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Effect of Ingredients and Packaging on Color of High Pressure Processed Ground Beef

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Summary with Implications

High pressure processing is a non-thermal pasteurization technique to control pathogens, like E. coli. However, color changes in raw beef induced by processing restrict high pressure processing's use within the beef industry. The objectives of this study were to investigate the effects of adding curing agents (nitrite) and packaging with or without reducing compounds (ascorbic acid/erythorbate) on color retention in high pressure processed ground beef. High pressure processing resulted in a detrimental effect on the color of the beef patties for all treatments. Lightness and yellowness increased and redness decreased after high pressure processing. The effect remained the same throughout the course of the study (up to 21 days). However, there was less color change in samples treated with reducing compounds. Both inorganic and natural sources of nitrite and ascorbic acid/erythorbate performed similarly in terms of their ability to maintain redness. Treatments leading to formation of nitrosylmetmyoglobin (Fe^{3+}) had less color change as compared to the treatments leading to the generation of nitrosylmyoglobin (Fe^{2+}).

Introduction

A major challenge faced by the ground beef processors is microbial contamination such as *E coli* O157:H7 and other Shiga toxin producing *E. coli* (STEC). Sanitary handling, pre-harvest washing, and spraying the carcass with organic acids reduces the risk but does not completely eliminate STEC. In ground beef and other non-intact beef products, STECs are considered an adulterant by the USDA. These products are a greater food safety risk as pathogens can be introduced throughout the product, rather than just on the surface. High pressure processing (HPP) is a non-thermal pasteurization technique where between 300 and 800 MPa treatment ruptures the cell wall of bacteria. Use of HPP on raw meat products is uncommon due to high pressure-induced protein denaturation and discoloration. Therefore, to develop a HPP based pasteurization technique for raw ground beef products, it is important to find ways to stabilize meat color. The bright red color of nitrosylmyoglobin in anaerobically packaged raw meat is similar in color to oxymyoglobin but more stable and is formed with the addition of nitrite. Reducing agents, such as erythorbate or ascorbic acid, increase the reaction rate during curing and have also been shown to improve color stability in raw ground beef (2016 Nebraska Beef Report pp.158–160). The objective of this study was to determine the effects of differences in myoglobin state created by ingredient and packaging conditions and HPP treatment on the color stability of ground beef patties.

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Procedure

Patty preparation

Boneless, denuded USDA Select beef top rounds were ground through 1/2 in and 1/8 inch grinding plates, and subdivided into 5 lb batches for each of six treatments. The fine ground beef was mixed using a commercial kneader-mixer (RM-20, Manica USA, St. Louis, MO) with the following ingredients to convert myoglobin to different nitrosylmyoglobin states with or without the addition of reducing compounds (sodium erythorbate or ascorbic acid from cherry powder). The treatments (T1-T6) are as follows:

T1: Sodium nitrite 156 ppm/vacuum packaging (VP; anaerobic packaging)

T2: Sodium nitrite 156 ppm + Sodium erythorbate 547 ppm / VP

T3: Celery juice powder (VegStable 506, Florida Food Products, Inc., Eustis, FL; to add 100 ppm sodium nitrite equivalent) / VP

T4: Celery juice powder (equivalent to 100 ppm nitrite) + 0.43% cherry powder (VegStable 515, Florida Food Products, to add 469 ppm ascorbic acid) / VP

T5: Sodium nitrite 156 ppm/ oxygen permeable wrap (OPW; aerobic packaging)

T6: Sodium nitrite 156 ppm + Sodium erythorbate 547 ppm / OPW.

Four 113 g patties were prepared from each of the six treatments. Patties were formed using a 4.3 in diameter hand operated hamburger press. All T1, T2, T3 and T4 patties were vacuum packed using the vacuum sealer (Multivac Model C500; Multivac Inc., Kansas City, MO). Treatments T5 and T6 treated patties were placed on foam trays and overwrapped with oxygen permeable polyvinyl chloride. All patties were stored at 39°F for two days to allow for conversion to nitrosylmyoglobin (T1-T4) and nitrosylmetmyoglobn (T5-T6). After 48 hours, T5 and T6 were vacuum packaged just prior to HPP treatment. 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 (non-HPP treated) were HPP with three different conditions of 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.

