

## Research Note

## Reduction of Spoilage Microorganisms in Fresh Beef Using Hydrodynamic Pressure Processing

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## ABSTRACT

Hydrodynamic pressure processing (HDP) was investigated as a technology to reduce spoilage microorganisms found in fresh beef. In two separate studies (studies 1 and 2), retail ground beef and beef roasts were purchased (day 0). The roasts were divided into stew pieces (30 to 40 g). All meat samples, including control samples, were stored at 5°C for 20 h in a plastic film. After storage, designated samples were treated with HDP. In study 3, ground beef was treated with HDP (day 0) and stored aerobically (5°C) for 14 days with control samples. Each meat type was vacuum-packaged for HDP (100 g binary explosive, steel shock wave container). The pHs and the aerobic plate counts ( $\log_{10}$  CFU/g) were measured on day 0 (studies 1 and 2) and on days 0, 7, and 14 (study 3) for control samples and for HDP-treated samples. There was no pH difference between control and HDP-treated meat types (studies 1 and 2); HDP reduced bacteria in both meat types in study 1 (2 log) and study 2 (1.5 log) on day 0. In study 3, there was a significant difference ( $P < 0.05$ ) in pH between control meat (8.2) and HDP-treated meat (5.6) after storage. There was an immediate reduction (1.5 log) of microorganisms following HDP (day 0) and a 4.5-log difference between control samples (9 log) and HDP-treated samples (4.5) after 14 days of storage. With HDP, it is possible to reduce spoilage microorganisms found in or on different meat types (ground beef versus stew pieces), which could extend the shelf life of meat products.

Hydrodynamic pressure processing (HDP) was developed at the Food Technology and Safety Laboratory as an alternative, nonthermal process for tenderizing meats (5). Hydrodynamics is concerned with the motion of fluids and the instantaneous forces acting on solids immersed in these fluids (7). During HDP, a shock wave (created by the detonation of a high-energy explosive) moves through a liquid medium and causes a significant disruption of myofibrillar proteins found in muscle tissue (9). This finding led to the question of whether HDP could cause damage to indigenous spoilage microorganisms attached to fresh meats. HDP is an instantaneous process that occurs in milliseconds, in contrast to high hydrostatic pressure, which is not instantaneous and is characterized by liquids at rest and the pressure in a liquid or exerted by a liquid on an immersed object. Although the pressure (70 MPa) developed during HDP is not of the same order of magnitude as that of high hydrostatic pressure, it has been shown to reduce microorganisms in temperature-abused ground beef (8) and *Trichinella spiralis* (1) in fresh pork loins.

The purpose of this study was to determine the effect of HDP on indigenous spoilage microorganisms found in fresh ground beef and on beef stew pieces. Ground beef spoils in its entirety, whereas whole cuts of meat spoil by surface contamination (3). These two meat types were chosen to determine the penetration capability (internal versus

surface) of the shock wave created during HDP. A shelf life study with fresh ground beef was performed following HDP treatment to determine whether the process could increase the stability of ground beef stored aerobically at refrigerator temperature.

## MATERIALS AND METHODS

**Meat sample preparation: study 1.** Packages of fresh, lean (78%) ground beef and a whole boneless beef round rump roast muscle were purchased from a local retail store 4 days before their expiration date. The purchase date was established as day 0. The roast was cut into 2.54-cm-thick steaks and was further divided into smaller stew pieces (40 g each) and tumbled for 2 min. The ground beef was mixed for 2 min in a sterile metal pan by gloved hands. Subsamples of each meat type for control samples and HDP-treated meat samples were divided, placed in separate sterile metal pans, covered in oxygen-permeable plastic film and stored for 20 h in a refrigerator at 5°C. After storage, each meat type was removed from the metal pan and mixed again separately as described above. Control samples ( $n = 3$  each, 11 g each) of each meat type were placed in separate Ziploc snack bags (16.51 by 8.25 cm; Dow, Indianapolis, Ind.), which were folded, and stored at 5°C for 15 min. For HDP, each meat type ( $n = 3$  each, 40 g each) was double-packaged, first in separate Ziploc snack bags (Dow) that were folded but not evacuated, and then in a multilayer barrier bag with built-in boneguard protection (Cryovac B620 TBGW, Sealed Air Corp., Duncan, S.C.). The outer bag was evacuated of all air and sealed.

