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Effect of Myoglobin State on Color Stability of High Pressure Processed Ground Beef

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Summary with Implications

High pressure processing, a non-thermal pasteurization technique, can reduce E. coli in beef but the use is limited due to discoloration of raw beef after high pressure processing. Different states of myoglobin have inherently different color stability. The objective of this study was to determine the impact of myoglobin state on color stability of raw beef patties treated with high pressure processing. Modified atmosphere packaging (high oxygen-oxymyoglobin, carbon monoxide-carboxymyoglobin), vacuum packaging (deoxymyoglobin) or added potassium ferricyanide (metmyoglobin) treatments were used to prepare patties with desired myoglobin states. Color was measured (CIE L, a*, b*) before and after high pressure processing over a storage period of 21 days. Regardless of pressure and duration, beef patties lost redness after high pressure processing. However, carboxymyoglobin showed better color retention as compared to deoxymyoglobin, oxymyoglobin and metmyoglobin.*

Introduction

High pressure processing (HPP) is a non-thermal pasteurization technique where microorganisms are killed by cell wall or spore coat rupture at high pressure (300 to 800 MPa). However, use of HPP on raw meat products is limited due to the resulting loss of red color and, at times, a "cooked" appearance. High pressure-induced protein denaturation, loss of pigment, difficulty in pigment visualization by opaque flesh, and oxidation of myoglobin to metmyoglobin are possible causes.

Meat color is largely decided by the pigment myoglobin (Mb), where globin is attached to a porphyrin ring with an iron center. The oxidation state of iron (Fe^{2+}/Fe^{3+}) and bound ligands generate different Mb states with different visual color and color stability. The purple red color of the freshly cut beef is due to deoxyMb where Fe^{2+} is not bound to any ligand. Exposure to air binds oxygen to Fe^{2+} and develops bright cherry red color (oxyMb), which is accepted by the consumer as fresh meat color. Over time, oxyMb is oxidized to form brown metMb (Fe^{3+}). CarboxyMb, where carbon monoxide is bound to Fe^{2+} also imparts the desired bright cherry red color and is more stable. The objective of this study was to determine the effect of myoglobin state on color stability in HPP-treated ground beef.

Procedure

Patty preparation

Boneless, denuded USDA Select beef top rounds were ground through 1/2 in and 1/8 in plates, and subdivided into two batches of 5 lbs. One 5 lb portion was mixed with an aqueous solution of potassium ferricyanide (227 mL of a 0.01% solution) to oxidize myoglobin to metmyoglobin using a commercial kneader-mixer (RM-20, Manica USA, St. Louis, MO) and four patties (113 g) were formed using a 4.3 in diameter hand operated hamburger press. Patties were placed on styrofoam trays, overwrapped with oxygen permeable polyvinyl chloride wrap, and stored at 39°F for two days to form metMb. To prepare patties with the remaining myoglobin states, 12 patties (113 g) were prepared from the other portion of ground beef. Four of those were vacuum packed (deoxyMb), four were packed in an 80% oxygen atmosphere (oxyMb), and four were packed in a 0.4% carbon monoxide anaerobic atmosphere (carboxyMb). All patties were stored at 39°F for 2 days to allow for conversion to desired myoglobin state, vacuum packed, and sealed immediately before HPP. Three independent replications were produced.

High pressure processing treatment

Samples were processed using a large scale high pressure processing unit (Hiperbaric 55, Miami, FL) located in the food grade lab of the Food Processing Center, University of Nebraska–Lincoln. All samples, except controls, were HPP with three different conditions (pressure and hold time; 600 MPa / 3 minutes, 600 MPa / 6 minutes and 450 MPa / 3 minutes) and were subsequently stored at 39°F throughout the study. The three pressure combinations were chosen based on their effectiveness to reduce pathogens.

Colorimetry

Color of the patties was measured (CIE L*, a*, b*) through the vacuum pouch before HPP and on days 3, 7, 12, 14, 19 and 21 after HPP. A colorimeter (CR-300, MINOLTA, Japan) was used to determine the instrumental color which uses diffuse D65 illumination, 8 mm viewing port, and 0° viewing angle (specular component included). The system was calibrated to the included white calibration plate covered in the vacuum pouch before analyzing. The average of at least three measurements was taken from patty surface. Change in color, with respect to the control samples from the same myoglobin state, was expressed as ΔE, where $\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}$

Subscripts *i* and *f* represent before and after HPP.

