Letters in Applied Microbiology 2001, **32**, 205–210

Effects of chilling on sampling of bacteria attached to swine carcasses

S.-L. Yu, P.H. Cooke and S.-I. Tu
Microbial Biophysics & Biochemistry & Core Technologies, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, PA, USA

243/00: received 21 November 2000 and accepted 20 December 2000

S.-L. YU, P.H. COOKE AND S.-I. TU. 2001. Two microbiological sampling techniques, excision and sponge swabbing, were compared by determining counts of aerobic bacteria, coliforms and injured coliforms from 20 de-haired swine carcasses before and after chilling. Excised jowl skin produced significantly greater counts of the three types of bacteria than sponge swabs. Aerobic bacteria, coliforms and injured coliforms recovered by sponge swabbing carcasses before chilling were 11·6%, 0·9% and 11·0% of excised samples, respectively; the corresponding percentages recovered after chilling were 23·9%, 11·1% and 5·0%. Numbers of all bacteria present on the post-chill carcasses were substantially lower than on the pre-chill carcasses. Excision usually produced more countable plates for coliforms and injured coliforms on chilled carcasses than sponge swabbing and therefore, is more suitable in estimating low numbers of faecal bacteria on chilled carcasses. To explore the possible structural bases for these findings, skin samples were inoculated with 10^2–10^7 cfu cm^{-2} faecal bacteria and examined by scanning electron microscopy. Chilled samples showed bacteria and biofilm embedded in superficial crevices, which underlies a possible reason for the lower recovery of bacterial cells by the sponge swabbing. The study indicates that the differences between sampling techniques may be a result of the chilling process of swine carcasses.

INTRODUCTION

Accurate post-slaughter enumeration of microbial populations on meat carcasses is essential for reliable evaluation of the chilling process. Excision is considered to be the most effective bacterial sampling method for beef carcasses (Ingram and Roberts 1976; Anderson *et al.* 1987; Dorsa *et al.* 1996), but in meat processing facilities, excision is neither practical nor acceptable because it requires time and proficiency and devalues carcasses. Consequently, more practical, non-destructive and rapid sampling methods such as adhesive contact tape, swabbing, rinsing, direct agar contact, scraping and vacuuming from a moving processing line must be validated (Lee and Fung 1986). Unfortunately, none of the swabbing methods with sponge, griddle screen or 3M mesh yielded complete recovery of bacteria present on a beef carcass when compared with excision (Anderson *et al.* 1987; Dorsa *et al.* 1996). Though there was a significant difference between excision and sponge swabbing on inoculated beef carcasses at low inoculum levels, sponge sampling yielded bacterial populations closer to those of excision as the inoculum levels increased (Dorsa *et al.* 1996). Scholefield *et al.* (1981) reported that double wet and dry swabs recovered more psychrotroph counts on swine carcasses than single dry swabs. However, both methods gave lower recoveries when compared with excision, rinse and scrape techniques. The numbers recovered by swabbing have been reported to range from 1 to 89% of the numbers recovered by excision, which suggests that the nature and condition of the carcass surface may affect the numbers of bacteria recovered by the two sampling methods (Dorsa *et al.* 1996; Sharpe *et al.* 1996; Gill and Jones 2000).

Bacterial attachment and penetration into meat surfaces are of concern during slaughter and further processing of pigs into pork for consumption (Woody *et al.* 2000). Various mechanisms have been proposed to explain bacterial attachment to meat surfaces (Butler *et al.* 1979; Beachey 1981; Firstenberg-Eden 1981; Selgas *et al.* 1993). Although non-specific attachment of bacteria may occur during

Correspondence to: Dr S.-L. Yu, Microbial Biophysics & Biochemistry & Core Technologies, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA (e-mail: lyu@arserrc.gov).

