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THE EFFECTS OF ORGANIC ACIDS AND MICROCOLONY FORMATION ON THE ADHESION OF MEAT SPOILAGE ORGANISMS

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the degree of Masters of Science

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Short title

Effects of organic acids and microcolony formation on adhesion

Frederic D'Aoust

ABSTRACT

Beef tendons were sectioned into 60 µm-thick slices (1 cm^2) and deposited onto glass cover slips. These meat slices were flooded with a cell suspension of either *Pseudomonas fluorescens*, *Enterobacter agglomerans*, or *Moraxella osloensis* in distilled water and adhesion allowed to occur. The adhesion experiments were also conducted on agar-covered slides to evaluate the effect of the nature of the substratum on adhesion. The non-adherent organisms found on either surface tested (meat or agar) were removed by flushing liquid over the slide. The slides were then incubated in a moist atmosphere at 30°C. Once the presence of microcolonies had been established microscopically, the slides were mounted into flow chambers and the surface flushed with distilled water to ascertain the effects of bacterial proliferation on adhesion. In other experiments, the influence of acetic, citric, and lactic acid rinses on cell adhesion and subsequent cell proliferation was evaluated. Microcolony formation was shown to reduce the adhesion strength of *Enterobacter agglomerans* and, to a lesser extent, that of *Moraxella osloensis* while increasing that of *Pseudomonas fluorescens*. The probable determinant of adhesion strengths of microcolonies is exopolymer synthesis. A minimal decrease in bacterial adhesion and microcolony formation was observed with the use of organic acid rinses. The bactericidal activity and effect on bacterial proliferation increased with increasing concentration and rinse times of the organic acids. The extent of the adhesion reductions suggests that the preservation action of organic acids is due to cell death and not adhesion inhibition.

RESUME

Des tendons de boeuf ont été sectionnés en tranches de 60 µm d'épaisseur (1 cm^2) et déposés sur des lamelles de microscope. Ces sections de viande ont été inondées avec une suspension de *Pseudomonas fluorescens*, *Enterobacter agglomerans*, ou *Moraxella osloensis* tout en permettant à l'adhésion de se produire. Les experiences d'adhesion ont aussi été effectuées avec des lamelles recouvertes d'agar afin d'évaluer l'effet de la nature de la surface sur l'adhesion. Les bactéries non-adhérentes presentes sur les deux surfaces utilisées (viande, agar) ont été retirées en rinçant la surface des tranches avec de l'eau distillée. Les lamelles ont alors été incubées dans une atmosphère humide a 30°C. Une fois la présence des microcolonies établie au microscope, les lamelles ont été montées dans des chambres d'observation et la surface de ces lamelles a été subjectée à un rinçage d'eau distillée afin de constater l'effet de la prolifération bactérienne sur l'adhésion. Durant d'autres experiences, l'influence d'un rinçage d'acide acétique, citrique, et lactique sur l'adhesion bactérienne et l'éventuel prolifération cellulaire ont été évaluées. La formation de microcolonies a eu l'effet de réduire la force d'adhérence d'*Enterobacter agglomerans* et, à un moindre degré, celle de *Moraxella osloensis* tout en augmentant celle de *Pseudomonas fluorescens*. La synthèse d'exopolymer est probablement la cause déterminante de l'effet sur la force d'adhérence observé avec les microcolonies. Une diminution minimale a été observée dans la formation de microcolonies ainsi que dans la force d'adhérence, avec l'utilisation de rinçages d'acide organique. L'activité bactéricide et l'effet sur la prolifération bactérienne ont augmenté avec l'augmentation des temps de rinçage et la concentration des acides organiques. L'ampleur des

réductions d'adhérence suggère que l'action de conservation des acides organiques soit due à la mort des microorganismes et non pas à l'inhibition d'adhérence.

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INTRODUCTION

Microbial attachment is a widespread phenomenon in nature believed to be the initial step in infection. The phenomenon of bacterial adhesion is manifested in many forms and has been studied by scientists in many disciplines. Interest in this process has increased in the past 15 years because of the role adhesion plays in several animal and human diseases, in dental plaque formation, as well as participating in the fouling of man-made surfaces (Marshall, 1976). The process of adhesion is not always seen as detrimental to a system. In waste water treatment systems and biofermentation processes, bacterial adhesion is beneficial and research is being conducted to increase the occurrence and/or enhance the efficiency of this mechanism. However in terms of spoilage of food products and the study to increase the shelf life of these products, bacterial adhesion is a negative attribute.

The attachment of microorganisms has been investigated under various conditions, including extensive studies in aquatic and marine environments (Johnson and Sieburth, 1976; Fletcher and Floodgate, 1973), in terrestrial habitats (Balkwill and Casida, 1979; Marshall *et al.*, 1975) and on meat surfaces (Chung *et al.*,1989; Dickson and Crouse, 1989; Dickson and Koohmaraie, 1989). The terminology used by various authors to describe this phenomenon differs greatly and for this reason it is necessary that the various terms that will be used throughout this thesis be defined. The accumulation of microorganisms on a surface as a result of gravimetric forces is considered to be **deposition** or **sedimentation**. When force is required to separate the bacterium from a surface, the bacterium is said to be

adhered (adhesion) to the surface (Marshall, 1984). Thus the **strength of adhesion** of a microorganism can be quantified as the work required to remove that cell from the surface. Adhesion may be either **specific**, i.e. a lock-and-key mechanism of interaction between adhesins on the cell surface and receptors on the solid surface; or **nonspecific**, i.e. hydrogen, ionic, or hydrophobic bonding. Furthermore, when there is synthesis of an extracellular polymer, which has the effect of strengthening the adhesive bonds, the process is called **attachment** (Fletcher *et al.*, 1980). Our current understanding of the mechanisms of attachment of various bacteria is still very limited. Greater knowledge of these principles could lead to solutions to the problems that microbial attachment produces, notably in the area of food contamination and spoilage.

In terms of food protection and food preservation the microbial contamination of meat continues to be an important issue. One of the current methods employed to ensure good meat quality involves washing and spray-sanitizing carcasses with either water and/or organic compounds. For the method to be useful, adhered bacteria must be removed or destroyed. Information is available on adhesion rates and strengths of different individual bacteria to meat. However, microbial spoilage of meat is influenced not only by the initial interaction of bacteria to the substrate, but also by subsequent proliferation after adhesion. This study was undertaken to elucidate the influence of microcolony formation and organic acid rinses on bacterial adhesion.

LITERATURE REVIEW

MECHANISMS OF BACTERIAL ADHESION

General principle of bacterial adhesion

The process of bacterial adhesion to natural or synthetic substrata has been shown to be a complex phenomenon and has generated various theories to try and explain it. Among these theories we find adhesion explained in terms of a) thermodynamic interactions involving interfacial free energies, b) electrokinetic interactions based on the physicochemical properties of surfaces e.g. hydrophobicity, and c) specific molecular interactions e.g. lectin-like interactions (Busscher and Weerkamp, 1987).

The first substantive study of bacterial adhesion was conducted by Zobell (1943), who recognized the possibility of macromolecular conditioning films modifying surfaces prior to microbial adhesion. He proposed that the phenomenon of adhesion was biphasic. The initial step is believed to result in the loose reversible union of the cells to the surface mediated by London van der Waals attraction, electrostatic attraction of the two surfaces or by a gain in entropy. At this point the main process involved would be deposition (Marshall *et al.*, 1971). This initial step is followed by a time-dependent, irreversible association mediated by the production of extracellular polymers (Fletcher and Floodgate,

1973). Once the interaction becomes irreversible the process can then be defined as being attachment. However it is important to note that at anytime throughout the two stages the process can be referred to as adhesion, whether it be deposition or attachment. As previously discussed this has proved to be a fertile source of arguments between the different fields involved in studying adhesion. For the purpose of this study the terms adhesion, attachment and deposition will be used as defined previously.

A growing consensus is that adhesion is not mediated by a single interaction but rather by several different interactions, such as electrokinetic and thermodynamic forces (Bouttier *et al.*, 1997). In addition to these forces some bacteria are thought to be entrapped by small channels and crevices formed during meat processing (Notermans and Kampelmacher, 1983). Because of the production of extracellular substances, composed in large part of acidic polysaccharides (Fletcher and Floodgate, 1973), the strength of attachment during the second phase was shown to increase (Notermans *et al.*, 1979). It was also suggested by Costerton *et al.* (1978) that bacteria attach due to tangled fibres of polysaccharides or branching sugar molecules, referred to as the "glycocalyx", extending from the bacterial surface. In addition to providing a mechanism of adhesion this complex would also served to protect the microorganism against various sources of stress.

Subject to the laws of thermodynamics, bacterial adhesion would only be favoured if the process itself causes the thermodynamic function (i.e., total free energy of the system) to decrease. This function is expressed as:

$$\Delta adhG = G_{BS} - G_{BL} - G_{SL} < 0$$

where ΔadhG represents the Gibbs energy of adhesion ($J \cdot m^{-2}$), which is the change in the total free energy of the system induced by adhesion, and G_{BS}, G_{BL}, and G_{SL} are respectively the bacterium-surface, the bacterium-liquid, and the surface-liquid interfacial free energies (van Loosdrecht *et al.*,1989).This has lead to the development of two theories, thermodynamic and electrokinetic, that take into account the values of the mathematical models previously mentioned.

The thermodynamic theory

This theory implies that the potential free energy of bacterial adhesion will be minimized at equilibrium. In systems where both the electrostatic and specific biochemical interactions can be considered as negligible, the change in the free energy function (ΔF^{adh}) can be defined by the equation shown below:

$$\Delta F^{adh} = \Delta adhG = \gamma_{BS} - \gamma_{BL} - \gamma_{SL}$$

in which ΔF^{adh} is the change in the free energy function ($J \cdot m^{-2}$) and the bacterium-surface, the bacterium-liquid, and the surface-liquid interfacial tensions are expressed as γ_{BS}, γ_{BL}, and γ_{SL}, respectively (Absolom *et al.*, 1983).Therefore adhesion will occur if the process itself causes the thermodynamic function to decrease. If on the other hand the thermodynamic function is shown to increase then the process of adhesion would not be favoured.

The use of a fortran computer program (Neumann *et al.*, 1980b) permitted the generation of tables of the various interfacial tensions associated with different contact angles (Neumann *et al.*, 1980a). The interfacial tension relationship has also been suggested to be correlated with their polar and apolar components, represented either in the form of a geometric-mean equation (Fowkes, 1964) or a harmonic-mean equation (King *et al.*, 1985). Each of these two equations combined with Young's equation which characterizes the state of equilibrium of a liquid drop on a solid surface, make calculating γ_{BS}, γ_{BL}, γ_{SL} and ΔF^{adh} values possible.

The thermodynamic model proposes that the extent of bacterial adhesion depends on the magnitude of the change in the free energy of adhesion (ΔF^{adh}), in such way that increasing negativity of the value would result in the production of stronger van der Waals interactions, due to the spontaneous nature of the adhesion process. Therefore, theoretically in situations where the surface tension of the bacteria-vapour interface exceeds that of the liquid-vapour interface, bacterial adhesion would increase with increasing surface tension of the substratum-vapour interface, or, in other words, decreasing surface hydrophobicity. On

the other hand, in situations where these same two surface tensions are equal, the resulting free energy of adhesion would be zero, suggesting that bacterial adhesion under such circumstances is not dependent on the surface tensions related to the substratum (Absolom *et al.*, 1983). It would be important to note that this thermodynamic model does not predict the number of bacteria that will adhere to any given surface, but rather the extent to which the process will occur, i.e., greater or lesser (Absolom, 1988).

The thermodynamic theory stipulates that electrostatic forces are not involved in bacterial adhesion. However there is substantial experimental evidence to suggest that electrostatic forces, including hydrophobic interactions (McGuire and Krisdhasima, 1993; Svanberg *et al.*, 1984; Gibbons and Etherden, 1983), and electrical charge (Dickson and Crouse, 1989) play an important role in adhesion. Other data reported by Facchini (1990), demonstrated constant adhesion of microorganisms in situations where the bacterial surface energy equalled the surface energy of the liquid medium, hence disproving the suggestion of non adhesion stipulated by the thermodynamic theory. In addition the thermodynamic theory does not seem to take into account the reversible characteristic of adhesion observed during numerous experiments (van Loosdrecht *et al.*, 1989).

The electrokinetic theory

This theory is based on the fact that particles are subjected to forces. These forces can arise from the interaction of the charged electric double layers located around the

particles. In addition to these forces, interactive electrostatic forces can be generated when superficially located ionogenic groups of the particles are placed in an environment containing polar, ionizing substances whch are present in most biological cultures (Ho, 1986). Rutter and Vincent (1980) proposed an electrokinetic approach to the understanding of bacterial adhesion, they formulated the following expression:

$$Gi(h) = \int -fi(h)\, dh$$

in which the Gibbs free energy (Gi) is related to the particle-surface separation distance (h) of the particle-liquid-surface system. This expression is based on the previous work by Deryagin and Landau (1941); Verway and Overbeek (1948) that postulated the DLVO model of adhesion (Deryagin, Landau, Verway and Overbeek theory).

