

## Identification of Microorganisms in Duck Meat Products Available in Korea and the Effect of High Hydrostatic Pressure

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### Abstract

The objective of this study was to investigate the microbial count of duck meat and duck meat products commercially available in Korea. High hydrostatic pressure (HHP) treatment was applied at 0.1, 300, 400, and 500 MPa for 5 min to enhance the microbiological safety of duck meats. The levels of total aerobic bacteria were in the ranges of 3.53-6.19 and 3.62-6.85 Log CFU/g in raw and smoked duck products, respectively. By DNA sequence analysis, we identified microorganisms responsible for spoilage, with the most common species in the raw and smoked duck products being *Aeromonas* spp. or *Pseudomonas* spp. and *Leuconostoc mesenteroides*, respectively. HHP treatment significantly reduced the levels of total aerobic bacteria in raw and smoked duck products. This study demonstrates that HHP treatment may be used to effectively improve the safety of raw and smoked duck meat products.

**Keywords:** duck meat products, microbial quality, identification, high hydrostatic pressure

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### Introduction

Duck meat can be a good source of protein for humans (Adzitey *et al.*, 2012a) and is high in iron, selenium, and niacin, as well as containing fewer calories than many cuts of beef (Adzitey *et al.*, 2012b). Duck meat and duck meat products are relished and consumed by many people worldwide. The consumption of white meats, including duck meat, is gaining more attention owing to recommendations for a reduced intake of red meat due to its association with cardiovascular pathologies (Adzitey *et al.*, 2012b; Witak, 2008). The consumption of duck meat and duck meat products increased approximately 5-folds in Korea from 1997 to 2012 (Korea Duck Association, 2013).

The safety of food products has become a major issue

of concern (Kim *et al.*, 2014). The consumption of contaminated duck meat or products, like other types of meat, poses the risk of foodborne diseases; however, it has received little attention in terms of epidemiological studies. For instance, the consumption of duck meat and duck meat products has been associated with outbreaks of salmonellosis (Adzitey *et al.*, 2012b). Contact with young birds, including ducklings in a nursery school, has also been linked to outbreaks of *Salmonella* infections (Merritt and Herlihy, 2003). The Korea Centers for Disease Control and Prevention announced that the prevalence of *Salmonella* spp. in food, especially in poultry meat, is high in the South Korea (Bae *et al.*, 2013). In addition, incidences of *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, and enterococcus contamination in duck meat have been reported (Adzitey *et al.*, 2013; Hu *et al.*, 2011; Jamali *et al.*, 2015; Sánchez *et al.*, 2007).

High hydrostatic pressure (HHP) processing is a non-destructive and chemical-free food preservation technology that efficiently eliminates food spoilage microorganisms. In particular, HHP has good potential for application in

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the meat industry (Garriga *et al.*, 2004). The ability of HHP to eradicate microorganisms regardless of product geometry and without preservatives/additives (Zhang and Mittal, 2008) make this technology safe and consumer-friendly (Kruk *et al.*, 2014). In the meat sector, its application is increasing continuously, particularly for enhancing the shelf life and safety of raw and sliced cooked products (Khan *et al.*, 2014).

Therefore, the current study was designed with the objectives of evaluating microbial contamination and identifying microorganisms in raw and smoked duck products that are commercially available in the Korea, and for determining the efficiency of HHP in microbial reduction in raw and smoked duck meat products.

## Materials and Methods

### Sample preparation

Refrigerated raw duck meat (sliced and bone-in whole type), frozen raw duck meat (sliced, deboned, and bone-in whole type), and smoked duck meat products (sliced and bone-in whole type) were purchased from local markets (Seoul, Korea). Each product was labeled randomly by alphabet letters. The samples (approximately 5 g) were transferred into a sterilized oxygen-impermeable nylon bags (2 mL O<sub>2</sub>/m<sup>2</sup>/24 h at 0°C, 0.09 mm thickness; Sunk-yung Co. Ltd., Korea) with a sterilized knife and pincette on a clean bench. The packs were sealed and transferred to a refrigerated storage (4°C) before analysis. The frozen samples were thawed in a refrigerator at 4°C for 24 h before use.

### Microbial analysis

Each sample was cut into small pieces (approximately 0.5 × 0.5 cm) and homogenized for 2 min in a sterile Stomacher bag containing 45 mL of sterile saline solution using the Stomacher BagMixer<sup>®</sup> 400 (Interscience Co., France). Then samples were serially diluted in sterile saline (0.85%) solution, and each diluents (0.1 mL) was spread on plate count agar (Difco Laboratories, USA). Plates were incubated at 37°C for 48 h, and microbial counts were expressed as colony forming units per gram (CFU/g).

