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Microbial changes under packaging conditions during transport and comparison between sampling methods of beef

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Abstract

This study was performed to evaluate the microbial and temperature changes of boxed beef during transport and distribution under vacuum and modified atmosphere packaging (MAP), and to compare between excision and swab sampling for 15 days. The top round and striploin (quality grade 1) from Hanwoo steers at 2 days post-slaughter were obtained from a local meat processing plants and chilled at 4 ± 2°C in a cold room. The boxes were transported under refrigeration (4 ± 2°C) to the laboratory within half an hour. Vacuum and MAP packs were subsequently taken out from cool boxes, and microbiological examinations were carried out at 0, 6, 12, and 24 h of storage time. MAP was more effective than vacuum packaging for the inhibition of total aerobic, lactic acid bacteria and *Pseudomonas* ($p < 0.05$). Microbial loads of swab methods were slightly lower than those of excision ones ($p < 0.05$). The results of this study could be utilized by meat consumers in future studies as well as by manufacturers to determine the ideal storage conditions for cool boxed meat, thus ensuring reduced economic losses due to spoilage.

Keywords: Cool boxed beef, Microorganism, Packaging method

Introduction

HACCP system requires continuous monitoring, recording, and controlling of critical parameters throughout the entire manufacturing process from production through distribution and storage including domestic storage at the consumer level [1, 2]. However, conditions during transportation at the retail stage are out of manufacturer's direct control. In Korea, most boxed beef that is offered for delivery sales by telephone or internet order is dispatched from packing plants as a chilled, vacuum-packed, boxed product. The boxed product is fabricated into retail forms by cutting or grinding and is packed in cool boxes or trays in meat-cutting facilities at retail stores from which it is distributed to individual homes [3]. Thus, the microbiological quality and appearance of the product offered for sale are be substantially affected by the temperature and the duration the boxed product is in the distribution system [4]. Although many processors now stipulate that the product temperature must not exceed 5℃ at the time of delivery, and most products meet that criterion, processors consider temperature abuse during storage and transport to be the most likely explanation for the summertime increase in the numbers of bacteria in manufactured beef [5].

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Selection of proper packaging methods are crucial for the preservation of freshness in meat products after slaughter and processing, and several packaging methods have been developed [6]. In Korea, 80% O_2/20% CO_2-modified atmosphere packaging (MAP) or high oxygen-MAP is usually utilized for the display of raw beef at retail market. When compared with vacuum packaging (VP), MAP with 70%–80% oxygen and 20%–30% carbon dioxide is more effective for maintaining a stable bloomed red meat color and extending shelf-life by inhibiting bacterial growth [7]. Meat sold at the retail level is usually boxed meat with primal cuts produced and often vacuum packaged at a packing plant and distributed to retail centers [8]. Very little information is available on the effects of packaging conditions on the microbial and temperature changes that occur in boxed beef during transport and distribution.

The excision sampling method in microbiological testing of meats is exclusively recommended to assess the hygienic quality. Swabbing is also permitted, but only if a correlation has been shown between the excision and swabbing techniques [9]. Swabbing is acceptable only when substantial fractions of bacteria present on the sampled areas are recovered and when correlation with excision is high [10, 12]. It is necessary to know the percentage of bacteria recovered by different sampling methods to compare the microbiological data obtained using different techniques [13]. Yet, no comparison between excision and swabbing results has been established.

To obtain a better understanding of the microbiological conditions of cool boxed beef throughout commercial distribution systems, we conducted a study of the microbial changes of boxed Hanwoo beef during transport and distribution, simulating the chilled distribution chain under vacuum and MAP for 24 h. In addition, the present work aimed to compare the swabbing and excision methods for determining the microbial load changes in boxed beef during storage.

