EFFECT OF KOSHERING AND HYDRODYNAMIC PRESSURE ON BEEF COLOR, ODOR, AND MICROBIAL LOADS[1]

Z. HOLZER

Agric. Res. Organization
Institute of Animal Science
Newe Ya'ar Research Center
Ramat Yishay 30095, Israel

B.W. BERRY, A.M. CAMPBELL, A.M. SPANIER
and M.B. SOLOMON[2]

Food Technology and Safety Laboratory
Agricultural Research Service, USDA
Beltsville, MD 20705-2350

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ABSTRACT

Vacuum packaged koshered (salted) meat typically undergoes a rapid color change (turning brown) with the formation of objectionable odor during refrigeration. Alterations in muscle structure have been suggested to affect the oxidative activity of salt. The hydrodynamic pressure (HDP) has been shown to physically disrupt portions of myofibrils without distorting the physical shape of the piece of meat, which may alter the effect of the kosher processing on fresh meat properties. The objective of this study was to assess the effects of the koshering process and hydrodynamic pressure (HDP), either alone or in combination, on pH, microbial growth, color and odor properties of beef. Treatments were: (1) control (C); (2) koshering process (salted = S); (3) hydrodynamic pressure treated (H); (4) the combination of koshering followed by HDP treatment (SH). The H treatment was more effective in inhibiting the normal microflora than either the S or the combination of S with H. After 14 days of storage, S samples had greater than 80% surface discoloration (brown color) compared to less than 40% discoloration on the surface for the C, H and

[1] Mention of brand or firm names does not constitute an endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

[2] Corresponding author. USDA, ARS, FTSL, Bldg. 201, BARC-East, Beltsville, MD 20705. TEL: (301) 504-8400; FAX: (301) 504-8438; EMAIL: msolomon@anri.barc.usda.gov

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SH samples. The H treatment reduced the surface discoloration of koshered (salted) meat samples to a level similar to that of the control. It appears that hydrodynamic pressure treatment may diminish the undesirable pro-oxidant effects (discoloration) of koshering (salting) beef.

INTRODUCTION

According to Jewish dietary laws (Angel 1994), koshering is the last step to be carried out in the process undertaken to make meat suitable for consumption by Jewish observants. The koshering process, known as "melichah" (salting), is intended to remove blood from the meat and consists of soaking meat in water for half an hour, followed by salting with a coarse salt for an hour, followed by triple-rinsing (Regenstein and Regenstein 1988). It is known that salt exerts a drying effect on both foods and microorganisms, thus leading to food preservation (Jay 1992). Salting meat, in the koshering process, was recently found to be effective in reducing generic *E. coli* and *Salmonella* on beef briskets (Hajmeer *et al.* 1999). In spite of its microbiological safety merits, sodium chloride (NaCl) has been reported to act as a pro-oxidant (Ellis *et al.* 1968). Salt is often cited (Lee *et al.* 1997) as a factor which decreases the oxidative stability of muscle foods, resulting in discoloration. Sodium chloride may also accelerate muscle lipid peroxidation (Powers and Mast 1980; Salih *et al.* 1989; Kanner *et al.* 1991). One of the main problems of salted meat from the koshering process is the loss of red color. Because a high salt concentration promotes oxidation of myoglobin molecules, meat preserved by salting typically has an unattractive brownish-gray color. Vacuum packed koshered meat has been criticized for undergoing a rapid color change (turning brown) and for developing objectionable odors during refrigeration (Tal, Y., Marbek, Ltd., Kiryat Malachi, Israel, personal communication). The atmosphere in vacuum packaged fresh meat changes quickly after packaging (Hedrick *et al.* 1994). Low levels of oxygen entrapped in superficial muscle tissue layers result in some metmyoglobin formation (Hedrick *et al.* 1994). Furthermore, respiratory enzymes in the muscle and microorganisms use the remaining oxygen in the package and the respiratory activity results in carbon dioxide production and some pH lowering from carbonic acid formation (Hedrick *et al.* 1994).

Hydrodynamic pressure technology is a new strategy for tenderizing meat (Solomon *et al.* 1997; Solomon 1998). This tenderization process is capable of physical disruption of the ultrastructure of muscle myofibrilliar component (Zuckerman and Solomon 1998). As alterations in muscle structure have been postulated to affect the oxidative activity of sodium chloride (Osinchak *et al.* 1992; Kanner *et al.* 1991; Kanner and Kinsella 1983), it was our assumption that the HDP process, which alters the physical ultrastructure of the muscle

myofibrils, may interfere with tissue structure in a manner to possibly limit the undesirable effects from koshering meat. The HDP process has also been shown to reduce indigenous spoilage microorganisms found in meat products (Williams-Campbell and Solomon 2001, 2002).

