THE SPOILAGE MICROFLORA OF CANADIAN RETAIL BEEF STEAKS

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Abstract – Four types of beef steaks collected from retail stores in four Canadian cities were stored at -20 °C. When the 598 steaks were thawed, a major surface (≈ 100 cm^2) of each steak was sampled by swabbing and the swabs were processed for enumeration of total aerobic counts (TAC), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), *Brochothrix thermosphacta* (BTH), coliforms (COL) and *Escherichia coli* (ECO). The fractions of steaks from which TAC, PSY, LAB, PSE and BTH were recovered at numbers ≥ 2 log cfu/steaks, and from which COL and ECO were recovered at numbers ≥ 0 log cfu/steak were 97, 88, 92, 25, 49, 44 and 8%, respectively. For ≥ 90% of steaks, the log numbers recovered per steak were < 6 for TAC, PSY and LAB, < 5 for PSE, < 4 for BTH, and < 2 for COL; and 99% ECO counts were < 1 log cfu/steak. Numbers of bacteria recovered from steaks of different types, from different cities or from different groups of stores were not substantially different.

Key Words – Retail sale, Microbiological contamination, Spoilage.

I. INTRODUCTION

Surveys of the microbiological conditions of retail meats have usually been focused on the detection of pathogens. Consequently, information on the numbers and compositions of the spoilage microflora present on premium cuts of beef offered for retail sale is largely lacking. The times for which consumers may wish to retain chilled meat after it is purchased will vary widely with their circumstances. Thus, for many consumers, a product storage life of several days in a domestic refrigerator is likely desirable. The time for which chilled meat can be retained without spoilage under any particular conditions of handling and storage by a consumer will depend upon the status of the spoilage flora at the time the meat is purchased. Therefore, for better understanding of the time for which prime Canadian beef can be retained by Canadian consumers without spoilage, the microbiological conditions of steaks displayed for retail sale in four Canadian cities were investigated.

II. MATERIALS AND METHODS

During August and September 2009, steaks were obtained from 113 retail stores in the Canadian cities Montreal, Toronto, London and Calgary; with the collection of 150 of each of inside round (IR), top sirloin (TS) and striploin (SL) steaks, and 148 cross rib (CR) steaks. When each steak was collected the type of steak, the store name and location, the date of collection and an identification number were recorded. Each steak was removed from its retail packaging, tagged with the identification number, zip-sealed in a plastic pouch, and frozen at -20 °C.

Between 5 and 8 months after their collection, each frozen steak was placed in a polystyrene tray, overwrapped with plastic film and thawed overnight at 4 °C. The whole upper surface of the thawed steak, i.e. about 100 cm^2, was swabbed using a sterile gauze sponge (Curity gauze sponge, Kendall Canada, Peterborough, Ontario, Canada) moistened with 0.1% (w/v) peptone water (Difco, Becton-Dickinson, Sparks, MD, USA). Each gauze was placed in a plastic bag and frozen at -80 °C within 20 min of being collected. The swab samples were processed for enumeration of bacteria between 3 and 9 months after they were frozen. Bags containing gauzes were immersed in water of 30 °C for 15 min to thaw the gauzes, and 5 ml of peptone water was added to each bag. After stomaching, dilution of each stomacher fluid were prepared, and 0.1 ml portions of the undiluted fluid and each dilution were spread on duplicate plates of tryptone soy agar (TSA; Difco) and on single plates of de Man, Rogosa, Sharpe agar (MRS; Difco), cephaloridine fucidin centrimide agar (CFC; Difco), and streptomycin thallous actidione agar (STAA; Difco). One set of

plates of TSA inoculated with the undiluted stomacher fluid and each dilution and plates of CFC and STAA were incubated aerobically at 25 °C for 48 h. The other set of TSA plates was incubated at 4 °C for 10 days. Plates of MRS were incubated anaerobically, at 25 °C for 72 h. Colonies were counted on plates bearing between 20 and 200 colonies for enumeration of total aerobic counts (TSA, 25 °C) and presumptive psychrotrophs (TSA, 4 °C), lactic acid bacteria (MRS), pseudomonads (CFC) and *Brochothrix thermosphacta* (STAA). Each gauze was squeezed, and all fluid in the bag was removed and mixed with 1 ml papain solution (EZ-Enzyme; Oxoid, Nepean, Ontario, Canada). The mixture was incubated at 25 °C for 20 min and filtered through a hydrophobic grid membrane filter (HGMF; Oxoid). The filter was placed on a plate of lactose monensin glucuronate agar (LMG; Oxoid) and incubated at 35 °C for 24 h. Squares on the filter that contained blue colonies were counted, and a most probable number (MPN) of coliforms was calculated from that count using the formula MPN = N $\log_n$ (N/N-X) where N is the total number of squares on the filter and X is the count of squares with blue colonies. The filter was then incubated on a plate of buffered 4-methylumbelliferyl-β-D-glucuronide agar (BMA), at 35 °C for 2 h. Squares containing blue colonies that fluoresced under UV light were counted, and a MPN for *E. coli* was calculated from the count.

