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ARTICLE

Contamination Level of Hygiene Indicator and Prevalence of Foodborne Pathogens in Retail Beef in Parallel with Market Factor

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Abstract In this study, the contamination levels of hygienic indicators and foodborne pathogens in retail meat products were investigated in relation to the various market factors including processing temperature, processing area, and market type. Ground beef samples (n=80) were purchased from 40 meat markets and investigated for microbiological quality. Beefs processed below 20°C had significantly lower numbers of total coliforms (TC) than these processed over 20°C (2.01 vs. 2.79 log CFU/g; $p<0.05$). Interestingly, separation of processing area did not affect the contamination levels. Remarkably, the contamination levels of hygienic indicator differ among market types, indicating that not only processing condition but distribution structure that is directly related with storage period could affect the final microbiological loads of the meat products. In addition, the prevalences of *Listeria monocytogenes* (a psychrotroph), *Enterococcus faecium*, and *Enterococcus faecalis* were 7.5% (6/80), 10.0% (8/80), and 20.0% (16/80), respectively, which is irrelevant to market factors except meat products from wholesale markets where no *L. monocytogenes* were found among 30 samples. The results of this study indicate that the contamination level of hygiene indicator and foodborne pathogens in retail beef is more related with processing temperature and storage period than other environmental factors.

Keywords meat market, HACCP (Hazard Analysis Critical Control Point), processing condition, hygienic indicator bacteria, foodborne pathogens

Introduction

Meat and meat products offer a highly favourable environment for the growth of pathogenic microorganism (Barros et al., 2007). For this reason, retail meat is frequently associated with foodborne illness if infective doses are reached at the time of consumption (Da silva et al., 2016; Perez-Rodriguez et al., 2010). Epidemiological and microbiological studies have focused on cross-contamination during distribution and processing and subsequent bacterial growth as one of the main causes of foodborne

illness (Bolcan et al., 2015; Park et al., 2002; Perez-Rodriguez et al., 2010).

During processing such as cutting and handling, retail meat is exposed to microbial contamination via contact with worker, utensil, and other processing environments (Marinho et al., 2013; Perez-Rodriguez et al., 2010). To ensure the microbiological safety of meat products, meat markets must monitor their processing conditions according to official standards or management systems (KAPE, 2013; KOLPHAS, 2015). For example, HACCP (Hazard Analysis Critical Control Point) is one of the internationally recommended process management systems for establishing safety control points and methods (Lee et al., 2012; Tomasevic et al., 2016). HACCP guidelines for meat processing are fundamentally focused on critical control points to maintain low temperature in meat processing areas, separated processing rooms, and washing utensils between processing procedures (Cintra et al., 2016; EC, 2004; KOLPHAS, 2015).

Market types could also affect the microbiological contamination levels of retail meat (Jeong et al. 2017; Ko et al., 2013). The distribution steps vary among market types, and can influence in contamination levels of incoming meat (Park et al., 2002). In addition, workers of large-sized markets typically process more meat than those at small and medium-sized markets (KAPE, 2013; Ko et al., 2013). Thus, large-sized markets have an increased opportunity for cross-contamination during processing (Ko et al., 2013; Park et al., 2002). For example, a previous study showed that meats handled in department store are associated with lower microbiological quality than other market types (Ko et al., 2013).

Although it has been generally postulated that these factors are closely related to microbial contamination levels of meats, the specific effects of each factor on the microbiological quality of meat products have never been addressed. In this study, beef samples obtained from various types of meat markets were investigated for the level of hygienic indicators (mesophilic aerobe [MA] and total coliform [TC]) and the prevalence of *Listeria monocytogenes*, *Enterococcus faecalis,* and *Enterococcus faecium*. These data were analysed in parallel with market factors including market type, separation of processing area, processing temperature, washing utensils.

Materials and Methods

Sample collection

In total, 80 ground beef samples (300 g each) were purchased from 15 single markets (small retail shops selling meat products with a complex distribution structure), 10 department stores (large stores selling various foods including meat products with a complex distribution structure), and 15 wholesale markets (group of wholesale establishments selling meat products directly from the manufacturers) in Seoul, Korea between July and September 2015. Ground beef samples were individually wrapped, stored in an ice chest, and transported to the laboratory within 3 h for immediate processing.

Investigation of market factors

Each meat market was profiled according to meat processing area (SP+, processing room separated from the outside; SP−, sale area exposed to the outside) and meat processing temperature (TEM+, below 20°C; TEM−, over 20°C), and whether the markets washed their knives before meat processing (WASH+, washing; WASH−, no washing). Overall experimental design is depicted in Fig. 1.

