



Microbial profiles of commercial, vacuum-packaged, fresh pork of normal or short storage life

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Abstract

The microbial ecology of fresh vacuum-packed pork cuts during storage at $-1.5\text{ }^{\circ}\text{C}$ for up to 45 days was examined to characterize rates of microbial growth and pH changes in commercially prepared products of normal storage quality. Pork loins in commercial distribution with odour defects were also studied to determine a possible cause of the defects and avoid future problems. In addition, microbial profiles of pork cuts from two plants were compared, after storage for 25 days at $-1.5\text{ }^{\circ}\text{C}$, to identify possible reasons for differences in the storage life of product from the plants. The effects of a change in sanitation procedures on the microbial populations of products stored for 25 days were also studied.

With normal product, microbial growth in different packages progressed at different rates, reflecting differences in initial levels of bacterial contamination. All samples in the study reached 8 weeks without apparent organoleptic change and samples carried $5.8 \pm 1.2 \log$ bacteria cm^{-2} (mean \pm S.D.). The flora of loins with the odour defect were predominately lactic acid bacteria (LAB) and carnobacteria, but they contained large fractions of Enterobacteriaceae <35 days after packaging. *Aeromonas* spp. and *Shewanella* spp. were likely responsible for the sulfide-putrid smell of these spoiled products, but species of Enterobacteriaceae and lactic acid bacteria could have contributed to spoilage. Comparison of microbial groups present in 16 other cuts, half from each of two commercial plants, which were stored for 25 days at $-1.5\text{ }^{\circ}\text{C}$, showed that larger fractions of Enterobacteriaceae were present in samples from the plant having difficulty achieving the desired storage life. Additional bacterial samples from 12 cuts supplied by the latter plant obtained after adoption of an acid sanitizer step in the plant cleaning regimen, and also stored for 25 days at $-1.5\text{ }^{\circ}\text{C}$, yielded few Enterobacteriaceae, *Aeromonas* or *Shewanella*. Use of an acid sanitizer in plant cleaning may be a means of controlling alkali-tolerant bacteria such as *Aeromonas* or *Shewanella* which can contaminate pork cuts and spoil vacuum-packaged product. The fraction of Enterobacteriaceae in bacterial populations on fresh pork stored for 25 days at $-1.5\text{ }^{\circ}\text{C}$ may be a useful indicator of the effectiveness of plant sanitation.

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1. Introduction

Canada is the world's largest exporter of fresh pork (Guan and Holley, 2003). Much of the meat is

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vacuum-packed and shipped to remote destinations at temperatures just above that at which the freezing of meat commences (-1.5 ± 0.5 °C). During recent years, the expected storage life has been increased from about 7 weeks (McMullen and Stiles, 1991; Nadon et al., 2001) to the maximum that may be possible with vacuum packaging, and an alternative technology such as packaging under O_2 -depleted CO_2 atmospheres may be needed to satisfy commercial requirements (Jeyamkondan et al., 2000). To achieve a storage life ≥ 7 weeks, the initial numbers of bacteria present on pork must be ≤ 2 log cfu bacteria cm^{-2} , and refrigeration systems must maintain temperatures near to -1.5 °C (McMullen and Stiles, 1993). Recently, Gill et al. (2000) found an industry average of 2.5 ± 0.5 log cfu cm^{-2} for the numbers of aerobes on cooled eviscerated pork carcasses, and contended that these results indicated that numbers < 2 log cfu cm^{-2} could be consistently obtained in commercial practice.

The commercial storage life attainable with vacuum packaging is being approached, because the psychrotrophic lactic acid bacteria (LAB) which predominate in the flora of vacuum-packaged pork will, by 8 weeks, usually number ≥ 6 log cfu cm^{-2} , which is the maximum number for acceptance of the products by some customers irrespective of whether or not the product is overtly spoiled. Spoilage of product at lower bacterial numbers can be caused by growth of other psychrotrophs such as *Brochothrix thermosphacta*, *Shewanella* spp. or *Aeromonas* spp., and/or by Enterobacteriaceae growing at chiller temperatures (Gill and Greer, 1993; McMullen and Stiles, 1993). *B. thermosphacta* can be controlled by packaging in films of very low oxygen permeability (Holley, 1999) but with *Shewanella* and *Aeromonas* a low meat pH, temperatures maintained at -1.5 °C and strict sanitation are necessary to avoid early spoilage. Successful attainment of long storage life is more difficult when customers prefer darker coloured meat, which is likely to have a high pH and be more prone to spoilage than meat of normal pH (Blixt and Borch, 2002).

