

Role of *Hafnia alvei* and a *Lactobacillus* Species in the Spoilage of Vacuum-Packaged Strip Loin Steaks

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ABSTRACT

A microbiological examination of vacuum-packaged strip loin steaks that were defective (gassy packages, hydrogen sulfide odor) revealed high total counts (10^7 - 10^8 /cm²) with *Hafnia alvei*, *Lactobacillus* and *Pseudomonas* spp. as major isolates. Re-inoculation experiments indicated that *H. alvei* was the likely cause of the hydrogen sulfide odor. Gas formation resulted from the activity of heterofermentative lactobacilli and *H. alvei*. Improvements in plant practices and temperature control eliminated the problem.

In July of 1978, a meat purveying company which distributes hotel, restaurant and institutional meat cuts to a national clientele received complaints from end-users in five states regarding spoilage of vacuum-packaged strip loin steaks (IMPS 1180) packed 10 to the pouch and in fiberboard boxes. According to customer complaints, the vacuum packages were gassy to severely puffed, an odor similar to that of rotten eggs was evident when the pouch was opened and, in some reports, the meat had a green surface color.

Reports in the literature show that some vacuum-packaged beef, usually after extended storage under refrigeration exhibits, upon opening of the pouch, an acid odor sometimes described as sour, lactic, cheesy, milky or butyric. These odors most likely result from the presence of a variety of volatile fatty acids which are produced at least in part by heterofermentative lactic acid bacteria (1,9). Greening of vacuum-packaged meat with or without a hydrogen sulfide odor occurs more frequently in meat with a pH of 6.0 or higher and is often attributed to the activity of *Pseudomonas* spp. (3,5). This report describes the conditions and microbial activities most likely responsible for spoilage of these vacuum-packaged strip loin steaks.

MATERIALS AND METHODS

Product history

By using code numbers on the fiberboard boxes, the production dates were narrowed down to a specific Thursday, Friday and following Monday two weeks before the first complaint. Evaluation of plant records revealed the following chronological history for the product: (a) vacuum-packaged, boxed boneless strip loins (IMPS 180) were purchased from a meat packer in September, 1977 and frozen-stored at -23 C until July, 1978, (b) product was removed from the freezer and placed in a -1 C tempering cooler in the original boxes for 1 to 3 days (exact time unknown), (c) frozen, tempered product was removed from boxes — vacuum packaging material remained intact — and placed into 1.2 × 1.5 × 1.2-m stainless steel vats, and (d) vats were filled with 26

to 30 C tap water and stored in a processing room where the temperature is maintained at 10 C for 19 h/day but which reaches 24 C during a 5-h cleanup period. At this time plant personnel were not certain as to how the product was handled. Under usual circumstances, the product would have been: (a) used the next day, at a time when internal product temperature was 0 to 5 C, or (b) if it was not used the next day, the vat would have been returned to the cooler, drained and held overnight at -2 to 0 C — product would have been moved back to the 10-C processing room and processed during that working day. Plant personnel now suspect that the meat might not have been returned to the -2 to 0-C cooler (as would have been standard operating procedure) after storage in the 10 C/24-C processing room for 2 days; product would then have remained in the 10 C/24-C processing room over the weekend and would have been processed on the following Monday.

Normal fabrication procedure was followed — vacuum packaging materials were removed from the product, the strip loins were passed twice through a blade tenderizing machine, steaks were cut, trimmed, weighed and placed in a vacuum pouch. Workmen would have handled unwrapped product in all of the fabrication steps described above.

Normal packaging-handling procedure was followed, a vacuum was drawn on the pouch, the pouch was heat-impulse sealed, the sealed pouch was placed in a fiberboard box and the box was stored in a -2 to 0-C holding cooler, boxes of product were stored for 3-8 days at the processing plant and shipped as far as 3200 km by refrigerated transport. Complaints were received as early as 14 days following fabrication and cutting; because complaints were received from end-users in several states, the problem is assumed to have originated in the processing plant and not in-transit or after receipt of the product by the customer.

