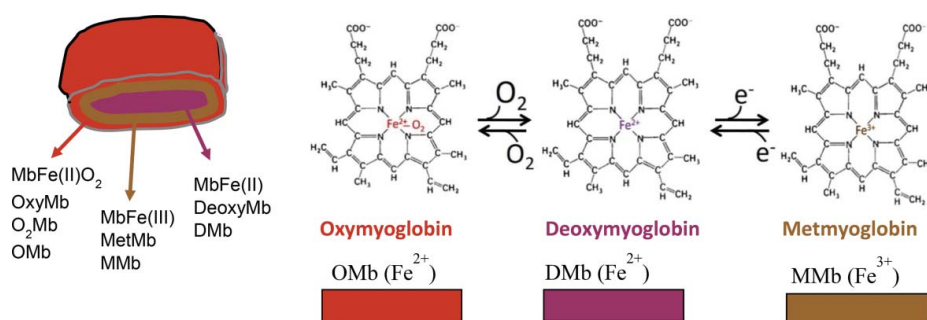
Figure 1. The color cycle of myoglobin; deoxymyoglobin/DeoxyMb, oxymyoglobin/O$_2$Mb, and metmyoglobin/MetMb. DeoxyMb and O$_2$Mb have iron in the ferrous (Fe^{2+}) state, while MetMb has iron in the ferric (Fe^{3+}) state. In meat, there is a constant interconversion between these three myoglobin species depending on the oxygen level/availability.

system towards a smaller molar volume, thereby forcing changes in the structure of proteins. It is suggested that the conformational changes during pressurization occur due to swelling of the folded polypeptide chain (by pressing water into the interior) and disruption of non-covalent interactions. In general, most globular proteins denature under pressure due to pressure-destabilization of the Van der Waals, hydrophobic, and electrostatic interactions depending on the concentration of protein and the intensity and duration of the pressure treatment. However, the complexity of HP-effects is high, and the exact action of HP, either under or after pressure, on the various molecular forces determining the protein structure is still largely unknown.

In the meat industry, high pressure processing is mainly used because treatment above 300 MPa at room temperature results in microbial inactivation, which improves microbiological safety and extends shelf-life (Carlez et al., 1994; Balasubramaniam and Farkas, 2008). Furthermore, HP treatment preserves flavor better than thermal treatment (Schindler et al., 2010). However, HP treatment affects the color of both raw and cured meat. As seen in Figure 3, HP treatment results in fading of color and beef becomes brownish, whereas pork and poultry become brighter/whiter. The hypotheses of pressure-induced color changes in meat are basically dependent on three main mechanisms: 1) denaturation of myoglobin, 2) modification or disruption of the porphyrin ring, and 3) changes in the myoglobin redox chemistry. The aim of the present review is to provide a detailed synopsis and assessment of the effect of HP on myoglobin in model systems and on the color of fresh and cured beef, pork, and poultry.

Myoglobin system

In order to understand the pressure effect on myoglobin as such, several studies have been conducted on myoglobin changes with or without various ligands either *in situ* under pressure or after pressure, see Table 2 and 3.

Table 1. The color and approximate myoglobin concentration of various meat types. Adapted from Young and West (2001) and Faustman and Suman (2017).

	beef	sheep	pork	turkey	chicken
Visual Color	cherry red	bright red	pink	pink-white	white
Mb (mg/g)	3.0–9.0	2.0–7.0	0.6–6.0	0.15–1.5	0.01–1.0

Hemoproteins are indeed of major interest due to their diverse range of biological functions, including oxygen transport, oxygen storage, and electron transfer. The structures of the polypeptide chain and the heme group are closely related to this function. During pressurization, both the chain and the heme may undergo conformational changes. Thus, early investigations focused on modifications of the protein-heme dynamics due to HP in order to control the levels of reactivity and functionality. Ogunmola et al. (1977) studied the changes in the visible spectrum and concluded that Mb underwent transformation from high-spin (open crevice) structure to low-spin (closed crevice) form under pressure up to 580 MPa. As the analytical methods developed, it became possible to perform detailed investigations of the pressure-induced structural perturbations such as subtle changes of the iron-ligand bond. With high-resolution NMR technique, Morishima, Ogawa, and Yamada (1980) found that pressure (up 200 MPa) caused a structural change in the five-coordinated heme, possibly due to displacement of the heme plane at the distal side. Later, Raman spectroscopy gained insight into pressure-induced changes of the heme's active site and the linkage between the iron-atom and the proximal histidine. It was found that pressure resulted in conformational change affecting the tilt angle between the heme plane and the proximal histidine and the out-of-plane iron position, as shown in Figure 4 (Alden et al., 1989; Schulte et al., 1995; Galkin et al., 1997).

At the macromolecular level, it has been known since the first study by Bridgman (1914) that pressure is able to affect protein conformation. One step in understanding the mechanism of pressure-induced changes of the Mb polypeptide chain is to measure the *in situ* effects. Smeller, Rubens, and Heremans (1999) showed that pressure-unfolding of myoglobin formed an intermediate form, often referred to as the molten globule form/state, with a conformation between that of the native protein and the completely unfolded state. Moreover, they observed that these intermediate forms are highly prone to aggregation upon pressure release, and suggested that intramolecular hydrogen bonds were broken under pressure. The increased number of available intermolecular hydrogen bonds thereby stabilized the newly-formed aggregates. Later, it was shown that HP resulted in a significant loss of α-helix structure and an increase in random structure (Meersman, Smeller, and Heremans, 2002). Moreover, they suggested that the tyrosines did not become solvent-exposed in the pressure-unfolded state
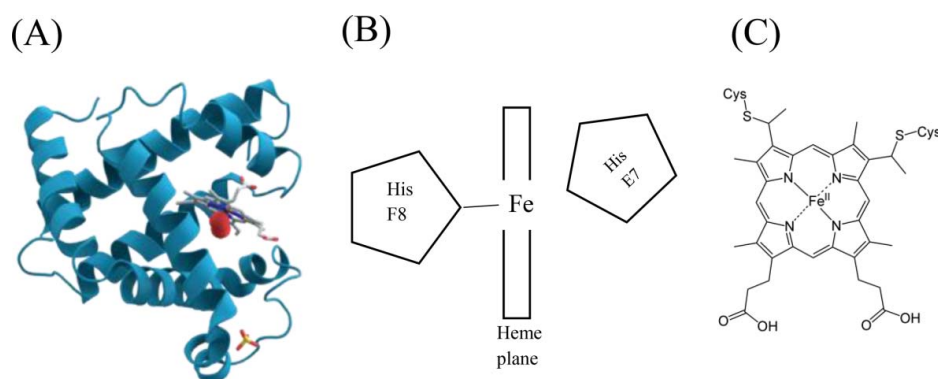
Figure 2. The myoglobin molecule consists of the globular protein globin (A) (AzaToth https://commons.wikimedia.org/w/index.php?curid=68596) and the prosthetic heme group with the central iron atom (B) (https://commons.wikimedia.org/wiki/File:Haem_c.png). The importance of the two histidine residues is shown (C).

due to the observed lack of any changes in the tyrosine ring vibration band position near the transition midpoint. It was concluded that pressure-unfolded states are only partially unfolded, and are similar to early folding intermediates with a high content of extended chain (50–60%), and that a rigid core of the G and H helices is maintained. Another approach for studying structural and dynamical features of HP-states of myoglobin was by using circular dichroism and site-directed spin labelling electronic spin spectroscopy (Lerch et al., 2013). It was found that Mb maintained a rigid, relatively incompressible structure at pH 6.0 under pressure up to 200 MPa. However, changing pH to 4.1 changed the HP effect on Mb, thus, the pressure-induced Mb molten globule experienced dynamic disorder in some fraction of the E and F helices, and a fluctuating tertiary fold in the remaining helices.

Regarding meat color, the irreversible pressure-induced changes of Mb that remain after pressurization are obviously of great concern. Indeed, there are great differences between how HP affects the pure Mb molecule in a model system and the various Mb states in a meat system with several other components, including a large variation in pH. Yet, Mb is the molecule responsible for meat color and it may be assumed that the changes of the myoglobin molecule as such have a significant effect on the resulting color of meat after HP treatment in relation to the three mechanisms (c.f. introduction). In this light, remarkably few studies on the post-pressure effect on Mb have been conducted (Table 3). Zipp and Kauzmann (1973) were the first to study the pressure denaturation of MMb and

presented an isobaric contour plot in the pH, T-plane. Moreover, they found that MMb precipitated under some specific P, T, pH conditions, and for most conditions the denaturation was 100% reversible. Later, Defaye et al. (1995) studied the renaturation and the nature of the precipitate of pressurized MMb. MMb at neutral pH (6.9) denatured during treatment at 750–800 MPa (20 min) and only partially reformed to the native state upon subsequent storage at 4°C. Both initial pH and ionic strength affected the degree and rate of renaturation, suggesting that electrostatic forces dominate the protein-protein attractive forces in the aggregate. These authors further suggested that the heme environment in the pressure-denatured state was typical of the ferric pigment of heat induced-denatured myoglobin. Interestingly, this study also provided information of the color of the pre-pressurized (brown) and post-pressurized (pink color like baked bean tomato sauce) and the precipitate (reddish/brown) MMb. Thus, the initial color of suspensions following pressure denaturation changed rapidly on standing and may indicate that the initial product of pressure treatment is a very unstable complex that rapidly converts to the denatured ferric pigment.

