CONTAMINATION OF BEEF CARCASSES WITH SPOILAGE BACTERIA DURING SLAUGHTER AND CHILLING

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SUMMARY
The extent of contamination of beef carcasses with Gram-negative spoilage bacteria during slaughter and chilling was determined at a commercial abattoir with the combined use of (1) determination of hygiene index, (2) identification of spoilage flora present on carcasses and (3) environmental sampling of spoilage bacteria.

Process operations found to be critical for the extent of the contamination with spoilage bacteria were dehiding of carcasses and the handling of carcasses in the rapid chiller. Operation of the hide-puller led to an air-borne contamination of psychrotrophic Pseudomonas along the slaughter line. During the handling of carcasses in the rapid chiller, a resident environmental microflora consisting mainly of Pseudomonas fluorescens contaminated the beef carcasses by direct contact and by aerosols.

INTRODUCTION

The shelf-life of meat is dependent on the initial level and composition of spoilage bacteria on the meat surface. Important spoilage bacteria of meat are Pseudomonas, Enterobacteriaceae and lactic acid bacteria. Pseudomonas spp. usually dominates on aerobically stored meat, while lactic acid bacteria dominate in vacuum and modified atmosphere packages with elevated CO_2 concentrations (Blickstad & Molin, 1983; Dainty et al., 1983,).

The spoilage bacteria originate from different sources: The animal carries psychrotrophic bacteria on its hide that will contaminate the carcass during slaughter. The plant harbours a resident psychrotrophic microflora in its refrigerated rooms. In addition to this, structural and work surfaces may be as important as the hide itself as sources of the psychrotrophic contamination of carcasses (Newton et al., 1978).

Total aerobic counts are frequently used for surveying the bacteriological status of carcasses along the processing line in meat production, (Roberts et al., 1980, Snijders, 1988; Stolle, 1988). However, monitoring the slaughter hygiene by means of the total bacterial count only provides the ability to detect aberrant hygiene practices (Johansson et al., 1983). Furthermore, no evidence has been demonstrated of a correlation with shelf-life (Roberts, 1980) and the total aerobic count cannot be used for estimating the contamination level of spoilage bacteria during processing (Borch et. al., 1988). Thus, in order to evaluate the influence of different process operations on the shelf-life of cut meat, an analysis reflecting the level of spoilage bacteria should be used.

In the present study, a bacteriological analysis reflecting spoilage bacteria was used in order to determine which critical process operations in beef production have an effect on the shelf-life of cut meat.

MATERIALS AND METHODS

Experimental design

The slaughtering and chilling processes for beef were examined at a commercial abattoir in Sweden. Sampling was done at two stages along the slaughter line (dehiding; carcass-splitting) and at three stages during chilling (before rapid-chiller; after rapid-chiller; after quarter-splitting and subsequent cold storage for 24 h).

A total of 17 carcasses were followed along the slaughter line during one production day. At the abbatoir under investigation hanging line and mechanical hide-puller were being used. A total of 10 carcass-halves representing 5 animals were followed during chilling on another production day. The carcass-halves were sprayed with cold water before entering the rapid chiller.

Analysis of carcasses
Surface samples were taken from four sites on the carcasses (Fig. 1). By using a cork-borer, sixteen meat pieces of diameter 2 cm were excised from each carcass at each investigated stage. The meat pieces were divided into four polyethene bags, each bag containing meat samples representing 12.5 cm^2. The bags were stored aerobically at 2°C. Microbiological examination was performed after 0, 5, 9 and 14 days (samples representing slaughter line): and after 0, 3, 7 and 14 days (samples representing chilling). Samples were homogenized with 25 ml of peptone-water (0.85% NaCl, 0.1% peptone, 0.1% Tween 80) in a stomacher for 35 s. The total aerobic count was determined on Tryptone Glucose Extract agar (TGE, Oxoid; incubated at 25°C, 3 d), the Enterobacteriaceae count on VRBD agar (Violet Red Bile Agar, Oxoid, supplemented with 1% glucose; 37°C, 1d) and the Gram-negative psychro- trophic bacteria count on VRBD agar (4°C, 10d).