© 2001 The Society for Applied Microbiology

post-mortem processing from dried or clotted animal blood, most researchers believe that polysaccharide-containing components on the cell wall, such as flagella and pili (Butler *et al.* 1979; Firstenberg-Eden 1981), cell surface charge (Dickson and Koohmaraie 1989) and hydrophobicity of bacteria (van Loosdrecht *et al.* 1987; Dickson and Koohmaraie 1989; Benito *et al.* 1997), and specific binding sites or receptors on animal cell membrane (Beachey 1981; Firstenberg-Eden 1981) are responsible for the adhesion process. Although bacterial attachment to meat at chill temperatures is generally regarded as a surface phenomenon, Gill and Penney (1977) reported that bacteria can penetrate the meat surface as a result of the breakdown of the connective tissue by bacterial proteolytic enzymes. In addition, the radial shrinkage of muscle fibres during the development of rigor was found to facilitate bacterial penetration of muscle tissue through the formation of gap regions (Gill *et al.* 1984).

In the present study, counts obtained by sponge swabbing swine carcasses were compared with those obtained by excision during air chilling. Swab and excision samples were plated to produce counts of aerobic bacteria, coliforms and injured coliforms to determine efficiency of each sampling method. The effect of chilling on the attachment and entrapment of faecal bacteria on pork skin using scanning electron microscopy (SEM) was also examined.

MATERIALS AND METHODS

Sample collection and sampling methods

Skin samples were obtained from 100 to 110 kg (live weight) pigs processed according to the following slaughter procedures. Animals were electrically stunned, exsanguinated, scalded in 60°C water, mechanically de-haired, singed, polished with 25°C water and further shaved with bell scrapers to remove residual hair. After evisceration, splitting and washing of carcasses, jowls were removed immediately after the final wash, before entering the chiller, and brought to the laboratory. Jowl samples were chosen for the experiments to avoid the devaluation of the carcasses. One jowl taken from each of five hogs on each of four days was placed skin-side up in a plastic tray. For the comparison among sponge swabbing, excision and excision after sponge swabbing, the sponge and excision samples were taken side-by-side on the jowls before and after chilling. Additional excision samples were taken after swabbing. The other jowl from the matched half of the same carcass was sampled after air chilling at 2°C overnight for the post-chill experiments.

Sponge swab method

A 10×10 cm^2 area of the jowls was swabbed using a pre-moistened Whirl-Pak sponge (Nasco, Ft Atkinson, WI, USA) with 10 ml sterile 0·1% peptone water. The jowl surface was swabbed 10 times from top to bottom, applying a firm pressure on the surface. After swabbing, the sponge was stomached for 2 min in a stomacher (Seward Stomacher 400, Tekmar, Cincinnati, OH, USA). Two 0·5 ml samples were surface plated on duplicate plates of Plate Count Agar (PCA; Difco), MacConkey (Difco) and Trypticase Soy Agar (TSA; Difco) for the recovery of aerobic bacteria, coliforms and injured coliforms, respectively. TSA plates were incubated at 25°C for 2 h before overlaying with MacConkey agar to recover injured coliforms (Yu *et al.* 1999). All plates were incubated at 37°C for 22–24 h before enumeration of bacterial colonies.

Excision and excision after sponge swab methods

Two 3·14 cm^2 areas (2 cm diameter) of skin and underlying fatty tissue of jowls were cut with a sterile cork borer. The skins (0·2–0·5 cm thick) were aseptically removed using a sterile scalpel and forceps and placed in a sterile Whirl-Pak bag. Additionally, two 3·14 cm^2 areas of skin and underlying fatty tissue were taken from the 10×10 cm^2 area that was swabbed by the sponge swab method mentioned above. Peptone water (10 ml) was added to each sample and the contents were stomached for 2 min. Two 0·5 ml samples were surface plated onto PCA, MacConkey and TSA plates in duplicate. TSA plates were overlaid with MacConkey agar after 2 h incubation at 25°C. All plates were incubated at 37°C for 22–24 h.

Inoculum preparation and sample inoculation

As faecal contamination is likely to occur during evisceration, jowl samples were inoculated with faecal bacteria to study microbial attachment using scanning electron microscopy. Caecal samples were obtained from slaughtered hogs immediately after evisceration. A sterile scalpel was used to make a small incision into the caecum, 1 g of the content was placed into a Whirl-Pak bag containing 9 ml sterile 0·1% peptone water and stomached for 2 min. Appropriate serial dilutions were made and surface plated (0·5 ml) on PCA in duplicate. Plates were incubated at 37°C for 24 h before enumeration of bacterial colonies.