The objective of the present study was to assess the effects of the koshering process and HDP, either alone or in combination, on pH, microbial load, color and odor properties of beef.

MATERIALS AND METHODS

An experiment, consisting of four treatments, was performed on boneless strip loins (*m. longissimus lumborum*) removed caudal to the 13th thoracic vertebrae from steer carcasses. The treatments were as follows: (1) control - nontreated (C); (2) koshering processed (salted) meat (S); (3) hydrodynamic pressure treatment (H); (4) the combination of koshering followed by HDP (SH). Boneless strip loins were removed from the left side of five Angus crossbred steer carcasses at 6 h postslaughter that were chilled in a cooler at 4C at the USDA-ARS Abattoir (Establishment #68). The animals (average live weight of 462 ($\pm$ 21 kg)) were part of a larger study which evaluated animal performance and growth physiology parameters (Fritsche *et al.* 2000). The animals were fed a total mixed diet, consisting of 40% silage and 60% concentrate mix (DM basis).

The koshering and HDP portion of this experiment started at 24 h postmortem. Each strip loin was cut into four equal sections. Each section, approximately 5 cm in length, was randomly assigned to one of the 4 treatments listed above. Following the application of the treatments, each section was cut into two steaks (2.54 cm thick), which served as 0-day and 14-day samples, respectively. The 14-day samples were vacuum packaged using Cryovac® B-620 bags [water vapor transmission, gm. at 38C 100% R.H. (645.2 cm^2, 24 h)/0.5-0.6] [oxygen transmission, cc at 23C (m^2, 24 h, 1 atm.)/30/50] (Cryovac® Sealed Air Corp., Duncan, S.C.), and stored at 4C.

The koshering process (Regenstein and Regenstein 1988) involved soaking the meat sections in cold (5C) tap water for 30 min, then applying coarse salt (Morton Coarse Kosher Salt, Morton Intern. Inc., Morton Salt, Chicago, Ill.). Salt was rubbed on all the surfaces by hand. The salt covered meat was placed on a cutting board angled at approximately 30°, in a 20-22C temperature room, to allow the samples to drain. After one hour the meat was rinsed with three changes of clean cold water and allowed to drain on the angled cutting board for 30 min. The nonkoshered samples were placed in a dry container (uncovered) and held under similar conditions (20-22C) during the koshering process.

Immediately following the koshering process, meat sections were vacuum packaged for the HDP treatment: first in a vacuum bag (Vector/Inovpack Vector Inc., Totowa, N.J.), and then in a multilayer barrier "TBG bone-guard" bag (B650TBGW, Cryovac®/Sealed Air Corp., Duncan, S.C.). Both bags were evacuated of all air and sealed. Double bagging was used for HDP treated samples to protect samples in case of bag failures. Samples were removed from these packages after HDP treatment.

Pressure processing (HDP) of strip loin sections was performed using 100 g binary explosive in a stainless steel, 54-L capacity, chamber filled with water. The explosive was immersed to a distance of 30.5 cm above the top of the packaged meat. The lid of the chamber was locked in place and the explosive detonated. The 10 samples for HDP treatment were randomly assigned to three HDP treatments, each HDP treatment using the conditions described above. Samples were packaged side by side with no stacking or overlapping.

pH Determinations

Eleven grams of muscle, from the experimental samples, was blended with 99 mL of sterile distilled water using a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.) and the pH determined with a glass electrode (Metrohm 744 pH meter, Brinkman Instr., Westbury, N.Y.). pH determinations were obtained at day 0 and after 14 days of storage at 4C, on samples from all treatments.

Microbiological Analysis

Bacterial numbers were determined before (controls) and after treatments with salt, HDP, or the combination of both treatments (SH), at day 0 and after 14 days of storage at 4C. Aerobic plate counts (APC) were determined by homogenizing 11 g meat samples (obtained from the surface of the sample), in 99 mL of sterile distilled water using a Stomacher 400 (Tekmar, Montclair, N.J.) for 2 min. Samples were serially diluted and surface plated on plate count agar (PCA - Difco, Detroit, Mich.) and incubated at 30C for 48 h. Colony forming units were converted to $\log_{10}$ numbers for data analysis.