**Meat sample preparation: study 2.** Lean (80%) ground beef and a whole boneless beef round rump roast were purchased

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from a local retail store and prepared as described above (day 0). Following storage for 20 h at 5°C, ground beef and stew pieces were processed as in study 1 and divided into subsamples (11 g for ground beef and 40 g for stew pieces) for a total of 60 samples (30 ground beef samples and 30 stew pieces). Five control samples for each meat type (11 g each) were packaged in a single layer of Cling Wrap (Glad, Danbury, Conn.) and stored at 5°C until evaluation with samples following HDP. For HDP treatment, all meat samples were individually wrapped in a single layer of Cling Wrap and then in a multilayer barrier bag (described above) that was vacuum-sealed. There were a total of 3 packages, with each package containing 10 ground beef samples and 10 stew pieces, for a total of 20 samples. There was only 1 package per HDP treatment, with three successive HDP treatments. In this study, a smaller sample size (11 g) was used to expedite sampling, whereas in study 1, 40-g samples were used.

**Meat sample preparation: study 3.** Fresh, lean (80%) ground beef was obtained from a local retail store and mixed thoroughly as described above. After mixing, lots were divided for untreated control samples ( $n = 15$ , 11 g each) and HDP-treated samples ( $n = 15$ , 11 g each). Control samples were individually wrapped in a single layer of Cling Wrap and stored aerobically in the refrigerator at 5°C for 14 days. HDP-treated meat samples were wrapped individually in single layers of Cling Wrap, and all 15 samples were placed in one multilayer barrier bag (described above) that was evacuated of all air and sealed. There was one barrier bag containing 15 samples for only one HDP treatment. Following HDP, the treated meat samples were removed from the sealed multibarrier bag and immediately assayed for pH and aerobic plate count ( $\log_{10}$  CFU/g) or were stored aerobically in Cling Wrap at 5°C for 14 days and assayed for pH and aerobic plate count on days 0, 7, and 14. This study was replicated three times.

**HDP.** Packaged meat samples were placed in the bottom of a stationary steel shock wave container (54-liter capacity). The container was filled with water. One hundred grams of a binary explosive (5) was immersed in the water 30.5 cm away from the front surface of the meat. The lid was placed on the container and locked, and the explosive was detonated.

**Microbiological analyses.** In all studies, intact packaged meat samples were removed from the shock wave container immediately following HDP treatment. Post-HDP treatment samples and control samples were assayed for pHs (Metrohm 744 pH meter, Brinkman Instruments, Inc., Westbury, N.Y.) and aerobic plate counts. Enumerations were carried out by homogenizing 11- or 40-g meat samples in sterile 0.1% peptone water for an initial 1:10 dilution in a Stomacher 400 (Tekmar, Montclair, N.J.) for 2 min. The samples were serially diluted 10-fold, spread plated in duplicate on plate count agar (Difco Laboratories, Detroit, Mich.), and incubated aerobically at 30°C for 48 h (study 1).

In study 2, control samples and HDP-treated samples were prepared as described above. Bacterial enumerations were carried out with a spiral plater (Microbiology International, Frederick, Md.). The incubation media, time, and temperature were the same as those in study 1. Bacterial counts were carried out with the Synbiosis Protocol Colony Counter (3.06 Beta; Microbiology International).

In study 3, untreated (control) and HDP-treated meat samples were evaluated for pH and bacterial numbers on days 0 (immediately following HDP), 7, and 14 by the same protocol as that used in study 1. During the refrigerated-storage period, when spoilage was indicated by a microbial population of 7  $\log_{10}$  CFU/g, analysis of the meat was terminated.

TABLE 1. Instantaneous effect of hydrodynamic pressure processing (HDP) on microorganisms ( $\log_{10}$  CFU/g) in fresh ground beef (studies 1 and 2)<sup>a</sup>

Sampling time	Ground beef (APC $\pm$ SEM)	Beef stew pieces (APC $\pm$ SEM)
Study 1		
Day 0 (purchase date)	4.69 $\pm$ 0.02	3.61 $\pm$ 0.03
20 h, control (5°C)	5.45 $\pm$ 0.05*	4.30 $\pm$ 0.04*
20 h, HDP	3.15 $\pm$ 0.02*†	2.48 $\pm$ 0.03*†
Study 2		
Day 0 (purchase date)	5.45 $\pm$ 0.02	5.42 $\pm$ 0.02
20 h, control (5°C)	6.88 $\pm$ 0.03*	6.29 $\pm$ 0.02
20 h, HDP	5.49 $\pm$ 0.03*	4.95 $\pm$ 0.04*

<sup>a</sup> Mean and standard error of the mean (SEM) of control and HDP-treated samples ( $n = 3$ ) in study 1 and control ( $n = 5$ ) and HDP-treated samples in study 2, replicated three times. \*Significant ( $P < 0.05$ ) compared with respective control. †Significant ( $P < 0.05$ ) compared with day 0 (purchase date).

