



## Effect of Extended Hanging Time on the Microbial Quality of Pork Carcasses and Pork Blade Steaks

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**Abstract:** To evaluate the effect of extended post-harvest hanging time on pork carcass sides ( $n=20$ ), aerobic plate count (APC), Enterobacteriaceae, yeast and mold populations, pH, and moisture content were determined. Pork carcasses were sampled on d 1, 7, 14, and 21 to determine their microbial quality from the lean tissue of 3 anatomical locations, the flank, shoulder, and jowl. After the 21-d extended hanging time, pork shoulder butts ( $n=17$ ) (Institutional Meat Purchase Specifications #406; USDA Agricultural Marketing Service, 2014) were fabricated from these carcasses into 2.54-cm pork blade steaks. Pork blade steaks were vacuum packaged, stored up to 35 d at  $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , and evaluated for APC, Enterobacteriaceae, yeast and mold populations, and pH on d 0, 7, 14, 21, 28, and 35. Pork carcass surface moisture content declined ( $P < 0.01$ ) from 65.1% on d 1 to 50.5% on d 21. The carcass pH was similar ( $P > 0.42$ ) at 5.88 on d 1, 7, and 14; however, the pH declined ( $P < 0.05$ ) to 5.72 at d 21. Pork carcass APC populations remained relatively low during the extended hanging time; however, the jowl had the highest ( $P < 0.05$ ) APC populations among all anatomical locations. In addition, the proportion of yeast populations above the detection limit for the jowl was found to be greatest ( $P < 0.05$ ) compared to the flank and the shoulder. Pork blade steak APC population was 5.06 log colony-forming units/g (CFU/g) on d 35; however, these counts were below  $10^8$  log CFU/g, which is when meat is considered spoiled. There was a day effect for Enterobacteriaceae and mold populations ( $P < 0.05$ ). These results indicate that pork carcasses and vacuum-packaged steaks fabricated from pork carcasses have acceptable microbial quality when they undergo an extended hanging time.

**Keywords:** pork blade steaks, microbial, pork carcass, extended storage

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## Introduction

The meat industry employs refrigeration as the air-chilling system to help maintain processing areas at low temperatures to delay microbial growth (Lovatt, 2014). In the pork industry, the time between harvest and fabrication is typically 24 to 48 h (Schweihofner, 2014); however, small processors may hold carcasses for an extended period of time prior to fabrication. Sebranek (2008) suggested that pork carcasses be stored in chill rooms with 85% to 90% relative humidity at  $0^{\circ}\text{C}$  to  $1^{\circ}\text{C}$ . Pork processors usually hold carcasses prior to fabrication using these parameters;

however, the time of extended storage of pork carcasses has not been well established.

At the pork subprimal level, research has demonstrated that vacuum-packaged pork cuts can be stored up to 9 wk at  $-1.5^{\circ}\text{C}$  without compromising the quality (Jeremiah and Gibson, 1997). Throughout the food supply chain, pork is typically held at  $4^{\circ}\text{C}$ , and some psychrotrophic bacteria—including *Lactobacillus* spp., *Pseudomonas* spp., *Alcaligenes-Enterobacter* spp., *Flavobacterium* spp., *Micrococcus* spp., and *Moraxella-Acinetobacter* spp.—can be present on pork at this temperature (Kotula, 1987).

Meat spoilage can be present when organoleptic properties no longer exist and bacterial degradation of amino acids triggers the formation of slime and off-flavors on the meat surface (Gill, 1997). Off-odors can be formed when growth reaches  $10^8$  log colony-forming units/cm<sup>2</sup> (CFU/cm<sup>2</sup>), resulting in an unmarketable and unacceptable product for consumers (Gill, 1982; Ellis and Goodacre, 2001).