Colorimetry

Color of the patties was measured (CIE L*a*b*) through the vacuum pouch before

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

		Color values					
Color traits	HPP (MPa/ min)	Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L*	0/0	40.78±0.48[b]	42.20±0.34[c]	43.13±0.30[c]	43.73±0.38[c]	42.06±0.43[b]	43.31±.034[c]
	450/3	53.58±0.50[a]	54.49±0.36[b]	54.87±0.31[b]	55.09±0.39[b]	55.30±0.45[a]	55.26±0.36[b]
	600/3	54.53±0.48[a]	55.62±0.34[a]	56.66±0.30[a]	56.2±0.38[a]	56.45±0.43[a]	55.61±0.34[ab]
	600/6	53.47±0.48[a]	55.84±0.34[a]	55.98±0.30[a]	56.20±0.38[a]	56.36±0.43[a]	56.29±0.34[a]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
a*	0/0	22.50±0.55[a]	21.98±0.56[a]	19.46±0.46[a]	19.33±0.44*	21.04±0.50*	19.39±0.62[a]
	450/3	21.33±0.57[ab]	18.18±0.58[b]	16.56±0.48[b]	14.91±0.46*	16.02±0.52*	16.38±0.64[b]
	600/3	18.17±0.55[b]	16.35±0.56[c]	14.31±0.46[c]	14.67±0.44*	14.62±0.50*	14.68±0.62[bc]
	600/6	20.43±0.55[c]	15.81±0.56[c]	14.46±0.46[c]	14.76±0.44*	13.77±0.50*	13.21±0.62[c]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b*	0/0	8.95±0.17[d]	9.50±0.18[c]	8.70±0.14[c]	8.34±0.16[c]	9.20±0.20[b]	8.90±0.17*
	450/3	11.14±0.18[c]	10.67±0.19[b]	10.67±0.15[b]	10.44±0.16[b]	11.39±0.20[a]	11.00±0.18*
	600/3	11.67±0.17[b]	11.32±0.18[a]	11.27±0.14[a]	11.39±0.16[a]	11.95±0.20[a]	11.55±0.17*
	600/6	12.34±0.17[a]	11.12±0.18[ab]	11.44±0.14[a]	11.49±0.16[a]	11.72±0.20[a]	11.65±0.17*
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔE	450/3	13.14±0.89	13.29±0.54	12.84±0.62	12.95±0.52	15.07±0.73	13.17±0.62
	600/3	15.00±0.85	14.90±0.52	14.92±0.59	14.01±0.50	16.54±0.71	13.98±0.60
	600/6	13.90±0.85	15.32±0.52	14.34±0.59	14.14±0.50	16.57±0.71	14.94±0.60
	P-value	0.322	0.026	0.056	0.209	0.259	0.134

[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.

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, 8mm 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 the cut surface. Change in color, ΔE, was calculated with respect to the control samples (non-HPP treated) within each of the six treatments, where

$$\Delta E = [(L_f\text{-}L_i)^2 + (a_f\text{-}a_i)^2 + (b_f\text{-}b_i)^2]^{1/2}$$

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

Statistical analyses

Statistical analyses were run on color data (L, a*, b*, ΔE) using SAS software version 9.4 (SAS Cary, NC) to see the main effects of ingredient/packaging conditions (T1-T6) and HPP treatment and their interactions within each day of storage. Treatment interaction and main effects were determined using PROC GLIMMIX. When significant interactions or main effects were identified ($P \leq 0.05$) , separation of least square means was conducted.

Results

Regardless of the ingredients/packaging treatment, HPP had a detrimental effect on the color of the beef patties for all three pressure and time combinations (Table 1). Lightness (L*) and yellowness (b*) increased and redness (a*) decreased ($P < 0.001$) due to HPP treatment for all days of storage. Within each day, color change with respect to control samples (ΔE) was similar ($P > 0.05$) for all three HPP conditions. Table 2 represents the effect of different ingredients/packaging on the color parameters. Within a particular day, all six differently treated samples had similar lightness (L*, $P > 0.05$, except for day 21) and yellowness (b*, $P > 0.05$, except for day 3 and day 21), but showed differences in redness (a*, $P < 0.001$). Samples treated with reducing compounds (T2, T4 and T6) showed greater redness (higher a*) than the counterparts without reducing compounds (T1, T3 and T5) and this pattern was maintained throughout the course of the study. Reduction of oxidized myoglobin (nitrosylmetmyoglobin) to nitrosylmyoglobin may be responsible for increasing the redness. Among the color parameters evaluated, a* had an interaction of treatment (T1-T6) × HPP effects ($P \leq 0.004$) for days 14 and 19 only (data not shown) and b* had an interaction of treatment (T1-T6) × HPP effects ($P = 0.012$) for days 21. On these days, treatments with reducing compounds had redness values that were more similar to the non-HPP treated control samples than