Bacteriological examination of packaged product

Total counts of the meat surface and purge (fluid which accumulates around the cut inside the package) were made by the spread-plate method on tryptic soy agar (TSA, Difco), lactobacilli MRS broth with 1.5% agar (MRS, Difco) and peptone iron agar (PIA, Difco). A 2.5-cm² area of the meat surface was swabbed with a dacron swab moistened in sterile 0.1% peptone. The swab then was placed in 9 ml of 0.1% peptone and shaken 20 times. Appropriate dilutions were made with sterile 0.1% peptone. Plates were incubated for 4 days at 25 C. Representative colonies of countable plates were picked, placed on TSA slants and incubated for 2-3 days at 25 C. Diagnostic schemes and procedures to identify the isolates have been reported by Vanderzant and Nickelson (10).

Reinoculation of steaks with bacterial isolates

In reinoculation experiments, beef steaks were inoculated with the major isolates of the defective steak: a homofermentative *Lactobacillus*, a heterofermentative *Lactobacillus*, two strains of *Hafnia alvei* (lactose +, lactose -) and a *Pseudomonas* sp. Blade chuck steaks (11 × 9 × 2 cm) were fabricated from beef wholesale chucks. Two steaks were inoculated with each test organism and two similar steaks served as controls (non-inoculated). *H. alvei* and the *Pseudomonas* sp. were grown for 24 h at 25 C in brain heart infusion; the homofermentative *Lactobacillus* was grown in MRS broth and the heterofermentative *Lactobacillus* in APT broth. Counts of these cultures (on TSA plates,

incubated for 4 days at 25 C) after incubation ranged from 1.6×10^8 to 2.0×10^9 per ml. A 0.1-ml aliquot of broth was placed on a steak and spread with a sterile glass rod over the entire surface of the steak. Each steak was placed in a laminated nylon/saran/polyethylene pouch (oxygen transmission rate 32 cc/m²/24 h; moisture vapor transmission rate of 0.8-1.8 g/m²/24 h). The pouches were evacuated of air, and heat-impulse sealed with a chamber-type vacuum packaging machine; pouches were then stored at 1-3 C for 3 weeks.

After storage, the vacuum packages were opened with a sterile scalpel and a sterile aluminum template (50 cm²) was placed on the meat. A sterile cellulose sponge (7 × 4.5 × 1 cm), moistened in sterile 0.1% peptone, was drawn across the exposed meat surface. The sponge then was placed in 50 ml of sterile 0.1% peptone and squeezed five times. Appropriate dilutions were made with sterile 0.1% peptone. Agar plate counts were made on TSA, MRS, APT (BBL) and PIA with the spread plate method. Plate incubation was as described earlier.

RESULTS AND DISCUSSION

A pouch containing 10 steaks was opened under aseptic conditions and one steak representative of the defective lot, was obtained for further study. A strong hydrogen sulfide odor was noticed upon initial opening of the pouch; this odor dissipated in a few minutes and was followed by a "sour" odor which persisted for some time. No greening of the meat was observed. A gram stain of organisms on the meat surface and in the purge showed large ($1.2 \times 8 \mu\text{m}$) gram-positive rods in palisades and smaller ($0.8 \times 3 \mu\text{m}$) gram-positive rods in chains. Counts of the meat surface and purge ranged from 3.2×10^7 to 6.5×10^8 per cm² or ml. Nearly all of the isolates from countable plates consisted of *Lactobacillus* spp. Homofermentative types were more numerous (about 5:1) than heterofermentative types. Colonies of homofermentative lactobacilli on MRS agar were 2-3 mm in diameter, convex, white and entire; heterofermentative types were somewhat smaller (0.5-1 mm), raised, white and entire. *Hafnia alvei* and *Pseudomonas* spp. also were isolated but the concentration of these organisms was about 2 logs lower (3.6×10^5 - 2.0×10^6 per cm² or ml) than that of the lactobacilli. Based upon the procedures and classification of Sharpe et al. (8) the homofermentative isolates were atypical streptobacteria, resembling *Lactobacillus plantarum*, the heterofermentative isolates were atypical betabacteria, resembling *Lactobacillus viridescens*. Characteristics of *H. alvei*

were as follows: gram-negative rods ($0.5 \times 1.5 \mu\text{m}$); oxidase -; urease -; OF glucose, fermentative, lysine decarboxylase +; arginine dihydrolase -; ornithine decarboxylase +; phenylalanine deaminase -; indole -; MR -; VP +; citrate -; H₂S (PIA) +; gelatin -; TSI K/A; nitrate +; esculin -; motility +; acid from lactose ±; sucrose -; sorbitol -; adonitol -; inositol -; raffinose -; malonate -. *Pseudomonas* isolates were typical motile gram-negative rods ($0.5 \times 1.5 \mu\text{m}$); oxidase +; TSI K/K; NH₃ from arginine +.