The electrokinetic theory, unlike its thermodynamic counterpart, takes into account the presence of a reversible and irreversible phase, characteristic of most bacterial adhesions (Fletcher,1988). In addition this theory supports the findings that Gibbs free energy will decrease with increasing ionic strength. Therefore bacterial adhesion is favoured with increasing ionic strength due to the fact that the double electrical diffusible layer decreases when the ionic strength is increased. This relation between adhesion and ionic strength was demonstrated by Stanley (1983) and also by Jones *et al.* (1981). However, the Hamaker constant (A) necessary to obtain the value of the van der Waals interactions has been shown to be difficult to evaluate. To solve this problem, plausible values of A are used in order to

produce curves related to the variations of the free energy with the distance of separation of the particle and surface. These curves permitted the observation of the two-step adhesion kinetics.

The problems of the electrokinetic theory are mostly related to the inability to properly describe the irreversible adhesion observed by Fletcher (1988) and Jones *et al.* (1981) at greater distances (> 10 nm) of separation . Moreover the theory takes into account only long and medium-range interactions and does not take into consideration short-range interactions (Busscher and Weerkamp, 1987).

Potential model

The limitations of the theories provided thus far have lead scientists to reevaluate the models of adhesion, new models are presently being proposed that take into account these restrictions (Busscher and Weerkamp, 1987; Ho, 1986). It is important to note that none of these models have been successfully verified experimentally, and so remain theoretical. One of these models is represented in Figure.1.

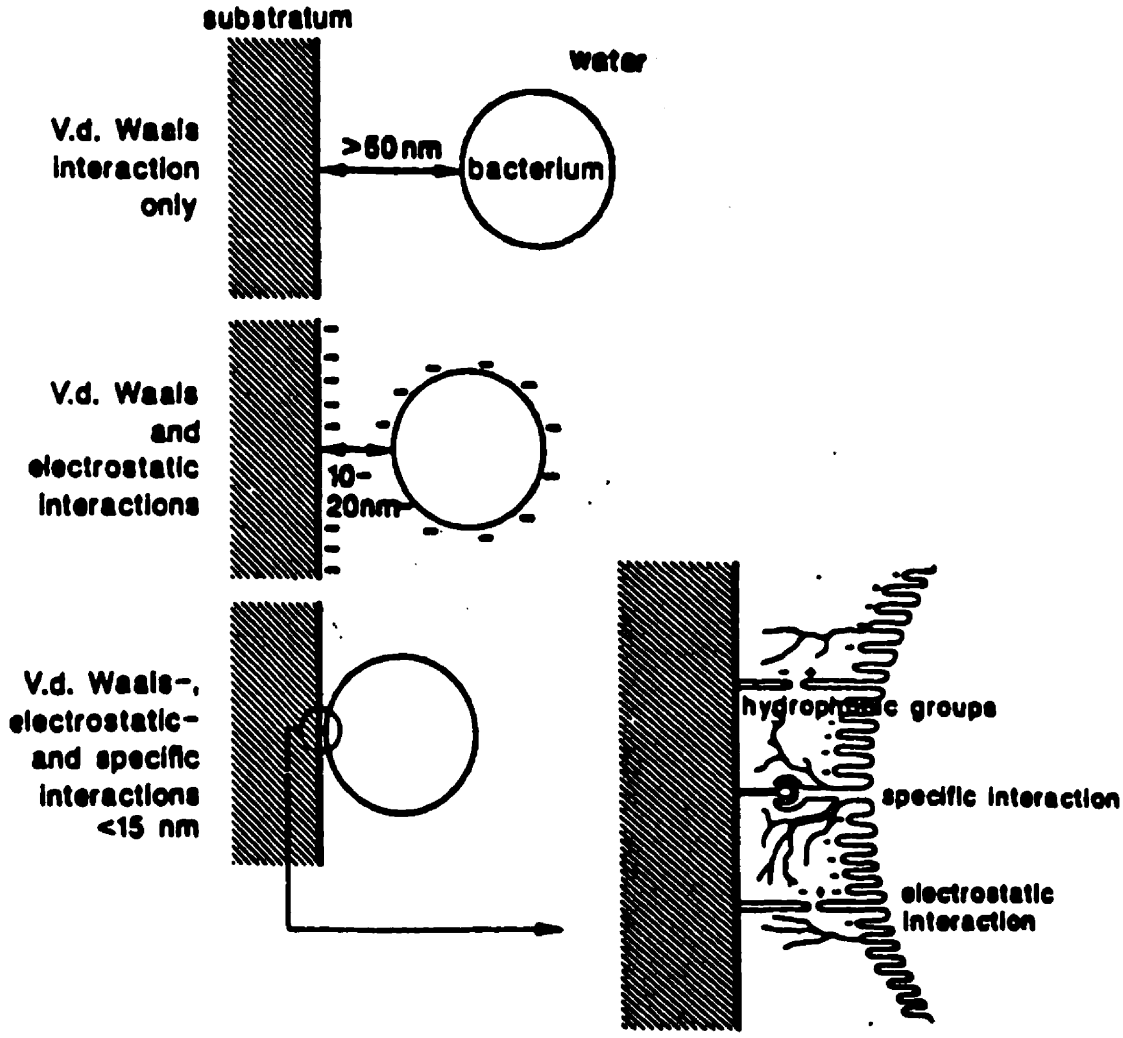

Figure 1. Schematic representation of interactions involved in bacterial adhesion to solid substrata. (Busscher and Weerkamp, 1987)

This model postulates that only van der Waals forces are involved in adhesion when the separation distance is greater than 50 nm. When the separation distance is in the range of 10 and 20 nm, the presence of additional electrostatic repulsion forces result in a reversible secondary minimum adhesion. The process becomes irreversible after a certain length of time due to the rearrangement on the bacterial cell leading to specific short-range interactions. It is at this point that hydrophobicity plays its role by removing the water film present between the interacting surfaces and thus allowing for a greater amount of specific short-range interactions to occur. These specific short-range interactions will lead to irreversible bonding when the potential energy barrier has been overcome at a separation distance less than 1.5 nm (Ho, 1986).

For this model or any other model proposed in the future to be acceptable it will have to account for the occurrence of adhesion despite positive values of Gibbs free energy, adhesion in the presence of electrostatic repulsion, strain-specific relations between the number of adhering cells and the surface free energy of the substratum, hydrophobicity, specific surface components, and the two-step kinetics of bacterial adhesion.

MEASUREMENTS OF BACTERIAL ADHESION

Interfacial tensions

The complexity of the adhesion mechanism and the theories involved have generated a variety of measurements and methodologies in order to calculate the different variables. In respect to the thermodynamic theory, the interfacial tension between liquid and air are simply measured with the use of a tensiometer, resulting in values from 20 $mJ \cdot m^{-2}$ to 80 $mJ \cdot m^{-2}$, water being situated at approximately 73 $mJ \cdot m^{-2}$ (Gerson and Zajic, 1979). In addition to surface tension the thermodynamic theory requires the determination of the contact angles on the solid surfaces, which can be accomplished in a variety of ways. Most of the time the measurements are acquired through sessile drop techniques (Defrise and Gekas, 1988; Busscher *et al.*, 1984; Fletcher and Loeb, 1979). Other methods employed to calculate contact angles include the liquid-liquid method (Schurch *et al.*, 1981; Gerson, 1980) and the captive bubble technique (Andrade *et al.*, 1979).

Hydrophobicity

Contact angles can also be used as an estimate of bacterial cell surface hydrophobicity because of the common association with van der Waals forces (van Oss *et al.*, 1980). There are conflicting opinions as to whether the hydrophobicities of surfaces were important in the process of bacterial adhesion (Al-Makhlafi *et al.*, 1995; Rijnaarts *et al.*,

1993; Dickson and Koohmaraie, 1989; Absolom, 1988; van Loosdrecht *et al.*, 1987) (see p.24). As a consequence several researchers thought it important to definitively establish the role, if any, this factor has on bacterial adhesion. To this end several different methods were developed to calculate this parameter. These include phase partition, hydrophobic interaction chromatography, and salt aggregation (Benito *et al.*, 1997; van der Mei *et al.*, 1987; Gerson and Zajic, 1979). The standard method for the determination of bacterial hydrophobicity remains BATH (Bacterial Adhesion To Hydrocarbons) (Vanhaecke *et al.*, 1990). This method relies on the principle that hydrophobic cells will tend to associate with liquids of greater hydrophobicity.

Cell surface properties

The importance of the electrical properties of the cell surface in the adhesion process was briefly discussed in the section on the electrokinetic theory. The methods used to quantify these properties are, once again, numerous, and include electrofocusing, electrophoresis, and electrostatic interaction chromatography. (Pederson, 1980; Sherbet and Lakshmi, 1973; Einolf and Carstensen, 1967).

Adhesion strength

One of the increasingly important variables to be measured in adhesion remains the strength of adhesion which is defined as the force to be neutralized in order to free an adherent particle from the surface to which it is bound. Two methods of measuring adhesion strength of bacteria to surfaces are available. The first, proposed by Fowler and Mckay (1980), involves the constant flow of a bacterial suspension through a radial flow chamber composed of two parallel discs separated by a small gap. Since the shear force exerted on the walls decreases, from the centre outwards, a point is reached where bacteria will no longer adhere. This point correlates to the shear stress representative of the strength of adhesion. The second method consists in colonizing a capillary tube with adherent cells, followed by rinsing with water at increasing flow rates. This whole process is observed under light microscopy. The shear force associated with the flow rate where the total bacterial population is released is a measure of the maximum strength of adhesion of that population (Powell and Slater, 1982).

Bacterial adhesion quantification

Direct population counts remain the most utilized method to study bacterial adhesion. By itself direct bacterial quantification is not very indicative of bacterial adhesion. However, when bacterial enumeration is coupled with treatments designed to influence the adhesion process, the outcome is a quantification of bacterial adhesion. In general, cells on the

substratum before and after the treatment are counted and the difference related to adhesion. Great care should be taken in designing the experiment to ensure that the differences in counts can be attributed to a single variable.

Standard light microscopy has been used to enumerate adherent bacteria to glass (Piette and Idziak, 1991a; Uyen *et al.*, 1985), eukaryotic cell monolayers (Hood and Zottola, 1988; Kleeman and Klaenhammer, 1982), and meat (Piette and Idziak, 1992, 1991a, 1991b,1989; Thomas and McMeekin, 1984). To facilitate the enumeration process bacterial stains are often employed, e.g., basic fuchsin (Piette and Idziak, 1989). When light microscopy is ineffective, cells could be treated with fluorescent dyes (acridine orange) and enumerated using standard epifluorescence microscopy (Fletcher and Loeb, 1979). In addition microorganisms can be coated with a thin layer of gold and examined by scanning electron microscopy (Frantamico *et al.*, 1996).

Nonmicroscopic methods include standard viable count enumerations. Bacteria can also be labelled with tritium and their numbers estimated using liquid scintillation (Fletcher, 1986) or spectrophotometry (Wood, 1980). The numbers of microorganisms can also be determined by evaluating the amount of protein (Delaquis *et al.*, 1989), lipopolysaccharide (Dexter, 1979) or adenosine triphosphate (Vanhaecke *et al.*, 1990). It is important to note that all these techniques fail to distinguish between entrapped cells and adhered cells.

BACTERIAL ADHESION TO MEAT

Immersion experiments.

The fact that bacteria adhere to chicken skin and muscle surfaces has been well documented in large part using immersion experiments. Notermans and Kampelmacher (1975, 1974) were the first to study this phenomenon in studies describing bacterial attachment to poultry skin during washing of the carcasses. The experiment involved immersing the poultry meat in bacterial suspensions for various times. The carcasses were then rinsed for a 30 s period with water to remove the loosely or nonadherent bacteria from the surface. The bacteria left on the meat, considered to be adherent bacteria, were quantified using standard dilution and plating methods. They observed a linear relationship between immersion time and bacterial adhesion (Figure 2, Notermans and Kampelmacher, 1975). Another important observation was the presence of bacteria in the residual water film surrounding the meat surface after immersion. When this water film surrounding the carcass was not removed (no 30 s rinse), it was observed that the plot did not intersect the origin; whereas, when this water film was removed by rinsing the sample with water, the origin was intersected. Therefore, the point where the ordinate and the solid line intersect in Figure 2. represents the amount of bacteria present in the water film. This was the first indication that not all bacteria found in close proximity to the meat interacted with the surface.