Enumeration of indicator microorganisms

For detection of indicator organisms, 25 g of each 300-g ground beef sample was diluted 10-fold in 225 mL of Butterfield's

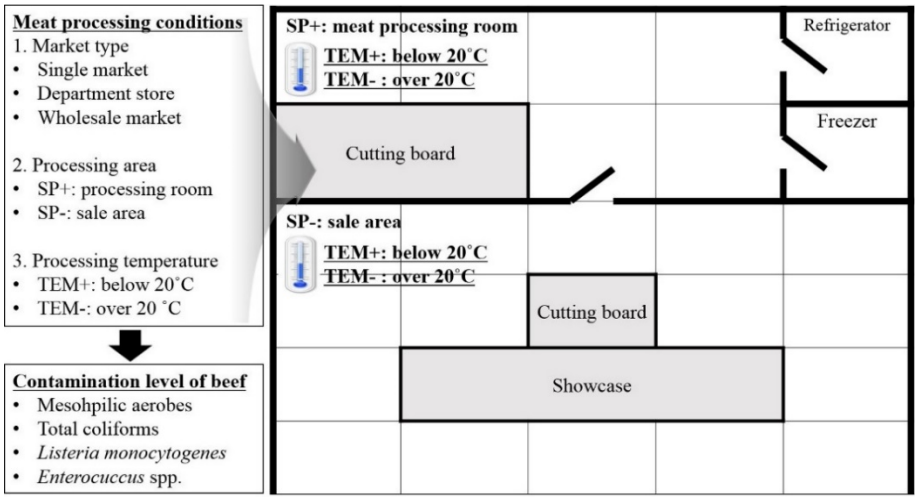

Fig. 1. Overall experimental design in this study.

phosphate-buffered water (Difco, Sparks, MD, USA) and plated on Petrifilm Aerobic Count (3M, St. Paul, MN, USA) and *Escherichia coli*/coliform Petrifilm medium for enumeration of MA and TC, respectively. All films were incubated at 35℃ for 24 h to 48 h, and the resulting colonies were counted according to the phenotypic characteristics of each group (MA, formation of red colonies after 48 h; TC, formation of red colonies associated with gas production after 24 h).

Detection of *L. monocytogenes* and *Enterococcus* spp.

For isolation of *L. monocytogenes*, samples (25 g) were diluted 1:9 (w/v) in University of Vermont Modified *Listeria* Broth (UVM; Difco), homogenised in a laboratory stomacher for 1 minute, and incubated at 30℃ for 48 h. The enriched culture (100 μL) was then transferred into Fraser Broth (Difco) and incubated at 37℃ for 48 h for selective enrichment, and a loopful of the resulting enrichment culture was streaked onto Oxford Agar (Oxoid, Ltd.) and incubated at 35℃ for 48 h.

For isolation of *E. faecalis* and *E. faecium*, samples (25 g) were diluted 1:9 (w/v) in sterile Azide-Dextrose Broth (Merck, Darmstadt, Germany), homogenised in a laboratory stomacher for 1 minute, and incubated at 37℃ for 24 h. The enriched culture (1 mL) was then transferred into 9 mL of Bromocresol Purple Azide Broth (Oxoid Ltd) and incubated at 37℃ for 48 h for selective enrichment; a loopful of the resulting culture was streaked onto Enterococcosel Agar (Oxoid, Ltd.) and incubated at 37℃ for 24 h (Sung et al., 2013). All presumptive *L. monocytogenes* and *Enterococcus* colonies were subsequently confirmed using a Vitek-2 Compact Microbial Identification System (bioMérieux).

Data analysis

Associations between market type and SP+, TEM+, and WASH+ were determined using Fisher's exact test in pairs with GraphPad Instat software (GraphPad Software, Inc., San Diego, CA, USA). The levels of MA and TC contamination were compared between beef samples using student's *t*-test and one-way analysis of variance (ANOVA; Duncan method). The prevalence of *L. monocytogenes*, *Enterococcus* spp. were compared between beef samples using Fisher's exact test in pairs with GraphPad Instat software (GraphPad Software, Inc.). All statistical analyses were conducted using SPSS version 19.0 software (SPSS Statistics, Inc., Chicago, IL, USA); $p<0.05$ was considered statistically significant.