Materials and Methods

Collection and packaging of beef

For this study, top round and striploin (representative parts used in boxed beef) from 10 Hanwoo (Korean cattle) steers were obtained from the same plant and aged for 10 days at 4 ± 2℃ in a cold room. Portions of the samples were then aseptically sliced (approximately 100 g each) using a slicer (WMC-330; Watanabe, Kawaguchi, Japan). The meats used were approximately 700 g and were packaged by either VP or MAP. One set of samples was individually vacuum-packaged in nylon/polyethylene bags (20 × 30 cm; Sunkyung Co. Ltd., Seoul, Korea) with a packaging machine (Watanabe Co., Kawaguchi, Japan). The other samples were filled with a modified atmosphere containing 80% oxygen and 20% carbon dioxide and automatically heat sealed with a packaging unit (HFV-7800D; Fuji, Japan) with an 89.4-μm thick polyamide/polyethylene (PA/PE) film. The PA/PE film had an oxygen permeability of 6.04 cm^3 per m^2 per 24 h at 1 atm and 23℃, and a carbon dioxide permeability of 16.42 cm^3 per m^2 per 24 h at 1 atm and 23℃. The MAP treatments (80% O_2/20% CO_2) were packaged in PA/PE gas impermeable trays (maximum O_2 transmission rate: 0.1 cc/cm^2 per 24 h at 23℃, RH 0%, maximum moisture vapor transmission rate: 7.87 mg/cm^2 per 24 h at 38℃, RH 100%; SCB00-096, Cryovac Sealed Air Corp., Duncan, SC, USA), with O_2 barrier films (maximum O_2 transmission rate: 0.002 cc/cm^2 per 24 h at 4.4℃, RH 100%, maximum moisture vapor transmission rate: 0.39 mg/cm^2 per 24 h at 4.4℃, RH 100%; Lid 1050; Cryovac Sealed Air Corp.), and a MAP machine (MAP-HA2; HyperVac Co., Hwaseong, Gyeonggi, Korea) equipped with a gas mixer (MAP Mix 9001 ME; PBI Dansensor A/S, Ringsted, Sjælland, Denmark). The samples were placed into polystyrene barrier foam trays and then packaged with permeable intact films (100247492; Cryovac Sealed Air Corp.) and a vacuum skin packaging machine (VSP-S100; Samhwa Co., Hwaseong, Gyeonggi, Korea) before packaging with MA. After samples were vacuum-packaged, the packs were dipped in a water bath at 65℃ for 5 sec to shrink the packaging. They were put on gel-type ice packs (2 × 1.8 × 2 cm). Samples were placed on a self-absorbent expanded polystyrene box (4.9 × 3.2 × 2.2 cm) and 2 cm thickness, and the total weight of the case-ready unit was approximately 400 g. The boxes were transported under refrigeration (4 ± 2℃) from the plant to the laboratory within 30 min. Packages (VP and MAP packs) were subsequently removed from the cool boxes, and microbiological examinations were carried out after 0, 6, 12, and 24 h of storage.

Sample preparation

A total of 10 top round and striploin samples (quality grade: 1) from Hanwoo (Korean cattle) steers at 2 days post-slaughter were obtained from a local meat processing plant and chilled at 4 ± 2℃ in a cold room. The samples were collected in accordance with the MFDS Food Code of Practice for microbiological food sampling [14]. For the swab sampling, 25 mL of chilled Butterfield's Phosphate Buffer (BioMérieux, Baulkham Hills, NSW) was added to a sterile polyurethane sponge (Nasco Whirlpak; Nasco, Fort Atkinson, WI) in a sterile bag, and the sponge was allowed to fully hydrate. Excess buffer solution was squeezed from the sponge, and two designated sites (top round and striploin) were swabbed using 10 horizontal and 10 vertical passes. The sampled sites for the surface swabs were a 100-cm^2 surface area of meat samples, and samples were obtained on days 5, 7, 10, and 15. The sponge was then returned to the bag containing the diluent and squeezed to release the organisms. All swab samples were kept in an icebox (4 ± 2℃) and quickly transported to the laboratory for microbiological

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analysis. Each sponge was squeezed through the plastic bag, and serial dilutions of the swab fluid were prepared in 0.1% peptone water (Oxoid Ltd., USA). For excision sampling, a 25-cm^2 area was excised using a sterilized knife. Excised tissues were placed in a sterile plastic bag for transport to the laboratory. At each plant, samples of minced meat were obtained for analysis. Approximately 50 g of minced meat was placed in a sterile bag using a sterile spoon for transport to the laboratory. Samples were stored on ice and transported to the laboratory for testing, usually within 4 h, but never more than 24 h after sampling. Immediately upon arrival at the laboratory, samples were analyzed, on the day of collection. Analyses started within 2 h after arrival at the laboratory.

