

Research Article

Effect of Differing Ingredients and Packaging Technologies on the Color of High-Pressure Processed Ground Beef

Jhinuk Gupta,¹ Chad G. Bower,² Gary Sullivan,² and George Cavender¹ ³

¹Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

²Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

³Department of Food Science and Technology, University of Georgia, Athens, GA 30602, USA

Correspondence should be addressed to George Cavender; cavender@uga.edu

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High-pressure processing (HPP) is a nonthermal pasteurization technique to control pathogens, like *Escherichia coli*. However, color changes in raw beef induced by HPP restrict its 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. Color was measured (CIE $L^*a^*b^*$) before HPP and on days 3, 7, 12, 14, 19, and 21 after HPP. Statistical analysis (SAS GLIMMIX) was run to identify the main effects of adding curing agents, packaging, and reducing agents on color retention. HPP resulted in a detrimental effect on the color of the beef patties for all treatments. Lightness and yellowness increased ($P < 0.001$) and redness decreased ($P < 0.001$) after high-pressure processing. The effect remained the same throughout the course of the study. However, there were less color changes in samples treated with reducing compounds. Both synthetic 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 severe color change compared to the treatments leading to the generation of nitrosylmyoglobin (Fe^{2+}).

1. 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, preharvest washing, and spraying the carcass with organic acids reduce the risk but do not completely eliminate contamination [1]. In ground beef and other nonintact beef products, STECs are considered an adulterant by the USDA [2]. These products are at 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 nonthermal pasteurization technique where pressure treatment between 300 and 800 MPa kills bacteria [3] by cell wall/spore coat rupture [4] or denaturation of critical proteins/enzymes [5, 6]. The process is most effective on Gram negative bacteria followed by yeasts/molds, Gram positive bacteria, and spores [4]. *Salmonella*, *Listeria*, and

E. coli are the major meat pathogens which can be effectively controlled by HPP. A 2.0 to 6.0 log CFU/g reduction of these pathogens is achievable by HPP treatment [7–9].

Several HPP-treated food products are in the market including fruit jellies and jams, fruit juices, pourable salad dressings, raw squid, rice cakes, foie gras, ham, and guacamole [3]. However, the use of HPP on raw meat products is uncommon due to high-pressure-induced protein denaturation and discoloration [10–15]. Therefore, it is important to find ways to stabilize the bright red color (oxymyoglobin) of fresh meat to develop a HPP-based pasteurization techniques for raw ground beef products. Effectiveness of HPP to control pathogens in cooked and uncooked meat and poultry items has been studied in great detail [7–9, 12, 14]. However, there are only very few reports available about the effect of HPP on the appearance of raw beef [3, 13, 16, 17].

Use of curing agents, such as nitrite salts, is well known to retain the bright red meat color. Nitrite salt generates