Result and Discussion

Investigation of various market factors of meat markets

Of the 40 meat markets evaluated in this study, 40% (16/40), and 55% (22/40) were SP+, and TEM+, respectively (Table 1), with these indices being most prevalent ($p<0.05$) in department stores [SP+, 90.0% (9/10); TEM+, 100.0% (10/10); WASH+, 0% (0/10)], followed by wholesale markets [SP+, 33.3% (5/15); TEM+, 46.7% (7/15); WASH+, 0% (0/15)], and single markets [SP+, 13.3% (2/15); TEM+, 33.3% (5/15); WASH+, 0% (0/15)] (Table 1). Notably, however, none of the sampled markets was observed to wash knives before meat processing [WASH+, 0% (0/40)].

Retail meat market is one of the major potential distributors of foodborne illness (Barros et al., 2007; Jeong et al. 2017). To date, many researches have focused on the contamination level of retail meat products remaining the market factors of the origin market uninvestigated (Ko et al., 2013; Park et al., 2002; Samadpour et al., 2006). To the best of our knowledge, this is the first study investigating the on-site market factors of retail meat markets.

Table 1. Market factors of 40 retail meat markets in Seoul Korea, examined in this study

Meat market[1)]		Sampling date	Market factors[2)]	
			Processing area	Processing temperature
Single market	A	2015.07.21	SP–	TEM–
	B	2015.07.21	SP–	TEM–
	C	2015.07.21	SP–	TEM–
	D	2015.07.21	SP–	TEM+
	E	2015.07.21	SP–	TEM–
	F	2015.08.26	SP–	TEM+
	G	2015.08.26	SP–	TEM–
	H	2015.08.26	SP+	TEM+
	I	2015.08.26	SP–	TEM–
	J	2015.08.26	SP–	TEM+
	K	2015.08.26	SP–	TEM–
	L	2015.08.26	SP–	TEM–
	M	2015.08.26	SP–	TEM–
	N	2015.09.15	SP+	TEM+
	O	2015.09.15	SP–	TEM–
Department store	A	2015.07.21	SP+	TEM+
	B	2015.07.21	SP+	TEM+
	C	2015.07.21	SP+	TEM+
	D	2015.07.21	SP+	TEM+
	E	2015.07.21	SP+	TEM+
	F	2015.08.26	SP–	TEM+
	G	2015.08.26	SP+	TEM+
	H	2015.08.26	SP+	TEM+
	I	2015.08.26	SP+	TEM+
	J	2015.09.15	SP+	TEM+

Table 1. Market factors of 40 retail meat markets in Seoul Korea, examined in this study (continued)

Meat market[1]		Sampling date	Market factors[2]	
			Processing area	Processing temperature
Wholesale market	A	2015.08.18	SP+	TEM+
	B	2015.08.18	SP+	TEM+
	C	2015.08.18	SP+	TEM+
	D	2015.08.18	SP–	TEM+
	E	2015.08.18	SP–	TEM–
	F	2015.08.18	SP–	TEM–
	G	2015.08.18	SP–	TEM–
	H	2015.08.18	SP–	TEM–
	I	2015.09.15	SP–	TEM–
	J	2015.09.15	SP–	TEM–
	K	2015.09.15	SP+	TEM+
	L	2015.09.15	SP–	TEM–
	M	2015.09.15	SP–	TEM+
	N	2015.09.15	SP–	TEM–
	O	2015.09.15	SP+	TEM+

[1] Single market, small retail shops selling meat products with a complex distribution structure; Department store, large stores selling various foods including meat products with a complex distribution structure; Wholesale market, group of wholesale establishments selling meat products directly from the manufacturers.
[2] SP+, meat processing room separated from the outside; SP–, meat sale area exposed to the outside; TEM+, processing temperature maintained below 20°C; TEM–, processing temperature maintained below over 20°C.

In present study, department stores showed the highest facility level of processing area among the three market types, indicating market type influence not only distribution steps and market scale, but their facility level of processing area. However, no markets washed utensils between meat processing. It is widely known that poor utensil-washing practices can lead to increased levels of cross-contamination and to increased bacterial biofilm formation and proliferation (Goulter et al., 2008; Perez-Rodriguez et al., 2010; Tomasevic et al., 2016). According to the official criteria of many countries, including Korea, the knives used during the slaughter and dressing of carcasses must be sanitised by brief submersion in 82°C water or via equivalent science-based procedures (EC, 2004; Goulter et al., 2008). However, specifications for the type of sanitizer used and the submersion temperature/time for knife sanitisation in meat markets are limited (KOLPHAS, 2015). Future studies are therefore necessary to develop effective methods for washing knives to reduce cross-contamination during meat processing.