Microbiological analysis

To compare the excision and swab sampling methods, each sample homogenate (excision or swab) was placed in a stomacher bag with 10 mL of 0.1% peptone water, and pummeled with the diluent in a stomacher (STOMACHER® 400 CIRCULATOR; Seward, Ltd., UK) at low speed for 3 min. To determine the microbial changes in boxed beef under VP and MAP during transport and distribution, 25 g of packaged meats from each cool box was weighed into sterile stomacher bags and then homogenized with 225 mL of buffered peptone water using a stomacher (STOMACHER® 400 CIRCULATO) for 3 min at room temperature. Total aerobic plate counts (TACs) were analyzed according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant, and Fisheries Quarantine and Inspection Agency Notification [15]. Homogenized microbial extracts were serially diluted 10-fold with distilled water. Portions of the samples (0.1 mL) were spread plated. TACs were enumerated on plate count agar (Difco Laboratories, MI, USA) and colonies were counted after incubation at 35 ± 1℃ for 48 h. Lactic acid bacteria (LAB) counts were determined by plating with overlay on BCP plate count agar (Difco Laboratories), and colonies were counted after incubation at 35 ± 1℃ for 72 h. *Pseudomonas* spp. were assessed by the spread technique on *Pseudomonas* Agar (Difco™ Laboratories) after incubation at 30 ± 1℃ for 48 h. All analyses were performed in duplicate, and the results were expressed as the logarithm of colony-forming units per gram or cm^2 of sample (Log CFU per g or cm^2).

Statistical analysis

All experimental data were analyzed by Analysis of Variance (ANOVA) using SPSS (2011) program [16]. The significance of differences among the means of different treatments at the same storage time was determined using Duncan's multiple range tests at $p < 0.05$.

Results and Discussion

Microbial changes in boxed beef under vacuum and modified atmosphere packaging (MAP) during transport and distribution

Changes in the microbial populations in boxed beef under VP and MAP during transport and distribution are indicated in Table 1. Microbial loads showed differences between packaging conditions during storage time ($p < 0.05$). The population of total aerobic bacteria, lactic acid bacteria, and *Pseudomonas* significantly increased during transport and distribution, regardless of cut or packaging

Table 1. Microbial changes in boxed beef under vacuum and modified atmosphere packaging during transport and distribution (n=10)

	Cut	Packaging methods	Storage time (h)			
			0	6	12	24
Total plate counts (Log CFU/g)	Top round	VP	5.42 ± 0.01^{dA}	5.48 ± 0.02^{cA}	5.59 ± 0.02^{bA}	6.03 ± 0.03^{aA}
		MAP	4.60 ± 0.02^{dB}	4.73 ± 0.05^{cB}	4.97 ± 0.01^{bB}	5.30 ± 0.01^{aB}
	Striploin	VP	5.24 ± 0.02^{dA}	5.42 ± 0.01^{cA}	5.60 ± 0.03^{bA}	5.91 ± 0.02^{aA}
		MAP	4.37 ± 0.01^{cB}	4.61 ± 0.04^{bB}	4.71 ± 0.06^{bB}	5.37 ± 0.01^{aB}
Lactic acid bacteria (Log CFU/g)	Top round	VP	4.77 ± 0.02^{dA}	5.00 ± 0.01^{cA}	5.39 ± 0.01^{bA}	5.69 ± 0.04^{aA}
		MAP	3.96 ± 0.01^{dB}	4.27 ± 0.04^{cB}	4.44 ± 0.01^{bB}	4.89 ± 0.03^{aB}
	Striploin	VP	4.55 ± 0.03^{dA}	5.20 ± 0.04^{cA}	5.46 ± 0.02^{bA}	5.59 ± 0.05^{aA}
		MAP	4.05 ± 0.01^{dB}	4.29 ± 0.03^{cB}	4.57 ± 0.02^{bB}	4.77 ± 0.03^{aB}
Pseudomonas (Log CFU/g)	Top round	VP	5.42 ± 0.03^{dA}	5.69 ± 0.01^{cA}	6.03 ± 0.02^{bA}	6.31 ± 0.02^{aA}
		MAP	4.44 ± 0.06^{dB}	4.84 ± 0.01^{cB}	5.07 ± 0.02^{bB}	5.37 ± 0.01^{aB}
	Striploin	VP	5.79 ± 0.01^{cA}	5.79 ± 0.02^{cA}	5.84 ± 0.01^{bA}	6.15 ± 0.01^{aA}
		MAP	4.52 ± 0.03^{dB}	4.73 ± 0.03^{cB}	4.91 ± 0.01^{bB}	5.46 ± 0.01^{aB}

$^{a-d}$Means ± SD in the same row with different superscripts differ significantly ($p < 0.05$).

^{A,B}Means ± SD in the same column with different superscripts differ significantly ($p < 0.05$).