Visual Appraisal (Panel Assessment)

A three-member trained panel assigned scores independently for color and off-odor, using AMSA Guidelines for Meat Color Evaluation (AMSA 1991). After opening each package samples were removed and stored under refrigeration (5C) for 30 min during bloom time. Surface color and off-odor were scored after this 30 min storage period. For the internal lean color evaluation, a slice was cut out of the sample and the cut surface was exposed to air for 30 min

under refrigeration. The following properties were evaluated: external and internal *lean color - oxygenated pigment* state (1 = extremely dark red and 8 = extremely bright cherry-red; lean *surface discoloration* [1 = no discoloration (0%) and 7 = total discoloration, (100%)]; *off-odor* (1 = extreme off-odor and 5 = no off-odor).

Instrumental Color Measurements

Color measurements on the external (outside surface of the muscle) and internal (inside cross section) cut surface of steaks at days 0 and 14 of storage were carried out by means of a Minolta Chroma Meter CR-200 (Minolta Corp., Ramsey, N.J.) equipped with an 8 mm aperture opening (illuminant C, calibrated with a brown plate, C = 26.5. X = 0.366 and Y = 0.340). Instrumental color values were assessed at 6 locations per surface. Measurements of CIE (Commision International de l'Eclairage) color coordinates, L*, a* and b* were obtained. Saturation index and hue angle were calculated according to Little (1975).

Surface reflectance analysis of the 14-day refrigerated vacuum packed stored samples were evaluated using a Shimadzu 2101 PC UV-VIS scanning spectrophotometer fitted with an integrating sphere assembly (Shimadzu Scientific Instr., Columbia, Md.) at room temperature (25C). Absorbance scans (400-700 nm) at 1 nm intervals were performed on 2.54 × 2.54 × 0.32 cm thick slices which were wrapped in oxygen impermeable plastic film wrap to prevent oxygenation (blooming).

Statistical Analysis

For each of the quantitative dependent variables, a statistical model was developed to test significance of the main effects and identify any interactions (SAS Institute, 1997). The independent variables used were treatment, day, and surface (animal was modeled as a random effect). Since the number of pair wise comparisons of means of interest exceeded the allowable degrees of freedom for contrasts (implying that some combinations of pairwise comparisons were not independent), an adjusted alpha level of 0.002 (using methodology proposed by Sidak 1967) was used so as not to exceed the experiment wise error rate of (P = 0.05. Dependent variables from the panel data were of the ordinal type.

RESULTS AND DISCUSSION

pH and Microbial Growth

The pH of the S, the H, and the combined SH treated samples was lower ($P < 0.05$) than the control at both day 0 and after 14 days of refrigerated storage (Table 1). However, the numerical differences (range 0.11 to 0.20 pH units) between treatments were minimal. The addition of salt to meat has been shown to reduce pH and aid in meat preservation (Hedrick *et al.* 1994). Williams-Campbell and Solomon (2002) reported that pH was not affected by H treatment for ground beef (pH ranged from 5.5 to 5.8).

TABLE 1.
EFFECTS OF KOSHERING (SALTING) AND HYDRODYNAMIC PRESSURE, ALONE OR IN COMBINATION, ON pH AND NATURAL SPOILAGE MICROORGANISMS (APC = AEROBIC PLATE COUNT), OF BEEF STRIP LOINS

Storage Parameter	Control (C)	Koshered (Salted) (S)	Hydrodynamic pressure (H)	Koshered and Hydrodynamic pressure (SH)	SE of differences
Day 0					
pH	$5.64^{a,x}$	5.53^{b}	5.46^{c}	$5.44^{c,x}$	0.0235*
Day 14					
pH	$5.55^{a,y}$	5.49^{b}	5.48^{b}	$5.51^{a,y}$	0.0235
Day 0[1]					
APC (log)	$3.63^{a,x}$	$2.11^{b,x}$	$2.12^{b,x}$	$2.12^{b,x}$	0.0421
Day 14[2]					
APC (log)	$5.02^{a,y}$	$3.76^{b,y}$	$2.40^{d,y}$	$3.24^{c,y}$	0.0421

*Standard error of difference across days is the same as that across treatments.

[a, b, c, d] Values in a row with different superscripts are different ($P < 0.05$).