Contamination levels of hygienic indicator bacteria in beefs

The contamination levels of MA and TC in beefs are shown in Table 2. Beefs purchased from TEM+ markets exhibited lower levels of contamination in ground beef samples than these from TEM− markets (Table 2). Notably, TEM+ markets exhibited significantly lower levels of TC than TEM− markets ($p<0.05$). Conversely, there were no significant differences between SP+ and SP− markets in the contamination levels of MA and TC in beefs (Table 2). For market types, beefs purchased from wholesale markets showed significantly lower levels of MA and TC contamination than these from department stores and single markets ($p<0.05$, Table 2).

Table 2. Comparison of mesophilic aerobe and total coliform contamination levels by market factors

Market factors[1)]		Number of indicator bacteria (Mean±SD, log CFU/g)[2)]	
		Mesophilic aerobe	Total coliform
Processing temperature	TEM+	5.07±0.67 ^{A}	2.09±1.31 ^{A}
	TEM−	5.23±1.10 ^{A}	2.79±1.71 ^{B}
Processing area	SP+	5.10±0.77 ^{A}	2.15±1.43 ^{A}
	SP−	5.17±0.96 ^{A}	2.58±1.59 ^{A}
Market type	Single market	5.48±1.08 ^{A}	3.47±1.39 ^{A}
	Department store	5.30±0.62 ^{A}	2.73±0.74 ^{B}
	Wholesale market	4.69±0.60 ^{B}	1.13±1.10 ^{C}
Total		5.14±0.89	2.41±1.53

[1)] SP+, meat processing room separated from the outside; SP–, meat sale area exposed to the outside; TEM+, processing temperature maintained below 20°C; TEM–, processing temperature maintained below over 20°C; Single market, small retail shops selling meat products with a complex distribution structure; Department store, large stores selling various foods including meat products with a complex distribution structure; Wholesale market, group of wholesale establishments selling meat products directly from the slaughterhouse.
[2)] Different letters within a column indicate significant differences ($p<0.05$, Student's *t*-test or ANOVA; Duncan method).

The level of indicator microorganisms such as MA and TC in meat products provide an estimate of the overall population of microorganisms present in meat, as well as in the environment and on the items used for processing of meat products (Costa Sobrinho et al., 2012; Da Silva et al., 2016). In particular, TC has been used as an indicator of fecal contamination and as a suitable marker for noncompliance of cold-chain guideline in meat industry (Barros et al., 2007; Nieri et al., 2014). Cold-chain continuity is a mainstream method used to limit microbial multiplication in meat products, as low temperature can reduce microbial, chemical, and enzymatic activities that can alter overall food quality (Cintra et al., 2016; Nieri et al., 2014; Tomasevic et al., 2016). Although the meat processing steps last only a few minutes, continuous temperature control of the processing area can prevent microbial multiplication within the environment and on the processing utensils (Cintra et al., 2016). Consistently, our data showed that low processing temperature is one of the most effective factors to limit microbial multiplication, remaining the effect of separation of processing area and washing knives unknown.

For market types, notwithstanding their highest ratio of SP+, and TEM+, meat markets in department stores showed higher levels of MA and TC contamination than wholesale markets. This result is consistent with previous finding that department store is a large store processing more meat than other market types, and can provide an increased chance for cross-contamination in poor hygienic conditions such as WASH− markets in this study (Ko et al., 2013). Meanwhile, wholesale markets had the shortest distribution process among the three market types tested because meat was directly supplied from individual farms without complex distribution steps (KAPE et al., 2013). Multiple distribution and/or handling steps were previously shown to result in gradual increases in contamination (Park et al., 2002). Thus, this simplified distribution network might partially explain the low levels of MA and TC contamination in the beef from wholesale markets, compared with those observed at department stores and single markets.

Prevalence of *L. monocytogenes*, *E. faecium*, and *E. faecalis* in beefs

In total, 6, 8, and 16 strains of *L. monocytogenes*, *E. faecium*, and *E. faecalis* were isolated from the 80 ground beef samples tested, respectively. Notably, there was no significant association between market factors (including market type, separation of processing area, processing temperature, washing utensils) and the prevalence of these three microorganisms (Table 3). However, TEM+ markets were associated a significantly higher prevalence of *L. monocytogenes* than TEM−

Table 3. Prevalence of *Listeria monocytogenes, Enterococcus faecium*, and *Enterococcus faecalis*. in the 40 meat markets (80 ground beef samples) evaluated in this study