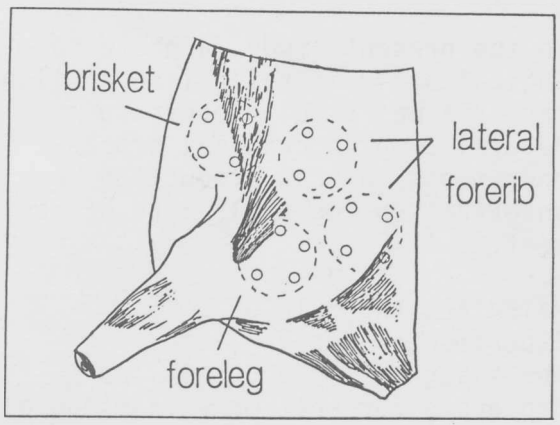


Figure 1: Sampling sites (o) on the front-quarter. The meat samples were distributed in such a way that each dotted marked circle area was represented in every microbiological analysis.

Analysis of environment
Environmental samples were collected simultaneously with the excision of the meat samples during slaughtering and chilling at the respective stages. Petri plates were exposed for 10 min along the slaughter line. The following counts were determined: total aerobic (TGE, 25°C, 3 d), total psychrotrophic (TGE, 8°C, 8 d) Enterobacteriaceae VRBD, 37°C, 1 d) and Gram-negative psychrotrophic (VRBD, 4°C, 10 d). Samples were taken from surfaces in the chilling rooms using the contact plate method (Favero, 1978) using TGE agar plates (25°C, 3 d). Samples from surfaces and equipment were collected by using cotton wool swabs moistened with 0.9% NaCl solution and subsequently swabbed on to petri plates (VRBD, 4°C, 10 d).

Identification
Single bacterial colonies were isolated from plates of the Gram-negative psychrotrophic count of meat samples stored for 14 days at 2°C; an average of 5 colonies per plate was isolated. Colonies from environmental samples were isolated from plates of the total aerobic count and the Gram-negative psychrotrophic count; an average of 10-20 colonies per plate was isolated. In total, 640 isolates were examined.

All isolates were examined for Gram reaction (KOH method; Gregersen, 1978); morphology and motility (phase contrast microscope); growth at 4°C on TGE; pigmentation on TGE. The schemes of Molin et al. (1983) were followed when grouping the organisms down to family level. All Gram-negative isolates were further tested for acid production from glucose incubated anaerobically (Gas Pak Anaerobic System, BBL) at 30°C for 3 days (Hugh & Leifson, 1953). Pseudomonadaceae were examined for oxidase reaction (Kovacs, 1956);

Table 1. Criteria used for the separation of *Pseudomonas* spp. into species.

Species	Acid production from maltose	Assimilation of malonate	Assimilation of D-xylose
Pseudomonas lundensis	+	-	-
Pseudomonas fluorescens	-	+	v
Pseudomonas fragi	v	-	+

+: positive reaction
-: negative reaction
v: variable reaction

production of fluorescent pigment (King *et al.*, 1954); acid production from maltose (Hugh & Leifson, 1953); utilization of D-xylose, malonate, D-arabinose and meso-inositol (Molin & Ternström, 1982) and were divided into species according to Table 1.

Hygiene index

Growth curves for the increase in bacterial counts during the storage of meat samples at 2°C were determined, each growth curve representing a single processing stage. The hygiene index was determined as the time taken for the total aerobic count to reach 10^7 or 10^5 cfu/cm^2; for the *Enterobacteriaceae* count to reach 10^5 cfu/cm^2; for the Gram-negative psychrotrophic count to reach 10^7 cfu/cm^2. If the determined cfu level was not reached within 14 days of storage, exponential regression was used in order to estimate the hygiene index.