TABLE 1: Least square means (\pm 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 traits	HPP (MPa/min)	Color values ¹					
		Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L^*	0/0	40.78 \pm 0.48 ^b	42.20 \pm 0.34 ^c	43.13 \pm 0.30 ^c	43.73 \pm 0.38 ^c	42.06 \pm 0.43 ^b	43.31 \pm .034 ^c
	450/3	53.58 \pm 0.50 ^a	54.49 \pm 0.36 ^b	54.87 \pm 0.31 ^b	55.09 \pm 0.39 ^b	55.30 \pm 0.45 ^a	55.26 \pm 0.36 ^b
	600/3	54.53 \pm 0.48 ^a	55.62 \pm 0.34 ^a	56.66 \pm 0.30 ^a	56.2 \pm 0.38 ^a	56.45 \pm 0.43 ^a	55.61 \pm 0.34 ^{ab}
	600/6	53.47 \pm 0.48 ^a	55.84 \pm 0.34 ^a	55.98 \pm 0.30 ^a	56.20 \pm 0.38 ^a	56.36 \pm 0.43 ^a	56.29 \pm 0.34 ^a
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
a^*	0/0	22.50 \pm 0.55 ^a	21.98 \pm 0.56 ^a	19.46 \pm 0.46 ^a	19.33 \pm 0.44 [†]	21.04 \pm 0.50 [†]	19.39 \pm 0.62 ^a
	450/3	21.33 \pm 0.57 ^{ab}	18.18 \pm 0.58 ^b	16.56 \pm 0.48 ^b	14.91 \pm 0.46 [†]	16.02 \pm 0.52 [†]	16.38 \pm 0.64 ^b
	600/3	18.17 \pm 0.55 ^b	16.35 \pm 0.56 ^c	14.31 \pm 0.46 ^c	14.67 \pm 0.44 [†]	14.62 \pm 0.50 [†]	14.68 \pm 0.62 ^{bc}
	600/6	20.43 \pm 0.55 ^c	15.81 \pm 0.56 ^c	14.46 \pm 0.46 ^c	14.76 \pm 0.44 [†]	13.77 \pm 0.50 [†]	13.21 \pm 0.62 ^c
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b^*	0/0	8.95 \pm 0.17 ^d	9.50 \pm 0.18 ^c	8.70 \pm 0.14 ^c	8.34 \pm 0.16 ^c	9.20 \pm 0.20 ^b	8.90 \pm 0.17 [†]
	450/3	11.14 \pm 0.18 ^c	10.67 \pm 0.19 ^b	10.67 \pm 0.15 ^b	10.44 \pm 0.16 ^b	11.39 \pm 0.20 ^a	11.00 \pm 0.18 [†]
	600/3	11.67 \pm 0.17 ^b	11.32 \pm 0.18 ^a	11.27 \pm 0.14 ^a	11.39 \pm 0.16 ^a	11.95 \pm 0.20 ^a	11.55 \pm 0.17 [†]
	600/6	12.34 \pm 0.17 ^a	11.12 \pm 0.18 ^{ab}	11.44 \pm 0.14 ^a	11.49 \pm 0.16 ^a	11.72 \pm 0.20 ^a	11.65 \pm 0.17 [†]
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔE	450/3	13.14 \pm 0.89	13.29 \pm 0.54	12.84 \pm 0.62	12.95 \pm 0.52	15.07 \pm 0.73	13.17 \pm 0.62
	600/3	15.00 \pm 0.85	14.90 \pm 0.52	14.92 \pm 0.59	14.01 \pm 0.50	16.54 \pm 0.71	13.98 \pm 0.60
	600/6	13.90 \pm 0.85	15.32 \pm 0.52	14.34 \pm 0.59	14.14 \pm 0.50	16.57 \pm 0.71	14.94 \pm 0.60
	<i>P</i> 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 ($P > 0.05$). [†]A significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day. ¹Each experiment was carried out in triplicate.

HNO₂ and is reduced to NO in the presence of reducing agents or by other endogenous pathways [18, 19]. NO reacts with myoglobin in anaerobic and aerobic conditions to generate bright red nitrosylmyoglobin and nitrosylmetmyoglobin, respectively. These two are more stable than oxymyoglobin and thus impart greater color stability. Reducing agents, such as erythorbate or ascorbic acid, increase the nitrosylation rate and have shown to improve color stability in raw ground beef [20]. Excess reducing agent plays a dual role by inhibiting lipid oxidation and by increasing stability of cured meat color through shifting the equilibration between nitrosylmyoglobin and oxymyoglobin [21].

With the recent trend of using natural food ingredients, synthetic nitrite salt has been greatly replaced by plant based nitrite sources, such as celery juice powder (CJP), swiss chard, spinach, and broccoli [22]. CJP is advantageous due to absence of any strong color or flavor [23]. Similarly, cherry powder (CP) is a rich source of ascorbic acid and potential alternative to sodium ascorbate/erythorbate [24, 25]. The objective of this study was to study the effects of adding different nitrosylating agents in the presence and absence of reducing agents and aerobic and anaerobic packaging on the color stability of HPP-treated ground beef patties.

2. Materials and Methods

2.1. Patty Preparation. Boneless, denuded USDA Select beef top rounds were ground through 1.27 cm and 0.32 cm grinding plates (Model 4732, Hobart Manufacturing, Troy, OH) and subdivided into six batches of 2.27 kg. 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 formed from each portion. Patties were formed using a 10.92 cm diameter hand operated patty press. All T1, T2, T3, and T4 patties were vacuum packed (Clarity 3 mil standard barrier nylon/polyethylene pouches, Bunzl Processors Division, North Kansas City, MO; OTR = 0.007 ml/cm²/24 hr at 23°C and 0% relative humidity) using the vacuum sealer (Multivac Model C500; Multivac Inc., Kansas City, MO). Treatments T5- and T6-treated patties were placed on foam trays (13.3 × 25.6 × 1.4 cm, Styro-Tech, Denver, CO) and overwrapped with oxygen permeable polyvinyl chloride (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm²/24 hr at 23°C and 0% relative humidity; water vapor transfer rate = 496 g/m²/24 hr at 37.8°C and 90%