Faecal slurry or a dilution (0·1 ml) was inoculated onto duplicate 3·14 cm^2 round pieces of jowl sample from freshly slaughtered hogs to achieve 10^2–10^7 cfu cm^{-2}, and bacteria were allowed to attach for 30 min at 25°C. One set of the inoculated skin was then removed aseptically with a sterile cork borer, scalpel and forceps, and placed in 100 ml 2% glutaraldehyde–0·1 mol l^{-1} imidazole HCl solution. After sampling, the other set of inoculated skin was stored at 4°C for 24 h before the skin was removed and prepared using the same procedures described above.

Observation of bacterial attachment using scanning electron microscopy (SEM)

For viewing under SEM, both inoculated and uninoculated skins were immersed in 0·1 mol l^{-1} imidazole buffer (pH 7) for 1 h, dehydrated in a graded series of ethanol solutions (50, 80 and 100%) and critical point dried with carbon dioxide. Dried skins were mounted on aluminium stubs with colloidal silver adhesive, coated with a thin layer of gold by DC sputtering and viewed using a JEOL Model 840 A scanning electron microscope.

RESULTS

The chilling procedure reduced counts of aerobic bacteria, coliforms and injured coliforms on swine carcasses regardless of the sampling technique employed. Differences in bacterial numbers between sampling techniques varied as a result of chilling. Pre-chill carcasses had 2·48 log cfu cm^{-2} more aerobic bacteria, 1·34 log cfu cm^{-2} more coliforms and 0·08 log cfu cm^{-2} more injured coliforms than post-chill carcasses by the excision method (Table 1). A consistently higher recovery of aerobic bacteria, coliforms and injured coliforms was observed with the excision technique as compared with the sponge-swabbing technique. Sponge swabbing removed only 11·6%, 0·9% and 11·0% of aerobic bacteria, coliforms and injured coliforms, respectively, from pre-chill carcasses as compared with the skin excision technique. The percentages of sponge swab *vs* excision from post-chill carcasses were 23·9%, 11·1% and 5·0% for aerobic bacteria, coliforms and injured coliforms, respectively. Low numbers of coliforms and injured coliforms were recovered by sponge swabbing (≤ -0.7 log cfu cm^{-2}). Only countable sponge swab samples for aerobic bacteria were found, at 1·61 and 1·07 log cfu cm^{-2} levels from pre-chill and post-chill carcasses, respectively. The bacterial counts obtained by excision taken from sponge-swabbed skin represented the populations of firmly attached bacteria which were not removed by swabbing alone (Table 1).

Table 1 Micro-organisms recovered from skin excision and swab samples of pre-chill and post-chill swine carcasses, $n = 20$

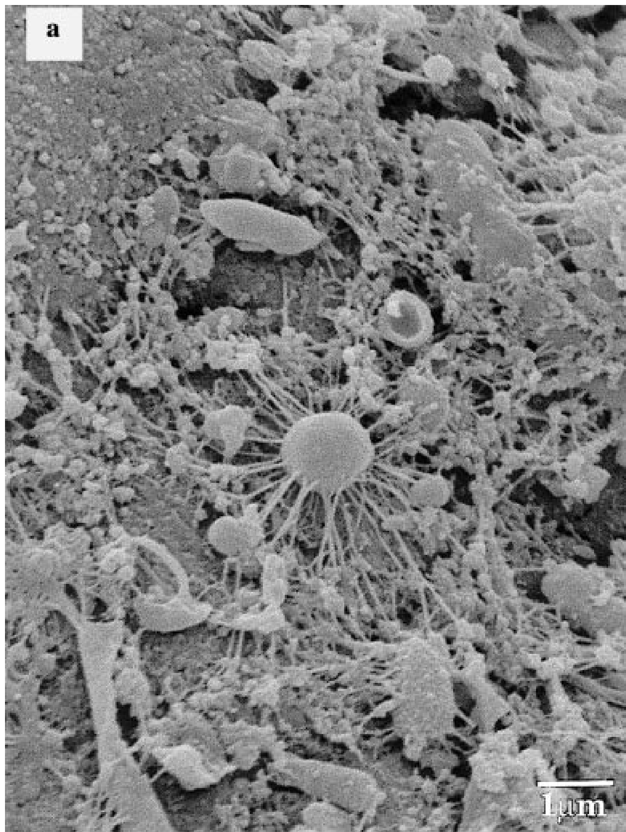
Type of micro-organisms	Numbers of micro-organisms recovered by different sampling methods (log cfu cm^{-2}) at pre-chill and post-chill processes					
	Excision		Swab		Excision after swab	
	Pre	Post	Pre	Post	Pre	Post
Aerobic bacteria	2·54	1·69	1·61	1·07	2·08	0·75
Coliform	1·35	−0·05	−0·70	−1·00	−0·10	−0·20
Injured coliform	0·26	−0·22	−0·70	−1·52	−0·05	−0·20