### Identification of microorganisms

Each strain was identified by using the 16S rDNA sequencing method. Briefly, each single colony from the purified isolates on the plate count agar plates were trans-

ferred to 10 mL tryptone soy broth (Difco Laboratories), and the cells were grown overnight at 37°C. The chromosomal DNA of isolated strain was separated by using the SolGent Genomic DNA prep kit (SolGent, Korea). The DNA extracts were used for the polymerase chain reaction (PCR) with the universal primers [27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3')] (Marchesi *et al.* 1998). PCR was carried out in a programmable therma cycler (SolGent EF-Taq, Korea), according to the following steps: one cycle of denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20 s, 50°C for 40 s, and 72°C for 90 s. The final extension was carried out at 72°C for 5 min. The purified PCR product obtained by using a SolGent PCR purification kit (SolGent, Korea) was used for sequencing by Basic Local Alignment Search Tool (BLAST) search of the National Center for Biotechnology Information (NCBI) (Maidak *et al.*, 2001).

### High hydrostatic pressure treatment

The vacuum-packed samples (-650 mmHg in 10 × 10 cm low-density polyethylene/nylon vacuum bags with oxygen permeability of 22.5 mL/m<sup>2</sup>/24h atm at 60% RH/25°C and water vapor permeability of 4.7 g/m<sup>2</sup>/24h at 100% RH/25°C) were transported to the Korea Food Research Institute (Korea) in a cooled container and were immediately subjected to HHP. Samples were placed in a pressure vessel submerged in hydrostatic fluid (Quintus food processor 6; ABB Autoclave Systems, Inc., USA) and pressurized at 300, 400 and 500 MPa for 5 min with the initial temperature of the pressure vessel set at 15 ± 3°C. The rate of pressurisation was 5-7 MPa/s and the pressure in the chamber was released within 10 s. Control samples were maintained under atmospheric pressure at 4°C while the other samples were treated. Immediately after treatment, all samples were transported on ice to the Laboratory, Seoul, Korea.

### Statistical analysis

All experimental procedures were conducted in triplicate with 3 observations. In the results of microbial contamination, mean values and the standard deviation were calculated using a SAS Software and reported. The result of HHP treatment was performed using a one-way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by performing the Duncan's multiple comparison test at a confidence level of  $p < 0.05$ .

## Results and Discussion

### Microbial quality of raw duck and smoked duck products

To evaluate microbiological contamination level of commercial duck meat products available in Korean market, total aerobic bacteria numbers were monitored. The total aerobic bacterial populations in commercial raw duck meats ranged from 3.53 to 6.19 Log CFU/g (Table 1). For refrigerated samples, bone-in whole raw duck meat showed significantly lower total aerobic bacterial counts than those in sliced raw duck meats. Samples stored frozen did not consistently show differences by distribution method. Levels of aerobic bacteria in smoked duck products ranged from 3.62 to 6.85 Log CFU/g (Table 2). Samples did not consistently show differences by distribution method.

Several studies have been carried out to assess the contamination levels of duck meat and duck meat products in Korea. Sung *et al.* (2013) reported numbers of total aerobic bacteria of 4.30, 3.43, and 3.84 Log CFU/g for fresh, fresh-torched, and freeze-thawed types of duck breast meats, respectively. Chae *et al.* (2006) showed that the numbers of total aerobic bacteria and *E. coli* were 3.26 and 0.43 Log CFU/g, respectively. The initial microbial load of meat depends on the physiological status of the duck at slaughter, contamination at slaughterhouses, and contamination during processing. The temperature and storage conditions during distribution also influence the rate of spoilage (Nychas *et al.*, 2008). The different microbial groups that potentially contribute to meat spoilage depend on the storage conditions applied and their competition. Our results indicated that raw and smoked duck meat products were not safe enough for consumption. Therefore, an improvement in the safety of raw and smoked duck meat and duck meat products is needed.

### Identification of microorganisms in raw duck and smoked duck products

The microorganisms that can colonize fresh meat depend highly on the characteristics of the meat and the way that it is processed and stored (Huisin't Veld, 1996). Sequence analysis identified spoilage microorganisms, such as *Aeromonas* spp., *Burkholderia* spp., *Pseudomonas* spp., and *Leuconostoc mesenteroides* in raw and smoked duck meat products (Tables 3-5).

Previous studies examined the levels of foodborne pathogens, i.e., *Salmonella* spp. and *Campylobacter* spp., in duck meats. However, very few detected spoilage micro-

**Table 1. Microbial population (Log CFU/g) of the raw duck meats commercially available in Korea**

Type	Products	Total aerobic bacteria	
Refrigerated raw duck meats	Sliced	A <sup>1)</sup>	4.56±0.17 <sup>2)</sup>
		B	5.33±0.25
		C	5.75±0.12
	Bone-in whole	D	4.40±0.26
		E	3.68±0.10
		F	3.53±0.24
Frozen raw duck meat	Sliced	G	4.12±0.09
		H	6.19±0.05
	Deboned	I	5.39±0.03
		J	4.97±0.04

<sup>1)</sup>Alphabet letters were randomly labeled for different commercial products.