By comparing the hygiene index of meat samples removed after the different processing stages, the extent and significance of contamination during slaughter and chilling could be estimated. A low hygiene index indicated that a substantial amount of psychrotrophic bacteria were present before storage.

RESULTS AND DISCUSSION

Slaughter line

Mean total aerobic counts obtained from carcasses along the slaughter line exhibited no significant difference after dehiding, as opposed to after evisceration and carcass-splitting (Fig. 2). Grau (1974) found no significant changes in mean total counts when mutton carcasses were examined after dehiding and evisceration. In contrast to these results, Nortjé and Naudé (1981), found significant changes at different positions along the slaughter line. However, they used a special sampling technique and did not transform their bacterial counts into log units and this may have affected their conclusions.

The mean hygiene index of beef carcasses was lower after evisceration and carcass-splitting than after dehiding (Fig. 2). This indicated an increased contamination with psychrotrophic bacteria along this part of the slaughter line. This contamination with psychrotrophic bacteria of the carcasses was not evident from the analysis of unstored samples. In fact, initial plate counts for *Enterobacteriaceae* and Gram-negative psychrotrophic bacteria were below the detection limit (<1.0 log cfu/cm^2) for all samples.

Storage of the excised meat samples permitted proliferation of *Enterobacteriacae* and Gram-negative psychrotrophic bacteria on a few samples from certain carcasses. Calculation of hygiene indexes from these samples for these types of bacteria proved to be difficult because of the large variation in

Table 2. Microbial counts of environmental samples from the slaughter line

Location	Contamination level [a]			
	Total aerobic count		Entero-bacteriaceae count	Gram-neg. psychro-trophic count
	mesophilic	psychro-trophic		
in front of hide-puller	210	46	2	1
behind hide-puller	500	250	65	160
beside hide-puller	200	32	0	0
beside split-saw	270	10	2	1
split-saw sprinkles	130	40	67	0

a) Colony forming units per petri plate (58 cm^2) exposed horizontally for 10 minutes, except for sprinkles that were collected by vertical exposure of the petri plate for 30 s.

contamination level between excised samples. Nevertheless, the hygiene indexes indicated that psychrotrophic bacteria were contaminating the carcasses along the slaughter line, which conventional plate counts of unstored samples never did. Gram-negative psychrotrophic bacteria were extensively spread to the area behind the hide-puller (Table 2), indicating that the hide was a prevalent source of spoilage bacteria. The hide's influence on the contamination of the carcasses has been shown by several other authors (Grau, 1974; Newton *et al.*, 1978; Patterson & Gibbs, 1978). *Pseudomonas* species prevailed in the Gram-negative psychrotrophic count of the environmental sample taken from the area behind the hide-puller where. *P. fluorescens* constituted 30%, *P. fragi* 5% and *P. lundensis* 5% (Fig. 3a). An equivalent distribution of *Pseudomonas* species was found on the aerobically stored meat samples (14 d, 2°C) taken simultaneously along the slaughter line where *P. fluorescens* constituted 50%, *P. fragi* 17%, and *P. lundensis* 17% (Fig. 3b). The equivalence of spoilage flora between the environmental samples and the stored meat samples gave strong indications that the operation of the hide-puller was critical in the contamination of spoilage bacteria.