The present work was undertaken to identify how current fresh pork fabrication/distribution systems might be altered to obtain a longer storage life for products.

2. Materials and methods

2.1. Sources of meat and microbial cultures from plants

Materials used in the study came from three commercial pork processing plants. Plant A received dressed carcasses while Plants B and C had slaughter operations. Plant C sent carcasses to Plant A for breaking and packaging.

Vacuum-packed boneless loin pieces (1 kg) which were stored ≤ 56 days at near freezing were packaged at Plant C and sent the day of fabrication to the University laboratory where they were immediately entered into the storage study.

Market samples of bone-in pork loins were fabricated and packaged at Plant B. They had been distributed to retail but were recovered by company personnel following complaints of a sulfurous-fecal odour. These were sent to the University laboratory for microbiological analysis. Two of four bags had been opened for inspection but were resealed without vacuum before being received by the laboratory.

Changes in microbial content of meat cuts vacuum-packed and stored at Plants A and B at -1.5 °C ± 0.5 °C were studied by examination of bacterial colonies on Standard Plate Count (SPC; BBL division of Becton Dickinson, Cockeysville, MD) agar plates prepared at the plants from cuts which were either 25 or 45 days old. Previously incubated plates were sent to the University laboratory where colonies were isolated and identified. At Plant B, following establishment of a new sanitation procedure, meat cuts were vacuum-packed and held at the plant for 25 days at -1.5 ± 0.5 °C. SPC plates were prepared by plant personnel, incubated, counted and sent to the University laboratory for bacterial identification. The new sanitation procedure included use of a peroxy-acetic acid sanitizer (Divosan Activ, Johnson Diversey Canada, Oakville, ON), a stabilized solution of 10–30% (w/w) hydrogen peroxide, 3–7% (w/w) peracetic acid and 10–30% (w/w) acetic acid. It was applied after a cleaning sequence consisting of a high pressure rinse with 1–4% (w/w) Endurochlor (Johnson Diversey), a chlorinated alkaline foam cleaner, applied at temperatures between 49 and 60 °C for 10–20 min; and then a post-treatment rinse with water at 49–57 °C. The last step was exposure

to a solution of Divosan Activ at 220 ppm for 30 min, 2–4 h before work started.

2.2. Microbial numbers during vacuum-packaged chill storage of commercial pork

Fresh boneless pork loin pieces (1 kg), vacuum-packed with an adsorbent pad on the lower side in film with an O₂ transmission rate of $\leq 6 \text{ cm}^3 \text{ O}_2 \text{ m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$ at 4.4 °C, were delivered to the laboratory on the day the loin cuts were fabricated from carcasses at slaughter Plant C. The loin pieces were stored at -1.7 ± 1.0 °C. Five pieces were sampled initially and at approximately weekly intervals from 30 to 56 days of storage. The pH and numbers of bacteria per square centimeter were determined for each sample. Meat carcass sample kits from Nasco (Fort Atkinson, WI) were used for surface sampling. After swabbing a 10-cm² area of meat surface, the sponge was placed in a sterile stomacher bag and 100 ml 0.1% (w/v) peptone water was added. The sample was pumelled for 1.5–2 min (Stomacher 400 Lab Blender, A.J. Seward, London, UK) and serial dilutions were prepared using 0.1% peptone water. Sample dilutions of 0.1 ml were spread on pre-poured plates of SPC and these were incubated at 35 °C for 48 h. Meat surface pH was determined using a model IQ240 pH meter (IQ Scientific Instruments, San Diego, CA) equipped with a surface contact electrode. The pH at four points on the surface of each piece of meat was determined at each time, and the mean value was reported. Appearance and odour of raw meat portions were noted upon opening packages and flavour was evaluated after cooking by an untrained panel in the Department of Food Science.

2.3. Analysis of market samples

Four market samples of vacuum-packed bone-in loins with sulfurous-fecal off odour were analyzed. The packaging material that had been used with the cuts had an O₂ transmission of $15\text{--}30 \text{ cm}^3 \text{ O}_2 \text{ at } 23 \text{ °C m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$.