Several studies have been made on the pressure effects on ligand binding to Mb both under and after pressure treatment (Table 2 and 3). The binding and release of small molecules to hemoprotein are controlled by the nature of the ligand and by the protein structure. Pressure can affect the interactions between the ligand and the peripheral amino acid residues through the structural changes. Therefore, investigations of the
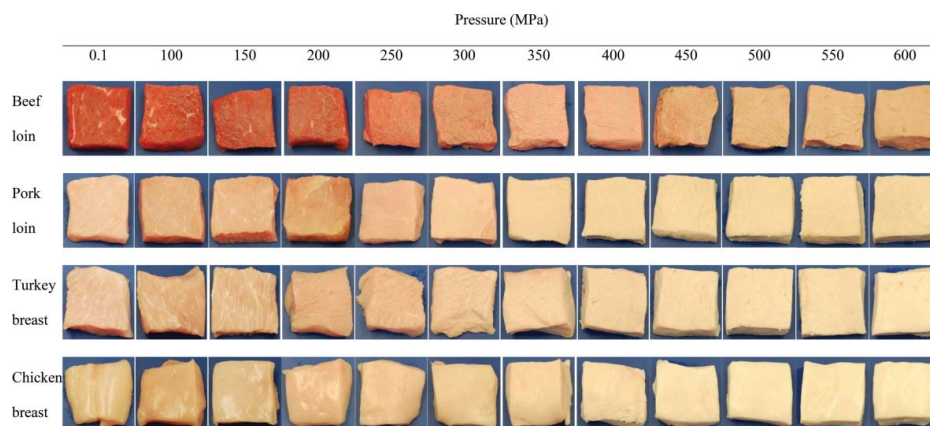
Figure 3. Color changes of different red and white meats upon high pressure processing (0–600 MPa) at the specific pressure for 5 min at room temperature (20°C).

Table 2. *In situ* pressure effect on myoglobin during HP treatment.

System[1]	HP Conditions (MPa)	Effect	Reference
Sperm whale, horse heart, and dog muscle OMb & COMb (0.1 M Tris pH 7.8)	0.1–200 (20°C)	The pressure effect on the ligand rebinding rate for Mb is very sensitive to each step of the multiple reaction processes as well as to the amino acid constituents around the ligand path channel.	Adachi and Morishima (1989)
Sperm whale Mb, OMb, COMb (3–5 mM, Tris pH 7)	100–3400	Mb: High to low spin transition at 700 MPa. Collapse of the heme pocket. O_2 dissociates from the heme. CO remains bound to the heme.	Alden et al. (1989)
Wild type (*E. coli*) and mutant apoMb (0.5 mM/10 mM, pH 5.4)	0.1–500	Wild type apoMb is resistant up to 80 MPa, denatures to an intermediate between 80 and 200 MPa, following denaturation of the intermediate up to 500 MPa. The mutants show that denaturation is sensitive to changes in the packing of the protein interior.	Bondos Sligar and Jonas (2000)
Horse muscle Mb (0.7 g D_2O/g protein)	100–700	Mb denatures at 300 MPa. Enhanced protein-solvent interactions in the unfolded form may destabilize the native state at high pressure.	Doster and Gebhardt (2003)
Horse Mb, COMb (2–4 mM)	0.1–200 (35–295 K)	A conformational change affecting the tilt angle between the heme plane and the proximal histidine and out-of-plane iron position. The heme assumes a more planar form, thereby increasing the rebinding rate.	Galkin et al. (1997)
Sperm whale OMb, COMb (0.1 M PO_4 pH 7.0)	0.1–276	The volumes of activation for the binding of O_2 was 8 cm^3/mol and of CO was -9 cm^3/mol, indicating that the interactions of these two ligands may be different in the transition state.	Hasinoff (1974)
E. coli apoMb (700μM, 20 mM MES pH 6.0)	3–300 (35°C)	apoMb exists as an equilibrium mixture of the N, I, MG, and U conformers at all pressures.	Kitahara et al. (2002)
Sperm whale & Horse heart MbCN (3 mM & 100 mM Tris, pH 7.5 and 8.6, respectively)	0.1–300 (25°C)	The heme peripheral structure as a whole was compressed by pressure. The distal His residue moved toward the outside of the heme pocket, but remained in the pocket even at 300 MPa. This conformational change was larger in horse heart MbCN.	Kitahara, Kato, and Taniguchi (2003)
Sperm whale & Horse heart Mb, apoMb (500μM, 20 mM MES pH 6.0 / 10 mM CH3COONa pH 4.1)	0.1–200	The molten globule state at 200 MPa has the same helical content as the native state. Horse heart Mb is less stable. ApoMb has large-scale collective motions of particular helices at 200 MPa.	Lerch et al. (2013) Lerch et al. (2014)
Horse heart MbN_3			Le Tilly et al. (1992)
Horse heart MMb (75 mg/mL, 10 mM Tris-HCl pH 8.2)	0.1–1000 (25°C)	Unfolded states are only partially unfolded and the rigid core of the G and H helices is maintained.	Meersman, Smeller, and Heremans (2002)
Horse heart Mb (3.5 mM, 10 mM Tris-HCl pH 7.6)	0.1–1000 (−25–140°C)	(P, T) state diagram.	Meersman, Smeller, and Heremans (2005)
Sperm whale Mb, MMb, MMbCN, MMbIm, MMbpyrazole, MMbF, MMbHCOO (5 mM, 50 mM PO_4 pH 7.5)	0.1–200 (32°C)	The primary pressure effect is to shift the spin equilibrium in favor of the low-spin state. Displacement of the heme iron from the heme plane.	Morishima, Ogawa, and Yamada (1980)
Horse heart & sperm whale MbCN (7 mM, 0.1 M Tris-HCl pH 7.8)	0.1–120 (30°C)	Pressure affects the heme environmental structure at the distal side. Horse heart MbCN is more pressure sensitive.	Morishima and Hara (1983)
Sperm whale MMb, $MMbN_3$, MMbF, MMbIm, MMbHCOO (50 mM in buffer)	0.1–196 (25°C)	The observed volume change reflects changes on the protein molecule other than those in the heme group.	Ogunmola, Kauzmann, and Zipp (1976)
Sperm whale MMb, Mb, MMbCN, COMb	0.1–780 (20°C)	The primary pressure effect with open crevice structure is to shift the equilibrium to the closed crevice structure. Ligands with high affinity for the heme iron stabilize the open crevice configuration and prevent denaturation.	Ogunmola et al. (1977)
Sperm whale OMb, COMb (5 mM Tris pH 8.5)	0.1–150 (25°C)	O_2 movement up the heme pocket is rate determining, while CO rapidly equilibrates with the heme pocket followed by rate-determining Fe-CO bond formation.	Projahn et al. (1990)
Horse heart MB (75 mg/mL, 10 mM Tris-D_2O pD 7.6)	0.1–1200 (30–90°C)	The pressure-induced unfolding of myoglobin forms two different states of aggregation: one that can be dissociated by HP, and another that is insensitive to HP. The aggregates are stabilized by intermolecular antiparallel β-structures stabilized by hydrogen bonds.	Smeller, Rubens, and Heremans (1999)
Horse Mb (4 mM, PO_4 pH 7)	0.1–175	A conformational change in the protein decreases the tilt of the proximal histidine from the heme normal and the out-of-plane iron position.	Schulte et al. (1995)

Table 2. (Continued).

System[1]	HP Conditions (MPa)	Effect	Reference
Sperm whale MbL, L = MeNC, f-BuNC, CO, O_2 (20μM, 50 mM Tris pH 7.0)	5–150 (25°C)	The volumes of activation indicate that bond formation is the rate-determining step only for carbon monoxide, while for oxygen, isocyanides, and l-methylimidazole almost no bond formation occurs in the transition state.	Taube et al. (1990)
Horse muscle apoMb (30μM, 10 mM bis-Tris pH 6.0 / 10 mM CH3COONa pH 4.2)	0.1–260 (21°C)	The volume change for the transition from the native state to an intermediate was −70 mL/mol. Complete unfolding of the protein revealed an upper limit for the unfolding of the intermediate of −61 mL/mol.	Vidugiris and Royer (1998)

[1]Mb: (deoxy)myoglobin, OMb: oxymyoglobin, COMb: CO-myoglobin, *E. coli*: myoglobin expressed in *E. coli* cell line, apoMb: apomyoglobin, MMb: metmyoglobin, MMbCN: cyanide complex of metmyoglobin, MMblm: imidazole complex of metmyoglobin, MMbpyrazole: pyrazole complex of metmyoglobin, MMbF: fluoride complex of met-myoglobin, MMbHCOO: format complex of metmyoglobin

pressure-induced structural changes in the heme peripheral amino acid residues are of importance for understanding how the protein structure in the heme environment controls such a dynamic process and for understanding the structure-function relationship of the protein.

The reaction of Mb (sperm whale) with O_2 under pressure up to 200 MPa resulted in an activation volume of 7.8 cm^3/mol (Hasinoff, 1974). The partial molar volume of a protein consists of contribution from the constitutive volume (van der Waals radii of all atoms), the conformational volume (voids and compressed regions) and the solvation volume (Hasinoff, 1974). Chemical reactions involving proteins are accompanied by changes in conformation and hydration of the protein, thus, affecting the molar volume of the protein. Therefore, changes in the activation volume may provide a useful probe for conformational changes and if pressure favors or counteracts the reaction in accordance to Le Châtelier's principle. A more detailed study showed that the overall activation volume for recombination of oxygen to sperm whale-Mb, horse heart-Mb, and dog-Mb was 4.6, 3.8, and 0.0 cm^3/mol, respectively. Thus, the rate constant for O_2 binding is roughly independent of pressure (Adachi and Morishima, 1989). However, the diffusion rate of the O_2-molecule from solvent to the protein matrix and approach/recombining rate to the heme iron varied considerably among Mb from the three different animal species, suggesting that the specific heme environments control the process (Adachi and Morishima, 1989). The difference was explained by the differences in the amino acid sequences of the Mb around the heme in the three species, especially the distal side

amino acid constituents were suggested to be of central importance. A comparable value of activation volume 5.2 cm^3/mol for the formation of OMb under pressure was found by Projahn et al. (1990). Based on a comprehensive discussion of the volume profile analysis, the authors concluded that the volume increase occurs prior to the binding of O_2 to the Fe(II). Thus, the passage of oxygen through the heme pocket is rate-determining in line with the findings of Adachi and Morishima (1989). In addition, it was found that the deoxygenation also had a high positive volume of activation (23.3 cm^3/mol), indicating that the process of O_2 escape proceeds via a dissociative mechanism and the O_2-Mb bond is almost completely broken in the transition state (Projahn et al., 1990).