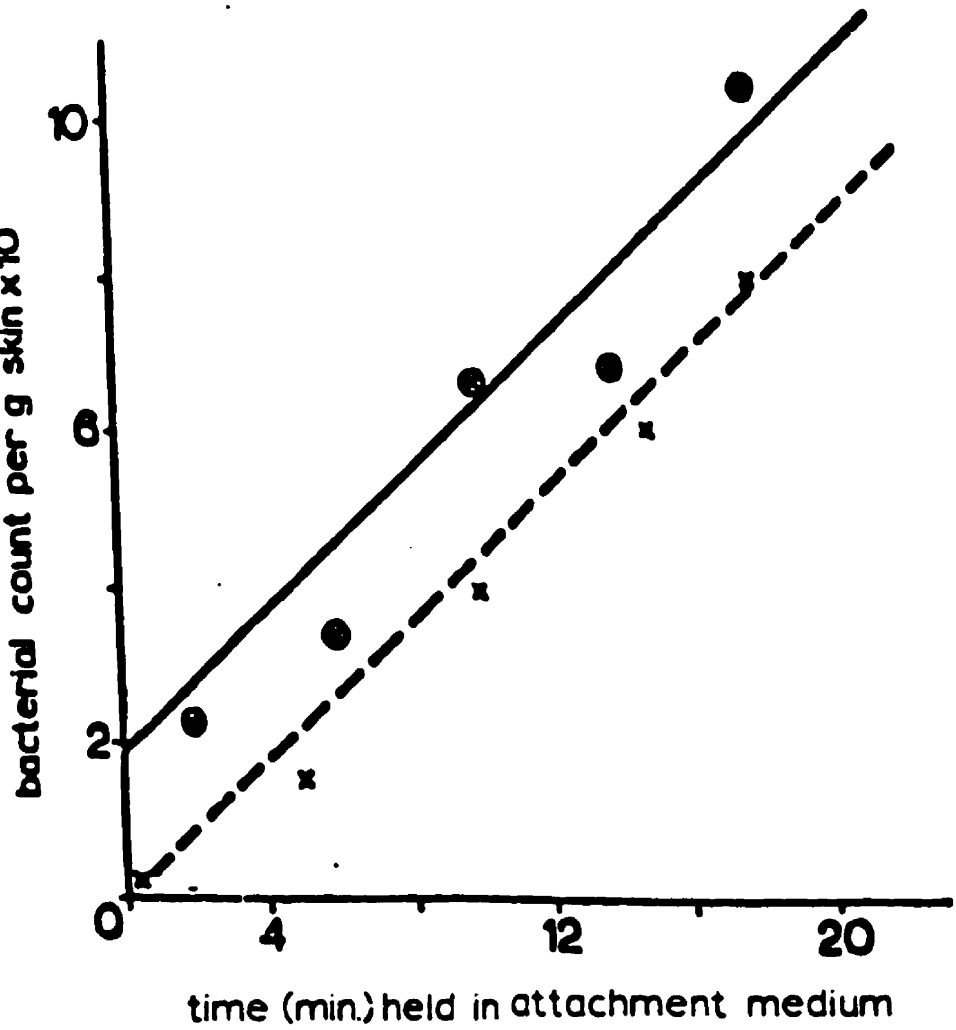

Figure 2. Attachment of *Pseudomonas putrefaciens* to skin of broilers at 21 °C (—) without removal of the water film (---) with removal of the water film. (From Notermans and Kampelmacher, 1975).

The technique was slightly modified and was used to quantify the extent of bacterial adhesion on various other types of meats (Lillard, 1988; Farber and Idziak, 1984; Thomas and McMeekin, 1984). All the methods discussed thus far, failed to established the true nature of the association between the bacteria and the surface of the meat after immersion. No particular attention was placed on whether or not the bacteria were in actual contact with the meat surfaces or just in the residual liquid film left on the meat after immersion.

Electron microscopy studies

The use of scanning electron microscopy (SEM) has provided information on the microbial morphology of food-borne bacteria as well as emphasizing a possible role of the skin microtopography in the process of bacterial adhesion. Although initial SEM studies produced results supporting the Notermans and Kampelmacher (1975) theory that the bacteria not rinsed off are adherent, the interpretation of the data can be questioned. In order to use this methodology the sample must first be fixed and dried, and as a consequence nonadherent bacteria or very loosely adherent microorganism can also be fixed to the substratum. As a consequence this technique alone is insufficient to study bacterial adhesion.

The use of SEM has nevertheless made possible the observation that bacteria are present throughout the suspending fluid film when fixation was carried out with osmonium tetroxide vapours (Thomas and McMeekin, 1981). With the use of SEM, Butler *et al.* (1980) noticed the formation of erosion troughs or craters surrounding the adherent bacteria. At the

time it was suggested that enzymatic degradation of the host surface by the bacteria resulted in the production of these crevices. They also observed the formation of an extensive matrix of extracellular material interconnecting the bacterial cells, resulting in an increase of the strength of adhesion. These formations are now known to be caused by the sample preparation, because these structures were not found during cryoscanning electron microscopy (McMeekin, 1986). Using SEM techniques, Thomas and McMeekin (1981) were able to observe the various microtopographic characteristics of meat surface, resulting in the discovery of effects generated by immersion in water of the meat. They concluded that this processing step gave rise to the formation of large channels and crevices and also the production of a dense mat of swollen collagen fibres absent before immersion. These large crevices would have the effect of physically entrapping the nonadherent organisms and protecting them from subsequent removal. Hence most of the methodology employed to date would considered these entrapped bacteria as adherent when in fact they are not.

Entrapment of bacteria on meat surfaces

The formation of crevices, channels and swollen collagen fibres, previously reported as the result of immersion, contributed to the entrapment of bacteria. The entrapped bacteria, not being exposed to the surface, were protected against rinsing, hence would appear as adherent even though they may not be in contact with the meat surface. With this new finding it became evident that there were now two different mechanisms in which bacteria could resist removal by conventional cleaning techniques. Since both entrapped and "truly"

adhered bacteria will eventually cause the spoilage of meat, it was important to determine the quantitative proportions of bacteria found to be adhered as opposed to being simply entrapped to help in the development of effective food sanitation systems. Experiments conducted by Thomas and McMeekin (1984) supported by results obtained by Lillard (1988), demonstrated that most of the bacteria were retained by means of entrapment. In order to elucidate the mechanism of bacterial adhesion, a new technique was proposed by Piette and Idziak (1989), and involved the use of a flow chamber and measurable shear forces coupled with staining and light microscopy techniques. The use of thin slices of meat transparent to light make this method possible. The thin meat slices also eliminate the possibility of having crevices or channels entrapping bacteria. The specially designed flow or observation chamber is presented in Figure 3. The chamber is composed of two polycarbonate plates (C and D) fastened to an aluminium base plate (E) by means of several screws. The central polycarbonate plate (D) was cut to create a channel to allow introduced liquids to circulate through the chamber. The external plates (C and E) are equipped with windows composed of a glass cover slip fixed (tape and silicone) to a polycarbonate frame. Gaskets cut from Parafilm sealing material (American Can Co., Greenwich, Conn.), using the central plate as a template, were inserted between the plates to prevent leakage. The shear force was shown experimentally to be even across the chamber as well as being directly proportional to the fluid flow rate. In addition, the flow was characterized as being laminar (Piette and Idziak, 1989).

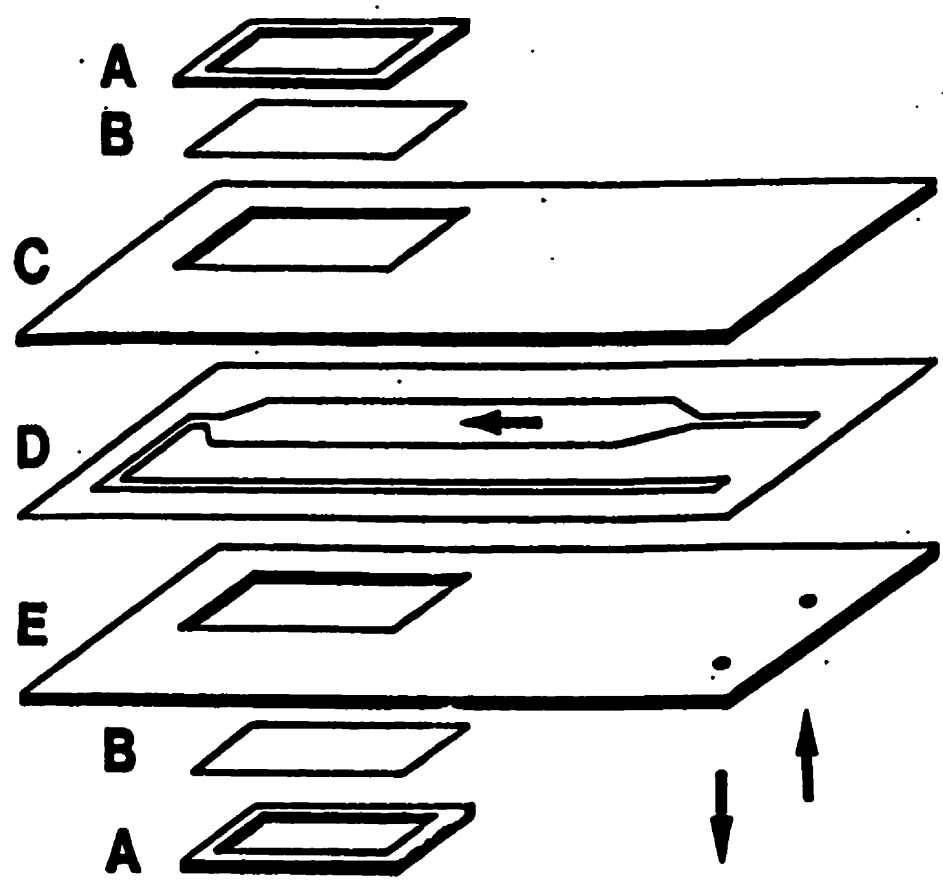

Figure 3. Expanded view of the observation chamber. Windows: A. Window frame; B. Glass cover slip. Main body: C. External polycarbonate plate (2.4 mm thick); D. Central polycarbonate plate (0.5 mm thick); E. External polycarbonate plate (2.4 mm thick). Arrows indicate the direction of the flow. (Piette and Idziak, 1989).

Strength of adhesion

As previously discussed the strength of adhesion remains one of the most interesting and important variables to study. Several different equations have been employed over the years to try and quantify bacterial adhesion strength. All of these equations share the common characteristic of comparing the number of loosely attached to the total number of bacteria present on the meat surface. One of the first of these parameters, S-value, was introduced by Firstenberg-Eden *et al.* (1978) and expressed as:

$$S = \log_{10} (\text{total bacterial}) - \log_{10} (\text{loosely attached bacteria})$$

The total number of bacteria and the number of loosely attached bacteria are calculated experimentally. The total bacterial population is usually determined by the blend method. This technique involves the homogenization of the meat sample and subsequent enumeration of the bacterial population present in the resulting suspension. On the other hand, the number of loosely attached organisms are estimated using the rinse method. In this case the meat sample is agitated in a known volume of peptone water. The suspension is then subjected to bacterial enumeration using standard dilution and plating techniques. The logic behind these calculations is that only with the use of the blend method can the total population be quantified; whereas loosely attached bacteria are the only organisms counted when employing the rinse method (Notermans *et al.*, 1979).

A high S-value would be associated with strong attachment, whereas a low S-value would indicate weak attachment. This method to determine the S-value was later slightly altered to account for microorganisms present in the liquid film surrounding the meat surface. In this instance the S-value was calculated as:

$$S = \log_{10}C - \log_{10}[A - (B - C)]$$

where: A= the number of loosely attached bacteria including those contained in the water film surrounding the meat sample.
B= The total population present on the meat as well as in the water film surrounding the meat sample.
C= the total population present minus the number of organisms found in the water film surrounding the meat sample (Farber and Idziak, 1984).

By taking into account the number of bacteria in the water film, it was felt that the values obtained using this equation better represented the adhesion strength of the organism studied. Finally Dickson and Koohmaraie suggested the use of the S_R value to measure the strength of adhesion.

$$S_R = \frac{\text{(firmly attached bacteria)}}{\text{(total bacteria)}}$$

With this method the results would range between zero for loosely attached bacteria, and 1 for firmly adhered organisms (Dickson and Koohmaraie, 1989).

Factors involved in bacterial adhesion to meat

Because not very much is known on the mechanisms of adhesion and attachment, most of the factors that influence this phenomenon are also poorly understood. The general consensus is that the following factors exert an influence on the process of adhesion of bacteria to meat surfaces: temperature, pH, salts, the nature of the meat surface, the organisms and growth conditions, and motility.

Temperature appears to have a minimal effect on adhesion by indirectly affecting other factors such as the growth conditions or even motility. Adhesion experiments with pork skin and five different bacterial cultures (*Escherichia coli*, *Pseudomonas putrefaciens*, *Erwinia herbicola*, *Lactobacillus* sp., and *Staphylococcus* sp.) generally demonstrated little effect of temperature variations from 2.5 to 37°C on adhesion. *E. herbicola* however adhered more at 26 to 37°C than at 3-7.5°C (Butler *et al.*, 1979). Similar results were obtained by Firstenberg-Eden (1981). The slight effect observed was associated with increases in bacterial activity and flagellar activity. Increases in both of these activities would have the effect of increasing the incidence of contact of bacteria with the surface and therefore increase adhesion (Notermans and Kampelmacher, 1974). Similarly, the influence of pH on bacterial adhesion acts indirectly by affecting the organism and meat surfaces rather than directly acting on the process of adhesion. Initially it was believed that differences in adhesion to broiler skin as a result of varying the pH of the adhesion medium was due to change in the mobility of the organisms (Notermans and Kampelmacher, 1974). Subsequent microscopic

observation indicated that the differences were the result of marked changes in the surface characteristics of the skin of the broiler chickens (Butler *et al.*, 1979). This new interpretation of the effects of pH on adhesion was further supported when only minor differences in bacterial adhesion to pork skin were recorded with five bacterial cultures (*Escherichia coli*, *Pseudomonas putrefaciens*, *Erwinia herbicola*, a *Lactobacillus* sp., and a *Staphylococcus* sp.) over a pH range of 4.0 to 8.8 (Butler *et al.*, 1979). Whether or not these differences could have beeen attributed to variations in surface characteristics of the two different meat surfaces was never investigated.