Meat surfaces were sampled by shaving ≤ 3 mm thick slices from the loins. A homogenate was made by pumelling 10 g of meat with 90 ml 0.1% peptone for 1–2 min in the stomacher. Serial dilutions were made in 0.1% peptone, and 0.1 ml portions were

spread on the following media and incubated as indicated: SPC for 48 h at 32 °C, for enumeration of total aerobic bacteria; de Man Rogosa Sharpe Agar (MRS, Difco Division of Becton Dickinson, Sparks, MD) for 48 h at 25 °C, for lactic acid bacteria; Violet Red Bile Agar (BBL) with 1% (w/v) glucose (VRBG) for 24 h at 35 °C for Enterobacteriaceae; Sulfite, Polymixin, Sulfadiazone Agar (SPS, BBL) for 48 h at 25 °C, aerobically for enumerating aerobic spore-formers, and in anaerobic jars for anaerobic spores; Streptomycin Thallous Acetate Actidione Agar (STAA, Gill and Greer, 1993) for 48 h at 25 °C, aerobically for *B. thermosphacta*; Peptone Iron Agar (PIA) for 72 h at 25 °C, anaerobically for hydrogen sulfide production (Gill and Greer, 1993); Cresol Red Thallium Acetate Sucrose Inulin Agar (CTSI, Wasney et al., 2001) for 48 h at 25 °C followed by 48 h at 8 °C was used for carnobacteria.

A total of 30 isolated colonies of different morphologies from PIA and VRBG plates prepared using meats from the two packages with intact vacuum were re-streaked on SPC Agar, purified and kept for further testing to determine their generic identity. These isolates were examined for Gram reaction using 3% KOH (Gregersen, 1978), catalase using 3% hydrogen peroxide and oxidase using 1% tetra methyl-*p*-phenylenediamine dihydrochloride. Characteristics used to assign isolates to major bacterial groups are shown in Table 1. Glucose and lactose utilization were determined using Hugh and Liefson's medium (Gill and Greer, 1993). Arginine dihydrolase/decarboxylase activity was determined according to the Bacteriological Analytical Manual (Elliot et al., 1998). Motility and morphology were determined by examination of wet mounts under phase contrast optics (Zeiss photomicroscope). Additional biochemical reactions of isolates were determined using the API 20E system (bioMérieux Vitek, Hazelwood, MO).

Table 1
Characteristics used to assign isolates to major bacterial groups

Group	Morphology	Gram reaction	Catalase reaction	Oxidase reaction
Lactic acid bacteria	rod	+	–	–
Enterobacteriaceae	rod	–	+	–
<i>Pseudomonas</i> -like	rod	–	+	+
<i>Micrococcus</i> -like	coccus	+	+	+/–

2.4. Identification of isolates from fresh pork after 25 days storage

Boneless backs and collar butt cuts of fresh pork, which had been vacuum-packed as a part of normal production runs at Plants A and B, were studied. The meat had been stored at -1.5 ± 0.5 °C for 25 days and then sampled for bacterial analysis by plant QA personnel. At both plants, samples were obtained by the excision of thin (<3 mm thick) pieces of meat from the surface. At Plant A, 25 g meat was mixed with 225 ml 0.1% peptone water and pumped in the stomacher for ≤ 60 s. The stomacher fluid was diluted in peptone water and pour-plated using SPC plates which were incubated at 35 °C for 48 h. At Plant B, 20 g of meat was mixed with 180 ml buffered peptone water (Difco) and was treated in the stomacher as at Plant A. The stomacher fluid was diluted in buffered peptone water and pour-plated in SPC containing 0.005% triphenyl tetrazolium chloride. Plates were incubated as at Plant A. After enumeration of colonies, plates were refrigerated and shipped to the University laboratory for colony isolation and bacterial identification.