Statistical analyses

Statistical analyses were conducted on color data (L, a*, b*, ΔE) using SAS software version 9.4 (SAS Cary, NC) to see the main effects of myoglobin states and HPP treatment and their interactions within each day of storage. Treatment interaction and main effects were determined using

Table 1. Least square means (± SE) for main effect of high pressure processing (HPP) on color (L*, a*, b*) and change in color (ΔE) during storage of ground beef patties.

		Color values						
Color trait	HPP (MPa/min)	Before	Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L*	0/0	43.13±0.50	42.65±0.54^{b}	41.95±0..43^{b}	41.79±0.38^{b}	41.24±0.44^{b}	37.86±0.45^{c}	38.87±0.40^{c}
	450/3	43.03±0.50	54.75±0.54^{a}	55.88±0.43^{a}	56.42±0.38^{a}	56.60±0.44^{a}	56.55±0.45^{a}	56.45±0.40^{a}
	600/3	42.16±0.50	54.13±0.54^{a}	55.79±0.43^{a}	56.00±0.38^{a}	56.27±0.44^{a}	55.22±0.44^{b}	54.95±0.40^{b}
	600/6	42.95±0.50	54.16±0.57^{a}	56.00±0.46^{a}	55.73±0.40^{a}	55.80±0.47^{a}	54.99±0.47^{b}	54.65±0.43^{b}
	P-value	0.206	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
a*	0/0	15.24±0.46	13.19.±0.45	9.92±0.34^{b}	9.10±0..29^{b}	9.10±0.24^{c}	9.99±0.370^{b}	9.59±0.24^{c}
	450/3	14.59±0.46	12.28±0.45	11.39±0.34^{a}	10.58±0.29^{a}	10.23±0.24^{b}	10.34±0.37^{b}	10.52±0.24^{b}
	600/3	14.89±0.46	12.34±0.45	11.39±0.34^{a}	11.15±0.29^{a}	11.21±0.24^{a}	11.54±0.37^{a}	11.07±0.24^{b}
	600/6	14.46±0.46	12.68±0.48	11.36±0.36^{a}	11.06±0.31^{a}	11.32±0.25^{a}	12.04±0.39^{a}	11.91±0.26^{a}
	P-value	0.352	0.480	0.010	<0.001	<0.001	0.001	<0.001
b*	0/0	7.48±0.40	5.97±0.22^{c}	7.51±0.14*	6.49±0.17^{b}	6.01±0.15*	6.65±0.22^{c}	6.34±0.25^{b}
	450/3	7.18±0.40	11.34±0.22^{b}	11.39±0.14*	11.44±0.17^{a}	11.37±0.15*	11.96±0.22^{ab}	11.58±0.25^{a}
	600/3	7.36±0.40	11.86±0.22^{ab}	11.64±0.14*	11.63±0.17^{a}	11.51±0.15*	12.45±0.22^{b}	11.93±0.25^{a}
	600/6	7.15±0.40	12.01±0.23^{a}	11.54±0.15*	11.48±0.18^{a}	11.50±0.16*	11.70±0.24^{a}	11.79±0.26^{a}
	P-value	0.820	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔE	450/3		13.45±0.74	14.60±0.53	15.56±0.61	16.35±0.52	19.49±0.749	18.44±0.57
	600/3		13.16±0.74	14.64±0.53	15.38±0.61	16.26±0.52	18.50±0.49	17.22±0.57
	600/6		13.19±0.79	14.85±0.56	15.21±0.65	15.83±0.55	18.26±0.52	17.09±0.61
	P-value		0.954	0.941	0.928	0.767	0.203	0.209

a-c LS means with a column and within a color trait with a common superscript are similar ($P > 0.05$).
* Signifies a significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day.

PROC GLIMMIX. When significant interaction or main effects were identified (P ≤ 0.05), separation of Least Square Means was conducted.

Results

Regardless of the pressure conditions and myoglobin state, HPP had a detrimental effect on the color of the beef patties (Table 1). Of all color traits measures on each day, only b* values on days 7 and 14 had a significant myoglobin state by HPP treatment interaction ($P < 0.050$) where HPP treated samples were more yellow in color. Lightness (L*) and yellowness (b*) increased ($P<0.001$) after HPP on all days of storage. The redness of the samples was similar ($P = 0.48$) on day 3 of storage but non-HPP treated samples were less red on all subsequent days of storage. Within each day of storage, color change with respect to control samples (ΔE) was similar for all three HPP conditions (P>0.05).