VP, vacuum packaging; MAP, modified atmosphere packaging (80% N_2, 20% CO_2).

types ($p < 0.05$). The initial microbial counts at 0 h were 5.42 ± 0.01 Log CFU/g for total aerobic bacteria, 4.77 ± 0.02 Log CFU/g for lactic acid bacteria, and 5.42 ± 0.03 Log CFU/g for *Pseudomonas* for top round samples in VP. At the end of storage (24 h), the microbial counts of total aerobic bacteria reached 6.03 ± 0.03 Log CFU/g in vacuum-packaged samples of top round, and 5.30 ± 0.01 Log CFU/g in MAP samples of top round. A similar pattern was observed for lactic acid bacteria, and the counts of total aerobic bacteria and lactic acid bacteria closely paralleled, although the lactic acid bacteria counts were slightly lower than the total aerobic bacteria counts (Table 1). Lactic acid bacteria (LAB) constitute a substantial part of the natural microflora of MAP meats, and LAB are able to grow under high concentrations of CO_2 [17]. The initial *Pseudomonas* spp. counts (0 h) in vacuum-packaged top round and striploin ranged from 5.42 to 5.79 Log_{10} CFU/g. The numbers of *Pseudomonas* increased until the end of storage (24 h), reaching 6.31 and 6.15 Log CFU/g, respectively, in vacuum packaged top round and striploin.

At all storage time points, MAP samples showed significantly lower total aerobic bacteria, lactic acid bacteria, and *Pseudomonas* counts than vacuum-packaged samples ($p < 0.05$). This result is in agreement with the result of Chung et al. who reported that the total bacteria counts of MAP samples were lower than those of vacuum-packaged samples [18]. Gill stated that 50% inhibition of psychrotrophic microorganism growth could be achieved in systems with atmospheres containing 20% CO_2 [19]. Kennedy et al. found that, for red meat packaged under MAP, the overall effect of CO_2 on microorganisms is an extension of the lag phase of growth and a decreased growth rate [20]. It has also been reported that *Pseudomonas* is the dominant genus on meat stored aerobically, and that storage under MAP suppressed *Pseudomonas* counts [21]. *Pseudomonas* growth was correlated with the O_2 concentration in the packs, and growth was delayed when the CO_2 concentration was increased [8].

A bacterial count of 7 Log CFU/g is the approximate point at which meat is considered to be spoiled or unacceptable [22]. In the present study, total bacteria, lactic acid bacteria, and *Pseudomonas* counts increased during transport and distribution ($p < 0.05$) (Table 1), but did not reach 7 Log CFU/g. Therefore, the meat packaged under VP or MAP in the present study remained within the acceptable limits established by the Korean MFDS during transport and distribution for 24 h. The maximum acceptable counts for packed meat, not matured, are less than 10^7 [14]. Therefore, the beef samples packaged both under MAP and VP stored in cool boxes during transport and distribution for 24 h would be safe.

The microbial changes in beef during cold storage and a comparison of swab and excision sampling

Changes in the numbers of microorganisms on chilled beef determined by swab and excision sampling during aging are presented in Table 2. During storage, the populations of total aerobic bacteria, lactic acid bacteria, and *Pseudomonas* on the aging in beef increased slowly, regardless of cut ($p < 0.05$). Total aerobic counts from swab samples of top round and striploin were 4.31 and 4.08 Log CFU/cm^2, respectively, after 5 days of storage. The population of total aerobic bacteria increased slowly during storage ($p < 0.05$); however, there were no significant differences in the top round samples. Total aerobic counts from swab samples of top round and striploin were 4.64 and 4.73 Log CFU/cm^2, respectively, after 15 days of storage. Therefore, all samples remained below the guidelines for the maximum limit of microbiological counts on meat (below 7 Log CFU/g) for 15 days [14]. Previous studies showed that total aerobic plate counts were ≤ 10^6 CFU/cm^2 from beef in retail shops [23]. Ko et al. reported that the total bacteria counts on meat samples in a butcher's shop, department store, and supermarket were 4.4×10^3 CFU/g, 3.9×10^5 CFU/g, and 1.0×10^4 CFU/g, respectively [24]. The lactic acid bacteria counts closely paralleled the total aerobic counts in this study (Table 2). Counts of lactic acid bacteria were slightly lower than those of total aerobic bacteria. Lactic acid bacteria counts from swab samples of top round and striploin were 4.30 and 4.37 Log CFU/cm^2, respectively, after 15 days of storage. The growth of *Pseudomonas* closely followed the sensory changes during storage; thus, a growth model for this group of bacteria could be used to predict the spoilage of aerobically stored meat [2]. As aging progressed, the *Pseudomonas* counts from swab samples of top round and striploin increased. The *Pseudomonas* counts were 3.88 and 4.17 Log CFU/cm^2 after 5 days of storage, respectively, and increased to 4.92 and 4.96 Log CFU/cm^2 after 15 days of storage, respectively. Total aerobic, lactic acid bacteria, and *Pseudomonas* counts during storage were similar to those reported by other authors [25].