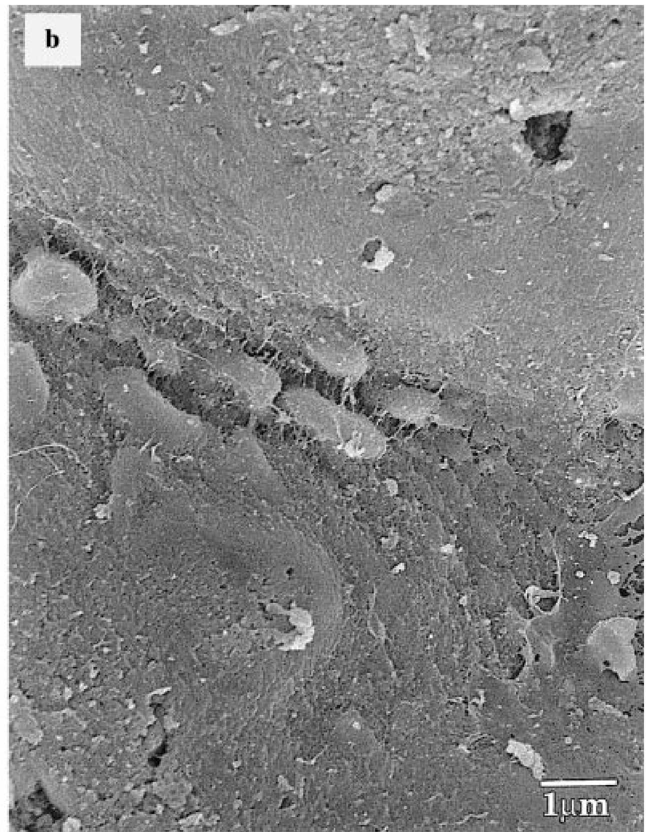
These results, with 10^2–10^7 cfu cm^{-2} faecal bacteria inoculated on pork skin, indicate that analyses of surface-attached micro-organisms by SEM requires a minimum of 10^6 cfu cm^{-2}. Scanning micrographs indicated that bacteria attached to the pre-chill skin surface by extracellular fibrils (Fig. 1a). On the surfaces of post-chill skin, clefts and crevices protect absorbed bacteria from being removed (Fig. 1b). The shapes of bacteria and their fit to the crevices seem to strengthen the attachment of bacteria to post-chill skin (Fig. 1c). Attachment of faecal bacteria to pork skin after air chilling also shows slime production and biofilm formation (Fig. 1d). Changes in muscle structure occurring during post-mortem rigor and the chilling process could allow bacteria to enter deeper layers of the surface tissue (Fig. 2a, b).