[x, y, z] Values in a column, within the same parameter with different superscripts are different ($P<0.05$).

[1]Prior to vacuum packaged storage.

[2]Vacuum packaged and stored at 4C for 14 days.

The microbial flora (APC) determination at day 0, which was immediately after treatment applications, revealed a reduction of 1.5 log in microorganisms as a result of each treatment application, compared to the control (Table 1). Following 14 days of refrigerated storage, log bacterial numbers increased by 1.39, 1.65, 0.28 and 1.12 log for C, S, H and SH treatments, respectively. Thus, the data suggests that hydrodynamic pressure was more effective at inhibiting the normal microbial flora during the 14 days of vacuum packaged storage than the S process or the combination of S and H treatments. Studies have shown that rubbing salt onto the surface of meat, in the koshering process, reduced *Escherichia coli* and *Salmonella* spp. on beef briskets (Hajmeer *et al.* 1999). Salt application during koshering of meat might reduce or inhibit microbial growth including pathogens due to associated changes in water activity and ionic strength (Hajmeer *et al.* 1999). It appears that hydrodynamic pressure technology, a new strategy developed by this laboratory for tenderizing meat, may also have the ability to increase the safety and/or shelf-life of meat by inactivating and/or reducing microorganisms. One might have expected the microbial load to have increased for the S, H, and SH treatments due to the extra handling of these samples. Although, for the S and SH treatments, these samples also included a series of water rinses which might have aided in washing away some microorganisms. Nevertheless, all three treatments resulted in significant microbial reductions which were sustained throughout the 14 day storage trial compared to controls. Williams-Campbell and Solomon (2001, 2002) found that the H treatment alone is capable of reducing microorganisms found on the surface of meat as well as throughout meat products (e.g., ground beef).

Lean Color, Surface Discoloration and Odor Development

No significant lean color or surface discoloration differences among treatments were found on the external lean surface at day 0 (Table 2). It was surprising to find no differences in lean surface color at day 0 among the treatments since the samples that were koshered and treated with salt also were rinsed with water. One might expect the lean surface color to have been lighter in appearance in these samples due to some of the water soluble proteins being leached from the lean surface of these samples. Due to a high degree of surface discoloration for all treatments following 14 days of storage, external surface lean color could not be accurately scored. The S samples had over 80% surface discoloration (brown color) compared to less that 40% discoloration on the surface of the C, H and SH samples. These data suggest that the HDP treatment may have a dramatic effect in lessening the occurrence of undesirable discolorations resulting from salting meat. All samples were handled in a similar manner in the period immediately preceding the color measurements so sample handling

TABLE 2.
EFFECT OF KOSHERING AND HYDRODYNAMIC PRESSURE, ALONE AND IN COMBINATION ON COLOR AND ODOR PROPERTIES

Storage, Surface, Parameter	Control (C)	Koshered (Salted) (S)	Hydrodynamic pressure (H)	Koshered and Hydrodynamic pressure (SH)	SE of differences
Day 0					
External surface					
Lean color[1]	4.7	4.8	4.9	4.1	0.4664*
Surface discoloration[2]	1.0^{x}	1.0^{x}	1.0^{x}	1.3^{x}	0.3796
Off-odor[3]	5.0	5.0	4.0	4.9	0.2816
Day 14					
External surface					
Lean color[1,4]	-	-	-	-	-
Surface discoloration[2]	$3.2^{a,y}$	$6.2^{b,y}$	$3.4^{a,y}$	$3.6^{a,y}$	0.3796
Off-odor[3]	$4.2^{a,b}$	4.9^{a}	3.8^{b}	4.0^{b}	0.2816
Day 14					
Internal surface					
Lean color[1]	5.4^{a}	4.3^{b}	5.4^{a}	4.2^{b}	0.4664
Surface discoloration[2]	1.3	1.0	1.0	1.1	0.3796
Off-odor[3]	4.9	4.9	5.0	5.0	0.2816

*Standard error of difference across days is the same as that across treatments

[a,b]Values in a row with different superscripts are different ($P < 0.05$)

[x,y]Values in a column, of the same parameter with different superscripts are different ($P < 0.05$)

[1] Lean color scale: 1 = extremely dark red; 2 = dark red; 3 = moderately dark red; 4 = slightly dark cherry-red; 5 = slightly bright cherry-red; 6 = moderately bright cherry-red; 7 = bright cherry-red; 8 = extremely bright cherry-red