The mechanism by which sodium chloride influences bacterial adhesion remains poorly understood. When adhesion is carried out on inanimate surfaces, increasing the amount of cations in the adhesion medium was shown to increase the rate of adhesion of several organisms (Stanley, 1983; Jones *et al.*, 1981; Marshall *et al.*, 1971). It has been speculated that the cations are absorbed by the generally negative surface of bacteria. Increasing the concentration of these cations would have the effect of reducing the electrical double layer, hence allow closer contact between the surfaces before repulsion takes place (Fletcher, 1988). However this mechanism is far more complex when dealing with adhesion to animate surfaces such as meat. Adhesion rates of *Lactobacillus* sp. (Barriga and Piette, 1996) and *Salmonella typhimurium* (Walls *et al.*, 1993) to collagen casings were shown to decrease with increasing salt concentrations; whereas, *Pseudomonas fluorescens* adhesion to tendon slices increased with increases in NaCl concentration (Piette and Idziak, 1992). It is important to note that none of the differences were significantly different. Significant variations in adhesion rates

were only reached with salt concentrations exceeding 100 mM (Piette and Idziak, 1992; Vanhaecke *et al.*, 1990; Lillard, 1988; Campbell *et al.*, 1987; Thomas and McMeekin, 1981). The reduction in bacterial adhesion at high salt concentration has been attributed to denaturation of the outer bacterial cell layer (Fletcher and Floodgate, 1976). Even though bacterial attachment is prevented from occurring, sodium chloride does not prevent subsequent bacterial proliferation and spoilage of the meat after initial contamination, because it has little if no effect on entrapped bacteria.

Bacterial hydrophobicity was found to play a crucial role in bacterial adhesion, where greater hydrophobicity of the organism resulted in greater attractive forces and higher levels of adhesion (Absolom, 1988; van Loosdrecht *et al.*, 1987). These findings were further supported by Dickson and Koohmaraie (1989) who found a correlation between hydrophobicity and adhesion. In contrast, Al-Makhlafi *et al.* (1995) observed that an increase in surface hydrophobicity could not be correlated to an increase in the entropic driving force for adhesion. A possible mechanism to explain the influence of hydrophobicity could involve removal of water between surfaces and hence diminishing separation distances. The surfaces in closer proximity would allow for short-range specific interactions to occur (Ho, 1986).

The adhesion of bacteria has been observed on various surfaces of meat, from pork skin (Butler *et al.*, 1979), sausage casings (Barriga and Piette, 1997), to beef fat (Bouttier *et al.*, 1997; Chung *et al.*, 1989) and teats of cows (Notermans *et al.*, 1979). Because of variations in the methodologies used no definitive correlations could be proposed between

the different factors under investigation. It has been suggested, in instances where comparisons were possible, that the nature of the meat plays an important role in the extent of bacterial attachment (Hood and Zottola, 1997a; Firstenberg-Eden, 1981). However, when comparing the level of bacterial adhesion to beef adipose as opposed to muscle tissues no significant differences were recorded (Dickson and Frank, 1993; Chung *et al.*, 1989; Dickson and Khoohmaraie, 1989). Similarly, no differences were found in bacterial adhesion rates of *Acinetobacter* LD2, *Pseudomonas fluorescens*, and *Lactobacillus* sp. to tendon or fat surfaces (Piette and Idziak, 1989). In addition, the number of adherent *Escherichia coli* O157:H7 cells was determined not to be significantly different ($P > 0.05$) on adipose tissue (5.31 ± 0.08 CFU·cm^{-2}) and muscle tissue (5.48 ± 0.09 CFU·cm^{-2}) (Cabedo *et al.*, 1997). A better understanding of the surface characteristics of meats would help in elucidating the influence, if any, that the nature of the meat surface has on microbial adhesion.

The actual microorganisms used in the adhesion experiments are believed to be the predominant factor influencing bacterial adhesion. Large differences in strength and amount of attachment have been reported for different bacteria. The mechanism to account for these differences has still not been elucidated. Morphology and Gram type of the organisms have been discounted as contributing factors, although Dickson and Koohmaraie (1989) have suggested that the degree of surface hydrophobicity, hence the cell surface charge of the bacteria, influences adhesion. The findings of Piette and Idziak (1991a) do not agree with this hypothesis. They reported slight differences in adhesion strength of several bacteria on fat and tendon surfaces but these differences could not be correlated to the hydrophobicity or surface

charge of the organisms. Thus the principal attributes of bacteria influencing their adhesion to surfaces remains in doubt.

Regardless of the type of surface used as a substratum it has been well established that the amount of adherent microorganisms is directly proportional to the initial bacterial concentration of the inoculum (Dickson, 1991; Chung *et al.*, 1989; Firstenberg-Eden, 1981; Butler *et al.*, 1979). Several studies have concluded that bacteria may alter their cell surface characteristics and adhesion properties with physiological age (Fletcher, 1977; Zvyagintsev *et al.*, 1977). These findings were contested by results demonstrating no differences in adhesion rates of organisms in their in mid to late or stationary phases of growth. In addition, none of the bacteria tested demonstrated any changes in hydrophobicity or electrophoretic mobility with growth (Piette and Idziak, 1991a). Even death of the cell by UV, γ rays, or heat did not produce any differences in bacterial adhesion (Piette and Idziak, 1992).

Growth conditions of the organisms were also investigated to determine their possible role in bacterial adhesion. No significant differences in adhesion were found with various organisms when grown in laboratory media or on chicken meat (Notermans *et al.*, 1980). In contrast recent findings concluded that along with the type of organism used the growth conditions employed also had a significant influence on bacterial adhesion to inanimate surfaces (Hood and Zottola, 1997b). Microorganisms were grown in either tryptic soy broth (TSB), 1% reconstituted skim milk (RSM), or RSM with 1% sucrose (RSM + S). *P. fragi* and *L. monocytogenes* cells adhered in greater numbers when grown in RSM + S; whereas,

S. typhimurium demonstrated higher adhesion rates when grown in TSB. These findings support the previous findings in which the adhesion rates of seven meat spoilage organisms were found to be different when observed in deionized water and in spent growth media (Piette and Idziak, 1991a).

The possible role of flagella and fimbriae in the mechanism of bacterial adhesion has been the focus of a number of studies (Bouttier *et al.*, 1997; Notermans *et al.*, 1980; Notermans and Kampelmacher, 1974). The adhesion of *S. typhimurium* to poultry skin was observed using cells either with flagella present or with flagella removed. The results led to the conclusion that fimbriae and flagella are not directly involved in the adhesion process, though a combination of these and other factors may be involved (Lillard, 1986). In another study it was showed that although deflagellated cells of *Pseudomonas aeruginosa* adhered extensively to tendon slices, cells with flagella adhered in greater numbers under similar conditions (Piette and Idziak, 1991b). Microscopic observations, revealed that motility indirectly influences attachment by increasing the number of bacteria reaching the surface and providing first contact with the meat surface. The adhesion resulting from flagella interaction is reversible, and motility is not essential for motile bacteria to adhere to surfaces. Thus the flagellum does not appear to posses specific binding characteristics.

It is a well established fact that fresh meat offers bacteria a good environment for growth (Roberts, 1990). Because of its innate characteristics and the circumstances of its production, it is recognized that raw meat will always have a certain bioload on its surface

unless it has been subjected to some form of biocidal process. While most of the microbial population that ends up in association with the meat consists of nonpathogenic organism, the possibility for the presence of pathogenic organisms still remains. Most of the contamination observed during the slaughter/dressing process has been attributed to the removal of the hide or pelt and viscera (Roberts, 1980). To extend shelf life (Lambert *et al.*, 1991) and also to protect the public from possible foodborne illness (Bean and Griffin, 1990), hygienic practices in the preparation and storage of meat products are essential (Kochevar *et al.*, 1997). The argument that many food products cannot be made safe for human consumption simply by attention to hygenic processing and attention to maintaining good sanitary practices during processing has been advanced by Mossel and Drake (1990). This statement stimulated an increase in the research for possible biocidal agents used in the decontamination of meat.

The decontamination of food animal carcasses by washes containing a variety of sanitizing agents has been reviewed by Dickson and Anderson (1992). Some of the techniques under scrutiny include the use of organic acids, trisodium phosphate, hot water, and hyperchlorinated water (Gorman *et al.*, 1995a,b; Kenney, 1992; Smith and Davey, 1990). Amongst these possible methods of decontamination, short chain organic acids have been designated as the most logical agents to spray directly onto carcasses to produce an antimicrobial effect. The reasons that make organic acids favourable candidates are that they are inexpensive, are GRAS compounds (generally regarded as safe), are environmentally friendly and are also naturally occurring. Taking these factors into account, acetic acid has been approved for commercially decontaminating carcasses in the USA (Anderson and

Marshall, 1989). In addition to acetic acid, the most commonly investigated acids have been citric and lactic(Smulders *et al.*, 1986). All three of these acids have been demonstrated to reduce bacterial populations, upon their application on animal carcasses (Cutter and Siragusa, 1994; van Netten *et al.*, 1994; Ockerman *et al.*, 1974).

The bactericidal activity of organic acids is attributed to the concentration of the undissociated acid present (Cutter and Siragusa, 1994). It is the undissocited acid that is readily diffusible through the cell membrane (Ouattara *et al.*, 1997). Meat spoilage bacteria like most organisms associated with foods are neutrophilic. Therefore they must retain an internal pH slightly higher than that of the growth medium to maintain their pH gradient that provides energy to the cells. To accomplish this, the cells must continuously remove protons from their cytoplasm to ensure the maintenance of this pH gradient (Booth, 1985). Organic acids disrupt this balance by lowering the internal pH of the cell. The cell is eventually lysed when it can no longer compensate. Along with the amount of organic acid found in undissociated form, the magnitude of the effect has also been attributed to the acid concentration, increasing concentrations being associated with greater bactericidal activity. However concentrations should never exceed 3%, since this level is associated with production of off flavours and colours (Smulders *et al.*, 1986; Eustace *et al.*, 1979).

The results obtained on the effectiveness of organic acids as sanitation agents are summarized in Table 1. The range of reductions in the number of surviving bacteria after organic acid treatment is between 1 to 3.5 $\log_{10}$ CFU/area.

Table 1. Examples of the studies conducted on the use of organic acids to decontaminate meat carcasses.

Acid Type	Tissue type	Concentration	Effect	Reference
Lactic + acetic mix	lamb	6 - 24% (v/v)	≤ 1 $\log_{10}$ APC[a] reduction	Okerman *et al.* 1974
Acetic + proprionic mix	swine	60:40 mix	2 $\log_{10}$ APC reduction	Reynolds and Carpenter 1974
Lactic	calves	0.75 - 2.5% (v/v)	no significant reductions	Woolthuis and Smulders 1985
Lactic, acetic, ascorbic, citric, mixture	beef	1% lactic, 2% acetic, 0.25% citric, 0.1% ascorbic	no significant reductions	Acuff *et al.* 1987
Lactic	veal tongues	2% (v/v)	▪ 3 $\log_{10}$ APC reduction	Visser *et al.* 1988
Lactic	beef	3% (v/v)	▪ 1.8 $\log_{10}$ APC reduction @ 70°C ▪ 1.1 $\log_{10}$ *E. coli* reduction @ 70°C ▪ 1.2 $\log_{10}$ APC reduction @ 25°C ▪ 0.4 $\log_{10}$ *E. coli* reduction @ 25°C	Anderson and Marshall 1990
Acetic	beef	2% (v/v), 55°C	≥ 2 $\log_{10}$ APC reduction	Dickson and Anderson 1991
Lactic	swine	1% (v/v), 55°C	▪ 1 $\log_{10}$ APC reduction	Prasai *et al.* 1991
Lactic	beef	1% (v/v), 55°C	▪ 1 $\log_{10}$ APC reduction	Prasai *et al.* 1991
Lactic	beef	1% (v/v), 55°C	▪ 1 $\log_{10}$ APC reduction	Prasai *et al.* 1991
Acetic	beef	1.5 - 3% (v/v)	< 1.0 $\log_{10}$ APC reduction	Anderson *et al.* 1992
Lactic	beef	1.5 - 3% (v/v)	< 1.5 $\log_{10}$ APC reduction	Anderson *et al.* 1992
Acetic + lactic	beef	2% acetic + 1% lactic	< 1.0 $\log_{10}$ APC reduction	Anderson, Marshall, and Dickson 1992
Acetic + lactic	beef	2% acetic + 1% lactic	< 1.0 $\log_{10}$ APC reduction	Anderson *et al.* 1992
Acetic	beef	2% (v/v) in alginate gel	▪ 1.5 $\log_{10}$ APC reduction	Siragusa and Dickson 1992
Lactic	beef	1.7% (v/v) in alginate gel	▪ 1.3 $\log_{10}$ APC reduction	Siragusa and Dickson 1992
Lactic and acetic	beef	1, 3, 5% (v/v)	≤ 1.75 $\log_{10}$ APC reduction	Cutter and Siragusa 1994
Lactic and acetic	beef	1, 3, 5% (v/v)	▪ 3.5 $\log_{10}$ APC reduction	Cutter and Siragusa 1994

[a] APC: aerobic plate count

(From Siragusa, 1996)

From the data in Table 1, it becomes evident that the effects of organic acids in reducing bacterial loads on carcasses are not consistant. The studies that support the use of short chain organic acids as effective meat carcass sanitizing agents show reductions up to 3.5 log (Outtara *et al.*, 1997; Frantamico *et al.*, 1996; George *et al.*, 1996; Breen *et al.*, 1995). However these results reflect a reduction in viable organisms and do not necessarily imply removal of the microorganisms from the meat surface. In addition, a majority of the investigations that reported more than a 2 log decreases in microbial population employed acid concentration exceeding 3%. With the use of lower acid concentrations (0.1 to 1%) very limited bactericidal activity, if any, was observed on meat surfaces (Acuff *et al.*, 1987). No significant difference was also noted by Prasai *et al.* (1991) when comparing acid sprayed meat cuts with controls. Organic acids have been shown to be less effective against certain organisms, e.g. *Salmonella typhimurium* (Greer and Dilts, 1992). This finding is problematic, since the bacterium mentioned is a pathogen. The meat industry would never consider a sanitizing agent that only reduces nonpathogenic giving a competitive advantage to pathogenic organisms. Therefore even though the large portion of the data on organic acids found in the literature showed some level of bactericidal activities the debate on the potential use of organic acids in the industry still continues.