In addition, different ligands other than O_2 have been investigated. In 1974, Hasinoff reported that the binding of CO to Mb was assumed to be different from binding of O_2 because the volume of activation was negative (−9 cm^3/mol), opposite to the positive volume change for O_2 (Hasinoff, 1974). He suggested that the interactions of the two ligands, O_2 and CO, are different in the transition state. In addition, the overall reaction volume was negative (from −9.2 to −18.8 cm^3/mol dependent on the Mb species) for the CO binding to Mb (sperm whale, horse heart, and dog) due to the rate-determining bond formation step with the variations explained by the differences in the amino acid constituents around the ligand path channel (Adachi and Morishima, 1989). Taube et al. (1990) supported the negative volume of activation for the CO addition (−10 cm^3/mol) to sperm whale Mb, and added that the pressure-induced high-to-low spin transition would increase the rate of

Table 3. Pressure effect on myoglobin after HP treatment.

System[1]	HP Condition (MPa)	Effect	Reference
Horse MbNO (0.1 mM, aq pH 6.8)	0.1–300 (15°C)	The rate of oxidation of MbNO decreases with pressure corresponding to a volume of activation of 8 mL/mol.	Bruun-Jensen and Skibsted (1996)
Horse OMb (0.08–0.14 mM, aq pH 5.65 and 5.21)	0.1–250 (25°C)	The rate of autoxidation of OMb decreased with pressure.	Bruun-Jensen, Sosnieckia, and Skibsted (1997)
Horse Mb(IV)O (0.05 mM Tris pH 8.25)	0.1–350 (30°C)	The rate of autoreduction of Mb(IV)O decreases with pressure corresponding to a volume of activation of 7 mL/mol.	Bruun-Jensen, Sosniecki, and Skibsted (1998)
Horse heart Mb (0.2%, aq pH 6–9)	750–800 (20 min, T_{amb}[2])	Mb denatured and only partially reformed the native state. The pH and ionic strength dependency suggested that electrostatic forces dominate the protein-protein attractive forces in the aggregate, but a marked temperature dependence indicated a hydrogen bond stabilization of the aggregate.	Defaye et al. (1995)
Sperm whale MMb (pH 4–13)	0.1–600 (5–80°C)	Denaturation surfaces in the (P, T, pH) space	Zipp and Kauzmann (1973)

[1]MbNO: nitrosylmyoglobin, Mb(IV)O: ferrylmyoglobin (MbFe(IV) = O), others same as in Table 2.
[2]T_{amb}: ambient temperature.
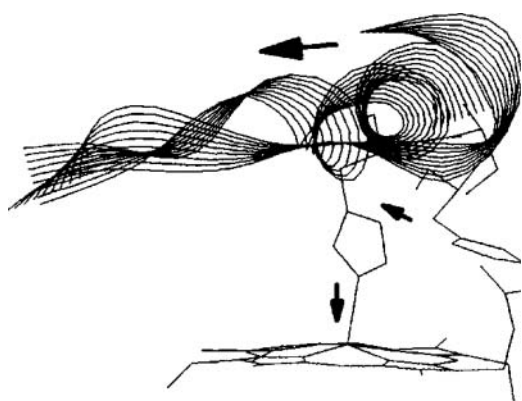
Figure 4. Schematic representation of pressure-induced changes in the proximal heme pocket. Arrows indicate motion of the heme iron, proximal histidine tilt angle, and F-helix. From (Galkin et al., 1997) with permission.

the association reaction. A similar activation volume (-8.9 cm^3/mol) for the Mb carbonylation was found by Projahn et al. (1990) who concluded that the negative volume resulted from the change in spin state and movement of Fe into the porphyrin plane upon the direct bonding between CO and the Fe(II) center. CO was found to stabilize the open crevice structure of COMb (sperm whale), thus preventing pressure denaturation (Ogunmola et al., 1977). The cyanide ion was also observed to be effective in stabilizing the low-spin CNMb (sperm whale) against denaturation due to the high affinity of CN for the heme iron (Ogunmola et al., 1977). However, Morishima and Hara (1983) showed pressure induced structural changes at the heme coordination site, but it was also found that sperm whale CNMb is more pressure-resistant than horse heart CNMb. Kitahara, Kato, and Taniguchi (2003) found that the distal histidine imidazole ring undergoes a rotational displacement towards the outside of the heme pocket, and that this conformational change of His64 was larger in horse heart CNMb than for the sperm whale CNMb in agreement with Morishima and Hara (1983).

In relation to the pressure effect on myoglobin redox chemistry, only investigations of the oxidation of OMb and nitrosylmyoglobin (NOMb) have been conducted according to our knowledge. The volumes of activation were found to be 8.3 and 12.7 cm^3/mol for oxidation of NOMb and OMb, respectively, thus increasing the pressure, decreased reaction rate of oxidation (Bruun-Jensen and Skibsted, 1996; Bruun-Jensen, Sosniecki and Skibsted, 1997). The positive activation volume of formation of nitrate within the heme pocket was suggested to arise from the expansion of the coordination sphere upon transferring four electrons to oxygen in the transition state (Bruun-Jensen and Skibsted, 1996). The pressure-induced oxidation of the red fresh meat color due to OMb, to the brown color due to MMb formation, was suggested to reflect both an expansion in the transition state and an increased acid catalysis (Bruun-Jensen, Sosniecki, and Skibsted, 1997).

The dominant features of the HP effects on myoglobin to pass on to the next section concerning HP effects on meat color can be summarized as: 1) severe changes in the secondary structure, 2) structural modification of the heme porphyrin ring, 3) reversible denaturation, but some aggregation, 4) rate of oxidation of OMb to MMb is decreased by increasing

pressure (yet only at high pH), and 5) oxidation of NOMb to MMb is protected by pressure.

Beef

The HP-induced color change in beef is similar in appearance to the color change upon cooking (i.e. redder meats are darker after cooking), although the mechanisms underlying the color changes under temperature and pressure are different (Bolumar et al., 2016). Generally, when pressure-treating beef, lightness increases, redness decreases and yellowness remains more or less unchanged. The specific color changes in beef upon HP-treatment depend both on the initial particular physical state and chemical conditions of the meat and Mb, as well as the changes taking place during pressurization. Table 4 gives an overview of the investigations of the effect of HP on beef color together with the suggested mechanistic reasons. These studies employed different HP conditions (P, T and t) of a single or multiple HP treatment(s) alone or in combination with other processing steps prior or during HP such as freezing/thawing and/or addition of different additives that impact color state and stability, and the resulting outcome of beef color is surely dependent on this. In addition, Table 5 gives an overview of the effect of HP on cured beef products.

Carlez and co-workers conducted the first detailed investigation about changes in color and myoglobin content and state of minced beef due to HP treatment (Carlez, Veciana-Nogues, and Cheftel, 1995). The effect of pressure level (100–500 MPa, at 10°C for 10 min) was investigated in conjunction with other factors affecting beef color such as packaging atmosphere (vacuum, air, oxygen), depth underneath of meat surface (surface, intermediate and center) and addition of different chemical compounds (nicotinamide, ascorbic acid, cysteine, NaCl, and sodium nitrite). This study established some of the fundamental knowledge of what is known about the effect of HP on beef color. They observed an increase in lightness (L* value) already at 200 MPa, whereas pressure higher than 400 MPa were required before redness (a* value) decreased. Moreover, they found that the ferrous OMb was oxidized to ferric MMb, especially at pressures higher than 300 MPa accounting for the observed developed gray-brown color. This mechanism was supported by the fact that exclusion of oxygen from the packaging using an oxygen scavenger protected OMb from oxidation, thereby preventing formation of MMb. Jung, Ghoul, and de Lamballerie-Anton (2003) found that the content of MMb in beef after treatment up to 300 MPa was reduced, whereas higher pressures resulted in an increase in MMb-content. This observation was explained by activation of the MMb-reducing enzyme system at low pressures and inactivation at high pressures (Jung, Ghoul, and de Lamballerie-Anton, 2003).

The partial denaturation of myoglobin occurring above 300 MPa and/or the displacement or separation of the heme from the globin was suggested as another important mechanism for the color change, and was termed a 'whitening effect' (Carlez, Veciana-Nogues, and Cheftel, 1995). However, this whitening effect was thereafter suggested to be a result of the pressure-induced denaturation of myofibrillar and sarcoplasmic proteins (Cheah and Ledward, 1996). In fact, an increase in the L* value of meat can be related to protein denaturation

Table 4. Color changes of HP-treated beef and lamb meats. ↑ indicates an increase of the color parameter, ↓ indicates a decrease of the color parameter, and → indicates no change. + indicates that the respective mechanism was suggested to cause changes in the color.