**Statistical analyses.** Bacterial plate counts were converted to logarithms and examined by analyses of variance; mean separation and least significant difference ( $P < 0.05$ ) assessments in one-to-one comparisons were performed with Statistical Analysis Systems software (SAS, version 6.12, SAS Institute, Inc. Cary, N.C.) to determine if the means of the bacterial numbers ( $\log_{10}$  CFU/g) for the HDP-treated meat samples were significantly different from those for control samples (studies 1 and 2). For study 3, the means were converted into logarithm values and analyzed using the general linear model procedure of SAS (4) to determine significant differences between control samples and HDP-treated samples.

## RESULTS AND DISCUSSION

**pH and aerobic plate count.** The pH data for studies 1 and 2 are not shown. There was no difference ( $P > 0.05$ ) in pH (5.5 to 5.8) between day 0 meat samples and meat samples stored for 20 h at 5°C for all meat types. The pH measurements were the same ( $P > 0.05$ ) for each meat type following 20 h of storage for control samples for meat treated with HDP. In study 3, the pH ranged from 5.8 to 8.2 for the control samples over a 14-day storage period. For HDP-treated meat, the pH range was 5.8 to 5.6 during storage. The bacterial numbers for studies 1 and 2 are presented in Table 1.

**Studies 1 and 2: ground beef.** In study 1, the initial aerobic plate count for ground beef on day 0 was 4.69  $\log_{10}$  CFU/g. Following storage for 20 h at 5°C, numbers had increased to 5.45  $\log_{10}$  CFU/g ( $P < 0.05$ ; a difference of 0.76  $\log_{10}$  CFU/g). Following treatment with HDP, the bacterial number decreased to 3.15  $\log_{10}$  CFU/g ( $P < 0.05$ ; a difference of 2.3  $\log_{10}$  CFU/g). The bacterial number was reduced to below the initial (day 0) number. In study 2, the initial aerobic plate count for ground beef was 5.45  $\log_{10}$  CFU/g. Following storage for 20 h, the number increased by approximately 1  $\log_{10}$  CFU/g to 6.88  $\log_{10}$  CFU/g ( $P < 0.05$ ). Post-HDP results showed a decrease to 5.49  $\log_{10}$  CFU/g ( $P < 0.05$ ; a difference of 1.39  $\log_{10}$  CFU/g). In study 2, the bacterial number was reduced to the initial (day 0) number.

**Studies 1 and 2: beef stew pieces.** For study 1, the initial (day 0) aerobic plate count was  $3.61 \log_{10}$  CFU/g. Following storage for 20 h at  $5^{\circ}\text{C}$ , the number increased to  $4.30 \log_{10}$  CFU/g ( $P < 0.05$ ; a difference of  $0.69 \log_{10}$  CFU/g). After HDP treatment, the bacterial number decreased to  $2.48 \log_{10}$  CFU/g ( $P < 0.05$ ; a difference of  $1.82 \log_{10}$  CFU/g). For study 2, the initial aerobic plate count was  $5.42 \log_{10}$  CFU/g. Following storage for 20 h, the number increased to  $6.29 \log_{10}$  CFU/g ( $P < 0.05$ ; a difference of  $0.87 \log_{10}$  CFU/g). After HDP treatment, the bacterial number decreased to  $4.95 \log_{10}$  CFU/g ( $P < 0.05$ ; a difference of  $1.34 \log_{10}$  CFU/g).

**Study 3: shelf life.** The effects of HDP on pH and the number of microorganisms in fresh ground beef stored aerobically for 14 days at  $5^{\circ}\text{C}$  are shown in Table 1 (study 3). There was an increase in the microbial population for control samples from  $5.22$  to  $9.11 \log_{10}$  CFU/g during storage; however, HDP-treated meat samples showed only a slight increase ( $P > 0.05$ ) in bacteria (from  $3.72$  to  $4.58 \log_{10}$  CFU/g). There was an instantaneous reduction ( $P < 0.05$ ) immediately following HDP (from  $5.22$  to  $3.72 \log_{10}$  CFU/g) on day 0. After 7 days, the control samples were determined to be spoiled ( $7.56 \log_{10}$  CFU/g); however, for HDP-treated meat, the bacteria did not reach the spoilage level for 14 days of aerobic storage.