Eight SPC plates containing 30–300 colonies were received at the laboratory from each of Plant A and Plant B. Plant A provided SPC plates from four different boneless backs and four collar butts. Plant B provided SPC plates from three boneless backs and five collar butts. From each plate, 20 colonies were randomly picked and each colony was transferred to 5 ml Brain Heart Infusion (BHI) broth (Difco) which was incubated for 48 h at 35 °C. Broth was incubated for a further period (≤ 7 days) at 25 °C if no growth occurred. Cultures were streaked on plates of All Purpose Tween Agar (APT, Difco) which were incubated at either 25 or 35 °C, and after growth was abundant, re-streaked on the same medium for assurance of purity. Isolated colonies were transferred to APT agar slants and transferred bi-weekly to maintain viability. Bacterial isolates were examined as previously described for their cellular morphology, and catalase, oxidase and Gram reactions. Rod-shaped Gram-positive organisms were examined for their ability to grow on CTSI agar which was used for carnobacteria and Rogosa SL agar (BBL) for lactobacilli and incubated 5 days at 30 °C. The production of

CO₂ from carbohydrates by lactic acid bacteria was monitored in tubes of APT broth containing inverted Durham tubes, which were incubated at 30 °C for 72 h. Gram-positive cocci were examined on Baird-Parker Agar (Difco) to distinguish between micrococci and staphylococci. Arginine hydrolysis by micrococci was also used to separate these genera (Gill and Greer, 1993).

Species of Gram-negative, oxidase-negative and catalase-positive organisms were identified using the API 20E system, but for some isolates, the characterization to the species level required conventional testing (Brenner, 1984). Both oxidase-negative and -positive Gram-negative organisms were identified using the key reactions noted in Table 2. Brilliant Green Sulfadiazine Agar (BGS, BBL), MacConkey Agar (BBL) and *Salmonella*–*Shigella* Agar (BBL) were also used to characterize Enterobacteriaceae genera. Indole production by Enterobacteriaceae and presumptive *Shewanella*/*Aeromonas* were monitored in Methyl Red-Voges Proskauer (MR-VP) Broth (Difco). Glucose utilization, either oxidatively or fermentatively, was studied in Hugh and Liefson's semi-solid medium with glucose (Gill and Greer, 1993), and lactose fermentation in the same medium with lactose instead of glucose. Presumptive *Vibrio* were examined by phase contrast microscopy for the presence of refractile poly beta-hydroxybutyrate (PHB) inclusions in cells (Bauman and Schubert, 1984). Cellular motility in wet mounts was examined microscopically. Colony pigmentation on SPC

Table 2
Main characteristics used to identify genera of Gram-negative isolates

Genera	Lactose utilization	Arginine metabolism	H ₂ S production
<i>Shigella</i> , <i>Alcaligenes</i> , <i>Serratia</i> , <i>Hafnia</i>	–	–	–
<i>Pseudomonas</i> , <i>Vibrio</i>	–	+	–
<i>Salmonella</i>	–	+	+
<i>Proteus</i> , <i>Shewanella</i>	–	–	+
<i>Enterobacter</i> , <i>Escherichia</i> , <i>Klebsiella</i> , atypical <i>Shewanella</i>	+	–	–
<i>Shewanella</i>	+	–	+
<i>Citrobacter</i> , atypical <i>Aeromonas</i>	+	+	–
<i>Aeromonas</i>	+	+	+

agar was also used to guide identification. Cultures of known identity were used to validate media performance and test results. These included: *Shewanella putrefaciens* ATCC 8071, *Carnobacterium piscicola* ATCC 43224, *Escherichia coli* ATCC 13706, *Lactobacillus delbrueckii* ATCC 11842 and Department isolates of *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Proteus vulgaris* no. 73, *Salmonella enterica* Typhimurium no. 98 and *Aeromonas hydrophila* c.

2.5. Influence of storage time and sanitation on microbial profiles

Twenty-four fresh portions of pork at Plant B (6 collar butts, 6 boneless backs, 6 Japanese belly and 6 tenderloin cuts) were divided into two groups and were vacuum-packed and stored at -1.5 ± 0.5 °C for either 25 or 45 days at the plant. Portions were sampled and SPC plates were prepared as previously described. Duplicate plates, half from each storage period, were sent to the laboratory for bacterial identification. These 25-day samples differed from those previously analyzed from this plant because they were taken after the plant had established a sanitation procedure which included use of peroxy-acetic acid (see Section 2.1).

Samples stored for 45 days were from a period where unmodified cleaning had taken place, with the use of an alkaline quaternary ammonium sanitizer. Ten colonies were picked from each duplicate set of plates unless the pair contained <10 colonies. Procedures used for isolation, purification, and identification of isolates were as previously described.