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L^*	a^*	b^*	Oxidation	Denaturation	Other	
Minced beef, frozen and unfrozen	300, 5 min, −10, −5, 0, 10, 20 °C	↑	HP: → freezing-thawing: ↓	→		+	+ Heme group displacement or release	Bulut (2014)
Raw patties of lean beef and pork back fat: low-fat (LF) (9.2%)	100, 300, 5, 20 min, 5 °C	LF: → HF: ↑	LF: ↓ at 300 MPa, → at 100 MPa HF:↓ except at100 MPa, 5 min HF:↓	LF: → HF: ↓ at 300 MPa, → at 100 MPa				Carballo et al. (1997)
high-fat (HF) (20.3%)								
Fresh minced beef muscle (round top rump)	50–500 (50 MPa intervals), 10 min, 20 °C	↑ for 150–350 MPa, → for 350–500 MPa	→ up to 300 MPa↓ from 350 to 500 MPa	→				Carlez et al. (1993)
Minced beef (M. semimembranosus)	200, 250, 300, 350, 400, 450, 500, 10 min, 10 °C	In air: ↑ in the range 200–350 MPa (paler color), → > 350 MPa	In air: ↓ in the range 400–500 MPa (from pink to gray-brown color)	In air: →	+	+	+	Carlez et al. (1995)
Effect of atmosphere: air, oxygen or vacuum (with or without oxygen scavenger (OS))	350, 400, 450, 500, 10 min, 10 °C	↑ at all pressures and atmospheres	In oxygen ↓ at all pressures	→	Oxidation of OMb into MMb at P > 300 MPa	Partial denaturation of Mb, especially above 300 MPa	Modification of the porphyrin ring	
Effect of depth: meat cubes (30–40 mm³) packaged in air (surface, intermediate and center zones)		Meat cubes: identical at the different depths	In vacuum without OS ↓ at 400–500 MPa	↑ under vacuum without OS	+	Denaturation of the globin	+	
Effect of addition of nicotinic acid, nicotinamide, ascorbic acid, cysteine, NaCl and sodium nitrite.			In vacuum with OS: → Meat cubes: ↓ at P > 350 MPa intermediate zone became gray-brown while the center and surface of the cube was pink. At 400, 450 and 500 MPa the grey-brown extended from the intermediate zone to the surface.		Exclusion of oxygen protect against oxidation		NaNO₂ at 100–200 ppm provided color protection.	
Beef longissimus dorsi (LD) and psoas major (PM) at 2, 7, 9 and 20 days pm	80–100, 20 min, ambient T	LD: ↑ at 2 days post-mortem. → at 7, 9 or 20 days pm PM: →	LD and PM: ↑ at 2 days post-mortem. →if HP treatment was applied at 7, 9 or 20 days pm. PM: ↑ after HPP HP at 2 days pm. → at 7–9 or 20 days pm.	LD: ↑ after HPP at 2 days pm. → at 7–9 or 20 days pm. PM: →	+		+ HP meat at 2-days pm activates the enzymes that consume oxygen	Cheah and Ledward (1997)
Beef Longissimus dorsi: raw, salted, air blast frozen (-30°C) raw and salted	650, 10 min, 20, −35 °C (samples	Raw: ↑	Raw: ↓	Raw: →	+	+	+	Fernandez et al. (2007)

Table 4. (Continued).

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L*	a*	b*	Oxidation	Denaturation	Other	
Beef *M. semitendinosus*	previously frozen using air blast; 200, 3 h, −20 °C	Salted: ↑ Raw frozen: → Salted frozen: → Non-freeze: ↑	Salted: ↓ Raw frozen: ↓ Salted frozen: ↓ Non-freeze: ↓	Salted: → Raw frozen: → Salted frozen: → Non-freeze: ↑		+	Water content changed due to drip loss + +	Fernandez-Martin et al. (2000)
Air-blast freezing (F), pressurized and non-freezing (P/NF), pressure-shift freezing (P/F)		Freeze: ↑	Freeze: ↓	Freeze: ↑		Denaturation for actin, myosin and other proteins. + Protein denaturation was greater when freezing occurred. +		
Minced beef	200, 300, 20 min, 20 °C Storage: 0, 2, 15 days 520, 260 sec, 10 °C	↑	↓	←	+			Hassan et al. (2002)
Beef *M. biceps femoris* and *longissimus dorsi*		↑	↑	←				Jung, Ghoul, and de Lamballerie-Anton (2000)
Bovine muscle (*biceps femoris*) (is a color unstable muscle)	2 days pm 50–600, 20–300 sec, 10 °C Subsequent storage for a week, color being measured on days 1, 3, 4, and 7. Some samples subsequent cooking for 1 h to a core of 65 °C).	Indirectly reported as total color change (ΔE). Considerable change r) P >300 MPa	↑ P ≤ 350 MPa ↓ P > 350 MPa After HP at 130 MPa, a* remains significantly higher than for controls during the first 4 days of storage, then a drastic decrease. After HP at 520 MPa, there is a significant decrease in a* from day 1–7 of storage probably due to accumulation of MMb	Idem as for L*		+	+ Inverse relationship between a* value and MMb content; maximum and minimum at P = 300–350 MPa, respectively +	Jung et al. (2003)
Ground beef (inside round)	300, 600, 5 min, 15 °C Vacuum bags opened and stored at 4 °C for 10 days	Raw samples: ↑	Raw samples: ↓	Raw: ↑	+	+	changes in MMb reductase +,	Jung et al. (2013)
without phosvitin (P), or with 500 or 1000 mg P/kg.		After storage: →	After storage: →	After storage: →				
Vacuum packed. Raw or cooked at 75°C for 30 min		Cooked: →	Cooked: →	Cooked: →				

Sample	Conditions							Reference
Beef loin (added or not with conjugated linoleic acid)	300, 450, 600, 5 min, 15 °C	↑ at all pressures: 300 MPa, 450 = 600 MPa	↓ similarly at all pressures	+	+	+	+	Kim et al. (2014)
Brine injected beef strip loin.	152, 303, 1 min, 0 °C	Without P: 152 MPa ↓ at 2 and 4% S; → at 0% S.	Without P: 152 MPa → at any S level. 303 MPa ↑ at all S levels.	+	+	+	+	Lowder and Dewitt (2014)
Sodium chloride (S): 0, 2 or 4%		303 MPa HPP → at 0% S, ↑ at 2 and 4% salt	With P: 152 MPa ↓ at all S levels. 303 MPa ↑ at all S levels.	+				
Sodium phosphate (P): 0 or 4%		With P: 152 MPa HPP → at 0% S, ↓ at 2 and 4% S					303 MPa reduced total and sarcoplasmic protein solubility by 24 and 32%, respectively	
Whole beef M. semitendinosus (inoculated with E. coli)	551, 4 min, 3 °C	C: ↑	C: ↓	+	+	+	+	Lowder, Waite-Cusic, and DeWitt (2014)
Chilled sample (C)		F: →	F: →	F: →			Conformational, oxidative and ligand binding state of Mb	
Frozen sample (F) (< −30 °C)				Mb oxygenation (blooming); C: ↓ F: →				
Beef M. longissimus dorsi	200, 400, 600, 20 min, 10, 20, 30 °C	All temp: ↑ at all pressures.	↓	+	+	+	+	Marcos, Kerry, and Mullen (2010)
		↑ from 200 to 400 MPa, further values for samples processed at 400 were similar to those at 600 MPa	values at 600 MPa were lower than at < 400 MPa	All temp: ↑ 400 = 600 MPa, → at 200 MPa			+	
							Correlations between solubility and denaturation of sarcoplasmic proteins and color	
Beef M. longissimus thoracis and lumbarum (LTL)	200, 400, 600, 20 min, 20 °C	↑ from 200 to 400 MPa, 400 = 600 MPa	→	↑ at 400 = 600 MPa	+	+	+	Marcos and Mullen (2014)
				→ at 200 MPa			Protein changes were correlated with L* and b*	+

(Continued on next page)

Table 4. (Continued).

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L^*	a^*	b^*	Oxidation	Denaturation	Other	
Steaks of beef *M. pectoralis profundus*	200, 300, 400, 20 min, 20, 40 °C	20 °C: ↑ 300 = 400 MPa, → at 200 MPa. 40°C: ↑ at 200 < 300 = 400 MPa	↓: 40 °C at 300 MPa. No other effect of HPP. More effect at 40 °C than at 20 °C	→ at 20°C or 40°C. Temp effect of HP: ↓ where 20°C < 40°C for all MPa. No temp effect of HP	+	+	+ Heme dis-placement or release. + changes in the water content and distribution	McArdle et al. (2010)
Steaks of beef *M. pectoralis profundus*	400, 600, 20 min, 35, 45, 55 °C Storage 30 days	↑ at all temp- and HP-levels. → of storage	↓ at all temp- and HP-levels. → of storage	→ of HP. → of storage	+	+		McArdle et al. (2011)
Lamb *M. pectoralis profundus*	200, 400 600, 20 min, 20, 40,60 °C Storage 30 days	20 °C: → at 200 MPa, ↑ at 400 = 600 MPa. 40 °C: ↑ at 200 < 400 = 600 MPa. 60 °C: ↑ at 200 < 400 = 600 MPa. After storage: mainly the same effects	20°C: →. 40 °C: ↓ at 400 = 600 MPa. 60 °C: ↓ at 600 MPa. After storage: mainly the same effects	20°C: → at 200 MPa, ↑ at 400 = 600 MPa. 40 °C: → at 200 MPa, ↑ at 400 = 600 MPa. 60 °C: ↑ at all MPa. After storage: mainly the same effects	+	+	+	McArdle et al. (2013)
Beef patties (ground meat)	Single-cycle: 400, 1, 5, 10, 15, 20 min, 12 °C. Multiple-cycle 400 (1 min (1, 2, 3 and 4 times) and 5 min (1, 2, and 3times), 12 °C)	Exterior: Single-cycle: ↑, higher with length of treatment. Multiple-cycle: ↑, higher with number of cycles. Interior: Single-cycle: ↑, length of treatment no effect; Multiple-cycle: ↑, number of cycles no effect	Exterior: Single- or multiple-cycle: →. Interior: Single-cycle: →. Multiple-cycle: ↓ in some (at 5-min cycles) 1 and 2 × 5-min cycles), → at 3 × 5-min cycles)	Exterior: Single and multiple cycles: ↑. Interior: Single or multiple cycles: →	+	+		Morales et al. (2008)

Sample	Conditions						Reference
Beef silverside	100–500, 10 min, 20 °C	Control:	Control:	Control		+	Ohnuma et al. (2013)
Sodium hydrogen carbonate (soaked in a solution of 0.4 mol/l NaHCO₃ for 40 min at 20 °C)		↑ at P > 300 MPa NaHCO₃ treatment: ↑ at P > 300 MPa	↑ (slightly) at P > 200 MPa NaHCO₃ treatment: ↑ at P > 400 MPa	↑ at P > 300 MPa NaHCO₃ treatment: ↑ at P > 400 MPa		+	
Fresh chevon (leg portion)	300, 600, 5, 10 min, 28 °C Chilling storage at 4 °C for 30 days	↑	↓	↑	Non-heme iron content increased	+ +	Reddy et al. (2015)
Beef M. longissimus lumborum et thoracis Control and papain injected	100, 200, 300, 10 min	↑ (data not shown)	↑ (data not shown)	↑ (data not shown)			Schenkova et al. (2007)
Cooked beef steaks of M. semimembranosus and M. biceps femoris	HP-heat and cook: 200, 20 min, 60 °C, plus cooked 30 min at 80°C Heat and cook: heating at 60 °C, 20 min + cooked 30 min at 80°C HP-heat: 200 MPa, 20 min, at 60, 64, 68, 72 or 76 °C Heat only: 20 min at 60, 64, 68, 72 or 76 °C	HP-heat and cook: ↑ compared with heat and cook HP-heat: → compared with heat only and ↓ compared with HP-heat and cook at 60°C	HP-heat and cook: → compared with heat and cook except at 60°C HP-heat: → compared with heat only and ↓ compared with HP-heat and cook at 60°C	→			Sikes and Tume (2014)
Frozen ground beef (round)	210, 280, 5, 15, 30 min Thawing meat from −15 °C using HP.	→ at 210 MPa ↑ at 280 MPa	→ at 210 MPa ↓ at 280 MPa	→			Zhao, Flores, and Olson (1998)

Table 5. Color changes of HP-treated cured beef. ↑ indicates an increase of the color parameter, ↓ indicates a decrease of the color parameter, and → indicates no change. + indicates that the respective mechanism was suggested to cause changes in the color.