TABLE 2: Least square means (\pm 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 traits	Mb state ¹	Color values ²					
		Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L^*	T1	50.64 \pm 0.62	52.25 \pm 0.44	53.55 \pm 0.39	53.06 \pm 0.49	53.32 \pm 0.56	53.40 \pm 0.44 ^a
	T2	51.17 \pm 0.58	52.72 \pm 0.42	52.63 \pm 0.37	52.83 \pm 0.46	51.59 \pm 0.53	52.65 \pm 0.42 ^a
	T3	49.74 \pm 0.58	51.61 \pm 0.42	52.58 \pm 0.37	53.49 \pm 0.46	52.49 \pm 0.53	52.84 \pm 0.42 ^a
	T4	50.37 \pm 0.58	51.86 \pm 0.42	52.09 \pm 0.37	52.23 \pm 0.46	52.01 \pm 0.53	52.28 \pm 0.42 ^{ab}
	T5	51.86 \pm 0.58	52.08 \pm 0.42	53.04 \pm 0.37	52.99 \pm 0.46	53.63 \pm 0.53	53.17 \pm 0.42 ^a
	T6	49.75 \pm 0.58	51.70 \pm 0.42	52.07 \pm 0.37	52.25 \pm 0.46	52.20 \pm 0.53	51.37 \pm 0.42 ^b
	P value	0.095	0.455	0.065	0.357	0.073	0.023
a^*	T1	20.51 \pm 0.72 ^b	17.28 \pm 0.72 ^b	13.42 \pm 0.60 ^c	14.49 \pm 0.57 [†]	13.99 \pm 0.65 [†]	13.88 \pm 0.80 ^b
	T2	23.29 \pm 0.68 ^a	19.94 \pm 0.68 ^a	18.34 \pm 0.57 ^{ab}	18.67 \pm 0.54 [†]	19.42 \pm 0.61 [†]	17.80 \pm 0.76 ^a
	T3	21.38 \pm 0.68 ^{ab}	17.28 \pm 0.68 ^b	14.23 \pm 0.57 ^c	13.21 \pm 0.54 [†]	14.13 \pm 0.61 [†]	14.47 \pm 0.76 ^b
	T4	23.13 \pm 0.68 ^a	20.53 \pm 0.68 ^a	19.83 \pm 0.57 ^a	19.23 \pm 0.54 [†]	18.90 \pm 0.61 [†]	18.14 \pm 0.76 ^a
	T5	14.31 \pm 0.68 ^c	14.45 \pm 0.68 ^c	13.71 \pm 0.57 ^c	12.50 \pm 0.54 [†]	13.20 \pm 0.61 [†]	13.28 \pm 0.76 ^b
	T6	21.03 \pm 0.68 ^b	19.00 \pm 0.68 ^{ab}	17.65 \pm 0.57 ^b	17.42 \pm 0.54 [†]	18.52 \pm 0.61 [†]	17.93 \pm 0.76 ^a
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b^*	T1	11.00 \pm 0.23 ^a	10.47 \pm 0.24	10.41 \pm 0.18	10.60 \pm 0.20	11.52 \pm 0.25	11.27 \pm 0.22 [†]
	T2	11.34 \pm 0.21 ^a	10.88 \pm 0.23	10.58 \pm 0.17	10.33 \pm 0.19	10.89 \pm 0.24	10.10 \pm 0.21 [†]
	T3	11.45 \pm 0.21 ^a	10.72 \pm 0.23	10.42 \pm 0.17	10.26 \pm 0.19	11.51 \pm 0.24	10.89 \pm 0.21 [†]
	T4	11.48 \pm 0.21 ^a	10.97 \pm 0.23	10.84 \pm 0.17	10.59 \pm 0.19	10.70 \pm 0.24	10.39 \pm 0.21 [†]
	T5	9.91 \pm 0.21 ^b	10.46 \pm 0.23	10.39 \pm 0.17	10.41 \pm 0.19	11.06 \pm 0.24	11.42 \pm 0.21 [†]
	T6	10.96 \pm 0.21 ^a	10.41 \pm 0.23	10.47 \pm 0.17	10.31 \pm 0.19	10.71 \pm 0.24	10.60 \pm 0.21 [†]
	P value	<0.001	0.362	0.431	0.724	0.056	<0.001
ΔE	T1	14.10 \pm 1.30	14.03 \pm 0.79 ^{ab}	13.3 \pm 0.90 ^{bc}	14.54 \pm 0.76 ^b	18.75 \pm 1.08 ^a	16.85 \pm 0.91 ^a
	T2	12.49 \pm 1.20	12.24 \pm 0.73 ^b	12.46 \pm 0.84 ^c	12.28 \pm 0.70 ^c	16.07 \pm 1.00 ^{ab}	13.13 \pm 0.84 ^{bc}
	T3	12.65 \pm 1.20	15.97 \pm 0.73 ^a	15.20 \pm 0.84 ^{ab}	17.60 \pm 0.70 ^a	16.63 \pm 1.00 ^{ab}	13.80 \pm 0.84 ^b
	T4	13.88 \pm 1.20	14.17 \pm 0.73 ^{ab}	13.08 \pm 0.84 ^{bc}	11.78 \pm 0.70 ^c	15.29 \pm 1.00 ^{bc}	14.25 \pm 0.84 ^b
	T5	13.36 \pm 1.20	15.26 \pm 0.73 ^a	16.51 \pm 0.84 ^a	13.34 \pm 0.70 ^{bc}	16.56 \pm 1.00 ^{ab}	14.92 \pm 0.84 ^{ab}
	T6	17.61 \pm 1.20	15.34 \pm 0.73 ^a	13.67 \pm 0.84 ^{bc}	12.65 \pm 0.70 ^{bc}	13.05 \pm 1.00 ^c	11.24 \pm 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 ($P > 0.05$). [†]A significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day. ¹T1: 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. ²Each experiment was carried out in triplicate.