Although there was an increase in bacterial numbers after 20 h of storage in studies 1 and 2, the pH did not change. This could be attributable to the buffering capacity of meats (3). In studies 1 and 2, the pHs for control samples and HDP-treated samples were measured immediately (within 15 min) following treatment. HDP did not show an effect on the pH of fresh meats on day 0 of treatment but did show an effect on fresh meat stored for 14 days. The increase in pH for control samples in study 3 was normal for aerobically stored fresh ground beef. The rise in pH is indicative of the proliferation of pseudomonads and the production of amines (3). The pH for HDP-treated meat samples remained constant during the 14-day storage period, which indicates a decrease in the predominant spoilage microflora (pseudomonads) and an increase in gram-positive bacteria.

The results of studies 1 and 2 showed that HDP treatment significantly reduced microorganisms in different meat types (ground beef versus beef stew pieces). The reduction of microorganisms in ground beef with HDP resulted in an extended shelf life for ground beef (study 3). HDP-treated meat samples showed no bacterial signs of spoilage following refrigerated aerobic storage after 14 days, and the bacterial population remained almost constant during storage. The bacterial numbers recovered were total counts; therefore, the evidence of no outgrowth could be attributable to the inhibition of gram-negative bacteria and the growth of gram-positive bacteria.

In these studies, HDP treatments were performed using a 100-g binary explosive placed 30.5 cm from the front surface of the meat. These conditions were shown to be more effective in tenderizing beef muscles (5) than 50 or 75 g at the same distance when a disposable plastic explo-

TABLE 2. Effect of hydrodynamic pressure processing (HDP) on microorganisms in fresh ground beef stored for 14 days at  $5^{\circ}\text{C}$  (study 3)<sup>a</sup>

Sam- pling time	Control samples		HDP-treated samples	
	APC $\pm$ SEM	pH $\pm$ SEM	APC $\pm$ SEM	pH $\pm$ SEM
Day 0	$5.22 \pm 0.47$	$5.8 \pm 0.02$	$3.72^* \pm 0.28$	$5.8 \pm 0.02$
Day 7	$7.56 \pm 0.12$	$7.4 \pm 0.10$	$4.01^* \pm 0.11$	$6.1^* \pm 0.03$
Day 14	$9.11 \pm 0.20$	$8.2 \pm 0.04$	$4.58^* \pm 0.08$	$5.6^* \pm 0.12$

<sup>a</sup> Mean and standard error of the mean (SEM) of control and HDP-treated samples ( $n = 5$ ) in study 3. \*Significant ( $P < 0.05$ ) compared to respective control.

sive container was used. This study was carried out to determine microbial reduction in meats resulting from the same HDP treatment parameters. Studies reported in this paper confirmed that the pressure front from an HDP shock wave is capable of inactivating bacteria on the surface (stew pieces) and beneath the surface (ground beef) of meat samples. The inactivation of microorganisms increases the shelf life of aerobically stored meat (Table 2; study 3). Investigations performed at our lab (8) have shown that the shock wave generated during HDP is capable of reducing numbers of microorganisms by 2 to  $3 \log_{10}$  CFU/g in temperature-abused ground beef immediately following HDP. The meat (8) was temperature abused to increase the initial microbial population.

The HDP process would be considered a nonthermal process, since there is no change in the pretreatment temperature of either the meat or the liquid medium ( $\text{H}_2\text{O}$ ) used in the process (8). It should be noted that there are some concerns about the use of HDP. The dangers include those involved in the handling, storage, and operation of the explosives (6). These results show that HDP is capable of reducing bacteria that cause spoilage on the surface (whole meat) and beneath the surface (ground beef) of fresh meats. Since pseudomonads are the predominant spoilage bacteria for fresh meats (3), it can be concluded that these microorganisms seem to be more susceptible to HDP. The reduction of bacteria results in an extended shelf life for ground beef. It is possible that HDP could be used as an alternative, nonthermal technology to reduce microbial contamination. Furthermore, the reduction of bacteria may possibly be enhanced by combining HDP with other preservation (natural antimicrobial) techniques.

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