Comparison among different Mb states (Table 2) revealed that redness (a*) is best retained by carboxyMb > deoxyMb > MetMb ~ OxyMb. This is likely directly correlated to the color stability of the various Mb states at formation. Carbon monoxide binds to Fe^{2+} 500 times stronger than oxygen, which imparts higher stability and redox resistance to carboxyMb. DeoxyMb can maintain its original purplish red color in anaerobic conditions such as vacuum packaging. Under high pressure conditions, cherry red oxyMb could be converted to metMb or the protein portion of myoglobin denatured resulting in loss of redness. MetMb cannot revert back to oxyMb due to the absence of a high oxygen atmosphere under vacuum packaging and possible loss in reducing enzymes.

During storage, redness (a*) of HPP treated carboxyMb and deoxyMb gradually decreased. The change was most prominent within the first week of storage (between day 3 and day 7). Gradual conversion of metastable carboxyMb to deoxyMb and metMb might be responsible for it. However high pressure processed metMb and oxyMb did not lose redness any further during storage.

Conclusions

Applying HPP treatment caused ground beef to be lighter and more yellow regardless of myoglobin state. CarboxyMb retained color better than other myoglobin states. However, controlling the state of myoglobin at the time of treatment does overcome color changes due to HPP.

Table 2. Least square means (± SE) for main effect of myoglobin (Mb) state on color (L*, a*, b*) and change in color (ΔE) during storage of ground beef patties.

		Color values					
Color trait	Mb state	Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L*	CarboxyMb	50.53±0.54[b]	51.58±0.43[b]	51.02±0.38[c]	51.50±0.44[b]	49.82±0.45[b]	50.09±0.40[b]
	DeoxyMb	51.11±0.57[b]	52.13±0.46[b]	52. 40±0.40[b]	52.16±0.47[b]	51.94±0.47[a]	50.79±0.42[b]
	MetMb	52.86±0.54[a]	54.00±0.43[a]	53.79±0.38[a]	53.80±0.44[a]	52.63±0.45[a]	53.18±0.40[a]
	OxyMb	51.18±0.54[b]	51.91±0.43[b]	52.72±0.38[ab]	52.45±0.44[b]	50.25±0.45[b]	50.86±0.40[b]
	P-value	0.028	0.002	<0.001	0.007	<0.001	<0.001
a*	CarboxyMb	17.38±0.45[a]	14.34±0.34[a]	13.44±0.29[a]	13.16±0.24[a]	13.91±0.37[a]	13.50±0.24[a]
	DeoxyMb	13.29±0.48[b]	11.05±0.36[b]	10.16±0.31[b]	10.49±0.25[b]	10.47±0.39[b]	10.67±0.26[b]
	MetMb	9.92±0.45[c]	9.59±0.34[c]	9.61±0.29[b]	9.71±0.24[c]	10.19±0.37[bc]	9.66±0.24[c]
	OxyMb	9.89±0.45[c]	9.09±0.34[c]	8.68±0.29[c]	8.50±0.24[d]	9.35±0.37[c]	9.25±0.24[c]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b*	CarboxyMb	9.27±0.22[b]	9.54±0.14*	9.50±0.17[b]	9.34±0.15*	10.10±0.22[b]	9.75±0.25[b]
	DeoxyMb	9.40±0.23[b]	10.16±0.15*	9.71±0.18[b]	9.55±0.16*	10.25±0.24[b]	10.16±0.26[ab]
	MetMb	11.22±0.22[a]	11.15±0.14*	11.04±0.17[a]	10.87±0.15*	11.40±0.22[a]	10.86±0.25[a]
	OxyMb	11.29±0.22[a]	11.23±0.14*	10.80±0.17[a]	10.63±0.15*	11.01±0.22[a]	10.87±0.25[a]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.006
ΔE	CarboxyMb	12.59±0.86	14.68±0.61	14.40±0.71	15.83±0.60	18.61±0.57	16.41±0.66
	DeoxyMb	15.10±0.93	14.57±0.66	16.95±0.76	17.118±0.65	19.22±0.61	17.88±0.71
	MetMb	12.17±0.86	13.73±0.61	14.91±0.71	15.34±0.60	18.74±0.57	18.76±0.66
	OxyMb	13.20±0.86	15.81±0.61	15.29±0.71	16.31±0.60	18.42±10.57	17.27±0.66
	P-value	0.135	0.153	0.117	0.257	0.801	0.109

[a-c] LS means in a column and within a color trait with a common superscript are similar ($P > 0.05$).
* Signifies a significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day.

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