<sup>2)</sup>Mean±standard deviation (n=9).

**Table 2. Microbial population (Log CFU/g) of the smoked duck meat products commercially available in Korea**

Type	Products	Total aerobic bacteria	
Sliced	K <sup>1)</sup>	6.41±0.33 <sup>2)</sup>	
		L	6.85±0.01
		M	4.90±0.04
		N	6.50±0.34
		O	5.61±0.09
		P	4.55±0.18
Bone-in whole	Q	3.62±0.29	
	R	6.84±0.03	
	S	5.33±0.24	

<sup>1)</sup>Alphabet letters were randomly labeled for different commercial products.

<sup>2)</sup>Mean±standard deviation (n=9).

organisms in duck meats (Adzitey *et al.*, 2012b; Jamali *et al.*, 2015). Bacteria developing in meat at cool temperatures are regarded as psychrotrophic and include *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, *Clostridium*, lactic acid bacteria (such as *Leuconostoc mesenteroides*), *Aeromonas*, and different genera of the family *Enterobacteriaceae* (Doulgeraki *et al.*, 2012; Labadie, 1999). Despite the large number of microorganisms detected, few species dominate to cause spoilage because of the storage temperature, time, and packaging atmosphere during storage of fresh meat (Casaburi *et al.*, 2015). *Pseudomonas fragi* can occur in meat stored in vacuum packaging and modified atmosphere packaging (Ercolini *et al.*, 2011; Pennacchia *et al.*, 2011), producing volatile organic compounds, recognized as active odor molecules, which are possibly responsible for off-odor release during meat storage at cool temperatures (Casaburi *et al.*, 2015).

**Table 3. Identification of microorganisms in refrigerated raw duck meats using 16S rDNA sequencing**

Type	Products	Microorganisms
Sliced	A <sup>1)</sup>	<i>Acinetobacter</i> spp., <i>Burkholderia</i> spp., <i>Enterobacter</i> spp., <i>Kocuria rhizophila</i>
	B	<i>Aeromonas</i> spp., <i>Raoultella terrigena</i> , <i>Pantoea ananatis</i> , <i>Streptococcus parauberis</i>
	C	<i>Enterobacter</i> spp., <i>Pseudomonas</i> spp., <i>Serrita</i> spp., <i>Serrita liquefaciens</i>
Bone-in whole	D	<i>Chryseobacterium</i> spp., <i>Chryseobacterium piscium</i> , <i>Kocuria rhizophila</i> , <i>Soonwooa buanensis</i> , <i>Staphylococcus pseudintermedius</i> , <i>Streptococcus parauberis</i>
	E	<i>Acinetobacter</i> spp., <i>Aeromonas</i> spp., <i>Burkholderia</i> spp., <i>Chryseobacterium indologenes</i> , <i>Soonwooa buanensis</i>
	F	<i>Dysgonomonas</i> spp., <i>Flavobacterium indicum</i> , <i>Kocuria rhizophila</i> , <i>Raoultella planticola</i>

<sup>1)</sup>Alphabet letters were randomly labeled for different commercial products.

**Table 4. Identification of microorganisms in frozen raw duck meats using 16S rDNA sequencing**

Type	Products	Microorganisms
Sliced	G <sup>1)</sup>	<i>Acinetobacter</i> spp., <i>Arthrobacter globiformis</i> , <i>Burkholderia</i> spp., <i>Chryseobacterium</i> spp., <i>Deinococcus aquaticus</i> , <i>Enterobacter</i> spp., <i>Kocuria rhizophila</i> , <i>Lactococcus</i> spp., <i>Pseudomonas</i> spp.
	H	<i>Burkholderia</i> spp., <i>Escherichia hermannii</i> , <i>Lactococcus lactis</i> , <i>Ralstonia pikettii</i>
Deboned	I	<i>Chryseobacterium</i> spp., <i>Chryseobacterium indologenes</i> , <i>Deinococcus</i> spp., <i>Kocuria rhizophila</i> , <i>Lactococcus garvieae</i> , <i>Moraxella</i> spp., <i>Pantoea ananatis</i>
Bone-in whole	J	<i>Enterobacter</i> spp., <i>Kocuria</i> spp., <i>Kocuria rhizophila</i> , <i>Pseudomonas</i> spp., <i>Spingobacterium</i> spp.

<sup>1)</sup>Alphabet letters were randomly labeled for different commercial products.