3. Results

3.1. Microbial numbers on chill-stored commercial pork

Numbers of bacteria on vacuum-packaged pork increased slowly to about $5 \log \text{cfu cm}^{-2}$ by day 36, following which fewer bacteria were recovered until day 56 (Table 3). Results from the five replicate samples at each time were highly variable. Variability was also high for pH measurements at day 51 (5.7–6.5). Meat pH was within the normal range (5.4–5.8)

Table 3

Numbers of aerobes and pH values at the surfaces of vacuum-packaged fresh boneless pork loins during storage at -1.7 ± 1.0 °C

Storage time (days)	Numbers of aerobes ($\log \text{cfu cm}^{-2}$)		Mean pH ^a
	Mean ^b	Range	
0	3.30	2.5–3.3	5.70
30	4.30	3.4–4.1	— ^c
36	5.16	2.7–5.5	6.02
42	3.41	2.0–3.7	6.04
46	3.11	2.6–3.5	5.89
51	4.07	3.2–4.4	6.02
56	5.78	3.3–6.4	6.03

^a S.D. ≤ 0.34 , $n=20$.

^b $n=5$.

^c Electrode failure.

initially and tended to be higher in samples measured at times in the later part of the study with all but one sample having values ≤ 6.04 . When numbers of bacteria on chill-stored samples at 30 days (Table 3) were compared with numbers present on plates from Plants A and B samples taken at day 25, all were similar at $4 \log \text{cfu cm}^{-2}$ or g^{-1} (data not presented). Numbers present on plates from Plant B were higher at $4.7 \log \text{cfu g}^{-1}$ than on 46 day stored meat ($3.11 \log \text{cfu cm}^{-2}$) (Table 3). Informal examination of loins from Plant C stored 56 days revealed that none had undesirable appearance or odour when raw, or undesirable flavour when cooked.

3.2. Market sample quality

Bacterial groups in market samples of bone-in loins are reported in Table 4. All had acceptable colour but smelled putrid when packages were first opened. Two loins (A and B) in unit 4 had a slight hydrogen sulfide odour. Samples had been packaged 25–35 days previously with loins in unit 4 being 35 days old. Numbers of aerobes, LAB, carnobacteria, Enterobacteriaceae and *B. thermosphacta* were all high. Numbers of *B. thermosphacta* were highest in units 3 and 4 which had been opened 16 days after being vacuum-packed, re-packed aerobically in plastic, then held refrigerated for an equal period before their analysis. No bacterial spores were detected ($< 1 \log \text{cfu g}^{-1}$) in any samples. Again, all samples had high but normal pH values with two loin portions

Table 4
Numbers of aerobes (log cfu g⁻¹) recovered from commercial bone-in pork loins rejected because of possible spoilage

Bacterial group	Unit ^a							
	1 ^b		2 ^b		3 ^c		4 ^c	
	A	B	A	B	A	B	A	B
Aerobes	8.32	8.26	8.52	8.11	7.88	8.79	7.56	7.79
Lactic acid bacteria	7.72	7.64	7.67	7.48	7.45	8.26	8.38	8.00
Carnobacteria	7.71	7.30	8.04	7.80	7.28	7.04	6.98	7.62
Enterobacteria	6.48	6.89	6.51	6.23	6.54	6.61	5.79	6.64
<i>B. thermosphacta</i>	5.49	5.57	5.74	5.88	6.80	6.69	6.53	5.93
pH	5.83	6.25	6.06	6.02	5.84	6.32	5.92	6.26

^a Each unit contained two loins, designated as A and B.

^b Units had not been opened before being returned.

^c Units had been opened before being returned.

from the rib area having pH values of 6.3 (3B, 4B). Twenty colonies from unit 1 VRBG plates and 10 colonies from unit 2 (five from KIA and five from VRBG plates) were identified further. Of the isolates examined, 12/20 and 3/10 were Enterobacteriaceae. Three pseudomonads were isolated from unit 1 but none were isolated from unit 2. A total of nine isolates from units 1 and 2 were *Shewanella* spp. and 3 were *Aeromonas* spp.