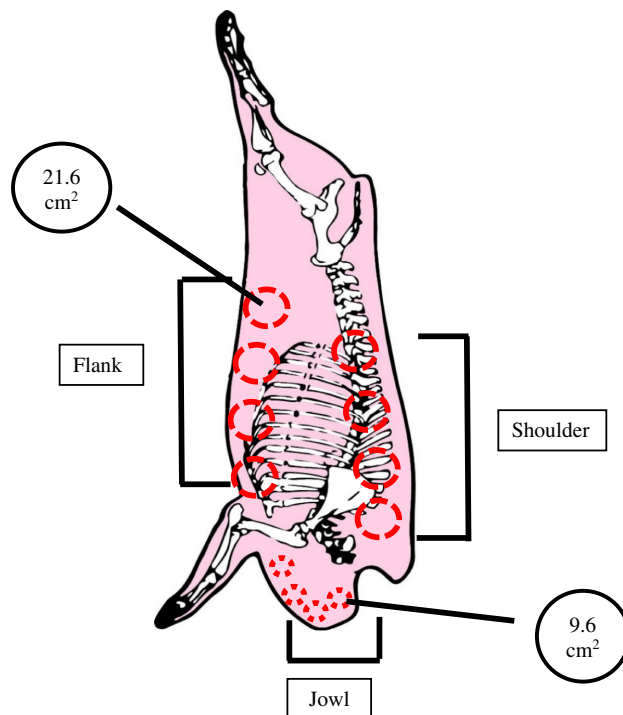
To the best of our knowledge, there is a lack of supporting documentation that can be used by small pork processors who hold pork carcasses for an extended period of time. Therefore, the objective of this study was to examine the microbial quality of pork carcasses and vacuum-packaged pork blade steaks fabricated from these carcasses during extended post-harvest cold storage.

## Materials and Methods

### Harvest and carcass sampling

Pigs ( $n = 20$ ) weighing approximately 130 kg were harvested on 2 separate days at the Kansas State University (KSU) Meats Laboratory (Manhattan, KS) under United States Department of Agriculture inspection. Pigs were rendered unconscious using electrical stunning (400 V for 15 s) and were immediately exsanguinated. The processing of pigs included scalding, dehairing, singeing, lymph node removal, bunging, evisceration, and carcass splitting. Carcass sides were trimmed for zero tolerance (free of feces, milk, and ingesta). After harvest, a hot-water intervention (77°C to 94°C) was applied to the carcasses, and the right sides were weighed, labeled, and stored for up to 21 d in a carcass cooler that averaged  $-0.7^\circ\text{C}$  and 87.3% relative humidity.

At 24 h, 7, 14, and 21 d postmortem, the flank, shoulder, and jowl of carcasses were sampled for aerobic plate count (APC), Enterobacteriaceae, yeast, and mold populations (Figure 1). At each location, 4 coring areas were randomly assigned for each sampling day. Two sterilized stainless-steel meat coring-devices were used to delineate the sampling area from the pork carcasses: a 21.6-cm<sup>2</sup> core for flank and shoulder locations and a 9.6-cm<sup>2</sup> core for the jowl location. Samples were excised at a depth of  $1.5 \pm 0.5$  mm using a sterile scalpel, placed into sterile plastic bags (Whirl-Pak bags, Nasco, Fort Atkinson, WI) containing 50 mL of sterile 0.1% peptone water (Bacto, Flankin Lakes, NJ), and immediately transported to the KSU Meat Microbiology Laboratory for microbiological analyses.



**Figure 1.** Carcass sampling locations on a pork carcass and core size used at each location

A calibrated pH probe (Model FC232, Hanna Instruments Inc., Woonsocket, RI) with a pH meter (Model HI 99163, Hanna Instruments Inc.) was utilized to determine pH on the side adjacent to the core taken from the shoulder location of the carcass for microbial sampling to obtain duplicate readings.

### Pork blade steak sampling

Pork shoulder butts ( $n = 17$ ) (Institutional Meat Purchase Specifications #406; USDA Agricultural Marketing Service, 2014) were separated from carcasses (3 carcasses were removed from the vacuum-packaged steak portion of the study and utilized for another research project) on d 21 postmortem, cut into six 2.54-cm-thick pork blade steaks using a Biro saw (Model 3334, Marblehead, OH), and vacuum packaged in 36×41 cm pouches (Prime Source Vacuum Pouches, 3 Mil Nylon/PE, Koch Supplies, Kansas City, MO), which had an oxygen transmission rate of 3.5 g/645.16 cm<sup>2</sup>/24 h at 21°C and 0.6 g/645.16 cm<sup>2</sup>/24 h at 0°C and a water vapor transmission rate of 0.6 cc/645.16 cm<sup>2</sup>/24 h at 37.8°C. Main muscles of pork blade steaks included *infraspinatus*, *supraspinatus*, *subscapularis*, *teres major*, *teres minor*, and *serratus ventralis*. The slicer was sterilized between samples using a hot-water (82°C) wash, and packages were randomly assigned within each pork shoulder butt to a sampling