CONCLUSION

It should be apparent at this time that still very little is known with regards to the actual mechanisms of bacterial adherence. Not very much is understood about adhesion to meat surfaces, and the little that is, is not understood completely. The new models that have been proposed to explain bacterial adhesion still have to be proven experimentally, in order to replace the inadequate thermodynamic and electrokinetic models. The factors influencing the attachment of microorganisms will have to be studied in greater detail, especially the surface characteristics of the bacteria and the surface to which they adhere. In recent years the focus of a majority of the research on bacterial adhesion to meat has been less on elucidating the mechanism and more on ways to interfere with it. This has lead to several investigations on the effectiveness of organic acids as possible sanitizing agents in the meat industry. However the methodology employed to evaluate the effectiveness of these compounds has relied on the number of viable organism recovered after treatment. The problem associated with this approach is that no information was acquired as to whether or not these compounds have indeed influenced bacterial adhesion and removed the adherent bacteria from the surface. The problem is further complicated by the knowledge that live and dead bacteria adhere equally well to meat surfaces. Therefore it would be a novel idea to study the effects of organic acids on meat spoilage organisms using a methodology that takes into account the total microbial population, both live and dead cells, present on the meat surface. This would provide useful information on the actual mechanism involved in treatment with organic acids.

OBJECTIVES

The objectives of this research are:

1. Study the effects of bacterial proliferation and microcolony formation on the adhesion strength of meat spoilage organisms.

2. Ascertain the efficacy of organic acids in the removal of adhered bacteria to meat.

3. Determine if the effectiveness of organic acids in reducing the microbial population is due to bactericidal activity or interference with the bacterial adhesion process.

MATERIALS AND METHODS

Organisms and cultures

Three different meat spoilage organisms were chosen on the basis of their strength of adhesion. The cultures were *Pseudomonas fluorescens*, *Enterobacter agglomerans* and *Moraxella osloensis* which represent strongly, moderately and moderately-weak attaching bacteria, respectively (Piette and Idziak, 1991). *Pseudomonas fluorescens* was isolated from beef tendons stored at room temperature. The cells were grown for three consecutive 24-h periods in Brain Heart Infusion (BHI, Difco Laboratories, Detroit, Mich.) to produce standardized cultures. Working cultures were obtained after a 16-h incubation of the standardized culture. All incubations were at 30°C without agitation. On the day of the experiment, the 16-h bacterial suspensions were harvested and subjected to two consecutive washing protocols. The procedure involved centrifugation of the culture at 10 800 × g for a period of 10 min and resuspension in distilled water. Standard dilution techniques were then used to prepare ~10^7 CFU·ml^{-1} suspensions.

Agar and Meat slides

Glass cover slips (24 × 60 × 0.15 mm) were flooded with 0.5 ml Brain Heart Infusion agar. The slides were used immediately after the agar had solidified to avoid dehydration of the media. Beef tendons acquired fresh from a local meat-processing plant were vacuum

packaged, frozen, and stored at -20°C. When needed, blocks of meat (1 × 1 × 0.5 cm) were removed and sectioned (60 µm thick slices) using a Tissue-Tek II cryostat (Ames, Div. Miles Laboratories, Inc., Naperville, Ill.) adjusted to -30°C. The slices were deposited onto glass cover slips and stored at -4°C for later use.

Organic Acids

Acetic acid (glacial, 99.7% [wt/vol]; Fisher Scientific, Nepean, ON), citric acid (monohydrate, 99.7%, AnalaR, Toronto, ON) and DL-lactic acid (63% [wt/wt], Sigma, St-Louis, MO) solutions were prepared to final concentrations of 0.1, 0.25 and 0.5% [wt/vol] and pH 5.0.

Design of the observation chamber

In previous experiments performed by Piette and Idziak (1989) a novel method of studying bacterial adhesion was introduced. The procedure required the use of a newly designed observation chamber composed of two polycarbonate plates fastened to an aluminum base plate by means of several screws. This chamber was slightly altered to increase the resistance to leakage and to improve the chambers durability. The observation chamber employed during this study consisted of three polycarbonate plates bound together using a strong adhesive. The central plate was cut to provide channels through which the introduced liquids (bacterial suspension, dyes, distilled water) could circulate. Similar to the old chamber,

the plates adjacent to the central plate are equipped with windows composed of glass cover slips fixed to polycarbonate frames held to the plates with tape and silicone. The operation of this chamber is described in previous adhesion studies (Piette and Idziak, 1989)

Effects of microcolony formation on bacterial adhesion

Microcolony adhesion experiments were conducted on two different substrata, agar and meat surfaces. All experiments were performed at room temperature (20 ± 2 °C). Slides with agar or meat slices were flooded with 2.5 ml of the desired bacterial suspension outside the chambers and left to stand for 10 min to allow bacteria time to adhere. The non-adherent cells were removed by flushing the slide with distilled water. The slides were then incubated at 30 °C for 0, 3, or 6h within a moist atmosphere. After incubation, the adherent single cells and microcolonies were counted (adherent cells before treatment) using direct light microscopy. The slides with adherent microorganisms were mounted in the adhesion flow chambers (Piette and Idziak, 1989). This was followed by a 10 min continuous rinse of distilled water (110 $\mu l \cdot s^{-1}$). For controls, inoculated and incubated agar or meat slides were mounted in the chambers, the chambers filled with water and then left standing for 10 min. After the elapsed time the chambers were drained. A staining solution (0.25% [wt/vol] basic fuschin) was then introduced and left in the chambers for 5 min. The excess dye was removed subsequently with a 1 min distilled water rinse. The slides were then removed and the number of adherent organisms counted (adherent cells after treatment). Care was taken in distinguishing between single cells and microcolonies. A schematic representation of this

adhesion experiment is shown below in Figure 4.

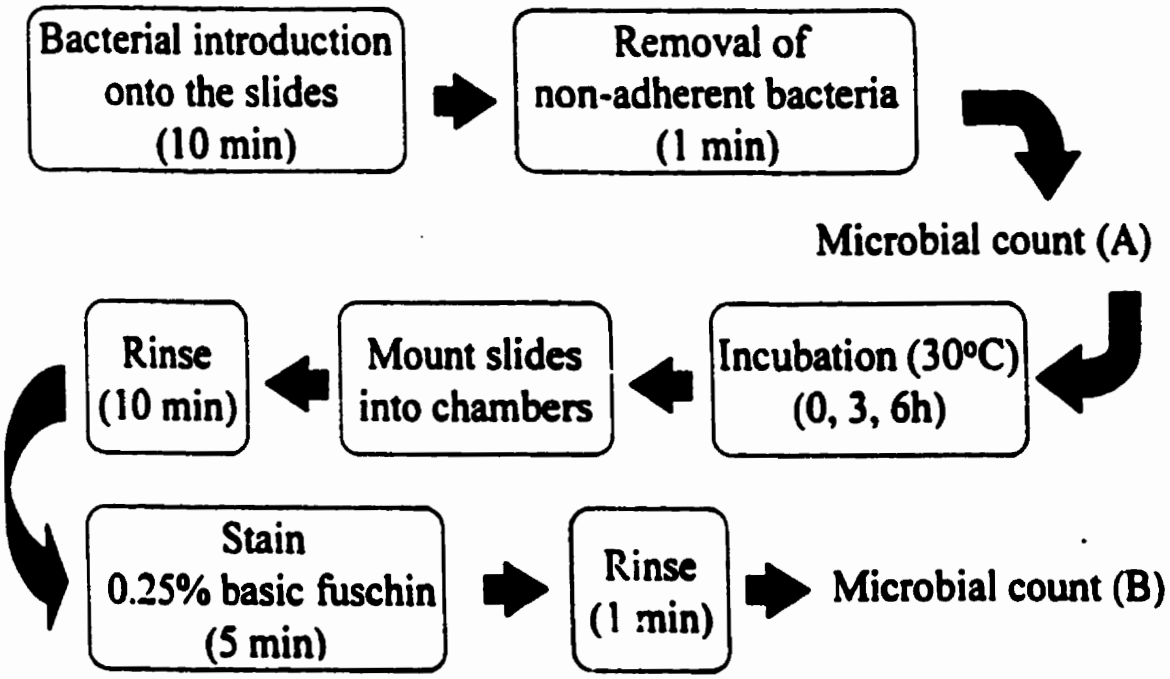

Figure 4. Schematic of experimental method to study the effects of microcolony formation on the adhesion strength of meat spoilage organisms.

Effects of organic acids on bacterial adhesion

Fresh meat slides were mounted and sealed in adhesion flow chambers as previously described (Piette and Idziak, 1989). The chambers were then filled with the *Pseudomonas fluorescens* bacterial suspension and left to stand for 10 min to allow adhesion to take place. The non-adherent cells were removed by rinsing the chamber with distilled water for 1 min (110 $\mu l \cdot s^{-1}$). At this point the number of adherent cells before treatment was determined by direct microscopic enumeration. The samples in the chambers were then subjected to organic acid rinses of 15, 30 and 45 s. As controls, inoculated meat slices were rinsed with distilled water for the same periods of time. The three concentrations of each of the organic acids tested were 0.1, 0.25, and 0.5% [wt/vol]. All meat slides were then stained and examined microscopically to establish the number of adherent cells after treatment. The schematic representation of this adhesion experiment is depicted in Figure 5.

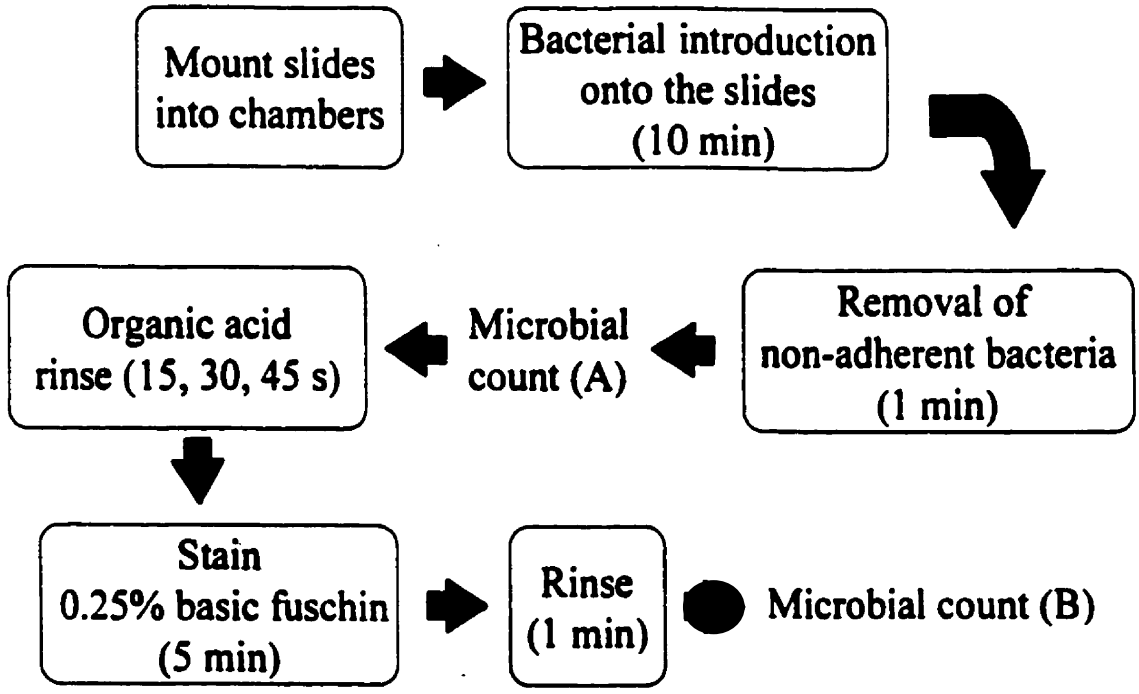

Figure 5. Schematic of experimental method to study the effects of organic acids on the adhesion strength of *Pseudomonas fluorescens*.

Effects of organic acids on microcolony formation

Newly prepared agar slides were flooded with a working culture of *Pseudomonas fluorescens* and adhesion was allowed to take place for a period of 10 min. As in previous adhesion experiments, the non-adherent cells were removed by flushing the slide with distilled water for 1 min. Once the samples with adherent single cells on their surface were mounted in the observation flow chambers, the various organic acid solutions were introduced for contact times of 15, 30 or 45 s. As controls, inoculated agar slides mounted in the chamber were treated with distilled water for the same periods of time. After the diverse treatments the samples were rinsed with distilled water and the chambers drained of their contents. A microscopic count was performed after the various contact times to determine the number of initial adherent microorganisms present before bacterial proliferation. All the agar slides were then incubated at 30°C for 1, 2, and 3h within a moist atmosphere to permit the formation of microcolonies. After each incubation time, the number of microcolonies present was established by direct microscopic enumeration. The schematic representation of this adhesion experiment is outlined in Figure 6.