3.3. Identification of bacterial isolates from pork stored for 25 days

Of 160 colonies picked from SPC Agar plates provided by each of Plants A and B, 131 from Plant

A and 148 from Plant B were successfully cultured, isolated and identified to the taxonomic groups or genera shown in Table 5. For meat from each plant, the majority of isolates were Gram-positive bacteria, about half of which were classified as LAB. However, many of these were carnobacteria with <5% being lactobacilli (Table 6). No leuconostocs or pediococci were isolated. Pseudomonad-like organisms were about 30% of the organisms recovered from both plants. In contrast, the Enterobacteriaceae recovered from meat from Plants A and B were 5% and 17% of the isolates, respectively. Substantially larger numbers of *Escherichia* were found among organisms recovered from meat samples from Plant B than from samples from Plant A. In contrast, *Alcaligenes* were more frequent in Plant A than in Plant B samples.

3.4. Influence of storage time and sanitation on bacterial profiles

Viable organisms were isolated from plates prepared from 21 of 24 meat samples used in this part of the study. Of 121 colonies from 25-day-old samples and 112 colonies from 45-day-old samples, 91 and 80, respectively, were isolated and studied. The percentage of Gram-positive isolates was higher than previously observed at 25 days (Table 5) and there were fewer Enterobacteriaceae and pseudomonad-like isolates in the microflora of samples from Plant B after sanitation crews used peroxyacetic

Table 5
The fractions of bacteria in major groups present in the microflora of vacuum-packaged pork cuts prepared before or after improvement of the equipment cleaning process, after storage at -1.5 ± 0.5 °C for 25 or 45 days

Bacterial group	25 days of storage						45 days of storage	
	Plant A ^a		Plant B ^a		Plant B ^b		Plant B ^a	
	No. of isolates	Percent	No. of isolates	Percent	No. of isolates	Percent	No. of isolates	Percent
Total	131	100	148	100	91	100	80	100
Lactic acid bacteria	63	48	74	50	65	71	68	85
Enterobacteriaceae	6	5	25	17	1 ^c	1	3 ^c	4
<i>Pseudomonas</i> -like ^d	44	34	45	30	12	13	3	4
<i>Micrococcus</i> -like	12	9	3	2	5	6	3	4
unidentified	—	—	—	—	8	9	3	4
Gram-positive	81	62	77	52	72	79	71	89

^a Without improvement of the equipment cleaning process.

^b After improvement of the equipment cleaning process.

^c Not identified to the genus level.

^d *Pseudomonas*, *Aeromonas*, *Shewanella*.

Table 6

Generic identities of bacterial isolates from fresh vacuum-packed pork held at -1.5 ± 0.5 °C for 25 or 45 days

Bacterial group	25 days of storage						45 days of storage	
	Plant A ^{a,b}		Plant B ^{a,c}		Plant B ^{d,e}		Plant B ^{a,f}	
	No. of isolates	Percent	No. of isolates	Percent	No. of isolates	Percent	No. of isolates	Percent
<i>Carnobacterium</i>	61	47	58	39	65	71	68	85
<i>Lactobacillus</i>	3	2.3	6	4.1	0	0	0	0
<i>Micrococcus</i>	13	9.9	3	2.0	5	6	3	4
<i>Brochothrix</i>	1	0.8	0	0	0	0	0	0
<i>Pseudo/Aeromonas</i>	– ^g	–	19	13	–	–	–	–
<i>Pseudomonas</i>	4	3	–	–	5 ^h	5.5	3	4
<i>Aeromonas</i>	8	6	10	6.8	6 ⁱ	6.6	3 ^j	4
<i>Shewanella</i>	17	13	11	7.4	1	1.0	0	0
<i>Alcaligenes</i>	15	11	4	2.7	–	–	0	0
<i>Escherichia</i>	3	2	32	22	–	<1.0	–	<4

^a Without improvement of the equipment cleaning process.^b Other genera recovered were *Acinetobacter* (1), *Moraxella* (1), *Proteus* (1), *Shigella* (1), *Staphylococcus* (1).^c Other genera recovered were *Salmonella* (3), *Vibrio* (1).^d After improvement of the equipment cleaning process.^e Other groups recovered were *Enterobacteriaceae* (1), corynebacteria (7).^f See Table 5 for other groups present.^g May have been present but either included within another taxonomic group or not identified.^h *Flavobacterium* (2), *Moraxella* (1).ⁱ *A. salmonicida* (4), *A. sobria* (2).^j *A. veronii* (2), *A. sobria* (1).