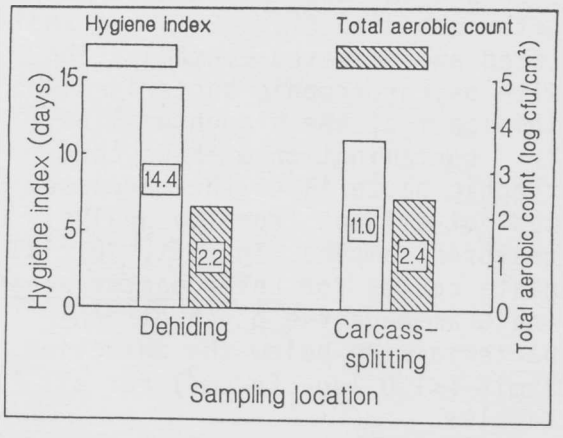
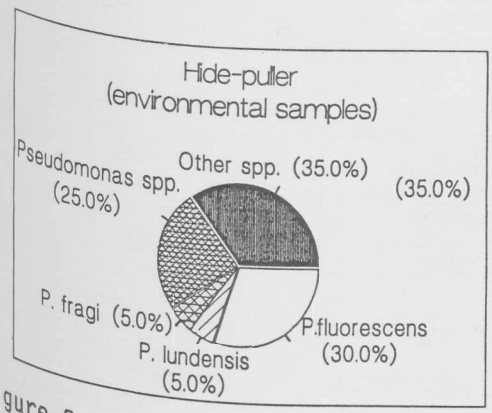


Figure 2: Hygiene index and total aerobic count after dehiding and carcass-splitting of beef carcasses. Hygiene index is expressed as no. of days for the total count of bacteria to reach 10^5 cfu/cm^2 during aerobic storage of excised meat samples at 2°C.

Chilling

Contamination with Gram-negative psychrotrophic bacteria had occurred in the rapid chiller, as indicated by the decrease in hygiene indexes (Fig. 4). Quarter-splitting and further storage for 24 hours in the



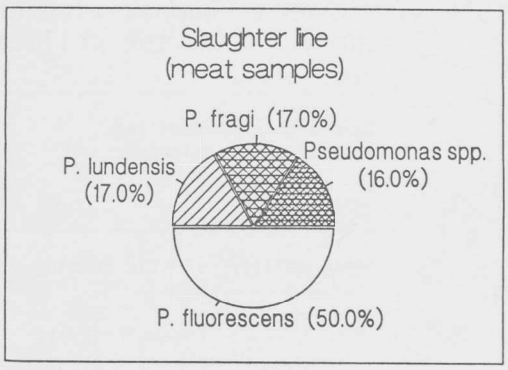


Figure 3: Distribution of Pseudomonas species on VRBD (4°C, 10 d) representing a) environmental samples from an area behind the hide-puller and b) meat samples excised after dehiding and carcass-splitting and stored at 2°C for 14 d.

quarter storage chiller did not affect the hygiene indexes (Fig. 4). During chilling, surface contamination is only expected to increase slightly and higher bacterial levels would indicate inappropriate handling of carcasses (Stolle, 1988).

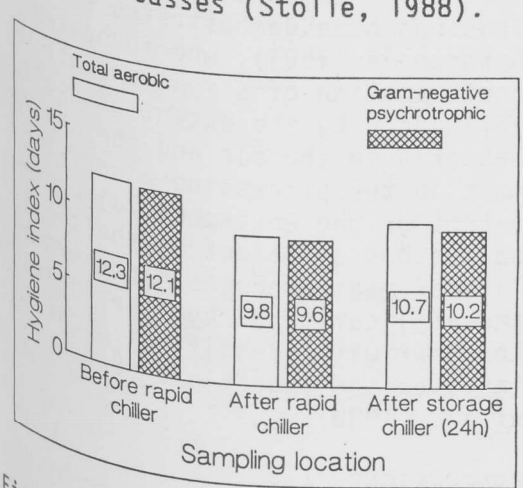


Figure 4: Hygiene indexes of beef carcasses before rapid chiller; after rapid chiller; after quarter-splitting and cold storage for 24 h (hygiene index = no. of days until total aerobic count reaches 10^7 cfu/cm^2; Gram-negative psychrotrophic count reaches 10^7cfu/cm^2 during storage of excised meat samples at 2°C).

The contamination level of spoilage bacteria at the end of the slaughter line (stage: before rapid chiller, Fig. 4) was relatively high on this occasion. The hygiene indexes based on Gram-negative psychrotrophic bacteria, were more consistent, as opposed to the previously described investigation when the slaughter line was studied (stage: after carcass-splitting, Fig. 2).