**Table 5. Identification of microorganisms in smoked duck meat products using 16S rDNA sequencing**

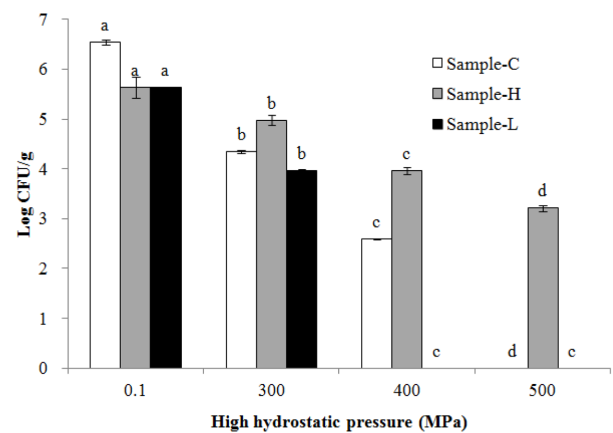
Type	Products	Microorganisms
Sliced	K <sup>1)</sup>	<i>Burkholderia cepacia</i> , <i>Leuconostoc mesenteroides</i> , <i>Ralstonia</i> spp., <i>Pseudomonas</i> spp.
	L	<i>Enterobacter</i> spp., <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Klebsiella</i> spp.
	M	<i>Burkholderia cepacia</i> , <i>Leuconostoc mesenteroides</i> , <i>Ralstonia pikettii</i> , <i>Pseudomonas brenneri</i>
	N	<i>Leuconostoc mesenteroides</i> , <i>Pseudomonas</i> spp.
	O	<i>Acinetobacter</i> spp., <i>Kocuria rhizophila</i> , <i>Leuconostoc mesenteroides</i> , <i>Stenotrophomonas maltophilia</i>
	P	<i>Bacillus</i> spp., <i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Microbacterium laevaniformans</i>
Bone-in whole	Q	<i>Burkholderia</i> spp., <i>Klebsiella</i> spp., <i>Pseudomonas</i> spp.
	R	<i>Burkholderia</i> spp., <i>Lactococcus lactis</i>
	S	<i>Burkholderia</i> spp., <i>Klebsiella pneumoniae</i> , <i>Leuconostoc mesenteroides</i>

<sup>1)</sup>Alphabet letters were randomly labeled for different commercial products.

### High hydrostatic pressure treatment

HHP treatment was evaluated for the ability to control microbial populations in duck meats by treatment of three products that have the largest microbial populations according to previous experiments. HHP treatment reduced the numbers of total aerobic bacteria in raw duck meats and duck meat products (Fig. 1). Compared to the control, the numbers of total aerobic bacteria in all samples at 300 MPa were reduced by about 2 Log CFU/g. The HHP treatment at 500 MPa inactivated the growth of aerobic bacteria in duck meats to undetectable levels (<1 Log CFU/g), except for in raw sample H.

The effect of HHP on the inactivation of microorganism was confirmed by previous studies. Pressures between 100 and 400 MPa efficiently reduced the numbers of bacteria of strains of *Salmonella* spp. (Malicki *et al.*, 2005).



**Fig. 1. Effects of high hydrostatic pressure processing on the number of total aerobic bacteria (Log CFU/g) of duck meat.** <sup>a-d</sup>Values with different letters differ significantly ( $p < 0.05$ ).

Pietrzek *et al.* (2011) reported that the proliferation of aerobic bacteria in chicken patties was suppressed by HHP treatment at 500 MPa, with decreases of 3 Log CFU/g and 6 Log CFU/g after storage at 4-6°C for 14 and 21 d, respectively. Khan *et al.* (2014) showed that a combination treatment at 200 MPa and 70°C for 10 or 20 min was sufficient to produce microbiologically safe duck breast products. Additionally, Kruk *et al.* (2011) and Jung *et al.* (2012) indicated that pressures of 450 to 600 MPa almost completely eliminated three major pathogens, i.e., *Salmonella* Typhimurium, *E. coli*, and *L. monocytogenes*. Microbial cellular membranes are affected by HHP, resulting in osmotic changes, lysis, alterations of nuclear material, and other modifications, which can result in cell death (Mackey *et al.*, 1994).

### Conclusion

The total numbers of aerobic bacteria in raw and smoked duck meat products were high in Korean commercial products and most of these bacteria were spoilage microorganisms. Even though there were no identified pathogens found in commercial duck meat products in the present study, it is necessary to implement sanitary step to minimize the growth of pathogenic and spoilage microorganisms. HHP treatment significantly reduced the levels of total aerobic bacteria in raw and smoked duck products. The results of the present study indicate that HHP treatment (300-500 MPa) is effective for ensuring the safety of duck meats and duck meat products.

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