acid to sanitize equipment. All Gram-positive rods recovered from meat packaged after this cleaning procedure was adopted were carnobacteria (Table 6). *Pseudomonas*, *Aeromonas* and *Shewanella* were also fewer than in samples from Plant B before improved cleaning and the percentages of *Pseudomonas* and *Aeromonas* isolates were similar to those reported for Plant A at 25 days, although acid sanitizing reduced the frequency of *Shewanella* isolations. Seven of eight organisms from the group after improved cleaning at Plant B were unclassified Gram-positive species (Table 5). These were catalase- and oxidase-positive rods or coccobacilli (perhaps corynebacteria) and the Gram-negative isolate (a rod) was both catalase- and oxidase-negative. Of 11 Gram-negative oxidase-positive isolates examined further, 6 were *Aeromonas* (4 *Aeromonas salmonicida*, 2 *Aeromonas sobria*), 2 were *Pseudomonas* spp. and 3 were other obligate aerobes (Neyts et al., 2000).

The 45-day meat samples were the only ones studied which were free from *Pseudomonas*, *Alcaligenes* and *Shewanella* spp. One of the three aeromonads isolated from these samples was identified as

A. sobria while the remaining two were *A. veronii* (Table 6).

4. Discussion

4.1. Microbial numbers in chill-stored commercial pork

Numbers of aerobes were determined by incubating agar plates at 35 °C for 48 h because some overseas customers require products to have numbers ≤ 6 log cfu g⁻¹ as determined under those conditions. Initial numbers of bacteria present on loin samples were, along with pH values, on the high end of levels usual for Canadian pork (McMullen and Stiles, 1991; Holley et al., 1994; Gill et al., 2000; Nadon et al., 2001). The decrease in bacterial numbers seen at 42 and 46 days of storage probably reflects the heterogeneity in numbers of bacteria present in different samples rather than real reductions in numbers. In their study of pork loin chops stored at -1.5 °C, Nadon et al. (2001) found bacterial numbers to reach an initial peak of 5 log cfu cm⁻² at 5 weeks, then dip

and return to $6.5 \log \text{cfu cm}^{-2}$ at week 7 and remain at that level until 9 weeks. In the present study, the average maximum number of bacteria, $\leq 6 \log \text{cfu cm}^{-2}$, was not reached until 8 weeks of storage. Obviously, lower initial numbers of bacteria on meat will tend to extend product storage life. McMullen and Stiles (1991) found that a 100-fold reduction in initial bacterial numbers present yielded 2 weeks greater storage life when pork was held at either 4 or -1°C . However, the numbers of organisms capable of growth at mesophilic temperatures cannot normally be used to estimate the storage life of refrigerated meat. If the dominant psychrotrophs present were carnobacteria, they may be accurately recovered at $32\text{--}35^\circ\text{C}$, but the exact role they play in determining product storage life is unknown at present. Additional work is needed to understand the influence of this group of organisms on the storage stability of fresh meat during extended storage at near freezing temperatures.

4.2. Market samples

The dominant populations of bacteria present on market samples were either LAB or carnobacteria. This agrees with results published by McMullen and Stiles (1993), but our use of CTSI, a medium selective for *Carnobacterium* (Wasney et al., 2001), showed the two groups were present in samples in almost equal numbers. These pork loins were probably spoiled by either or both *Shewanella* or *Aeromonas* spp. which were found on each of four loins in the two separate, unopened bags. Both organisms produce hydrogen sulfide, are capable of growth at -1°C , are facultatively anaerobic and are often found in vacuum-packed pork. McMullen and Stiles (1993) found *Aeromonas* to be a part of the dominant microflora in vacuum-packed pork stored at 10°C and also found them in similar samples stored at -1°C for 9 weeks. Neyts et al. (2000) isolated *Aeromonas* at levels of $3.2 \log \text{cfu g}^{-1}$ from ground pork. Gill and Greer (1993) regarded *Shewanella putrefaciens* as a potent meat spoilage organism, particularly at the pH values seen in samples studied here. Although *B. thermosphacta* does not produce hydrogen sulfide, they may have been able to contribute off odours at the levels found in these samples. Their presence suggests that samples had not been stored at well