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L^*	a^*	b^*	Oxidation	Denaturation	Other	
Beef carpaccio	450, 5, 10, 15 min, 12°C Stored for 30 days at 8°C	↑	→	→	+ Oxidation protected by nitric oxide	+	+ Structure and surface properties affected	de Alba, Bravo, and Medina (2012a)
Cured beef top inside round (*M. adductor femoris* and *semimembranosus*) with addition of NaCl, NaNO$_2$ and ascorbic acid (A) C = fresh meat not cured	150, 300, 600, 5 min, 20°C	↑ at 300 and 600 MPa	C: ↓ at 600 MPa, → at 150 and 300 MPa	C: ↑ at 600 MPa, → at 150 and 300 MPa				Gimenez et al. (2015)
A = with A WA = Without A Storage for 7 weeks		→ at 150 MPa	Cured samples: a^* tends to ↑ with pressure (A and WA), reaching maximum values in the samples which contained ascorbic acid	The value of b^* was not considered significant to represent the color variations of the samples.				
Dry cured beef "Cecina de Leon"	500, 5 min, 18°C Chilling storage at 6°C for up to 210 days	→ ↑	↑	↑				Rubio et al. (2007)
Beef carpaccio (*m. semitendinosus*) Samples treated as frozen (F) and thawed (T)	400, 500 600, 5 min, frozen (0 and 5°C) and thawed (5 and 20°C)	F: ↑ T: ↑↑	F: ↓ T: ↓↓	F: → T: ↓	+	+ Reduced denaturation of muscle proteins in frozen state		Szerman et al. (2011)
Cured beef carpaccio (*M. semitendinosus*)	400, 650, 1, 5 min, 20, −30°C	20°C: ↑ −30°C: →	20°C: → −30°C: →	20°C: ↑ −30°C: ↑	+	+		Vaudagna et al. (2012)

and increased light scattering (Hughes et al., 2014). It is also known that the degree of myosin denaturation in beef *semi-membranosus* muscle chilled at different rates, which will imply different extent of temperature-induced protein denaturation during chilling, can be related to L^* value (Hector et al., 1992). HP treatment affects myofibrillar proteins by decreasing their solubility due to protein denaturation following formation of larger insoluble protein aggregates, which also may affect the meat surface and the light reflectance (Olsen and Orlien, 2016). In addition, there is a consensus that HP treatment at pressures above 350 MPa trigger lipid oxidation in all types of meat, though the extent of lipid oxidation also depends on the treatment duration and pressure temperature, and on the type of meat (Guyon, Meynier, and de Lamballerie, 2016). Due to the autocatalytic nature of the lipid oxidation process a continuous radical formation and production of lipid hydroperoxides and secondary lipid oxidation products occurs. Especially, the generation of radicals in meat during HP treatments is of importance, as these short-life high-reactive molecules are initiators of lipid and protein oxidation. It was shown, that the formation of radicals under pressure is highly dependent on pressure level, duration and temperature, but also on meat type, since pressurization resulted in a higher level of radicals in beef compared to chicken meat (Bolumar, Skibsted, and Orlien, 2012; Bolumar, Andersen, and Orlien, 2014). It is likely, that the pressure-induced formation of radicals and lipid oxidation products will increase the oxidizing potential of the meat and, therefore play a role in the Mb redox system and related color changes.

Carlez, Veciana-Nogues, and Cheftel (1995) also found that the content of non-heme iron increased from 4.2 (untreated control) to 5.3–5.5 mg/kg meat (at 500 MPa), but concluded that this minor release was not of significant importance. The pressure-induced modification of the heme resulting in the release of the iron atom from the porphyrin ring is often proposed as one of the mechanism behind the color changes due to HP treatment. However, this has to our knowledge not been supported by other scientific studies, thus, is rejected as an important contribution to the pressure-induced color changes of meat so far. Hassan et al. (2002) found that HP treatment (200 and 300 MPa, 20°C, 20 min) of minced beef induced a strong discoloration as all tristimulus color values increased and concluded it was a result of denaturation of Mb and the myofibrillar proteins, myosin and actin. Notably, they reported this visual discoloration as a loss of redness though they reported a small increase in a^* value, which, however, decreased during subsequent storage. This observation expresses that meat color is in fact a complex combination of all visible colors and that the instrumental measurement may diverge from the visually perceived color. Interestingly, they observed that the meat was able to bloom as oxygen became accessible, explained by the oxygenation of the pigment (Hassan et al., 2002). These suggestions for the underlying mechanisms of the red discoloration are supported by the observation on pure Mb systems regarding pressure-induced structural modification of both the Mb molecule and the porphyrin ring reviewed in previous section.

The age of beef before HP treatment is found to be important for the color effect. HP treatment of beef two days post-mortem at 80 to 100 MPa increased the color stability during subsequent aerobic display in the dark at 4°C, whereas treatment of beef at seven or nine days post-slaughter did not affect the color stability (Cheah and Ledward, 1997). The formation of MMb was reduced in HP-treated meat after two days post-mortem. Accordingly, it was suggested that HP treatment resulted in inactivation of the enzymatic system in meat responsible for the oxygen consumption. Oxygen consumption in meat declines rapidly post-slaughter, while the MMb reducing system declines slowly with time, explaining the better color stability of HP-treated beef just two days post-mortem rather than after seven or nine days (Cheah and Ledward, 1997). To the best of our knowledge, the pressure level required to inactive oxygen consumption has not been completely elucidated yet. It is also likely that ageing affects color sensitivity towards pressure effects as a result of the possible changes of Mb state. More research to elucidate the molecular effects of HP treatment on the endogenoues enzymatic systems regarding MMb reducing activity and oxygen consumption is needed.

The temperature at which the meat is HP-treated is another important factor that affects beef color. In general, treatments at higher temperatures result in larger effect on the color. Thus, higher effect of HP on L^* and a^* was described when beef and lamb were treated at 40°C rather than at 20°C (McArdle et al., 2010, 2011, 2013). Even better, if the beef is treated in a frozen state the effect on the color will be even more reduced. Several studies have reported a protective effect on beef color of freezing at sub-zero temperatures prior to pressurization (Fernandez et al., 2007; Realini et al., 2011; Szerman et al., 2011; Vaudagna et al., 2012; Lowder and Dewitt, 2014). Fernandez et al. (2007) found that frozen beef pressurized (650 MPa, 10 min) at −30°C changed the HunterLab color values positively, as L- and a-values decreased and increased, respectively (darker and more red meat), compared to HP treatment at 20°C. It was concluded that the combination of a prior freezing and HP treatment at sub-zero temperatures protects meat against pressure-induced color deterioration, since the fresh meat color was recovered after thawing (Fernandez et al., 2007). Frozen meat has lower water activity than its fresh counterpart, and therefore, thermodynamic restriction on the water being "pressure facilitating". Thus, a frozen state restricts HP-induced modification of the protein spatial structure due to restricted molecular mobility, whereby protein denaturation is limited during pressurization. Consequently, this should minimize the denaturation of myoglobin as well as myofibrillar proteins, though this phenomenon has not been investigated at a molecular level. Indeed, a patent claims a protection of color of fresh meat and meat products (smoked, brined, marinated, salted, and dehydrated) provided that the product never thaws during the entire HP-cycle (compression and decompression) and never reaches temperatures above 0°C (Arnau et al., 2006). Contrary, a reduction of a as a result of the freezing-thawing process has been reported (Jeong et al., 2011). This is due partly to release of exudates, partial protein denaturation, and modification of the endogenous oxygen consumption and MMb-reducing activity systems of meat as a consequence of the freezing-thawing step. Overall, a freeze-thaw cycle led to reduced color stability on display (Jeong et al., 2011) but can minimize HP-induced color changes.

Furthermore, adding different compounds to meat in relation to the HP effect on the interrelation between the different Mb states and the color of meat has also been assessed. The addition of the reducing agent, cysteine (200 or 1000 mg/kg of minced meat, packaged in air) 1 h before processing at 450 MPa (10°C, 10 min) had no significant effect on the visual color, objective color measurements (L^*, a^* and b^* values), myoglobin content or the proportions of related pigments (compared with the pressurized control) (Carlez, Veciana-Nogues, and Cheftel, 1995). The addition of ascorbic acid (250 or 1000 mg/kg of minced meat, packaged in air), 1 h before processing at 450 MPa (10°C, 10 min) induced an additional decrease in a^* value and increase in MMb (as compared to the pressurized control) (Carlez, Veciana-Nogues, and Cheftel, 1995). This observation can be explained by a higher formation of radicals under pressure due to the presence of ascorbic acid (Bolumar, Andersen, and Orlien, 2014), which potentially can oxidize DMb and OMb to MMb resulting in a lower a^* value and increase of MMb. The addition of nicotinamide or nicotinic acid (244, 610 and 1220 mg/kg minced meat) 18 h before processing at 450 MPa (10°C, 10 min) did not provide color protection (Carlez, Veciana-Nogues, and Cheftel, 1995). The minor color changes observed (slightly lower a^* and slightly higher b^*) were ascribed to changes in pH induced by the addition of nicotinamide or nicotinic acid, and consequently, a pH-induced denaturation of myoglobin instead of a pressure-induced denaturation. In another study, phosvitin, a phosphoglycoprotein from egg yolk with a potent metal chelator ability, delayed both lipid- and protein oxidation in HP-treated beef, but did not protect against discoloration (Jung et al., 2013). Addition of phosphates to beef without adding salt (NaCl) increased redness (a^*) by two units upon pressurization at 303 MPa, which is beneficial for HP-treated beef (Lowder and Dewitt, 2014). Similarly, addition of sodium hydrogen carbonate resulted in beef with lower L^*, higher a^* and lower b^* than unpressurized beef (Ohnuma et al., 2013). It seems that compounds increasing the pH of meat can help protect beef color upon pressurization. It has been shown that Mb (in model buffer systems) is more stable to thermal denaturation at higher pH (Hunt, Sørheim, and Slinde, 1999). A similar protective effect of high pH on pressure-induced denaturation of Mb seems to prevail, though the mechanism for this stabilizing effect is unknown.