In addition, a group of 25 steaks from retail stores were tumbled together to homogenize their microflora. The steaks were frozen, thawed after 14 days, and sampled, and the gauzes used for swabbing steaks were frozen for 14 days then thawed, as described above. Stomacher fluids were diluted and spread on plates of TSA and the selective agars, as before; but a third set of TSA plates and sets of each of the selective agars overlayered with 10 ml of TSA and inoculated within 30 min of the TSA setting were also prepared. The additional plates of TSA were incubated at 25 °C for 4 h before they were incubated at 4 °C for 10 days. The overlayed plates were incubated as were the corresponding plates without overlay. Also a second group of 25 steaks were tumbled together before one surface of each steak was swabbed for enumeration of bacteria, as before. Each steak was placed in a plastic bag, frozen at – 20 °C and stored for 14 days. Then, each steak was thawed and the surface that had not been sampled previously was swabbed, as before. Each swab was frozen at -80 °C for 14 days, then thawed before it was used for enumeration of bacteria, as before.

All sets of log counts recovered from the two groups of 25 steaks were subjected to the Shapiro-Wilk test for normal distribution; and mean values for log numbers (mean log, $\bar{x}$) of the same organisms recovered with or without resuscitation or with or without freezing of steaks and swabs were separated using a t-test for paired comparison in SAS, version 9 (SAS Institute, Cary, NC, USA). A value for the log of the arithmetic mean was calculated for each set using the formula $\log A = \bar{x} + \log_n 10 \cdot SD^2 /2$, where SD is the standard deviation for the set.

III. RESULTS AND DISCUSSION

The media and conditions used for recovery of TAC, COL and ECO allow recovery of injured cells. The mean log numbers of each of the other

Table 1. Statistics for sets of 25 log numbers (log cfu/steak) of psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE) and *Brochothrix thermosphacta* (BTH) recovered from frozen steaks with (R) or without (N) resuscitation of injured cells.

Organisms	**$\bar{x}$[a] (SD[b])**			**Log A[c]**		
	R	N	R-N	R	N	R-N
PSY	4.18 (0.55)	4.31 (0.47)	-0.13	4.53	4.56	-0.03
LAB	4.55 (0.64)	4.33 (0.46)	0.22	5.02	4.57	0.45
PSE	3.55 (0.51)	3.74 (0.54)	-0.19	3.85	4.08	-0.23
BTH	2.57 (0.61)	2.51 (0.62)	0.06	3.00	2.95	0.05

[a] $\bar{x}$, mean log; [b]SD, standard deviation; [c]log A, log mean.
All sets of log counts are normally distributed (P> 0.05).
All differences (R-N) between $\bar{x}$ values are not significant (P> 0.05).

group of organisms recovered with or without resuscitation were not significantly different ($P > 0.05$); while log A values differed by < 0.5 log unit, and were larger for numbers recovered without than with resuscitation in some instances (Table 1).

Similarly, the mean log numbers of each group of organisms other than ECO recovered steaks without or after freezing of steaks and swab samples were mostly not significantly different ($p > 0.05$), while log A values differed by < 0.5 log units (Table 2). Log A values for numbers recovered from frozen steaks and swabs were mostly larger than the corresponding values for numbers recovered from steaks and swabs that were not frozen. The total numbers of ECO recovered from the not frozen and frozen steaks were 0.9 and 0.6 log cfu/ 25 steaks, respectively. The findings indicate that the numbers of bacteria recovered from the frozen steaks and swabs collected in the survey were unlikely to differ greatly from the numbers present on the steaks at the times they were frozen. TAC, LAB, PSY and PSE were recovered at numbers > 2 log cfu/steak from majorities of the steak that ranged from 97 to 75% but BTH were recovered at that level from only 49% of the steaks (Table 3). TAC, PSY, LAB and PSE were recovered from ≥ 50% of steaks at numbers ≥ 3 log cfu/steak (Table 3).