day. Packages were stored at  $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and were sampled on 0, 7, 14, 21, 28, and 35 d of storage. At the appropriate sampling time, packages were aseptically opened, pH was measured on the side not used for microbiological sampling, and 25 g of surface meat was collected and placed into a sterile plastic bag (Whirl-Pak bags, Nasco) with 225 mL of 0.1% peptone water (Bacto, Flankin Lakes, NJ).

### Microbial analyses

Excised pork carcass and pork blade steak samples were homogenized for 60 s using a blender (Model AESAP1064, AES Chemunex, Bruz, France). Serial dilutions of this homogenate were prepared using 9 mL of 0.1% peptone water (Bacto, Flankin Lakes, NJ) and plated in duplicate on Petrifilm (3M Co., St. Paul, MN) to enumerate APC, Enterobacteriaceae, and yeast and mold populations. Samples were incubated and enumerated according to manufacturer instructions. Counts were transformed logarithmically and reported as log CFU/cm<sup>2</sup> for carcasses and log CFU/g for pork blade steaks. Additionally, the detection limit (DL) of the microbiological samples was calculated.

### Moisture analysis

The moisture content of the pork carcass surfaces was determined using a 9.6-cm<sup>2</sup> stainless-steel corer to excise approximately 5 g of meat surface adjacent to the microbiological sample core taken from the shoulder location of the carcass. Moisture content was measured at each carcass storage time. Due to the small sample size, the meat core was manually chopped using a scalpel for 2 min for moisture determination. After chopping the sample thoroughly, moisture content was determined in the KSU Analytical Laboratory using a SMART System 5 (CEM Corporation, Charlotte, NC) procedure (AOAC, 2008).

### Statistical analyses

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). For pork carcass sides, APC was analyzed in “Proc MIXED” assuming a randomized complete block design. Slaughter day was considered a replication (i.e., random), and carcass was nested within slaughter day. Location and sampling day were treated as fixed effects, and sampling day was modeled as a repeated measure. Similarly, responses for moisture and pH were analyzed as a randomized complete block design with repeated

measures. Fisher’s protected least significant difference was used for pairwise comparisons. Because the majority of observations for Enterobacteriaceae and for yeast and mold were below the DL in the pork carcass and steak data sets, these variables were analyzed as binary responses (1 = above DL and 0 = below DL) in “Proc FREQ.” For pork blade steaks, APC was analyzed in “Proc MIXED,” and day was assumed to be a fixed effect modeled as a repeated measure.

## Results and Discussion

### Carcass

It is noteworthy to mention that relative humidity fluctuated from 66% to 100% during this study. Maintaining relative humidity from 85% to 90% is ideal and could diminish shrinkage (Sebranek, 2008). There was a sampling day main effect for pork carcass surface pH and moisture content ( $P < 0.02$ ; Table 1). Carcass surface pH was similar, at 5.84 to 5.88 on d 1, 7, and 14 ( $P > 0.42$ ); however, a slight decrease ( $P < 0.05$ ) of pH was observed from d 14 (with pH 5.84) to d 21 (with pH 5.72). The pH decline on d 21 was equivalent

**Table 1.** Least square means of carcass surface pH and moisture content, APC populations, and number of presumptive positive EB, yeast, and mold populations by sampling day above the DL<sup>1</sup> of hanging pork carcass sides<sup>2</sup> ( $n = 20$ )

	Storage days				SEM	P value
	1	7	14	21		
<i>Quality measures</i>						
pH	5.88 <sup>a</sup>	5.88 <sup>a</sup>	5.84 <sup>a</sup>	5.72 <sup>b</sup>	0.04	<0.02
Moisture content	65.10 <sup>a</sup>	60.90 <sup>b</sup>	58.87 <sup>c</sup>	50.52 <sup>d</sup>	1.39	<0.01
<i>Bacterial enumeration<sup>3</sup></i>						
APC	0.98	0.99	0.86	0.84	0.11	>0.55
<i>Number of samples<sup>4</sup> above the DL</i>						
EB	1	1	0	1		>0.61
Yeast	36 <sup>a</sup>	33 <sup>a</sup>	32 <sup>a</sup>	16 <sup>b</sup>		<0.01
Mold	2	1	1	1		>0.74

<sup>1</sup>Flank and shoulder DL = 0.06 log<sub>10</sub> CFU/cm<sup>2</sup>; jowl DL = 0.41 log<sub>10</sub> CFU/cm<sup>2</sup>.