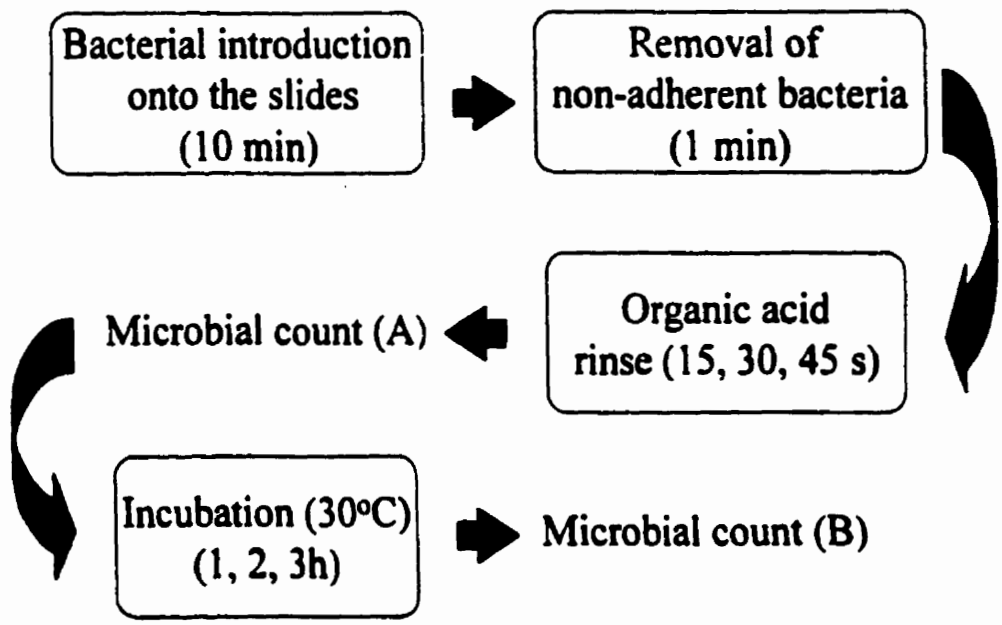

Figure 6. Schematic of experimental method to study the effects of organic acids on bacterial proliferation.

Sampling technique

All experiments were performed in triplicate. The distribution of the bacteria on the meat or agar surfaces was observed microscopically at 125-200X magnifications using standard light microscopy techniques. Furthermore the number of bacteria and/or microcolonies were counted in 20 different fields (800X ; 0.11mm^2 total area) evenly distributed over the different surfaces tested. The adherent bacteria present before and after treatment could be observed unstained. However this step was rendered much easier when the samples were subjected to a basic fuschin stain. Previous work conducted by Piette and Idziak (1989) demonstrated that colouration with basic fuschin had no effect on the adhesion process. Photographs were then taken for permanent records.

Data interpretation

The extent of bacterial adhesion was determined using a slightly altered version of the S_R equation (Dickson and Koohmaraie, 1989). Adhesion strength in this instance measures the magnitude of removal of bacteria by the various treatments and is reported as %Adhesion reduction. The %Adhesion reduction is calculated as follows:

$$\%\text{Adhesion Reduction} = \left[1 - \left(\frac{\text{Number of adherent bacteria after treatment (Microbial count B)}}{\text{Number of adherent bacteria before treatment (Microbial count A)}} \right) \right] \text{X } 100$$

where 0% indicates no bacterial removal and would therefore be indicative of very little influence on adhesion strength, and 100% total removal of the initial bacterial population present or a strong influence on the strength of adhesion.

The effects of the various organic acids on microcolony formation is presented in terms of %Inhibition (see below). The percentage of microcolonies formed with acid treatments was compared to the percentage reached with control treatments. This method was employed in order to take into account factors other then the organic acids used that might affect bacterial proliferation. Hence %Inhibition can be described as follows:

$$\%\text{Inhibition} = \left[\left(\frac{\text{Number of microcolonies formed after incubation times (control)}}{\text{Initial number of adherent bacteria after control treatment}} \times 100 \right) - \left(\frac{\text{Number of microcolonies formed after incubation times (acid)}}{\text{Initial number of adherent bacteria after acid treatment}} \times 100 \right) \right]$$

% Microcolonies formed under control conditions

% Microcolonies formed under acid conditions

The calculation reflects the difference in microcolony formation in the presence and absence of acids. In such a way that a greater %Inhibition value would be directly correlated to a greater influence of the acids tested on bacterial proliferation. In other words, for organic acids to be considered as effective sanitizing agents they must be associated with high %Inhibition values.

RESULTS

Effects of microcolony formation on adhesion of meat spoilage organisms.

Adhesion experiments were carried out on three meat spoilage organisms in an attempt to better understand the contribution of microcolony formation on bacterial adhesion. Adherent bacteria on meat and agar surfaces were allowed to proliferate for various periods of times (0, 3, and 6 h) with colony formation occurring after 3h of incubation. The proportions of microcolonies to single cells were determined by microscopic observation both before and after the application of the water treatments. The %Adhesion reductions associated with the different stages of microcolony formation (0, 3, and 6h incubation times) of the three organisms under investigation are shown in Figures 7 to 9. Lower %Adhesion reduction values would be associated with little effect on adhesion strength. On the other hand, increasing %Adhesion reductions would be indicative of greater influence on bacterial adhesion strength. The microcolony components of the microbial flora on the meat surface at the different stages in the procedure used to determine microbial adhesion are presented in Figure 10. We compared the %Adhesion reductions of the microflora growing on the meat surface for 6h with that of the microflora in contact with the meat surface for only 10 min. The 6h incubation resulted in a microflora composed solely of microcolonies; whereas, only single cells were observed to be in contact with the meat surface after the 10 min. In our investigation, with an increase in the number of microcolonies making up the microflora, *Pseudomonas fluorescens* showed a decrease in %Adhesion reduction (37.1 to 21.6%). Under similar circumstances,

both *Enterobacter agglomerans* (62.7 to 79.2%) and *Moraxella osloensis* (66.1 to 79.9%) displayed higher %Adhesion reductions when microcolony development was allowed to proceed to a greater extent.

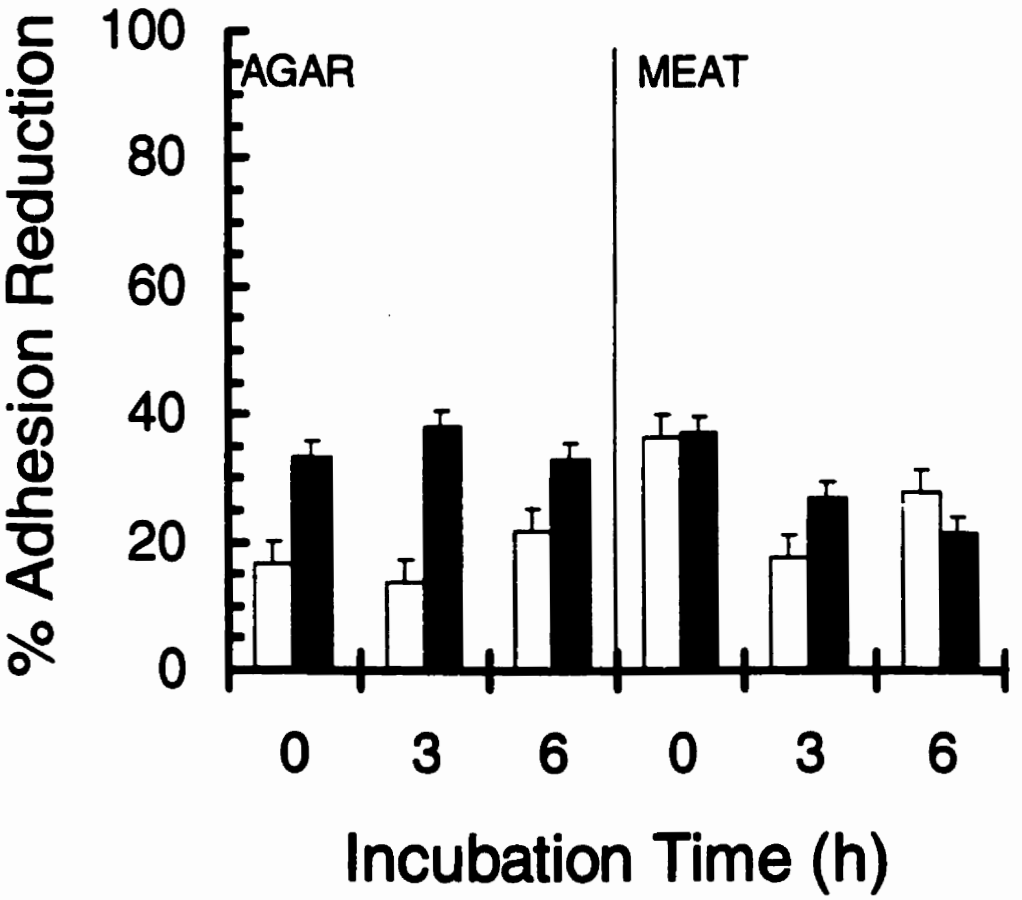

Figure 7. Effects of microcolony formation on the adhesion of *Pseudomonas fluorescens* to agar and meat surfaces. Treatment method: □ Non continuous rinse, ■ Continuous rinse.

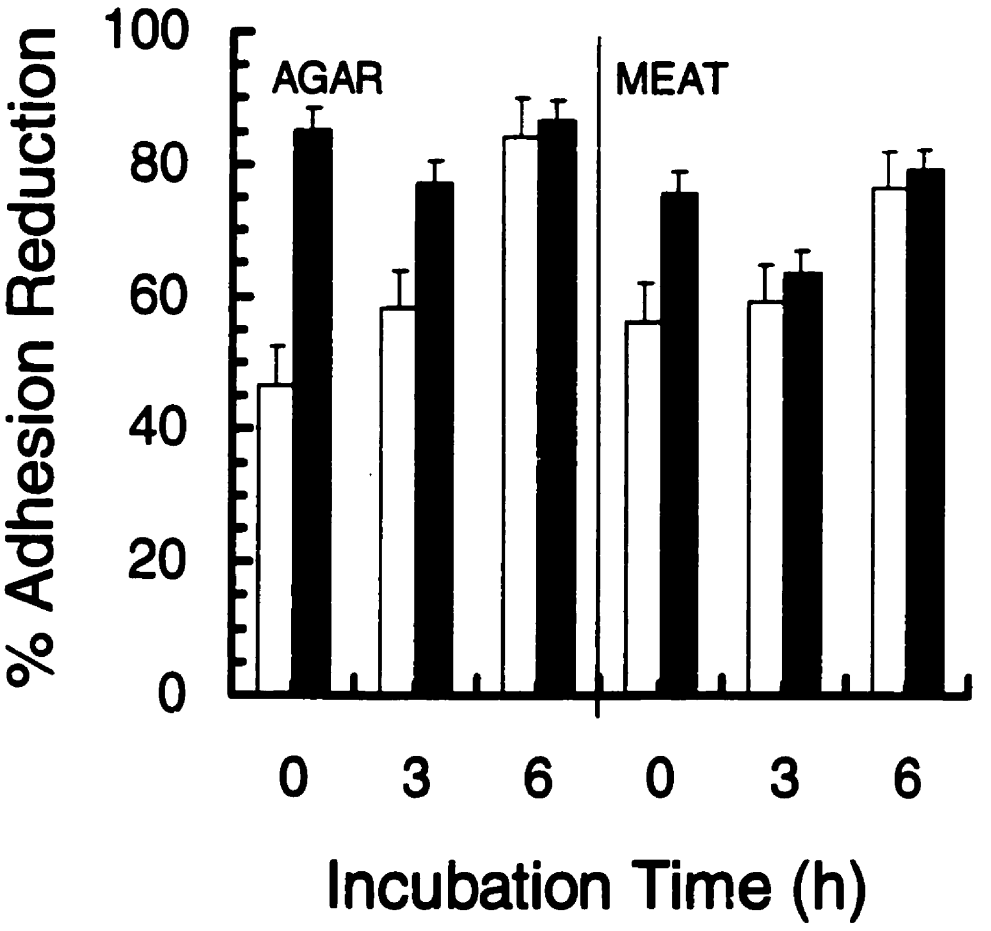

Figure 8. Effects of microcolony formation on the adhesion of *Enterobacter agglomerans* to agar and meat surfaces. Treatment method: □ Non continuous rinse, ■Continuous rinse.