The collection of environmental samples from contact surfaces, walls, floors and ceilings in the different chillers exhibited a prevalence of P. fluorescens. The plastic folding door used for entry into the rapid chiller was heavily contaminated with P. fluorescens (Table 3). The water used for spraying contained very few Gramnegative psychrotrophs and no P. fluorescens (data not shown). Aerosols were created when the wet carcasses were introduced into the existing air- flow in the rapid chiller, thereby facilitating the spreading of spoilage bacteria present on carcasses. A widespread occurrence of Pseudomonas species in refri- gerated areas has been found in other studies (Stringer et al., 1969; Newton et al.,1978; Patterson & Gibbs, 1978). The occurrence of P. fluorescens in the subsequent chillers, i.e. the quarter storage chiller, might reflect the adaptation of this species to, and the colonization of, these environments (Newton et al.,1978).

Although holding for 24 h in the quarter storage chiller did not affect the hygiene index (Fig. 4),

Table 3. Microbial composition of environmental microflora in samples from the chillers.

Sampling locations	Sampling places	% of *Pseudomonas* among isolated colonies	Prevalent *Pseudomonas* species
Rapid chiller	Plastic folding door	100	*P. fluorescens*
	Floor, concrete	50	*P. fluorescens*
	Floor, clinkers	40	*P. fluorescens*
	Wall, tiles	30	*P. fluorescens*
	Ceiling, pipes	100	*P. fluorescens*
Quarter storage chiller	Plastic folding door	25	*P. fluorescens*
	Floor, clinkers	5 5	*P. fluorescens* *P. fragi*

the composition of *Pseudomonas* spp. that proliferated on the aerobically stored meat samples (2°C, 14 d) changed. *P. fluorescens* prevailed after the rapid chiller and constituted 56% of the Gram-negative psychrotrophic flora, becoming further established during a period of 24 hours in the quarter storage chiller where *P. fluorescens* constituted 90% (Fig. 5). The changes in spoilage flora on carcasses moving along the slaughter line to the subsequent processes of chilling could be correlated to the environmental microflora where *P. fluorescens* also prevailed.

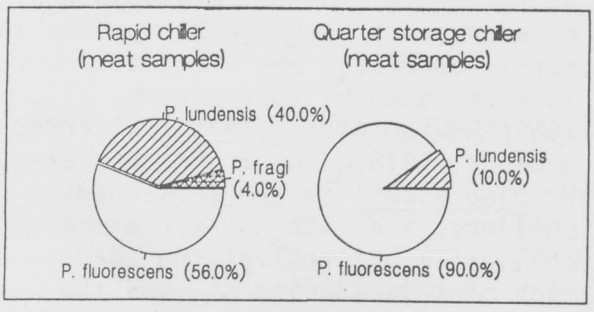


Figure 5: Distribution of *Pseudomonas* species from meat samples excised after the respective chilling stage and stored aerobically at 2°C:.

This has been demonstrated by Mäkelä & Korkeala (1987), who found that contamination of a meat product was influenced by the extent of spoilage bacteria in the air and the environment in the processing plant. The extent of the environmental contamination has an effect on the shelf-life of meat products, as shown by the application of hygiene indexes together with identification of the involved spoilage flora (Borch *et al.*, 1988).

CONCLUSIONS

In the present study, the combined use of (1) hygiene index, (2) identification of spoilage flora and (3) environmental sampling of spoilage bacteria made it possible to determine critical process operations during beef production. Process operations found to be critical for the contamination of spoilage bacteria were:

* dehiding led to airborne contamination of psychrotrophic pseudomonads (*P. fluorescens*, *P. fragi* and *P. lundensis*) of beef carcasses

* during the handling of beef carcasses in the rapid chiller, a resident environmental microflora consisting mainly of Pseudomonas fluorescens contaminated the beef carcasses through direct contact surfaces and by aerosols in the rapid chiller.

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