<sup>2</sup>Samples were stored at  $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 87.3% relative humidity for 21 d.

<sup>3</sup>Log<sub>10</sub> CFU/cm<sup>2</sup>.

<sup>4</sup>Total samples per day: 60.

<sup>abcd</sup>Numerals within a row with different superscripts differ ( $P < 0.05$ ).

There was no sampling day × location interactions ( $P > 0.54$ ) for APC.

APC = aerobic plate count; CFU = colony-forming units; DL = detection limit; EB = Enterobacteriaceae.

to 0.12 units. Although this difference was statistically significant, it may not have much effect on microbial growth. The recommended pH in pork carcasses after 24 h postmortem is between 5.7 and 6.1 (Towers, 2016). In our study, the pH ranged from 5.72 to 5.88. Very few studies have evaluated the extended hanging time on pork carcasses. Recently, Lee et al. (2016) found a final pH of 5.66 on the left sides of pork carcasses after being hung for 40 d at  $2^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 80% relative humidity. Moisture content of the pork carcass surfaces decreased gradually during the 21-d hanging time ( $P < 0.01$ ). The initial moisture content was 65.1%. At d 21, the moisture content of the surface of pork carcasses declined ( $P < 0.01$ ) to 50.5%. These 2 factors may pose unfavorable conditions for bacterial growth, resulting in low APC counts.

There was no carcass sampling day  $\times$  location interaction ( $P > 0.54$ ) for APC populations (Table 1). Day did not affect APC populations ( $P > 0.55$ ). There was an effect due to anatomical location ( $P < 0.01$ ) for APC. APC populations from the flank and shoulder were similar ( $P > 0.94$ ). Among the 3 anatomical locations, the jowl had the highest ( $P < 0.01$ ) APC populations. USDA Food Safety and Inspection Service (2017) reported that intact cuts from the jowl had the greatest incidence (43.8%) of *Salmonella*-positive samples among 6 anatomical locations from pork carcasses, indicating the high risk of contamination in this location. This microbiological increase in the jowl may be due to inherent dripping of water after the hot-water wash, resulting in water accumulation in that location. Additionally, this location is commonly used to handle pork carcasses, increasing the likelihood of cross-contamination. Overall, APC populations remained very low (0.84 to 0.99 log CFU/cm<sup>2</sup>) throughout the post-harvest extended hanging time. Large pork slaughter establishments process pork carcasses within 24 to 48 h postmortem (Schweihofer, 2014), whereas small pork facilities may experience microbial quality issues due to hanging pork carcasses for an extended time. Gill et al. (1995) found that initial populations for APC ranged from 2.50 to 3.00 log CFU/cm<sup>2</sup> on pork carcasses before entering the cooler at the polishing step. More recently, Janiszewski et al. (2018) reported 3.68 log CFU/cm<sup>2</sup> on half-carcass surfaces before chilling. Although these studies took microbiological samples from hot carcass, APC counts from the current study may be low because of the cold temperature on carcass surfaces after 24 h postmortem in the chilling room.

There were no carcass day or location effects for Enterobacteriaceae or mold populations ( $P = 0.61$ ;

**Table 2.** Least square means for APC populations and number of presumptive positive EB, yeast, and mold populations by anatomical location above the DL<sup>1</sup> of hanging pork carcass sides<sup>2</sup> ( $n = 20$ )

	Anatomical location				<i>P</i> value
	Flank	Shoulder	Jowl	SEM	
<i>Bacterial enumeration</i> <sup>3</sup>					
APC	0.77 <sup>a</sup>	0.76 <sup>a</sup>	1.21 <sup>b</sup>	0.12	<0.01
<i>Number of samples</i> <sup>4</sup> above the DL					
EB	0	2	1		>0.77
Yeast	30 <sup>a</sup>	36 <sup>a</sup>	51 <sup>b</sup>		<0.01
Mold	1	2	2		>0.81

<sup>1</sup>Flank and shoulder DL = 0.06 log<sub>10</sub> CFU/cm<sup>2</sup>; jowl DL = 0.41 log<sub>10</sub> CFU/cm<sup>2</sup>.