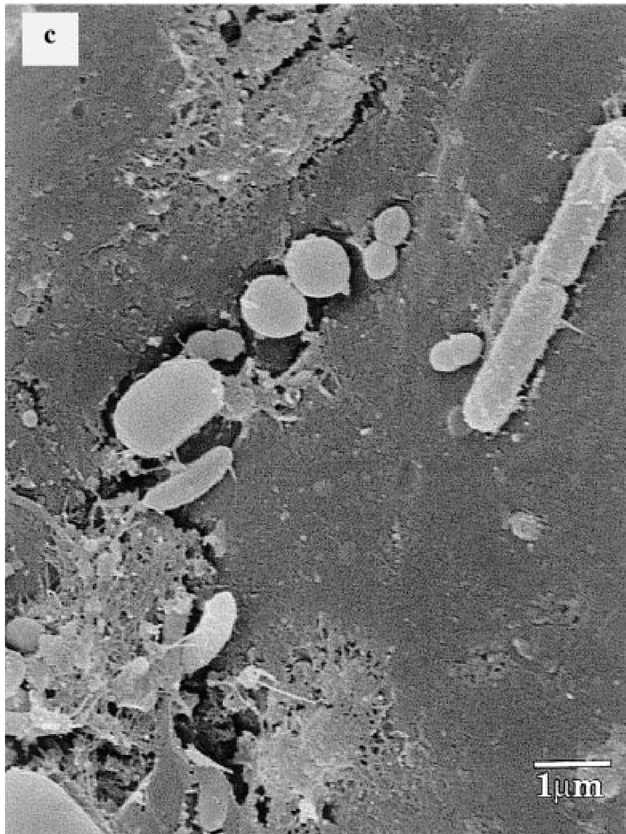
DISCUSSION

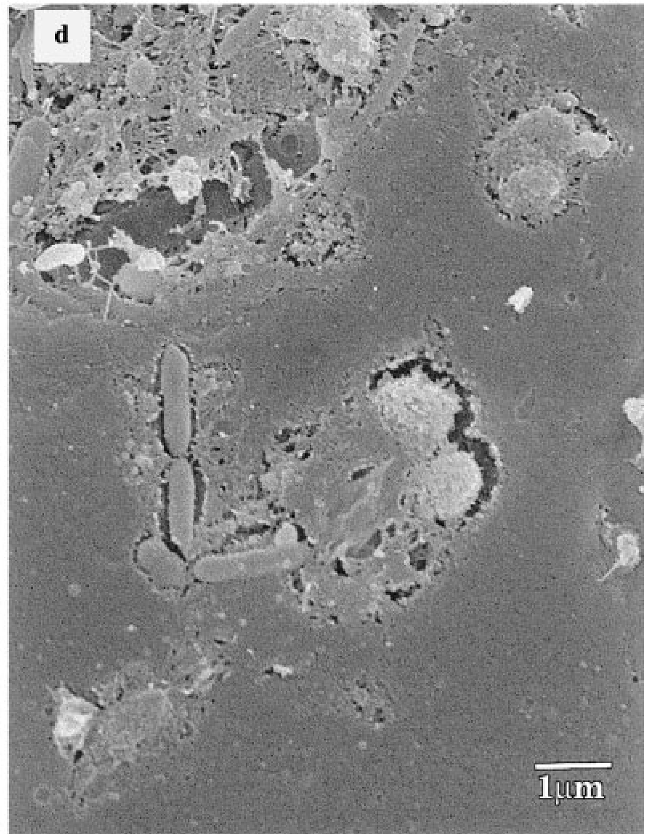
The mechanism of bacterial attachment to pork skin becomes important when attempting removal (Benedict 1988). Ojala (1964) reported that swabbing procedures recovered only 16% of the microbial population compared with cork borer sampling. Ingram and Roberts (1976) indicated that excised and blended surface produced 13–67% and 25–87% higher bacterial counts than swabs when fresh pork and chilled pork belly were sampled, respectively. Dorsa *et al.* (1997) reported that both swabbing and excision recovered similar levels of aerobic bacteria from beef carcasses after 24 h chilling. The present study also demonstrated similar aerobic bacteria counts on post-chill swine carcasses between excision and sponge swabbing (Table 1). An air-chilled swine carcass has experienced a degree of surface dehydration. Lahellec and Colin (1979) and Sauter *et al.* (1979) recovered significantly lower bacterial counts when dry carcass surface was examined by the swab technique. When a sampler device, Rotorinser, using a sponge swab was compared with the excision method for microbiological analysis of pork skin, pre-wetting skin significantly improved its efficiency for bacterial removal (Sharpe *et al.* 1996). The effectiveness of bacterial removal from pork skin by pre-moistened sponges depends on the abrasiveness of sampling and the forces by which the micro-organisms are held in, and released from the sponges during plating.