[2] Surface discoloration scale: 1 = no discoloration, 0%; 2 = slight discoloration, 1-19%; 3 = small discoloration, 20-39%; 4 = modest discoloration, 40-59%; 5 = moderate discoloration, 60-79%; 6 = extensive discoloration, 80-99%; 7 = total discoloration, 100%

[3] Off-odor scale: 1 = extreme off-odor; 2 = moderate off-odor; 3 = small off-odor; 4 = slight off-odor; 5 = no off-odor

[4] Due to a high degree of surface discoloration in day 14, lean color could not be scored accurately

would not account for the differences observed. As for the internal lean surface, after 14 days of storage, the S and SH treated samples were darker ($P < 0.05$) than the control and H treated samples which might indicate an effect from salt penetration or the additional water rinses.

Only a slight off-odor was detected by the panel at day 0 in the samples treated with HDP. After 14 days a slight off-odor was assessed by the panel for the C, H and SH treatments. The S treatment did not present any off-odors after 14 days of refrigerated storage. The criticism often mentioned as objectionable odor of vacuum packaged koshered meat may be more of a psychological bias due to the fact that undesirable color (typically noted in salted meat) is associated with objectionable odor.

Instrumental Color

The L* value of the external surface was lowest ($P < 0.05$), indicating darker color, for treatments SH and S, at day 0, and for treatments S and SH at day 14 (Table 3). This confirms that salting meat results in a loss of the red color associated with fresh meat. Hamm (1981) and Park *et al.* (1987) indicated that addition of salt to meat destabilizes the muscle proteins, accelerates protein denaturation and thus leads to an increased discoloration. The L* value increased for treatments C and H after 14 days of refrigerated storage. While L*-values increased after storage in treatments C and H, they decreased in the salted samples (S and SH). There were no significant differences at day 0 among treatments in a*-values on the external surfaces. After 14 days of storage the a*-values of the external surface of the salted samples were ($P < 0.05$) lower (less red) as compared with the C and H samples. The a*-values of the external surface of the SH treatment, although higher than the salted samples was not different for any of the treatments. These results concur with those observed for surface discoloration by the sensory panel (Table 2). It appears that hydrodynamic pressure treatment tends to lessen the development of brown color in the salted samples. The b*-value was highest (more yellow) for treatment C at day 0 and differed significantly on day 14 between S and SH, which suggests that the hydrodynamic pressure treatment led to a less yellow appearance of the samples.

Saturation index values tended to be lower (less intense red) for the S and SH treatments compared to the C and H treatments. Hue angle values of the salted samples were ($P < 0.05$) higher (less red and more brown color) at 14 days, compared with the other treatments, especially with SH samples, which had the lowest values. This may indicate a lessening of the excessive brown discoloration of the meat samples due to the koshering process as a result of the HDP treatment. It appears that the objectively measured data regarding surface browning of the meat samples, in the present experiment, is in full agreement

TABLE 3.
EFFECT OF KOSHERING AND HYDRODYNAMIC PRESSURE ALONE OR IN COMBINATION ON INSTRUMENTAL COLOR VALUES

Storage, Surface, Parameter	Control (C)	Koshered (Salted) (S)	Hydrodynamic pressure (H)	Koshered and Hydrodynamic pressure (SH)	SE of differences
Day 0					
External surface					
Lean surface color					
L* - value	$36.7^{a,x}$	$35.3^{a,b}$	$37.1^{a,x}$	34.3^{b}	0.7413*
a* - value	16.9	15.8	15.9	15.0	1.3726
b* - value	$7.9^{a,x}$	6.0^{b}	6.0^{b}	5.5^{b}	0.4206
Saturation index[1]	18.7	16.9	17.0	16.0	1.3329
Hue angle[2]	25.1	20.6^{x}	20.8	20.2	1.8923
Day 14					
External surface					
Lean surface color					
L* - value	$39.3^{a,y}$	33.9^{b}	$40.1^{a,y}$	34.8^{b}	0.7413
a* - value	17.4^{a}	13.8^{b}	17.7^{a}	$15.5^{a,b}$	1.3726
b* - value	$6.1^{a,b,y}$	7.3^{a}	$6.2^{a,b}$	5.2^{b}	0.4206
Saturation index	18.4	15.7	18.8	16.4	1.3329
Hue angle	19.2^{a}	$29.5^{b,y}$	19.3^{a}	18.9^{a}	1.8923
Day 14					
Internal surface					
Lean surface color					
L* - value	41.1	37.8	41.3	37.6	0.7413
a* - value	19.5	18.7	19.9	18.1	1.3726
b* - value	9.3	8.5	9.2	7.9	0.4206
Saturation index	21.6	20.6	21.9	19.7	1.3329
Hue angle	25.5	24.2	24.9	23.5	1.8923