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

		Color values					
Color traits	Mb state[1]	Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L*	T1	50.64±0.62	52.25±0.44	53.55±0.39	53.06±0.49	53.32±0.56	53.40±0.44[a]
	T2	51.17±0.58	52.72±0.42	52.63±0.37	52.83±0.46	51.59±0.53	52.65±0.42[a]
	T3	49.74±0.58	51.61±0.42	52.58±0.37	53.49±0.46	52.49±0.53	52.84±0.42[a]
	T4	50.37±0.58	51.86±0.42	52.09±0.37	52.23±0.46	52.01±0.53	52.28±0.42[ab]
	T5	51.86±0.58	52.08±0.42	53.04±0.37	52.99±0.46	53.63±0.53	53.17±0.42[a]
	T6	49.75±0.58	51.70±0.42	52.07±0.37	52.25±0.46	52.20±0.53	51.37±0.42[b]
	P-value	0.095	0.455	0.065	0.357	0.073	0.023
a*	T1	20.51±0.72[b]	17.28±0.72[b]	13.42±0.60[c]	14.49±0.57*	13.99±0.65*	13.88±0.80[b]
	T2	23.29±0.68[a]	19.94±0.68[a]	18.34±0.57[ab]	18.67±0.54*	19.42±0.61*	17.80±0.76[a]
	T3	21.38±0.68[ab]	17.28±0.68[b]	14.23±0.57[c]	13.21±0.54*	14.13±0.61*	14.47±0.76[b]
	T4	23.13±0.68[a]	20.53±0.68[a]	19.83±0.57[a]	19.23±0.54*	18.90±0.61*	18.14±0.76[a]
	T5	14.31±0.68[c]	14.45±0.68[c]	13.71±0.57[c]	12.50±0.54*	13.20±0.61*	13.28±0.76[b]
	T6	21.03±0.68[b]	19.00±0.68[ab]	17.65±0.57[b]	17.42±0.54*	18.52±0.61*	17.93±0.76[a]
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b*	T1	11.00±0.23[a]	10.47±0.24	10.41±0.18	10.60±0.20	11.52±0.25	11.27±0.22*
	T2	11.34±0.21[a]	10.88±0.23	10.58±0.17	10.33±0.19	10.89±0.24	10.10±0.21*
	T3	11.45±0.21[a]	10.72±0.23	10.42±0.17	10.26±0.19	11.51±0.24	10.89±0.21*
	T4	11.48±0.21[a]	10.97±0.23	10.84±0.17	10.59±0.19	10.70±0.24	10.39±0.21*
	T5	9.91±0.21[b]	10.46±0.23	10.39±0.17	10.41±0.19	11.06±0.24	11.42±0.21*
	T6	10.96±0.21[a]	10.41±0.23	10.47±0.17	10.31±0.19	10.71±0.24	10.60±0.21*
	P-value	<0.001	0.362	0.431	0.724	0.056	<0.001
ΔE	T1	14.10±1.30	14.03±0.79[ab]	13.3±0.90[bc]	14.54±0.76[b]	18.75±1.08[a]	16.85±0.91[a]
	T2	12.49±1.20	12.24±0.73[b]	12.46±0.84[c]	12.28±0.70[c]	16.07±1.00[ab]	13.13±0.84[bc]
	T3	12.65±1.20	15.97±0.73[a]	15.20±0.84[ab]	17.60±0.70[a]	16.63±1.00[ab]	13.80±0.84[b]
	T4	13.88±1.20	14.17±0.73[ab]	13.08±0.84[bc]	11.78±0.70[c]	15.29±1.00[bc]	14.25±0.84[b]
	T5	13.36±1.20	15.26±0.73[a]	16.51±0.84[a]	13.34±0.70[bc]	16.56±1.00[ab]	14.92±0.84[ab]
	T6	17.61±1.20	15.34±0.73[a]	13.67±0.84[bc]	12.65±0.70[bc]	13.05±1.00[c]	11.24±0.84[c]
	P-value	0.055	0.015	0.015	<0.001	0.015	0.003

[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.

[1] **T1**: Sodium nitrite 156 ppm / vacuum packaging (VP; anaerobic packaging); **T2**: Sodium nitrite 156 ppm + Sodium erythorbate 547 ppm / VP; **T3**: Celery juice powder to add 100 ppm sodium nitrite equivalent / VP; **T4**: Celery juice powder (equivalent to 100 ppm nitrite) + 0.43% cherry powder to add 469 ppm ascorbic acid) / VP; **T5**: Sodium nitrite 156 ppm/ oxygen permeable wrap (OPW; aerobic packaging); **T6**: Sodium nitrite 156 ppm + Sodium erythorbate 547 ppm / OPW.

treatments without reducing compounds which matches the significant main effect identified for a* for all other days. On day 21, HPP treated samples were more yellow than non-HPP treated samples. Others have reported that the addition of antioxidants containing cherry powder, a natural source of ascorbic acid, to ground beef resulted in greater red color in patties in simulated retail display (2016 Nebraska Beef Report pp. 158–160). Similar a* values of T2 and T4 within a particular day signifies that both inorganic and plant based sources of nitrite and reducing compounds had a similar influence on color. T1 had significantly higher a* than T5 on day 3, but the difference became less profound during storage. Although immediately after HPP, nitrosylmyoglobin is more red, it became less red and approached that of T5, likely due to the fact that nitrosylmetmyoglobin in T5 had already oxidized and it started with less red color. The ΔE of T6 was significantly higher than ΔE of T2 immediately after HPP but gradually decreased during storage. This signifies that T6 changes color after HPP, but color changes lessened during shelf storage. This is most likely due to the reduction of nitrosylmetmyoglobin (brown) to nitrosylmyoglobin (red) by sodium erythorbate. In the absence of reducing agents (T1 vs T5), the ΔE was similar throughout the course of the study.

Conclusions

While the addition of nitrite compounds alone did not stabilize ground beef color during HPP treatment, reducing compounds decrease the color change associated with HPP treatment of ground beef. The use of HPP provides potential to reduce the risk of *E. coli* O157:H7 and other STECs. These findings may allow processors to progress toward development of technologies that allow for the HPP treatment of raw ground beef without the negative color changes typically associated with the application of HPP.

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