Market factors[1)]		Number of positive samples/number of total samples (%)[2)]		
		L. monocytogenes	*E. faecium*	*E. faecalis*
Processing temperature	TEM+	6/44 (13.6)^{A}	6/44 (13.6)^{A}	10/44 (22.7)^{A}
	TEM−	0/36 (0.0)^{B}	2/36 (5.6)^{A}	6/36 (16.7)^{A}
Processing area	SP+	4/32 (12.5)^{A}	6/32 (18.8)^{A}	4/32 (12.5)^{A}
	SP−	2/48 (4.2)^{A}	2/48 (4.2)^{A}	12/48 (25.0)^{A}
Market type	Single market	3/30 (10.0)^{A}	3/30 (10.0)^{A}	7/30 (23.3)^{A}
	Department store	3/20 (15.0)^{A}	2/20 (10.0)^{A}	3/20 (15.0)^{A}
	Wholesale market	0/30 (0.0)^{A}	3/30 (10.0)^{A}	6/30 (20.0)^{A}
Washing knives	WASH+	-	-	-
	WASH-	6/80 (7.5)	8/80 (10.0)	16/80 (20.0)
Total (40)		6/80 (7.5)	8/80 (10.0)	16/80 (20.0)

[1)] SP+, meat processing room separated from the outside; SP–, meat sale area exposed to the outside; TEM+, processing temperature maintained below 20°C; TEM–, processing temperature maintained below over 20°C; WASH+, washing of knives before meat processing; WASH–, no washing of knives before meat processing; Single market, small retail shops selling meat products with a complex distribution structure; Department store, large stores selling various foods including meat products with a complex distribution structure; Wholesale market, group of wholesale establishments selling meat products directly from the manufacturers.
[2)] Different letters within a column indicate significant differences ($p<0.05$, Fisher's exact).

market, respectively ($p<0.05$, Table 2). Wholesale market showed the lowest prevalence of *L. monocytogenes* among the three market types.

We targeted *L. monocytogenes*, *E. faecium*, and *E. faecalis* in beef samples. There has been an increasing interest in these bacteria as *L. monocytogenes* is one of the most detrimental foodborne pathogens which has a zero tolerance policy in most countries, and *Enterococcus* spp. could act as a potent vector of antibiotic resistance genes (Chajęcka-Wierzchowska et al., 2016; Kim et al., 2014). In present study, All *L. monocytogenes* were isolated from single market and department stores. Meanwhile, wholesale markets showed the zero prevalence of *L. monocytogenes*, indicating simple distribution steps influence the prevalence of *L. monocytogenes* in incoming meat. Considering the lower level of indicator bacteria in TEM+ markets, the high prevalence of *L. monocytogenes* in TEM+ markets seem to indicate not that low temperature increased the prevalence of *L. monocytogenes*, but that the contamination level of original meat is more important factors than processing temperature control.

L. monocytogenes is capable of surviving and multiplying at refrigeration temperatures, tolerating harsh environmental conditions (Da Silva et al., 2016; Samelis and Metaxopoulos 1999), and maintaining nearly constant adhesion and biofilm formation at 4°C, 10°C, and 20°C (Bolocan et al., 2015). Indeed, *L. monocytogenes* can remain in meat and processing environment for months or even years due to its ability to form biofilms, leading to possible contamination of final products (Bolocan et al., 2015; Da Silva et al., 2016). Similarly, *Enterococcus* spp. can survive adverse environmental conditions such as extreme temperatures (10°C–45°C), pH values (4.5–10.0), and salinity (Marinho et al., 2013). The ability of *Enterococcus* spp. to grow at low temperatures can result in meat product decay during transport or storage (Chajęcka-Wierzchowska et al., 2016). These factors are also another reason why the low processing temperature (TEM+) was insufficient to effectively reduce the prevalence of *L. monocytogenes*, *E. faecium*, and *E. faecalis*, despite being associated with decreased contamination by hygiene indicator bacteria. Therefore, to reduce the prevalence of these microorganisms, HACCP models for meat markets should focus on not only low temperature maintenance, but stringent implantation of basic hygienic practices (i.e. utensil washing) in place of separated processing room.

Our findings indicate that temperature regulation during meat processing is a more important factor than separation of the processing area on the levels of indicator microorganisms in meat products. In addition to processing conditions, simple distribution structure is also identified as a key factor for reducing microbiological loads of meat products. Therefore, meat markets should focus on low temperature maintenance and the prevention of cross-contamination and proliferation during distribution.

Conflicts of Interest

The authors declare no potential conflict of interest.

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