Curing of beef is a less common practice in the meat industry than curing of pork. Nevertheless, there are cured beef products available, and some have been processed by HP (Table 5). Blending minced beef with a mixture of NaCl (1% in the final product) containing $NaNO_2$ (100–200 ppm in the final product) and stored 18 h at 5°C before HP (350–500 MPa, 10°C, 10 min) provided a protection of the red color as the L^* value increased but no changes of the a^* and b^* values irrespective of pressure level or nitrite concentration (Carlez, Veciana-Nogues, and Cheftel, 1995). The authors described the color of all pressurized samples to be pink, though the whitening effect was not possible to prevent. The time period (18 h, 5°C) before pressurization allowed the formation of nitrosylmyoglobin, which is protected against oxidation to the ferric form. In cured meat, the main pigments are nitrosylmyoglobin and its denatured form nitrosylmyochromogen, which has a stable pink color (Feiner, 2006). Studies have confirmed the evidence of a protective effect of the curing agent, nitrite, on beef color upon pressurization (Table 5). The increase of L^* value might be due to the fact that HP treatments produce changes in the structure of myofibrillar proteins. Moreover, an increase in a^* value after HP has also been described, and would indicate that the formation of nitrosomyoglobin is favored under pressure, which enhances the redness (Gimenez et al., 2015).

For several years, HP has been used in the food industry as a post-packaging intervention, namely to ensure food safety. In this sense, fresh cured beef products such as carpaccio have been given special attention (Szerman et al., 2011; de Alba Bravo and Medina, 2012a; Vaudagna et al., 2012). Carpaccio and similar products are not submitted to a cooking preservation step, and therefore, have a higher microbiological risk than other "microbiologically stabilized" cooked meat products. Hence, studies have focused on the potential of treating carpaccio with HP to minimize this microbial risk. Szerman et al. (2011) studied the application of HP on nitrite-salted (and other additives) beef carpaccio at three different pressure levels (400, 500 and 600 MPa) at low temperatures (0 and 5°C) and at medium temperature (20°C), and found that the negative effect on the chromatic parameters and water holding capacity were reduced when meat was frozen prior to HP treatment. This fact was explained by a reduced denaturation of sarcoplasmic and myofibrillar proteins when pressurized in frozen state. Similarly, the nitrification and freezing of beef prior to pressurization at sub-zero temperature (−30°C) provided protection of the color or a less severe effect on color than a treatment of the raw beef (de Alba, Bravo, and Medina, 2012a). Formation of nitrosylmyoglobin and a low water activity, due to frozen condition and salting, prevents oxidation of myoglobin and severe denaturation of muscle proteins upon HP, respectively, protecting beef color (Rubio et al., 2007).

In summary, the following general trends of the impact of pressure level on beef color as assessed by the instrumental color parameters L^*, a^* and b^* are emphasized: Lightness, as represented by the L^* value, increases already at pressure above 150 MPa, but no further change of L^* value is observed for pressure levels higher than 350 MPa. However, redness (a^*) is severely affected as the a^* value decreases markedly at pressures higher than 350 MPa. Yellowness (b^* value) is less affected by HP, and remains unchanged or increases slightly. The pressure-induced color changes of beef are suggested to be caused by denaturation of myoglobin and other muscle proteins inducing light scattering and lightness, oxidation of OMb to MMb developing gray-brown color and structural modification of the porphyrin ring resulting in fading of red color. The results presented do not unanimously point out one mechanism over the other as the main reason. Countermeasures to protect beef color upon pressurization can include strategies such as HP treatment of beef at sub-zero temperatures, increased pH of meat (e.g. adding sodium phosphates or sodium carbonate) and addition of nitrites allowing the formation of the stable nitrosylmyoglobin.

Currently, some HP-treated beef products exist in the market (Bajovic, Bolumar, and Heinz, 2012). Minced meats, which have a much higher risk of microbial poisoning than steaks, are the main applications in the industry so far. In this sense, the

company Zwaneberg based in the Netherlands uses HP as a cold pasteurization technique to combat hazardous bacteria in the production of steak tartare. The shelf life and food safety of this steak tartare is significantly extended without compromising quality or flavor levels while protecting freshness properties. The company Cargill from the USA uses HP in beef patties also for safety purposes (http://fressure.com/#home). Beef patties or hamburgers for food service are not displayed to consumer prior cooking, and thus, color is of less importance. Once cooked, the HP-treated patties have the same appearance and taste as a non-HP-treated patties. Thus, HP provides an extra decontamination step in the production of beef hamburgers that allows further assurance of food safety.

Pork

Numerous studies involving HP treatment have been carried out on both uncured and cured pork (Table 6 and 7). As the effect of pressure varies considerably between uncured and cured meat, these two types of pork are discussed separately.

Uncured pork

There seems to be two main reasons for the color changes of pressurized meat – HP conditions and Mb redox form prior to HP treatment.

Obviously, the conditions of the HP treatment (mainly pressure level and temperature) will influence the effect on pork color. Low pressures (below 300 MPa) have minor effects on color compared to treatment at higher pressures (Bak et al., 2012b). However, denaturation of myoglobin has been found to take place in porcine *longissimus dorsi* (LD) even at low pressures. Korzeniowski, Jankowska, and Kwiatkowska (1999) reported that after HP treatment at 100 MPa 28% of myoglobin was denatured, increasing to 66% denaturation of Mb after HP treatment at 400 MPa. The increase in the amount of myoglobin denaturation accounted for the observed color changes. Thus, HP treatment at 100 and 200 MPa resulted in a minor increase in brightness giving a light red color, but at 300 MPa, the color changed to pale pink, whereas 400 MPa resulted in a grayish white color (Korzeniowski, Jankowska, and Kwiatkowska, 1999). A change in color parameters in the form of an increase in L* and decrease in a* upon treating pork LD at low pressures (50, 100, and 200 MPa) was also observed by Massaux et al. (1998). However, Chun, Min, and Hong (2014) was not able to measure any color changes of LD after treatment below 150 MPa. Several studies have reported a threshold pressure around 300–400 MPa for the lightness effect. Accordingly, above 400 MPa, no further increase in lightness at increasing pressure can be measured (Shigehisa et al., 1991; Tintchev et al., 2010; Bak et al., 2012b). This is most likely an effect of the marked denaturation of the myofibrillar and sarcoplasmic proteins at pressures above 300–400 MPa (Cheah and Ledward, 1996).

It should be mentioned that cooking can eliminate or minimize the difference in L* between the HP-treated and control samples as seen in for example the study by O'Flynn et al. (2014a). The lightness of cooked (internal temperature 77°C), uncured breakfast sausages did not differ between non-HP-treated controls and sausages where the minced meat for sausage manufacture had been HP-treated (150 MPa or 300 MPa), presumably due to extensive heat-induced protein denaturation. It is noted, that the pressure-induced denaturation and the heat-induced denaturation of Mb proceed via different mechanisms. Accordingly, the resulting pigments are not completely identical, though they both share characteristics of ferric iron and denatured globin (Defaye et al., 1995; Bak et al., 2012b; Ma and Ledward, 2013).

The presence of different redox states of myoglobin prior to pressurization has a great impact on changes in a* as a result of HP treatment. It has been suggested that a color change can be mostly avoided when the OMb to DMb ratio is kept low prior to HP treatment (Wackerbarth et al., 2009). This suggestion is in agreement with the finding that OMb is more prone to pressure-induced denaturation than DMb in aqueous solution (Ogunmola et al., 1977). Along this line, when DMb was the main pigment at the meat surface, it was found that HP treatment (200–800 MPa) caused an increase in a* compared to non-treated pork LD when color was measured immediately after HP treatment. The highest a* values were measured at pressure above 500 MPa (Bak et al., 2012b). This increase in a* was attributed to the formation of a short-lived ferrohemochrome species at pressures from 300 to 800 MPa. However, concomitant with the increase in a*, a large increase in L* was also observed, hence, visually masking the red color (Bak et al., 2012b).

An initial large proportion of OMb can partly explain why most studies find a decrease in a* after HP treatment, especially at high pressure levels. Similar to what has been found for beef, oxidation of OMb to MMb has been given as a reason for the decrease of a* values for pork (Wackerbarth et al., 2009). The two effects of pressure on a* were found to be divided by pressure level as HP treatment of porcine LD at 150–200 MPa led to reduction of ferric MMb to the ferrous state, while HP treatment at 300 MPa led to a decrease in a* attributed to denaturation of myoglobin (Chun, Min, and Hong, 2014). In this study, pork samples 24 h postmortem were cut at about 2 cm from the muscle surface and kept vacuum-packaged for up to two hours prior to HP treatment. No information is given about the Mb redox states, but the conditions described make it reasonable to assume that the surface color was a mix of the different myoglobin redox states, but likely with DMb dominating prior to HP treatment (Figure 1).

Bak et al. (2012b) found that porcine LD HP-treated at 200 MPa (but not at 400 MPa nor at 700 MPa) had the ability to bloom in 30 minutes in the presence of oxygen after both one and six days of anaerobic storage. This emphasizes the importance of measuring color immediately after HP treatment, if the immediate effect of HP treatment is of interest, especially if the meat is re-packaged, thus introducing oxygen and potentially allowing blooming.