<sup>2</sup>Samples were stored at  $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 87.3% relative humidity for 21 d.

<sup>3</sup>Log<sub>10</sub> CFU/cm<sup>2</sup>.

<sup>4</sup>Total samples per anatomical location: 80.

<sup>a</sup><sup>b</sup>Numerals within a row with different superscripts differ ( $P < 0.05$ ).

APC = aerobic plate count; CFU = colony-forming units; DL = detection limit; EB = Enterobacteriaceae.

Table 2); however, location and day effects were found for yeast populations ( $P = 0.01$ ). The DL for Enterobacteriaceae and yeast and mold populations on pork carcass samples was 0.06 and 0.41 log CFU/cm<sup>2</sup> for the shoulder and flank and for the jowl, respectively. The proportion of yeast populations of pork carcass samples above the DL for the jowl was higher than the flank and shoulder ( $P < 0.02$ ), which were similar ( $P = 0.42$ ). The yeast population proportion on pork carcass samples above the DL on d 1, 7, and 14 did not differ ( $P > 0.58$ ); however, at d 21, yeast counts were reduced compared to all other days ( $P < 0.04$ ). Yeast populations are known to survive on dried-carcass surfaces at water activity values of  $\leq 0.95$  under aerobic conditions (Van Netten et al., 1995). Ahnström et al. (2006) reported that yeast populations increased during extended cold storage. Spoilage is present when organoleptic properties are lost and bacterial degradation of amino acids triggers slime formation and off-flavor development on the meat surface (Gill, 1997), and this typically occurs when bacteria growth reaches  $10^8$  log CFU/cm<sup>2</sup> (Gill, 1982). Overall, the microbiological results indicate that pork carcasses have acceptable microbial quality when stored for 21 d at  $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 87.3% relative humidity.

### Pork blade steaks

There was a sampling day main effect for pork blade steak pH ( $P < 0.01$ ; Table 3). The pH was similar ( $P > 0.31$ ) on d 0 and 7 at 5.73 and 5.78, respectively;



**Table 3.** Least square means of pH, APC populations, and number of presumptive positive EB, yeast, and mold populations by sampling day above the DL<sup>1</sup> of pork shoulder blade steaks<sup>2</sup>

	Storage days						SEM	P value
	0	7	14	21	28	35		
<i>Quality measures</i>								
pH	5.73 <sup>bc</sup>	5.78 <sup>b</sup>	5.89 <sup>a</sup>	5.70 <sup>bc</sup>	5.78 <sup>b</sup>	5.69 <sup>c</sup>	0.05	<0.01
<i>Bacterial enumeration</i> <sup>3</sup>								
APC	1.61 <sup>a</sup>	1.18 <sup>b</sup>	2.44 <sup>c</sup>	3.17 <sup>c</sup>	4.67 <sup>d</sup>	5.06 <sup>d</sup>	0.41	<0.01
<i>Number of samples</i> <sup>4</sup> <i>above the DL</i>								
EB	0 <sup>a</sup>	0 <sup>a</sup>	4 <sup>ab</sup>	7 <sup>b</sup>	16 <sup>c</sup>	10 <sup>b</sup>		<0.01
Yeast	9	11	11	11	7	9		>0.72
Mold <sup>5</sup>	0	0	0	3	4	2		<0.02

<sup>1</sup>DL = 0.70 log<sub>10</sub> CFU/g.

<sup>2</sup>Samples were stored at 0°C ± 1°C for 35 d.

<sup>3</sup>Log<sub>10</sub> CFU/g.

<sup>4</sup>Total samples per sampling day: 17.

<sup>5</sup>Statistical model did not detect differences between days for mold populations due to reduced sample size.

<sup>ab</sup>Numerals within a row with different superscripts differ ( $P \leq 0.05$ ).