The results of reinoculation experiments (Table 1) indicate that *Lactobacillus* spp. were predominant on both the control and inoculated vacuum-packaged steaks stored for 3 weeks at 1-3 C. This is in agreement with numerous reports on the microbial flora of vacuum-packaged beef. Gas production in the pouches was observed only with steaks inoculated with the heterofermentative *Lactobacillus* sp. (weak) and with *H. alvei* (stronger). The sulfide odor of the laboratory steaks inoculated with *H. alvei* was not as strong as that of the commercial meats. Factors which may be in part responsible for this difference include: type of meat sample (differences may exist, for example, in muscle pH between types of steaks), history of the sample (the commercial steaks had been subjected to wide fluctuations in temperature, whereas the laboratory steaks were not temperature-abused) and differences in the composition of the mixed microbial populations on the commercial vs. the laboratory steaks. The results indicate that *H. alvei* most likely was the cause of the hydrogen sulfide odor of the commercial vacuum-packaged meat. Gas production was likely a combination of CO₂ and H₂S generated by heterofermentative lactobacilli and *H. alvei*, respectively.

Production of hydrogen sulfide and sulfide-like odors by microorganisms in meats, poultry and fish has been reported in the literature (2,4,5). *Pseudomonas* spp., particularly *P. putrefaciens*, *P. mephitica*, *P. perolens*, *P. putida* and *P. fragi* are frequently involved. For example, Nicol et al. (5) reported that *P. mephitica* inoculated on packaged beef caused production of sulfmyoglobin if the pH was above 6.0 and if meat was maintained at low

TABLE 1. Agar plate counts and predominant bacterial types isolated from control and inoculated vacuum packaged steaks stored for 3 weeks at 1-3 C.

| Inoculum | Plating medium | Plate count per cm ² | Gas production in package ^a | Predominant isolates from countable plates ^b |
|-----------------------------------|----------------|---------------------------------|--|--|
| Control | TSA | 5.6×10^7 | — | <i>Lactobacillus</i> sp. (Ho); <i>Lactobacillus</i> sp. (He, dextran+) |
| | APT | 3.8×10^7 | — | <i>Lactobacillus</i> sp. (Ho) |
| | MRS | 3.4×10^7 | — | <i>Lactobacillus</i> sp. (He, dextran-); <i>Lactobacillus</i> sp. (Ho) |
| | PIA | 5.6×10^7 | — | <i>Lactobacillus</i> sp. (Ho) |
| <i>Lactobacillus</i> sp. 3 (Ho) | TSA | 6.5×10^7 | — | <i>Lactobacillus</i> sp. 3 (Ho) |
| | MRS | 9.8×10^7 | — | <i>Lactobacillus</i> sp. 3 (Ho) |
| <i>Lactobacillus</i> sp. 4 (He) | TSA | 7.5×10^7 | + | <i>Lactobacillus</i> sp. 4 (He) |
| | APT | 9.4×10^7 | + | <i>Lactobacillus</i> sp. 4 (He) |
| <i>Hafnia alvei</i> 5 (lactose+) | TSA | 4.1×10^7 | +++ | <i>Hafnia alvei</i> 5; <i>Lactobacillus</i> sp. (Ho) |
| | PIA | 4.7×10^7 | +++ | <i>Hafnia alvei</i> 5; <i>Lactobacillus</i> sp. (Ho) |
| <i>Hafnia alvei</i> 6 (lactose -) | TSA | 2.7×10^7 | +++ | <i>Hafnia alvei</i> 6; <i>Lactobacillus</i> sp. (Ho) |
| | PIA | 3.4×10^7 | +++ | <i>Hafnia alvei</i> 6; <i>Lactobacillus</i> sp. (Ho) |
| <i>Pseudomonas</i> sp. 9 | TSA | 1.2×10^8 | — | <i>Lactobacillus</i> sp. (Ho) |
| | PIA | 1.1×10^8 | — | <i>Lactobacillus</i> sp. (He, dextran-) |