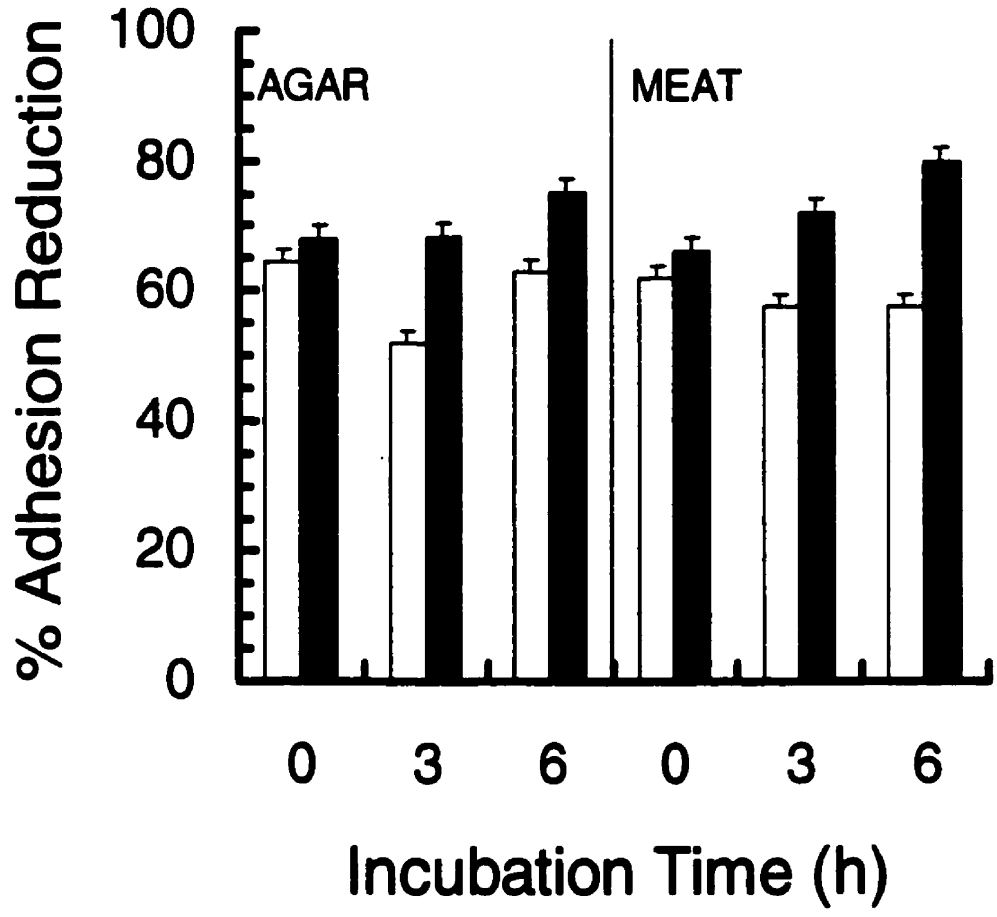

Figure 9. Effects of microcolony formation on the adhesion of *Moraxella osloensis* to agar and meat surfaces. Treatment method: □ Non continuous rinse, ■ Continuous rinse.

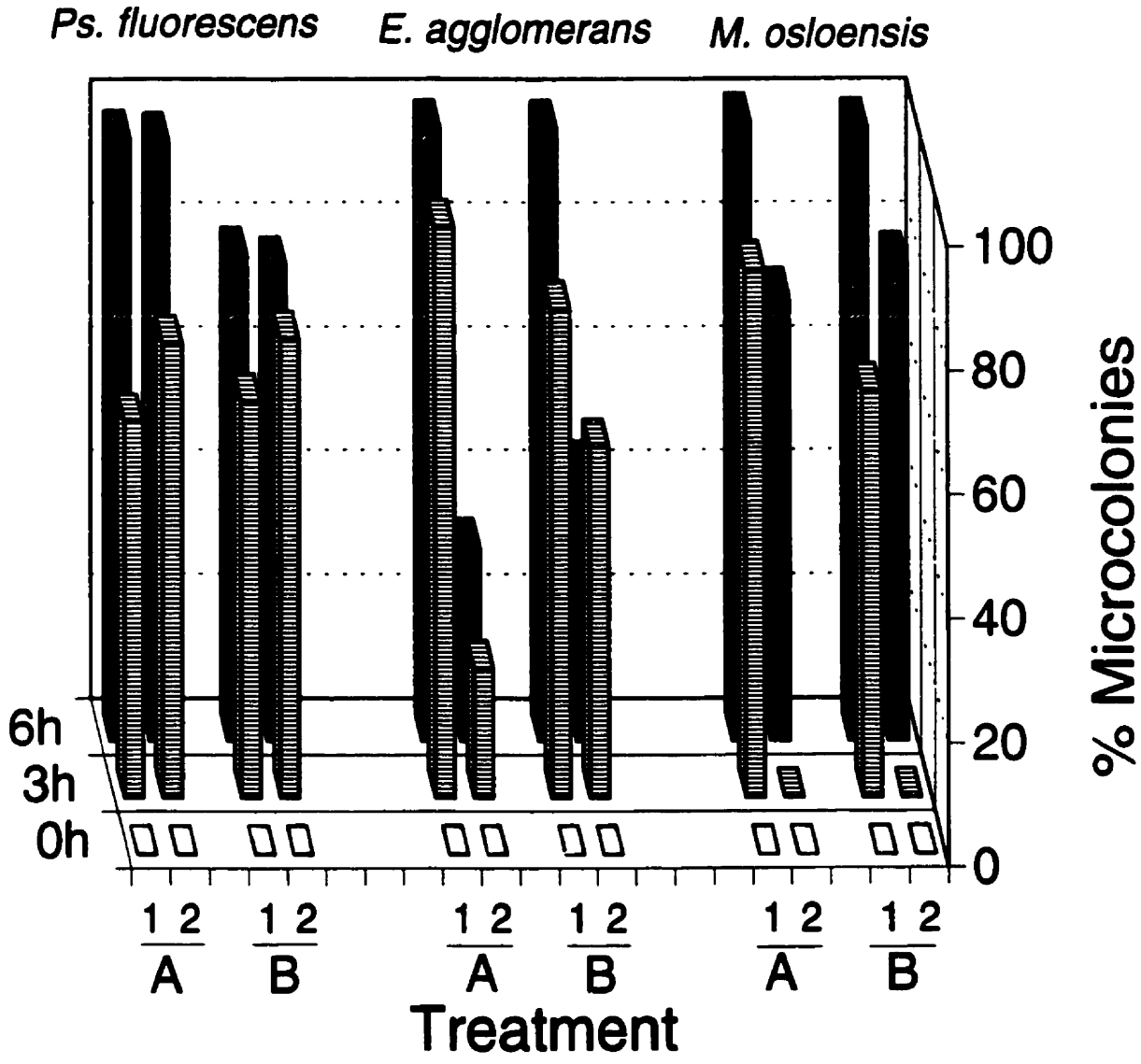

Figure 10. Microcolony component of the adhesion counts obtained during the study on the effects of microcolony formation on the adhesion strength of meat spoilage organisms. Incubation time: □ 0h; ■ 3h; ■ 6h. Treatment method: A. Continuous rinse; B. Non continuous rinse; 1. Microcolony component before treatment; 2. Microcolony component after treatment.

There was also a marked difference in the retention of microcolonies following rinsing. With *Pseudomonas fluorescens*, the %microcolonies of the adhered microflora before and after the application of one of the rinse treatments remained essentially the same. With *Enterobacter agglomerans* and *Moraxella osloensis* there were marked decreases in the colonial populations after the rinse treatments. *Enterobacter agglomerans* showed decreases of 78.1%, and 68.4% in the number of microcolonies present after treatment. With *Moraxella osloensis* the resulting decreases observed were in the order of 100%, and 28.3% in the number of microcolonies after treatment. With the use of the two different water treatments, continuous and non continuous, no differences were apparent between microcolony composition of the adhesion counts.

There was little difference seen in the %Adhesion reductions of all three organisms when the adhesion experiments were conducted using two different surfaces, i.e., agar and meat. The organism showing the least amount of variation in adhesion to the two surfaces was *Moraxella osloensis*. The %Adhesion reductions of this organism, when in contact with the two surfaces for 0, 3, 6h prior to assessing adhesion, were respectively 68.0%, 68.2%, and 75.3% on agar and 66.1%, 72.1%, and 79.9% on meat. When differences in %Adhesion reductions were noted, the values were usually lower when adhesion was carried out on meat surfaces rather than on agar surfaces. *Pseudomonas fluorescens* was shown to have differences in %Adhesion reductions ranging from 3.8% to 11.3% over the two surfaces, and a range of 7.4 to 22.4% was observed with *Enterobacter agglomerans*.

The adhesion experiment protocol employed during this investigation required that the chambers be drained prior to microscopic observation. This step had the effect of generating a water line that flowed gradually over the substratum. After enumerating the initial population present on the substratum after incubation, the adherent microbial population was then subjected to one of two treatments: either a continuous or non continuous rinse. The two different treatments were employed to evaluate the impact of the receding water line on bacterial adhesion. With the use of a non continuous rinse the force involved in the removal of the adherent bacteria would only be exerted by the receding waterline; whereas when a continuous rinse was applied to the system, removal of adherent microorganisms would be due to the combined forces of the receding waterline and the flow rate of the rinse. By comparing the %Adhesion reductions as a result of these two treatments, we would then be able to isolate the force generated by the receding waterline from the force of the rinse flow rate. As expected, the use of a non continuous rinse resulted in consistently lower %Adhesion reduction values than when a continuous rinse was utilized. When comparing the forces involved, we found that the force generated by the receding waterline is considerably stronger than the force associated with rinsing.

Effects of rinse time and concentration of organic acids on the adhesion of *Pseudomonas fluorescens* to meat surfaces.

In order to determine the impact of organic acids on bacterial adhesion, adherent bacterial cells were subjected to organic acid rinses for various periods of time (15, 30, and 45 s).The use of organic acids to rinse meat surfaces instead of water did not generally result in more detachment of adherent bacterial cells (Figures 11, 12 and 13). However, rinsing for 30 and 45 s with 0.1 and 0.25% acetic acid instead of with water removed more cells of *Pseudomonas fluorescens* from the meat surface (Figure. 11). A further increase in %Adhesion reduction was observed with 0.5% acetic acid as the rinse solution. This was not the case with lactic acid where rinsing with 0.1, 0.25 and 0.5% concentrations resulted in the same increase in %Adhesion reductions (Figure. 12). Rinsing with citric acid had the least effect on reducing adhesion (Figure. 13). It should be noted that reductions in %Adhesion using these organic acids instead of water as rinses did not exceed 20%.

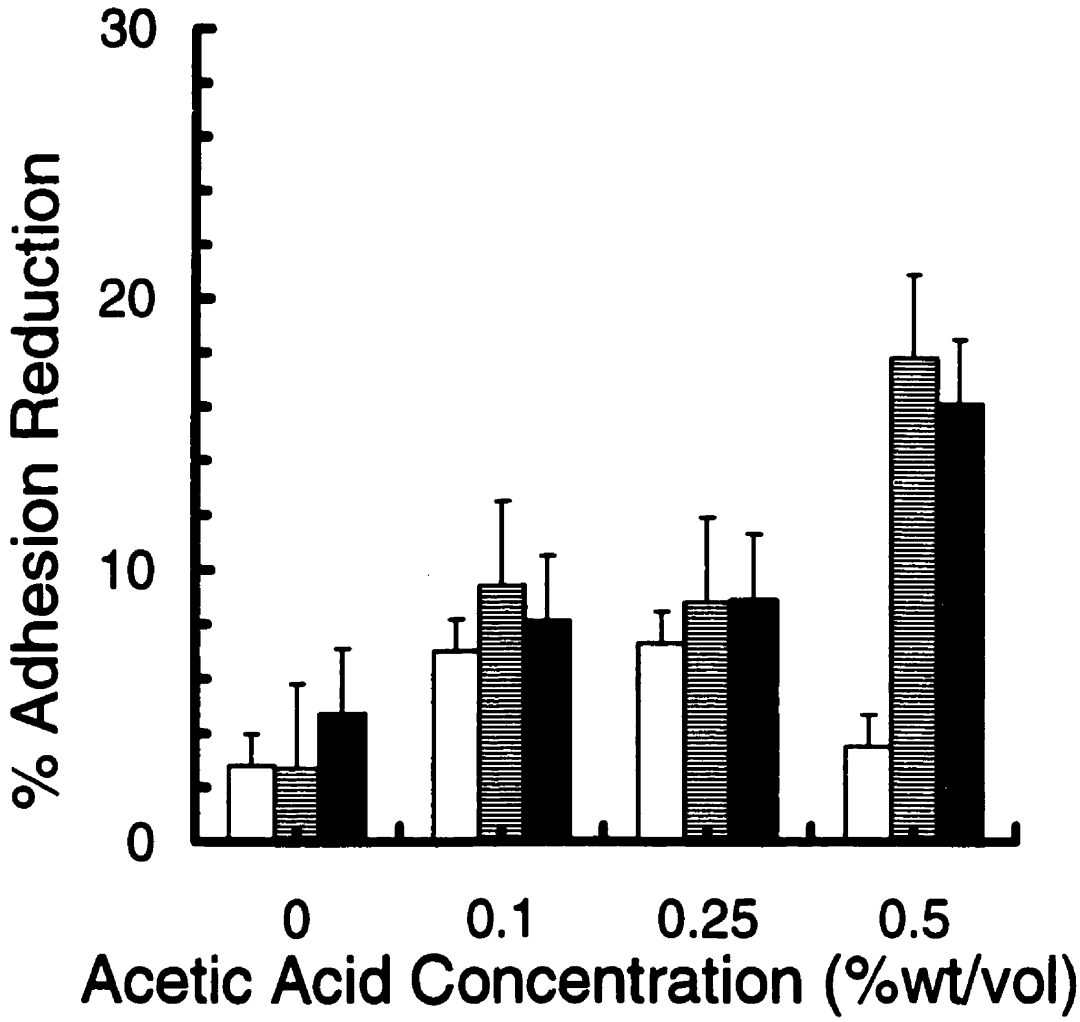

Figure 11. Effect of various acetic acid concentrations on the adhesion of *Pseudomonas fluorescens* to meat surfaces. Rinse time:□ 15 s,■ 30 s, ■45 s.