controlled temperatures. Although sometimes present, Enterobacteriaceae are not normally a significant part of the dominant microflora in pork stored at -1°C (McMullen and Stiles, 1993). Nevertheless, it is possible that Enterobacteriaceae or other lactics present may have been capable of producing the defect. Examination of Plant B production records showed that exposure of carcasses to a longer than normal period above 4°C may have occurred because of a malfunction in the carcass hot water wash system in the plant the day these meat cuts were processed. Since most *Aeromonas* and *Shewanella* isolates were weak hydrogen sulfide producers, Peptone Iron, Kligler Iron and Lead Acetate Agars (Fernandez-Coll and Pierson, 1985) yielded inconsistent results. The use of Bile Salts–Irgasan–Brilliant Green Agar to quantify *Aeromonas* (Neyts et al., 2000) and Lyngby Iron Agar (Oxoid, Basingstoke, England) for *Shewanella* spp. (Emborg et al., 2002) in future investigations of similar problems is recommended.

4.3. Bacterial isolates on pork stored for 25 or 45 days

There was no consistent pattern in the types of organisms from different cuts of meat. Perhaps this is not surprising in view of the work by Blixt and Borch (2002). These authors examined the growth of lactic acid bacteria, *B. thermosphacta*, Enterobacteriaceae and pseudomonads in three cuts of pork (collar, loin and entrécôte) and beef loins which were ground and stored at 4°C under vacuum for 8 weeks. They found that differences in microflora development during storage were related to the pH and fat content of each cut. Microflora developments were the same on pork loin and beef striploin, which had similar but lower pH and fat content than pork collars and entrécôte. Microbial growth was faster and more extensive on the cuts of higher pH and fat content. In contrast with the results of this study, meat pH dropped from 5.35–5.7 to 5.2–5.4 during storage. The difference may be due to the greater availability of glycogen, glucose and glucose 6-P in the ground meats than would be available at the surfaces of meat cuts examined here. The high pH of pork loins used in this study may have contributed to the defect seen in market samples.

In the present study, carnobacteria dominated microbial populations in most samples stored at

– 1.5 °C for 25 or 45 days, their proportion increasing with storage time. Under these conditions, this group of organisms is not known to spoil stored product. Thus, the large numbers of carnobacteria present in these samples probably has little significance for product storage life. There was heterogeneity in population profiles and carnobacteria were not found in two 25-day-old samples. Lactobacilli were present as a minor component in some 25 day meat samples but they were absent from others as well as from the 45-day-old samples (Table 6). Acid sanitizer use in Plant B reduced the frequency of Enterobacteriaceae isolations 17-fold and pseudomonad-like organisms by 50%.

Shewanella and *Aeromonas*, which were included with pseudomonad-like organisms, were a consistent part of meat microflora. McMullen and Stiles (1993) found *Aeromonas* were often co-dominant with lactobacilli in similarly stored products and Neyts et al. (2000) also found *Aeromonas* in fresh pork. Nonetheless, Gill and Greer (1993) stressed the important influence *Shewanella putrefaciens* can have in shortening meat storage life. The presence of *Aeromonas* and *Shewanella* is a potential threat to product storage life and steps for their reduction and elimination should be undertaken. The work presented here indicates that exposure to acid challenge in a regimen of cleaning and sanitizing that uses primarily alkali-based compounds can improve product storage life. *Aeromonas* and *Shewanella*, as well as carnobacteria, *Listeria* and *Yersinia* are all alkali tolerant. This work also showed that the fraction of Enterobacteriaceae in the total aerobic organisms present on stored vacuum-packed pork may be a useful indicator of the efficacy of plant sanitation. Zeitoun et al. (1994) suggested that Enterobacteriaceae could be used as an indicator of processing hygiene for freshly processed poultry. However, their use of Enterobacteriaceae as an indicator was different from the use proposed here. For predictive value for fresh pork storage life, the test should be done after 25 days of storage at $-1.5 \text{ °C} \pm 0.5 \text{ °C}$ and the proportion of Enterobacteriaceae present in the total established population calculated. Although results would not be available on the day of production, the test allows prediction of product quality ≥ 30 days in advance of the end of desired product storage life.

Enterobacteriaceae <5% would be considered a reflection of acceptable sanitation practice and hygiene.

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