^a+ weak, ++ fair, +++ strong

^bHo = homofermentative, He = heterofermentative.

oxygen tensions. At lower pH values the bacteria were unable to produce hydrogen sulfide. At low oxygen tensions, green reduced sulfmyoglobin is formed; at higher oxygen tensions oxidation to the red metsulfmyoglobin occurred. *Hafnia* spp. are reported to come from various sources associated with the animal and may be spread during slaughtering and dressing operations. Patterson and Gibbs (7) reported these organisms in water, hair, soil, feces, on the hands of workers, in air, on carcasses, in chill rooms and on tables in the boning room. Many of these isolates were capable of growing at 4 C. Patterson and Gibbs (6) also reported that *H. alvei* inoculated on meat of high pH (6.15) and stored at 4 C caused "cabbagey" odors when packed exposed to air and that of "slight pickles" when vacuum-packaged. No off-odors were detected when meat of normal pH (5.4-5.5) was inoculated with *H. alvei* and stored at 4 C.

Quality control procedures initiated by the meat purveying company after consultation with university personnel included: (a) discontinuance of water-thawing of cuts, (b) repeated sanitation with chlorinated water of all equipment and utensils, (c) tightened enforcement of use of disposable plastic gloves, and (d) increased surveillance to assure good manufacturing practice and compliance with regard to product temperature constraints. The problem has not recurred.

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Seven Elected to DFISA Board

Four new directors and three incumbent directors were elected by the membership of Dairy and Food Industries Supply Association to terms on the DFISA board of directors at the association's 60th Annual Meeting at Palm Beach, April 2-4, 1979.

New directors are Clyde Monda, president, Waukesha Foundry Div., Abex Corp., Waukesha, Wis., and Leonard Peterson, national dairy products sales manager, Burry Div., Quaker Oats Co., Elizabeth, N. J. New director for the chemicals and refrigerants group is H. Bruce Ellison, marketing manager for food industries, BASF Wyandotte Corp., Wyandotte, Mich. New director for the containers section is James McCullough, dairy industry manager, Soltex Polymer Corp., Houston, Texas.

Re-elected as at-large directors were Peter Miller, vice president, Chester-Jensen Co. Inc., Chester,

Pa., and Leroy Mommsen, president, CREPACO, Inc., Chicago. Re-elected as point-of-sale director was F. Heath Schroeder, regional manager, Kelvinator Commercial Products Inc., Lake Oswego, Ore.

NCI and ABI Elect Officers

The National Cheese Institute held its 52nd Annual Meeting at the Marriott O'Hare in Chicago April 23 and 24 and re-elected the following NCI corporate officers for a one-year term: Harold Steinke, Borden Foods Inc., President; and Vice Presidents, Arthur Jepsen, Land O'Lakes, Inc.; L. W. Arens, Pauly Cheese Co.; R. M. Bush, L. D. Schreiber Cheese Co., Inc.; and G. F. Heisinger, Kraft, Inc.

The American Butter Institute met for its 70th Annual Meeting at the same time. They honored their immediate Past President, John

DFISA is the national trade association of 500 equipment, supply and service firms serving the dairy, food, beverage and related processing industries.

Ringenberg, Mid-America Farms, Inc. and new officers elected for a one year term were: Claude Harper, Jr., Beatrice Foods Co., President, together with Vice Presidents Floyd Harris, Level Valley Dairy Co. and Jim Nieman, Wilsey Bennett of Oklahoma.

R. F. Anderson was re-elected Executive Director of both Institutes.

The theme for the 20th joint ABI/NCI Annual Meeting was "Strategies for the Future". Over 500 attendees heard speakers discuss topics as varied as productivity, strategic planning, butter cultures, low fat cheese characteristics, the future of dairy market orders, and international trade negotiations.