It should be noted that the pressure-induced changes in the b* value are usually a result of the same mechanisms underlying changes of a*. It was reported that changes in both a* and b* can be related to changes in the chemical state of myoglobin (Goutefongea et al., 1995).

Table 6. Color changes of HP-treated pork. ↑ indicates an increase of the color parameter, ↓ indicates a decrease of the color parameter, and → indicates no change. + indicates that the respective mechanism was suggested to cause changes in the color.

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L*	a*	b*	Oxidation	Denaturation	Other	
Fresh and cooked pork loin	414, 9 min, 2°C (A), 13 min, 25°C (B)	↑ for uncooked samples	→	↑ for uncooked samples				Anath et al. (1998)
LD	200–800, 10 min, 5, 20°C, ↑	↑	↑	↑	+ (a*)	+ (L*)	A short-lived HP-induced ferrohemochrome resulted in significant color change from day 0 to day 1	Bak et al. (2012b)
Minced pork from post-rigor pork leg	0, 100, 200, 300, 400, 600, 20 min, 20°C, storage at 4°C in air or nitrogen				+ ferrous Mb to denatured ferric Mb	+ at 400 MPa and above	Blooming possible for samples treated up to 300 MPa and packaged in nitrogen	Cheah and Ledward (1996)
LD	50, 100, 150, 200, 250, 300, 10 min, 2°C	→ up to 150 MPa; ↑ 200–300 MPa; ↓ during storage	→ up to 150 MPa and at 250 MPa; ↑ at 200 MPa; ↓ at 300 MPa; ↓ during storage	↑ up to 250 MPa; → at 300 MPa; ↑ during storage	+	+		Chun, Min, and Hong (2014)
Minced pork	600, 30 min, 20°C	↑	→	↓	+	+		Goutefongea et al. (1995)
Fresh pork sausage	414, 552, 0–16 min, 25, 50°C		→					Huang, Moreira, and Murano (1999)
LD	100, 200, 300, 400, 10 min, 20°C					+		Korzeniowski, Jankowska, and Kwiatkowska (1999)
Ground pork patties	300, 15 min, 5, 20, 35, 50°C	↓	→	↑ at 20 and 35°C	+	+		López-Caballero, Carballo, and Jiménez-Colmenero (2002)
Low-acid fermented sausage (chorizo)	300, 10 min, 17°C	↑	→ (possibly due to the addition of the colorants paprika and cayenne pepper)	↑	+	+	Possible loss of active pigment, affecting L*	Marcos, Aymerich, and Garriga (2005)
Low-acid fermented sausage (chorizo)	400, 10 min, 17°C	→ ↑	→	↑			HP after 28 days of ripening Slight decrease in color intensity detected by sensory analysis	Marcos et al. (2007)
LD	50, 100, 200	↓ →	→ ←	↑				Massaux et al. (1998)
Cooked breakfast sausage	150, 300, 5 min, 20°C	→	↑	↑		+		O'Flynn et al. (2014a)
Cooked breakfast sausage	150, 5 min, 20°C	↑ (low salt levels); → (high salt levels)	↓ (low salt levels); → (high salt levels)	→ ←		+	Changes in texture, fat content, size of fat globules, water content, may also result in color changes	O'Flynn et al. (2014b)

						References
Pork slurry	100, 200, 300, 400, 500, 600, 10 min, 25°C	←	→	↓↑	+		Shigehisa et al. (1991)
Pre-rigor fresh pork	215, 15 s, 33°C	↑ LD, TB, PM ↓ SM	↓ LD ↑ TB NS PM and SM		+	Pressure-induced changes in water-protein binding properties ⇒ changes in light scattering	Souza et al. (2011)
Pre-rigor fresh pork loins	215, 15 s, 15, 29°C	Prebloom: → Postbloom: →	Prebloom: ↓ Postbloom: ↓		+		Souza et al. (2012)
Parma ham	600, 9 min	↓→ ←	↓→ ←	+			Tanzi et al. (2004)
LD	0.1–700, up to 10 min, ambient T	←	↑→	+			Tintchev et al. (2010)
LD	600, 700, up to 10 min, ambient T	←	→	+			Wackerbarth et al. (2009)
Meat batters and cooked emulsion sausages	100, 200, 300, 400, 2 min, 10°C	←	→	+	+		Yang et al. (2015)

Table 7. Color changes of HP-treated cured pork. ↑ indicates an increase of the color parameter, ↓ indicates a decrease of the color parameter, and → indicates no change. + indicates that the respective mechanism was suggested to cause changes in the color.

System	HP Condition (MPa)	Color L*	Color a*	Color b*	Oxidation	Denaturation	Other	Reference
Dry-cured Iberian ham	200, 400, 15 min, 20°C	↑	↓		+	+	MbFe(II)NO sensitive to HP at 400 MPa	Andrés et al. (2006)
Dry-cured Iberian ham	200–800, 15 min, 20°C	↓ 200–400 MPa → 600–800 MPa	→	→	+	+		Andrés et al. (2004)
Minced cured restructured ham (dried 20% or 50%)	600, 5 min, 13°C	↑ 20% dried → for 50% dried	↓ 20% dried → for 50% dried	↓ 20% dried → for 50% dried	—	+		Bak et al. (2012a)
Minced cured restructured ham (dried 20% or 50%)	600, 5 min, 13°C	→	↑→ during storage	Varying effect depending on raw material	Not simple photooxidation	—	structural changes	Bak et al. (2013)
Dry-cured pork loin	300, 350, 400, 10 min, 20°C	↑	↓		+	+	change in the nitrosylmyoglobin; Presence of chili pepper may contribute to color	Campus et al. (2008)
Sliced vacuum-packed dry-cured Iberian ham and loin	200 and 300, 15 or 30 min, <14°C	↓ But difference eliminated w/ storage	→	→	+	(+)		Cava et al. (2009)
Sliced skin-vacuum packed dry-cured ham	600, 6 min, 15°C	↓	→↑ after 50 days of refrigerated light storage	→		+	Protective effect of HP on nitrosylmyoglobin	Clariana et al. (2011)
Sliced skin vacuum packed dry-cured ham	400, 6 min, 12°C	↑ after 50 days of storage	→ after 50 days of storage	→ after 50 days of storage		+		Clariana et al. (2012)
Dry-cured Serrano ham	400, 500, 600, 5 min, 12°C	↑→	→ 400 MPa ↓ 500–600 MPa	→ 400–500 MPa ↑ 600 MPa	↑	↑	Storage had a greater effect on color than HP	de Alba et al. (2012b)
Dry-cured restructured minced pork	500, 600, 7 min, 13°C	↑ 20% dried → for 50% dried	↓ 20% dried → for 50% dried	↓ 20% dried → for 50% dried	+	+		Ferrini et al. (2012)
Sliced vacuum-packed dry-cured Iberian ham	600, 6 min, 12°C. Storage at 2 ± 1°C in the dark for 0, 30, and 120 days	↑ after 0 and 120 days of storage	→ due to curing, vacuum packaging, and dark storage	→	—	+	Possible fat crystallization could also affect L*	Fuentes et al. (2014)
Restructured dry-cured ham	600, 3 min at 10°C → retail display light conditions at 4 +/− 2°C for 48 h.	↑ → after 48 h retail display	↑	↓ after 48 h retail display	(+)	(+)		Fulladosa et al. (2009)
Cooked cured ham	600, 30 min, 20°C	↑	↑	↑				Goutefongea et al. (1995)
Cured pork sausages	500, 600, 1 sec, 3, 6, 9 min, 40, 50, 60°C	→ ↓	→	→↑		+		Grossi et al. (2011)
Cured pork sausages	100, 300, 5, 20 min, 6–8°C	↑	→↓↑	→↓↑				Jiménez Colmenero et al. (1997)

Product	Conditions				Reference
Two types of cooked ham and one type of raw smoked pork loin	300, 400, 500, 10, 30 min	→↓ cooked ham ↑ smoked pork loin	↑		Karowski et al. (2002)
Sliced cooked cured ham	300, 15 min, 5, 20, 35, 50°C	→	↑		López-Caballero, Carballo, and Jiménez-Colmenero (2002)
Low-acid fermented sausage (fuet)	300, 10 min, 17°C	←	→	+	Marcos, Aymerich, and Garriga (2005)
Low-acid fermented sausage (fuet)	400, 10 min, 17°C	↑	↑		Marcos et al. (2007)
Cooked ham	600 MPa, 10 min, 20°C	↑	↑		Pietrzak et al. (2007)
Uncooked and cooked ham	500, 600, 1 min, 3°C	←	↑	+	Pingen et al. (2016)
Meat batter	300, 30 min, 7, 20, 40°C	←	→↓		Ruiz-Capillas et al. (2006)
Frozen GH and ERS hams	400, 600, 10 min, 10–20°C	GH hams: ↑ for BF →for SM ERS hams: →BF →↑SM	GH hams: ↑→ for BF →for SM ERS hams: ↑	+ protective action of curing and drying process	Serra et al. (2007)
Frankfurters	215, 15 s, 15.5, 29.4°C	↑	↑		Souza et al. (2012)
Cooked ham	600, 10 min, 10°C	→	↑		Vercammen et al. (2011)

Cured pork

Nitrite curing of pork is widely used in the meat industry due to the heat and shelf-life stable pink pigment nitrosylmyochromogen. It is generally accepted that cured meat color is particularly resistant to HP treatment. Therefore, subjecting cured pork to HP treatment is highly interesting, as evidenced by the huge amount of published research papers in this field (Table 7). The majority of the studies, 19 out of the total 25 investigations, reported no change in redness, supporting the protective role of nitrification on meat color. However, some color changes caused by HP treatment of cured meat have been reported. Overall, L^* tends to increase or remain stable, whereas a^* and b^* usually decrease or remain unchanged after HP treatment. In most studies, the increase in lightness is explained by changes in the myofibrillar component, i.e. protein denaturation leading to an increased light reflectance, similar to uncured meat. For cooked, cured meat, no further increase in L^* is observed due to HP treatment because of the initial heat denaturation of proteins (Goutefongea et al., 1995). Only two studies reported a decrease in L^* value of HP-treated pork. Namely, Andrés et al. (2004) for dry-cured ham, and Karowski et al. (2002) for cooked, cured ham. A potential explanation for the decrease in L^* is that HP-induced alteration of protein structure may affect the structure and surface properties in such a way that the ratio of reflected to absorbed light is decreased, hence, making the meat appear darker (Andrés et al., 2004). For dry-cured meat products, the HP effect on color is largely dependent on the production stage at which HP treatment is applied (Serra et al., 2007). Applying HP as late as possible in the production of dry-cured ham is recommended to allow full development of nitrosylmyoglobin and nitrosylmyochromogen (Serra et al., 2007). Another reason for HP-induced color changes may be that the nitrite to pigment ratio is too low to allow sufficient pigment conversion, though this is more relevant for beef than for pork, due to the higher myoglobin content of beef (Goutefongea et al., 1995).