The excision technique released both loosely and firmly-attached organisms, whereas other techniques, including double swab, agar contact and rinse, mainly recovered loosely adhered bacteria (Notermans and Kampelmacher 1983; Snijders *et al.* 1984). Marshall *et al.* (1977) found that different bacterial populations were obtained when colonizing organisms were removed by swabbing and excision.


a
1μm


b
1μm


c
1μm


d
1μm

◀

Fig. 1 (a) Attachment of a coccus and other rod-shaped faecal bacteria to pre-chill skin shows extracellular fibrils of the bacteria after 30 min at 25 °C; (b), (c) and (d) show how clefts and crevices on the surface of post-chill skin protect attached bacteria from removal, which in turn produce slime and biofilm. Magnification ×10 000

The kinetics of bacterial attachment depend on the bacterial species, meat surface and the temperature of the meat surface. Strength of attachment to beef surface was high for *Brochothrix*, *Clostridium*, *Staphylococcus* and *Yersinia* compared with *Enterobacter*, *Listeria* and *Salmonella* (Benito *et al.* 1997). *Pseudomonas* showed the fastest rate of attachment and *Salmonella typhimurium* the slowest rate on chicken and beef surfaces (Firstenberg-Eden *et al.* 1978). Attachment of *Escherichia coli*, *Ps. putrefaciens*, *Lactobacillus* and *Staphylococcus* spp. to pork skin also differs slightly over a temperature range of 2·5–37°C (Butler *et al.* 1979). After the micro-organisms have become firmly attached to the carcass surface through entrapment or biofilm formation, they resist removal and inactivation (Zottola 1994).

Relatively high inoculum levels ($\geq 10^6$), which normally would not be present in commercial meat processing, were needed in these experiments for satisfactory micrographs. A coccus and other rod-shaped faecal bacteria attached to pork skin by a mass of tangled fibrils of glycocalyx from the bacterial surface (Fig. 1a) (Costerton *et al.* 1978; Firstenberg-Eden 1981). The functions of glycocalyx are to anchor the bacteria to the surface and protect them against stresses such as disinfectant, heat and gamma irradiation (Firstenberg-Eden 1981; Notermans and Kampelmacher 1983; Cabedo *et al.* 1996). The initial attachment of micro-organisms involved in carbohydrate-containing surface components of the organism, and subsequent entanglement within clefts formed by shrinkage of surface tissue during post-mortem rigor of carcasses and the chilling process (Benedict *et al.* 1991; Woody *et al.* 2000), makes bacterial cells difficult to remove (Fig. 1b, c, d). Enlargement of crevices was observed on the post-chill skin, which may be attributed to the drying effect of the constant air blast used for chilling the carcasses (Fig. 2a, b). If a meat surface contains deep channels and crevices, bacteria can become trapped (Notermans and Kampelmacher 1983). From a hygienic point of view, both attached and entrapped bacteria have the same significance.

The attachment of bacteria to meat surfaces has been studied mostly with laboratory-cultured bacteria (Butler *et al.* 1979; Benedict *et al.* 1991; Dorsa *et al.* 1997). However, it is important in slaughter hygiene to use faecal bacteria because contamination of carcasses is mainly from this source. The present work with SEM indicates that faecal bacteria could have started to develop adhering structures as soon as 30 min, at 25°C, after contact with the surface. Butler *et al.* (1979) also showed that much of the bacterial attachment to pork skin occurs during the first few minutes after contamination, although continued attachment occurs over 30 min. The results of the present study demonstrated that microbial attachment and entrapment to pork skin are a function of the physical parameters of pork skin (temperature, moisture and crevices) and biological properties of bacteria (attachment fibrils and biofilm). Therefore, attached or entrapped bacteria cannot be counted adequately by the sponge swabbing method. A better understanding of the mechanisms of microbial attachment to the surface of meat carcasses would aid selection of adequate sampling procedures for estimating numbers and types of bacteria and development of interventions for the removal of spoilage and pathogenic bacteria.