As shown in Table 2, the numbers of total aerobic bacteria from swab samples were slightly lower than the numbers from excision samples ($p < 0.05$). This finding is in agreement with that of a previous study, which showed that the excision method recovers significantly higher numbers of bacteria from meat surfaces than swabbing [26]. On the contrary, sampling by swabbing or excision recovered similar numbers of bacteria, a finding comparable with that of Gill and Jones for samples obtained from beef and pork carcasses Sampling by swabbing is generally preferred to sampling by excision as it is non-destructive and easier to carry out under commercial conditions [26, 27]. Therefore, excision is the most effective carcass sampling method. In contrast, swabbing recovery is highly variable, ranging from 0.01% to 100% [27]. Nevertheless, swabbing is now a commonly used carcass sampling method in mandatory HACCP systems of red-meat abattoirs, according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant, and Fisheries Quarantine and

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Table 2. Microbial changes in beef detected by swab and excision sampling during storage at 4℃ (n=10)

	Cut	Methods	Storage period (d)			
			5	7	10	15
Total plate counts	Top round	Swabbing (Log CFU/cm^2)	4.31 ± 0.25^{B}	4.35 ± 0.10^{B}	4.50 ± 0.05^{B}	4.64 ± 0.04^{B}
		Excision (Log CFU/g)	4.98 ± 0.28^{A}	5.02 ± 0.21^{A}	5.13 ± 0.15^{A}	5.26 ± 0.18^{A}
	Striploin	Swabbing (Log CFU/cm^2)	4.08 ± 0.28^{bB}	4.62 ± 0.23^{aB}	4.63 ± 0.17^{aB}	4.73 ± 0.15^{aB}
		Excision (Log CFU/g)	4.71 ± 0.10^{bA}	5.24 ± 0.05^{aA}	5.38 ± 0.12^{aA}	5.49 ± 0.12^{aA}
Lactic acid bacteria	Top round	Swabbing (Log CFU/cm^2)	3.69 ± 0.21^{b}	4.02 ± 0.25^{a}	4.21 ± 0.14^{a}	4.30 ± 0.15^{a}
		Excision (Log CFU/g)	3.71 ± 0.22^{b}	4.08 ± 0.21^{a}	4.29 ± 0.11^{a}	4.37 ± 0.18^{a}
	Striploin	Swabbing (Log CFU/cm^2)	3.47 ± 0.45^{b}	4.23 ± 0.15^{a}	4.23 ± 0.13^{a}	4.37 ± 0.10^{a}
		Excision (Log CFU/g)	3.52 ± 0.39^{b}	4.31 ± 0.19^{a}	4.37 ± 0.21^{a}	4.42 ± 0.18^{a}
Pseudomonas	Top round	Swabbing (Log CFU/cm^2)	3.88 ± 0.10^{c}	4.44 ± 0.32^{b}	4.85 ± 0.22^{a}	4.92 ± 0.20^{a}
		Excision (Log CFU/g)	3.91 ± 0.12^{c}	4.49 ± 0.31^{b}	4.91 ± 0.21^{a}	4.99 ± 0.12^{a}
	Striploin	Swabbing (Log CFU/cm^2)	4.17 ± 0.47^{b}	4.66 ± 0.36^{a}	4.89 ± 0.20^{a}	4.96 ± 0.19^{a}
		Excision (Log CFU/g)	4.21 ± 0.39^{b}	4.72 ± 0.31^{a}	4.91 ± 0.28^{a}	4.99 ± 0.23^{a}

$^{a-c}$Means ± SD in the same row with different superscripts differ significantly ($p < 0.05$).

^{A,B}Means ± SD in the same column with different superscripts differ significantly ($p < 0.05$).

Inspection Agency Notification [15]. Several studies related to carcass surface microbiology have used non-destructive methods [28], and swabbing methods are commonly used in practice without previous assessment of the relationship between results obtained by excision and swabbing [13].

Conclusions

It could be concluded that MAP was more effective than VP for the inhibition of microbial growth. In addition, microbial loads of excision methods had higher than those of swab ones. The results of this study provide useful information for microbial risk assessment of boxed meat products that are sold in retail stores. Further study should be required for microbiological assessment of meat cut at all operational stages such as the slaughterhouse, processing line.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Yim DG.
Methodology: Yim DG.
Investigation: Jin SG.
Writing - original draft: Yim DG.
Writing - review & editing: Hur SJ.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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