Despite the stability of nitrosylmyoglobin and nitrosylmyochromogen to HP treatment, HP-treated cured pork is still susceptible to photooxidation (Andrés et al., 2006) if the meat is not stored under vacuum and in the dark (Fuentes et al., 2014). Still, it has been determined that HP treatment can improve color stability during display under retail conditions (Bak et al., 2013). This stabilizing effect is hypothesized to be caused by the formation of intermolecular hydrogen bonds with water, which further stabilize the nitrosylheme (Bak et al., 2013). When a decrease in a^* is found, it is not necessarily the result of a change in the heme group of the cured meat pigments as illustrated from the lack of change in the shape of the reflectance curves by Bak et al. (2012a), but may be the result of change in the conjugated system structure (Sun et al., 2009).

The color of meat is strongly related to the water content, thus, a decrease in water content commonly results in a decrease in L^* and an increase in a^* due to an increasing pigment concentration (Ferrini et al., 2012). In addition, the amount of water available also affects the outcome from the HP treatment, because water plays a key role in the pressure-induced disruption of inter- and intramolecular forces in proteins. A significant interaction between HP and water content has been found. At high water content (66%), HP treatment increased L^* and reduced a^* and b^*, while no effect of HP was found at low water content (44%) (Ferrini et al., 2012). Similar results were found for cured, restructured ham dried to water contents of 64% and 44% respectively by Bak et al. (2012a). However, as no change in the shape of the reflectance curve of the high water content ham (64%) was observed, the color changes were mainly attributed to denaturation of the protein part of nitrosylmyochromogen and/or changes in other sarcoplasmic proteins or in the myofibrillar component (Bak et al., 2012a). The unchanged color of the HP-treated restructured ham at 44% water was attributed to the low water content, which likely suppressed the role of water in the pressure-induced denaturation process of proteins, thereby having no effect on the color (Bak et al., 2012a; Ferrini et al., 2012).

The interest in HP treatment of the (already) stable-colored cured pork products is due to the potential to further stabilize the color. Even though a high water content in cured meat can result in some discoloration due to pressurization, simultaneously, a high water content has been found to stabilize the color during subsequent storage. Storing HP-treated (600 MPa) cured ham with either 64% or 44% water showed that only the color of the cured ham with 64% water content was stable during storage. This result was explained by a stabilizing effect of water on nitrosylmyochromogen during pressure due to formation of intermolecular hydrogen bonds (Bak et al., 2013). HP-treated (400, 500, or 600 MPa) sliced, vacuum-packaged, dry-cured ham was also found to be slightly more color stable during storage up to 60 days compared to untreated dry-cured ham (de Alba et al., 2012b). However, it was reported that a^* values were similar for untreated and HP-treated (200, 300 MPa) dry-cured Iberian ham and loin after 60 days of storage. Interestingly, it seems that high pressure levels (above 400 MPa) are needed to stabilize the cured meat pigments during storage.

In summary, the lightness (L^*) of uncured pork increases at pressures up to 400 MPa due to denaturation of the proteins resulting in an increased light reflection and scattering. The oxidation state of the iron in Mb is of outmost importance for the pressure effect on the red color. Though pressure may impair the red color immediately after HP treatment, pork treated at pressures lower than 300 MPa may still be capable of blooming under the right conditions. The pressure-induced discoloration can be counteracted by curing the pork prior HP treatment due to the general stability of nitrosylmyochromogen, and a beneficial effect of HP with long storage periods. The potential of the HP technology for cured meat is shown by the commercial availability of HP-treated cured meats worldwide. Products include both cooked products such as deli hams and tapas platters, and dry-cured hams such as serrano. Producers of such HP-treated pork products include the Spanish companies Espuña and NOEL. US company Hormel® has a range of HP-treated deli meats, including pork products, sold under the brand name Natural Choice®.

Poultry

While beef and pork are both considered as "red meats", poultry is considered "white meat". The normal color of raw

Table 8. Color changes of HP-treated poultry meat. ↑ indicates an increase of the color parameter, ↓ indicates a decrease of the color parameter, and → indicates no change. + indicates that the respective mechanism was suggested to cause changes in the color.

System	HP Condition (MPa)	Color			Discoloration mechanism(s)			Reference
		L^*	a^*	b^*	Oxidation	Denaturation	Other	
Minced chicken	500, 60 min, −10, +5, 20, 50°C	↑	↓					Beltran et al. (2004)
Breast fillets	400, 1–20 min + multiple cycles of 10 min, 5°C.	↑	↑	↑				Del Olmo et al. (2010)
Pieces of chicken	0.1–600. 1 min, 5°C.	↓						Knorr (2007)
Chicken breast fillets	300–600, 5 min, 15°C	↑	↑	↑	+	+		Kruk et al. (2011)
Thawed minced chicken breast,	300, 600, 800, 10 min, 5°C	↑	↓					Mariutti et al. (2008)
Minced chicken breast	400, 500, 600: 10 min, 15°C 400: 10, 30, 45 min, 15°C	↑	↓	↓				Massaux et al. (2000)
+ NaCl (0.5, 1, 2%)	400:10 min, 15, 30, 40°C							
Minced chicken fillet (sausage)	200, 400, 600	↑	↓	↑↓				Omana, Plastow, and Betti (2011)
+ NaCl, STPP, or BG	(20, 40, 60°C, 30 min							
Mechanically recovered poultry meat	500, 50, 60, 70, 75°C, 30 min	↑	↓		+	+	Hemoglobin	Yuste et al. (1999)

chicken breast is slightly pinkish, but can also appear bluish-white to yellow colored. Surely, this is due to the low content of myoglobin, which reduces the red color appearance compared to beef and pork (Table 1). Therefore, the effect of pressure, especially on redness, is not as pronounced for chicken meat as for beef and pork (Figure 3). The investigations of the pressure effect on beef and pork have established that high pressure results in a lightning of the meat. This should promote HP as a prospective processing technology for chicken meat, as the raw meat is already more light/white.

Indeed, as seen in Table 8, all studies but one reported that lightness increased upon pressurizing the respective chicken meat. When whole chicken breast fillets were pressurized (300–600 MPa) an increase in lightness and an increase in both redness and yellowness were found (Del Olmo et al., 2010; Kruk et al., 2011). The increase in the L^* value was attributed to the commonly noted HP lightness effect, thus namely due to denaturation of myofibrillar proteins (Kruk et al., 2011). However, contrary to the general observation for both beef and pork, a^* increased upon pressurization (it is noted, that the color measurements were performed several hours after pressurization). The increase in a^* was explained as a consequence of a (reversible) renaturation of pressure-denatured myoglobin from the time of HP treatment to the color measurement was performed (Kruk et al., 2011). It is more likely, though, that the vacuum packing has promoted the formation of DMb or a mix of the different myoglobin states, and the time period between HP treatment and color analysis allowed for blooming (*vide supra*).

Pressurizing minced chicken followed the general trend, thus, L^* increased and a^* decreased (Massaux et al., 2000; Beltran et al., 2004; Mariutti et al., 2008; Omana, Plastow, and Betti, 2011). Surely, the mincing process will introduce oxygen into the meat resulting in formation of OMb, thereby enabling oxidation of OMb to MMb resulting in the reduced redness. An addition of additives (sodium chloride, sodium tripolyphosphate and β-glucan, and combinations thereof) to the meat was found to have little impact on the color characteristics (Massaux et al., 2000; Omana, Plastow, and Betti, 2011).

Interestingly, Yuste et al. (1999) reported that such pressure-induced discoloration and paleness can be beneficial for mechanically recovered chicken meat sausages, as the HP-treated sausages were lighter and more yellow than the conventionally cooked sausages.

In summary, HP treatment of chicken meat increases lightness, while the impact on redness depends on whether the meat is treated as a whole meat part or as minced chicken. Examples of commercial HP processed poultry products are whole or sliced ready to eat/heat chicken meat products, e.g. Hormel's Deli Turkey and Chicken.

Conclusion

HP treatment of meat has a great impact on meat color irrespective of the type of meat ("red" or "white"), and meat discoloration is observed even when processing at low pressure levels (P < 350 MPa). Altogether, the pressure-induced color change is an interrelationship between modifications of the myoglobin molecules as such and alterations in the meat microstructure. Different factors such as P/T ranges and thresholds for protein denaturation, level of oxygenation of the myoglobin, presence of antioxidants, reducing agents, myoglobin binding ligands (e.g. NO or CO), and the physical state of the sample (e.g. pressurization of frozen or unfrozen sample) can impact color changes resulting from HP treatment.

In general

- Pressurization results in increased lightness.

This effect is ascribed to the denaturation of muscle proteins resulting in protein aggregation, in effect changing the meat surface properties, and, consequently, changing the absorbed, diffracted, and reflected light ratio, and creating a lightness effect.

- The pressure effect on redness varies.

This review showed that the oxidation state of myoglobin and the surrounding conditions during and after pressurization have a crucial role in the extent of the red color changes. The pressure-induced decrease in redness was mainly caused by the

loss of the active pigment by oxidation of the ferrous state to the brownish MMb as well as pressure-induced denaturation of Mb, also resulting in a ferric Mb-form.

Ultimately, a microbial inactivation using HP without a dramatic color change taking place could provide the meat industry with a powerful tool to extend shelf life, and thus, market boundaries globally.

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