Table 2. Statistics for sets of 25 log numbers (log cfu/steak) of total aerobic counts (TAC), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), *Brochothrix thermosphacta* (BTH), and coliforms (COL) recovered from steaks before freezing and without freezing of swab samples (NF) or after freezing and with freezing of swab samples (F).

Organisms	**$\bar{x}$[a] (SD[b])**			**Log A[c]**		
	NF	F	NF-F	NF	F	NF-F
TAC	7.38 (0.34)	7.39 (0.45)	-0.01	7.51[d]	7.62	-0.11
PSY	7.28 (0.35)	7.38 (0.44)	-0.10	7.46	7.60	-0.14
LAB	5.49 (0.47)	5.80 (0.35)	-0.31[e]	5.74	5.94	-0.20
PSE	6.03 (0.26)	6.24 (0.49)	-0.21	6.11	6.52	-0.41
BTH	6.29 (0.39)	6.25 (0.72)	0.04	6.46	6.85	-0.39
COL	2.76 (0.58)	2.32 (0.81)	0.44[e]	3.15	3.07	0.08

[a] $\bar{x}$, mean log; [b]SD, standard deviation; [c]log A, log mean.
[d] The set of counts is not normally distributed ($p < 0.05$).
[e] The difference is significant ($p < 0.05$).

Table 3. The frequencies, at intervals of 1 log cfu/steak, at which total aerobic count (TAC), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), *Brochothrix thermosphacta* (BTH), coliforms (COL) and *Escherichia coli* (ECO) were recovered from steaks collected from retail stores across Canada.

Organisms	**Frequency (%)**								
	ND[a]	0<1	1<2	2<3	3<4	4<5	5<6	6<7	7<8
TAC	3	-[b]	-	5	17	34	31	9	1
PSY	12	-	-	12	21	26	20	8	1
LAB	8	-	-	8	27	34	20	3	0
PSE	25	-	-	25	31	15	3	1	0
BTH	51	-	-	26	18	5	1	0	0
COL	56	19	18	6	1	0	0	0	0
ECO	92	7	1	0	0	0	0	0	0

[a]ND, not detected
[b]-, interval is below the level of detection.

Only TAC and PSY were recovered at numbers ≥ 7 log cfu/steak, from only 1% of the steaks. COL and ECO were recovered at numbers ≥ 0 log cfu/steak from 44 and 8% of the steaks, respectively. COL were recovered at numbers ≥ 2 log cfu/steak from only 6% of the steaks, while ECO were not recovered at that level from any steak. When the log numbers for each group of bacteria were arranged by steak type, city of origin and store group, most of the sets of log counts were not normally distributed. Although there were some significant differences between median

values for counts of the same type from different types of steak, steaks from different cities or steaks from different groups of stores, all the differences were < 0.5 log unit.

Over 80% of the beef offered for retail sale in Canada was produced at four (now three) large plants [1]; and all steaks examined in this study would have been cut from vacuum packaged primals prepared at those plants and stored for ≥ 14 days before dispatch. The flora recovered from steaks would then reflect the numbers of contaminants on primals, addition of contaminants during retail cut preparation, and growth of bacteria on the vacuum packaged and displayed products. The findings show that most of the bacteria on steaks were psychrotrophic LAB, which are the predominant organisms in the flora that grow on vacuum packaged primals [2]; while the numbers of PSE, which could be expected to spoil displayed meat were low. The area from which each sample was collected was about 100 cm^2, and numbers of PSE must approach about 8 log cfu/cm^2 before spoilage becomes apparent [3]. Therefore, the findings indicate that PSE numbers on > 90% of the steaks would have to increase by > 5 log units before spoilage became apparent. The few ECO recovered from steaks are comparable with the small numbers now found on product at one large Canadian beef packing plant [4]. The findings with steaks indicate that control over contamination of product with generic *E. coli* at Canadian plants has greatly improved in recent years; and that there is little or no contamination of product with such organisms during fabrication of primals to retail cuts.

IV. CONCLUSIONS

The microbiological conditions of steaks on retail sale in Canada are broadly similar for steaks of all types, in stores of all groups, in cities across Canada. The steaks are contaminated with very few *E. coli*, the accepted indicator for possible contamination with mesophilic enteric pathogens. The apparent level of microbiological safety attained for the steaks is then high. Extensive bacterial growth would be required before most steaks were spoiled. Therefore, the storage stability of the steaks generally would seem to be high and so should meet the requirements of consumers.

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