APC = aerobic plate count; CFU = colony-forming units; DL = detection limit; EB = Enterobacteriaceae.

however, on d 14, the pH increased ( $P < 0.01$ ) to 5.89 in comparison to d 0 and 7, which were similar ( $P > 0.09$ ) to d 21. Pork blade steak pH declined ( $P < 0.01$ ) to 5.7 in comparison to d 14. On d 21 and 28, pH tended to be different ( $P < 0.06$ ). The final pH on d 35 decreased ( $P < 0.01$ ) to 5.69 in comparison to d 28. Pork blade steak pH varied throughout storage time, but the initial pH was not different than the pH at the end of the study. The pH of muscle can vary among carcasses (Gill and Newton, 1978) as well as in different muscles within the same carcass (Topel et al., 1966). Yang et al. (2016) reported that pH of vacuum-packaged pork chops slightly increased after a 50-d storage period at 1°C. Similarly, Zhao et al. (2015) reported that pH of vacuum-packaged chilled pork increased from 5.72 on d 0 to 5.99 on d 21 in pork cuts stored at 0°C.

APC populations on pork shoulder blade steaks increased ( $P < 0.01$ ) throughout storage time. The initial APC population was 1.61 log CFU/g. On d 7, APC populations decreased ( $P < 0.02$ ); however, APCs on d 14 were higher ( $P < 0.01$ ) than d 0 and 7. On d 14 and 21, APC populations were not different ( $P > 0.11$ ). On d 28 and 35, APC populations reached 4.67 and 5.06 log CFU/g, respectively, which were similar ( $P > 0.90$ ) but higher than d 14 and 21 ( $P = 0.01$ ). Despite the increase of APC populations over time, bacterial growth did not exceed 10<sup>8</sup> CFU/cm<sup>2</sup>, a level

at which the development of off-odors and slime on meat has been documented (Gill, 1982; Ellis and Goodacre, 2001). Holley et al. (2004) conducted a similar study in which boneless pork loins were stored at  $-1.7^\circ\text{C} \pm 1^\circ\text{C}$ , and APC populations reached 5.78 log CFU/cm<sup>2</sup> on d 56.

Day did not affect yeast populations, but a day effect was found for Enterobacteriaceae and mold populations ( $P < 0.02$ ) on pork shoulder steaks. The DL for Enterobacteriaceae and for yeast and mold populations on pork shoulder blade steak samples was 0.70 log CFU/g. It is noteworthy that the 3M method for Enterobacteriaceae enumeration only tests for presumptive positive samples. On d 0 and 7, none of the samples were above the DL for Enterobacteriaceae populations, and they did not differ ( $P > 0.10$ ) from d 14, which had 4 samples above the DL and was similar ( $P > 0.46$ ) to d 21 with 7 samples above the DL for Enterobacteriaceae populations. At d 28, samples above the DL for Enterobacteriaceae populations on pork steaks increased ( $P < 0.01$ ) to 16 in comparison to d 21; however, at d 35, samples above the DL for Enterobacteriaceae populations declined ( $P < 0.04$ ) to 10. Enterobacteriaceae species that have been previously identified to grow in vacuum-packaged meat at temperatures between 0°C and 10°C included *Serratia liquefaciens* and *Hafnia* spp. (Labadie, 1999). Enterobacteriaceae populations were found to grow on pork stored at 0°C (Zhao et al., 2015) and on beef stored at 4°C (Ariyapitipun et al., 1999). In addition, Brightwell et al. (2007) reported that Enterobacteriaceae populations were responsible for the spoilage of vacuum-packaged lamb stored at 4°C. Although most of the pork blade steak samples were above the DL for Enterobacteriaceae populations toward the end of storage, none of the samples exceeded 4.40 log CFU/g (data not shown).

## Conclusions

APC populations were affected by the anatomical location within the pork carcass. The jowl had the highest APC population and highest number of yeast populations that were above the DL. On a pork carcass, water naturally drains downward because of gravity and resides on the jowl following a hot-water intervention, and the jowl is also the site where a carcass is commonly handled. These factors can play a role in increasing the likelihood of bacterial contamination, potentially leading to a high prevalence of APC and yeast populations on the jowl. Pork carcasses stored

at 0°C ± 1°C and 87.3% relative humidity may be held for up to 21 d, and pork shoulder blade steaks fabricated from these carcasses and then vacuum packaged could be stored for 35 d without compromising microbial quality.

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