L* - higher values = lighter; a* - higher numbers more red: b* - lower = less yellow
[1] Saturation index = $(a^2 + b^2)^{1/2}$, higher values are more intense red;
[2]Hue angle = $(\tan^{-1} b/a)$, higher values are indicative of less red color, more brown
* Standard error of difference across days is the same as that across treatments
[a,b] Values in a row with different superscripts are different ($P < 0.05$)
[x,y] Values in a column of the same parameter with different superscripts are different ($P < 0.05$)

with the data obtained from the sensory panel. Meat color was also examined by recording the spectrum of the surface scan for the strip loin treated samples. The surface scan (Fig. 1) of the S treated samples is similar to a scan of metmyoglobin, whereas the surface scan (spectrum) of the SH treated samples is similar to that of oxymyoglobin (Strange *et al.* 1974; Krzywicki 1979). Thus, the surface scan results confirm and explain the lean color differences observed for both the instrumental and visual appraisals, the differences being related to the state of myoglobin.

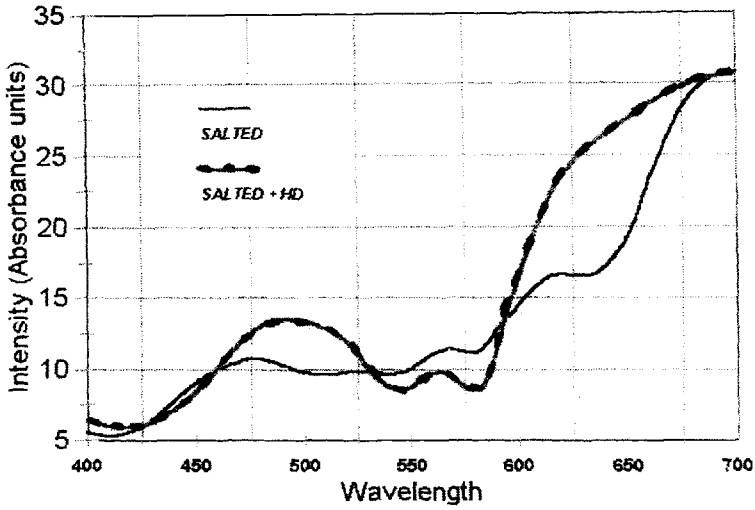

FIG. 1. SURFACE SCAN OF BEEF STRIP LOIN

Addition of salt to meat, either pre- or post-rigor, destabilizes muscle proteins and accelerates protein denaturation which increases discoloration (Hamm 1981; Park *et al.* 1987). The salting of meat has been recognized to have three effects on meat color: (1) acting as a pro-oxidant for heme pigment oxidation, causing meat to turn brown; (2) denaturating enzymes; and (3) increasing water-binding capacity of meat proteins, thereby rendering the tissues more translucent and darker (Price and Schweigert 1987).

Our results clearly indicate a reduction in surface discoloration of koshered meat as a result of hydrodynamic pressure treatment. HDP appears to reduce the undesirable color effects of the salting of meat. While the pro-oxidative effect

of sodium chloride has been postulated to be due to increased activity of lipid oxidation catalysts and/or alterations in muscle structure (Osinchak *et al.* 1992; Kanner *et al.* 1991; Kanner and Kinsella 1983), the effects of the hydrodynamic pressure treatment, as found in our study, may be due to the proteins and bonds tearing within the structure of the muscle tissue which occurs during the HDP process (Zuckerman and Solomon 1998). Catalase prevents the generation of some of the free radical species responsible for lipid oxidation. Perhaps there is a release of catalase during the HDP treatment which in-turn would have an effect on meat color stability. More studies are necessary to fully elucidate the mechanisms responsible for HDP prevention of brown discoloration.

CONCLUSIONS

The hydrodynamic pressure treatment has real potential to diminishing the undesirable color observed when salting (koshering process) beef that often occurs during the storage of koshered/salted beef. Therefore, the HDP treatment has potential for providing an antioxidant effect. Further research is needed to validate and explain this finding.

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