relative humidity). All patties were stored at 4°C for two days to allow for conversion to nitrosylmyoglobin (T1–T4) and nitrosylmetmyoglobin (T5–T6). After 48 hours, T5 and T6 were vacuum packaged just prior to HPP treatment. Three independent replications were produced.

2.2. High-Pressure Processing Treatment. Samples were processed using a 55 L HPP unit (Hiperbaric 55, Miami, FL) located in the food grade lab of the Food Processing Center, University of Nebraska Lincoln [26]. Processing of HPP-treated samples was performed at three different combinations of pressure, and the hold time (600 MPa/3 minutes, 600 MPa/6 minutes, and 450 MPa/3 minutes) that were chosen based on their effectiveness to reduce pathogens, according to previous research. During the course of the study, all samples were stored at 4°C to better simulate commercial refrigerated storage.

2.3. Colorimetry. Color of the patties was measured (CIE $L^*a^*b^*$) through the vacuum pouch prior to HPP and on days 3, 7, 12, 14, 19, and 21 after HPP [26]. 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 each measurement period. The average of at least three measurements was taken from randomly selected areas on the patty surface. Change in color, ΔE , was calculated with respect to the control samples (non-HPP treated) within each of the six treatments:

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

where subscripts *i* and *f* represent before and after HPP treatment, respectively.

2.4. Statistical Analyses. Statistical analyses were run on color data (L^* , a^* , b^* , ΔE) using a statistical software package (SAS 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 [26]. Treatment interaction and main effects were determined using the mixed mode general linear

model (PROC GLIMMIX). When significant ($P < 0.05$) interactions or main effects were identified, separation of least square means was conducted.

3. Results and Discussion

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) \times HPP effects ($P \leq 0.004$) for days 14 and 19 only (data not shown) and b^* had an interaction of treatment (T1–T6) \times 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 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 [20]. 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, and 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. ΔE of T6 was significantly higher than ΔE of T2 immediately after HPP but gradually decreased during storage. This suggests 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 versus T5), ΔE was similar throughout the course of the study.

4. Conclusions

While the addition of nitrite compounds alone did not stabilize ground beef color during HPP treatment, reducing compounds decreased the color change associated with HPP

treatment of ground beef. These findings may allow processors to progress toward the 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.

Data Availability

All data related to this article are described in Tables 1 and 2. Persons interested in the raw data may contact the corresponding author to receive a copy.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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