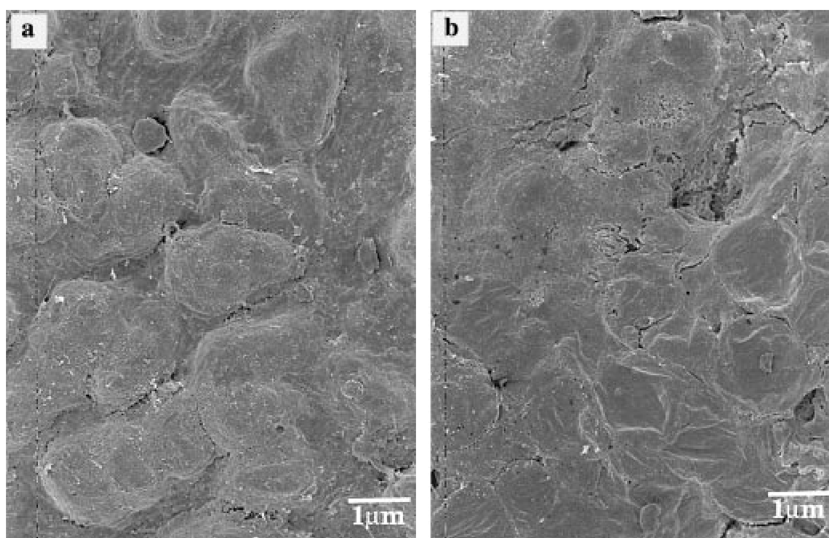


Fig. 2 Morphological changes in the surface structure of pork skin during chilling. (a) Pre-chill skin with fewer crevices; (b) post-chill skin with more deeper and larger crevices. Magnification ×250

REFERENCES

Anderson, M.E., Huff, H.E., Naumann, H.D. *et al.* (1987) Evaluation of swab and tissue excision methods for recovering microorganisms from washed and sanitized beef carcasses. *Journal of Food Protection* **50**, 741–743.

Beachey, E.H. (1981) Bacterial adherence: adhesion–receptor interactions mediating the attachment of bacteria to mucosal surfaces. *Journal of Infectious Diseases* **143**, 325–345.

Benedict, R.C. (1988) Microbial attachment to meat surfaces. *41st Annual Reciprocal Meat Conference Proceedings* **41**, 1–6.

Benedict, R.C., Schultz, F.J. and Jones, S.B. (1991) Attachment and removal of *Salmonella* spp. on meat and poultry tissues. *Journal of Food Safety* **11**, 135–148.

Benito, Y., Pin, C., Marin, M.L., Garcia, M.L., Selgas, M.D. and Casas, C. (1997) Cell surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. *Meat Science* **45**, 419–425.

Butler, J.L., Stewart, J.C., Vanderzant, C., Carpenter, Z.L. and Smith, G.C. (1979) Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. *Journal of Food Protection* **42**, 401–406.

Cabedo, L., Sofos, J.N. and Smith, G.C. (1996) Removal of bacteria from beef tissue by spray washing after different times of exposure to fecal material. *Journal of Food Protection* **59**, 1284–1287.

Costerton, J.W., Geesey, G.G. and Cheng, K.-J. (1978) How bacteria stick. *Scientific American* **238**, 86–95.

Dickson, J.S. and Koohmaraie, M. (1989) Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Applied and Environmental Microbiology* **55**, 832–836.

Dorsa, W.J., Cutter, C.N. and Siragusa, G.R. (1996) Evaluation of six sampling methods for recovery of bacteria from beef carcass surfaces. *Letters in Applied Microbiology* **22**, 39–41.

Dorsa, W.J., Siragusa, G.R., Cutter, C.N., Berry, E.D. and Koohmaraie, M. (1997) Efficacy of using a sponge sampling method to recover low levels of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and aerobic bacteria from beef carcass surface tissue. *Food Microbiology* **14**, 63–69.

Firstenberg-Eden, R. (1981) Attachment of bacteria to meat surfaces: a review. *Journal of Food Protection* **44**, 602–607.

Firstenberg-Eden, R., Notermans, S. and van Schothorst, M. (1978) Attachment of certain bacterial strains to chicken and beef meat. *Journal of Food Safety* **1**, 217–228.

Gill, C.O. and Jones, T. (2000) Microbiological sampling of carcasses by excision or swabbing. *Journal of Food Protection* **63**, 167–173.

Gill, C.O., Leet, N.G. and Penney, N. (1984) Structural changes developing with rigor that facilitate bacterial invasion of muscle tissue. *Meat Science* **10**, 265–274.

Gill, C.O. and Penney, N. (1977) Penetration of bacteria into meat. *Applied and Environmental Microbiology* **33**, 1284–1286.

Ingram, M. and Roberts, T.A. (1976) The microbiology of the red meat carcass and the slaughterhouse. *Royal Society Health Journal* **96**, 270–276.

Lahellec, C. and Colin, P. (1979) Bacterial flora of poultry: Changes due to variations in ecological conditions during processing and storage. *Archiv fur Lebensmittelhygiene.* **30**, 95–98.

Lee, J.Y. and Fung, D.Y.C. (1986) Methods for sampling meat surfaces. *Journal of Environmental Health* **48**, 200–205.

Marshall, R.T., Anderson, M.E., Naumann, H.D. and Stringer, W.C. (1977) Experiments in sanitizing beef with sodium hypochlorite. *Journal of Food Protection* **40**, 246–249.

Notermans, S. and Kampelmacher, E.H. (1983) Attachment of bacteria in meat processing. *Fleischwirtschaft* **63**, 72–73, 76–78.

Ojala, O. (1964) A comparison of sampling methods used for the estimation of surface contamination of meat. *Nordisk Veteriner Medicin* **16**, 231–240.

Sauter, E.A., Jacobs, J.A., Parkinson, J.F. and Ercanbrach, S.K. (1979) Effect of carcass weight and fat thickness of lamb carcasses on surface bacteria counts. *Journal of Food Science* **44**, 1430–1431, 1434.

Scholefield, J., Menon, T.G. and Lam, C.W. (1981) Psychrotroph contamination of pig carcasses. *Proceedings of the 27th European Meeting of Meat Research Workers* **2**, 621–624.

Selgas, D., Marin, M.L., Pin, C. and Casas, C. (1993) Attachment of bacteria to meat surfaces: a review. *Meat Science* **34**, 265–273.

Sharpe, A.N., Isigidi Bin Kingombe, C., Watney, P., Parrington, L.J., Dudas, I. and Diotte, M.P. (1996) Efficient nondestructive sampler for carcasses and other surfaces. *Journal of Food Protection* **59**, 757–763.

Snijders, J.M.A., Janssen, M.H.W., Gerats, G.E. and Corstiaensen, G.P. (1984) A comparative study of sampling techniques for monitoring carcass contamination. *International Journal of Food Microbiology* **1**, 229–236.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G. and Zehnder, A.J.B. (1987) The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology* **53**, 1893–1897.

Woody, J.-M., Walsh, R.A., Doores, S., Henning, W.R., Wilson, R.A. and Knabel, S.J. (2000) Role of bacterial association and penetration on destruction of *Escherichia coli* O157:H7 in beef tissue by high pH. *Journal of Food Protection* **63**, 3–11.

Yu, S.-L., Bolton, D., Laubach, C., Kline, P., Oser, A. and Palumbo, S.A. (1999) Effect of dehairing operations on microbiological quality of swine carcasses. *Journal of Food Protection* **62**, 1478–1481.

Zottola, E.A. (1994) Microbial attachment and biofilm formation: a new problem for the food industry? *Food Technology* **48**, 107–114.