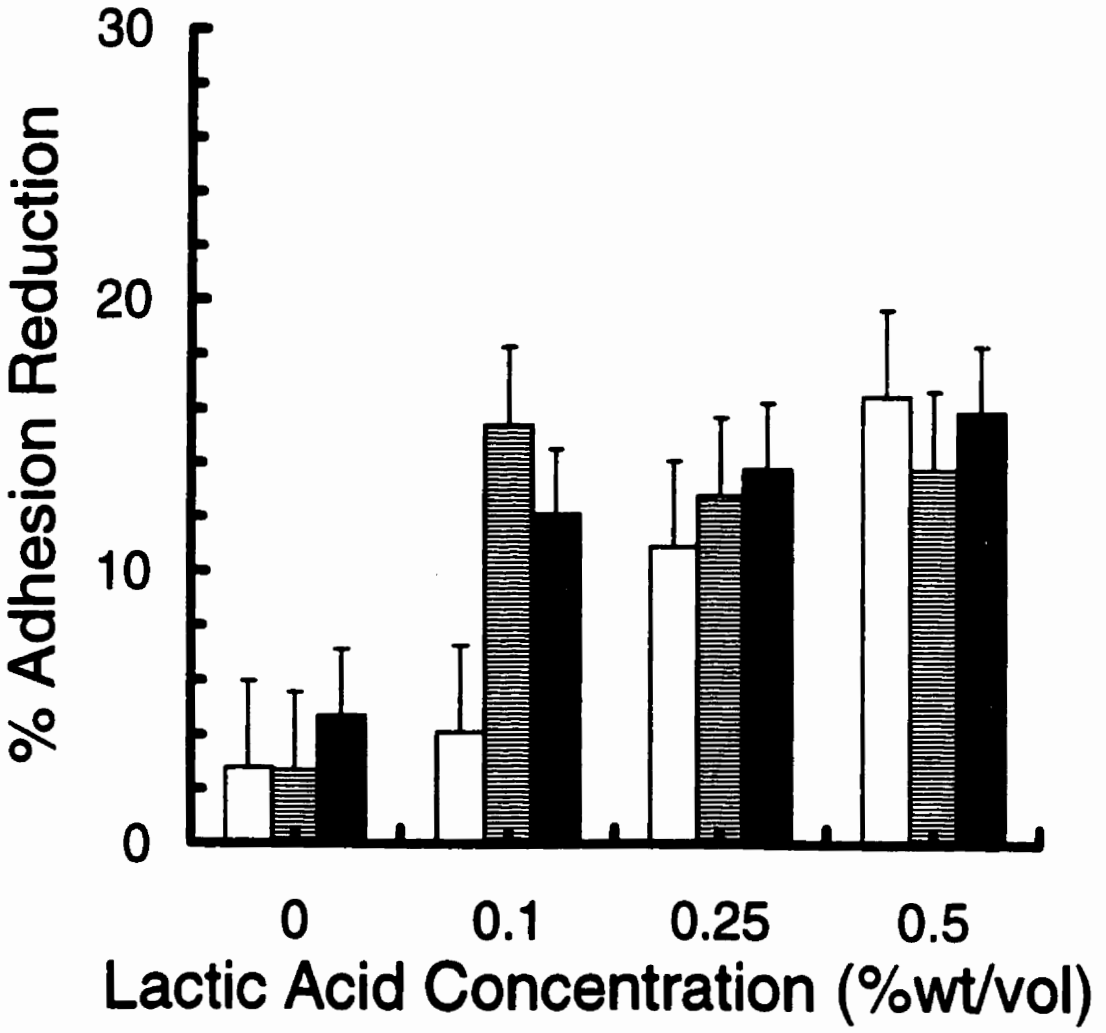

Figure 12. Effect of various lactic acid concentrations on the adhesion of *Pseudomonas fluorescens* to meat surfaces. Rinse time: □ 15 s, ■ 30 s, ■ 45 s.

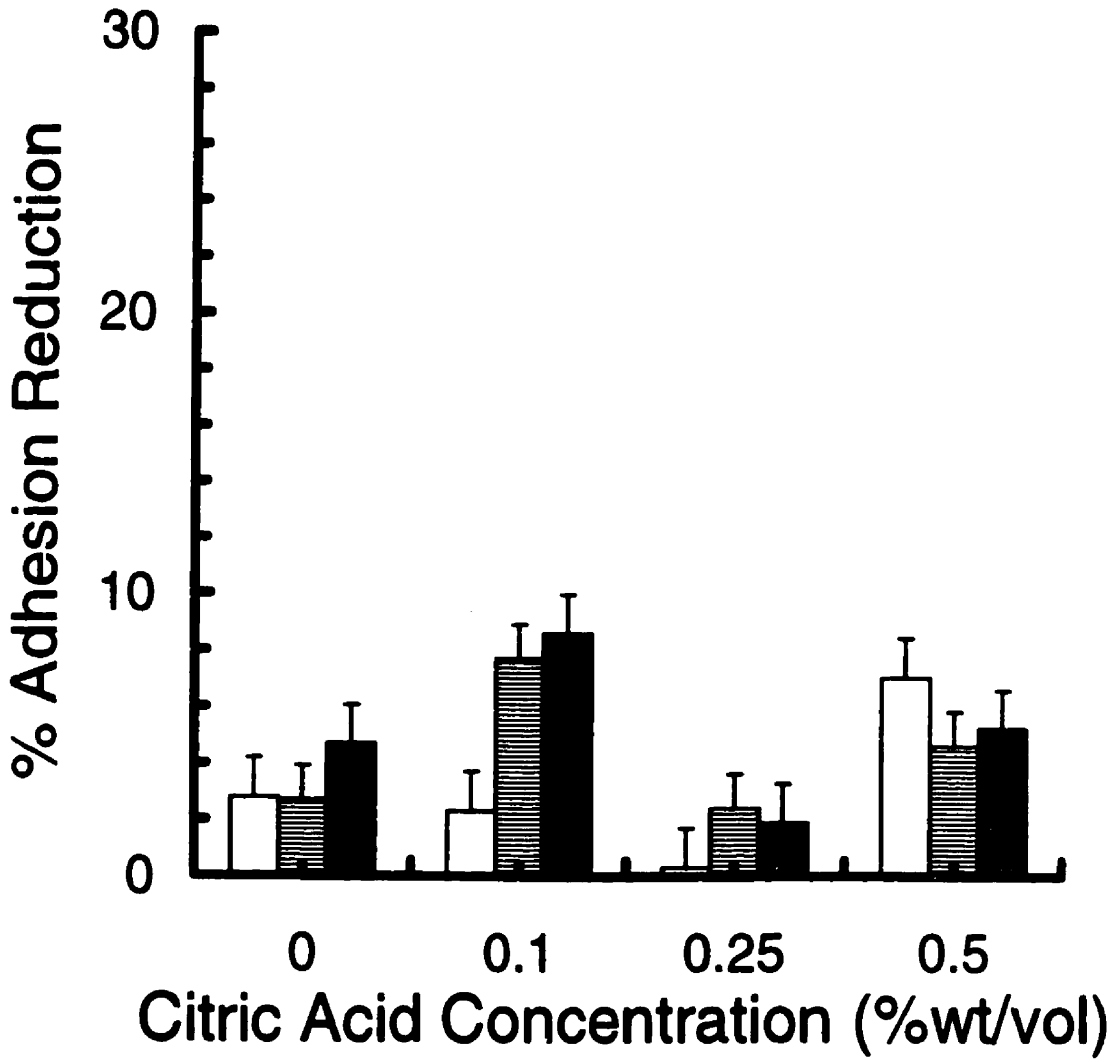

Figure 13. Effect of various citric acid concentrations on the adhesion of *Pseudomonas fluorescens* to meat surfaces. Rinse time: □ 15 s, ▩ 30 s, ■ 45 s.

Effects of organic acids on microcolony formation of *Pseudomonas fluorescens*.

In order to establish if the reduction in bacterial loads reported in previous studies associated with organic acid rinses (see Table 1, p.32) could be attributed to a bactericidal activity, their impact on microcolony formation was tested. Microcolony development by single adherent *Pseudomonas fluorescens* cells on agar surfaces rinsed with varying concentrations of organic acids (acetic, lactic, citric) for 15, 30, and 45 s is depicted in Figures 14-16. It is important to note at this time that the organic acid rinses were immediately followed by a 1 min distilled water rinse. Therefore, the activity of the acids was limited to the rinse times and the concentrations of acids on the agar surfaces during subsequent period of bacterial proliferation would be minimal.

It is quite obvious that the short contact times between the organic acids and the bacteria (15-45 s) did not affect the growth of the remaining adherent cells. The greatest inhibition of microbial proliferation was observed with acetic acid, range of %Inhibition of 0.73% to 7.54%; whereas, the organic acid demonstrating the least amount of effect on microcolony formation was found to be citric acid, having %Inhibition ranging from 0.26% to 2.81%. Lactic acid closely resembled acetic acid in terms of inhibitory effects toward bacterial proliferation with slightly lower values of %Inhibition (1.09% to 4.7%). Increasing the rinsing times with the different concentration of all three acids did not in general result in higher inhibitions of single cell proliferations. With acetic acid a three fold increase in inhibition of single cell proliferation was observed as the concentration of the acid increased from 0.1%

to 0.5%. With the other acid rinses the %Inhibition of microcolony formation remained unchanged with increasing concentration of the acid rinses.

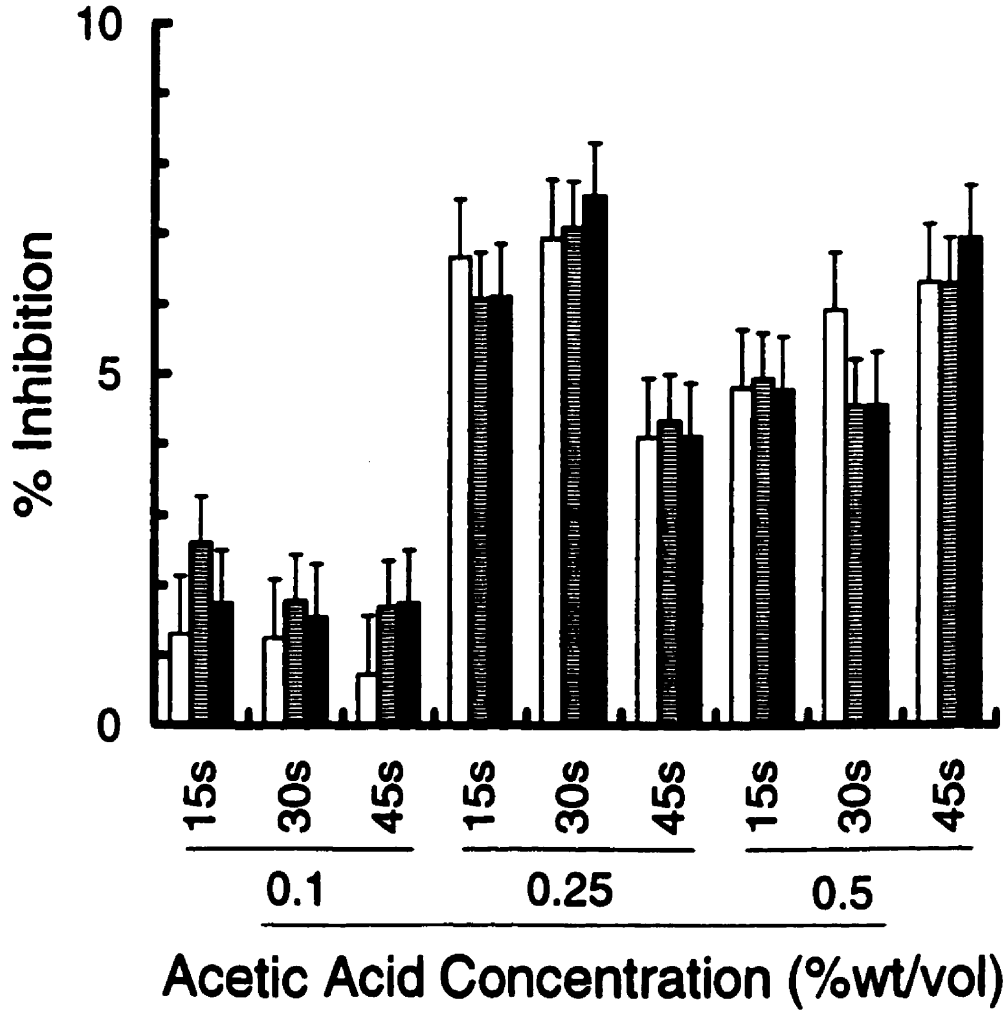

Figure 14. Effects of acetic acid concentration and rinse times on microcolony formation of *Pseudomonas fluorescens* on agar surfaces. Incubation times:□ 1 h,■2 h,■3 h.

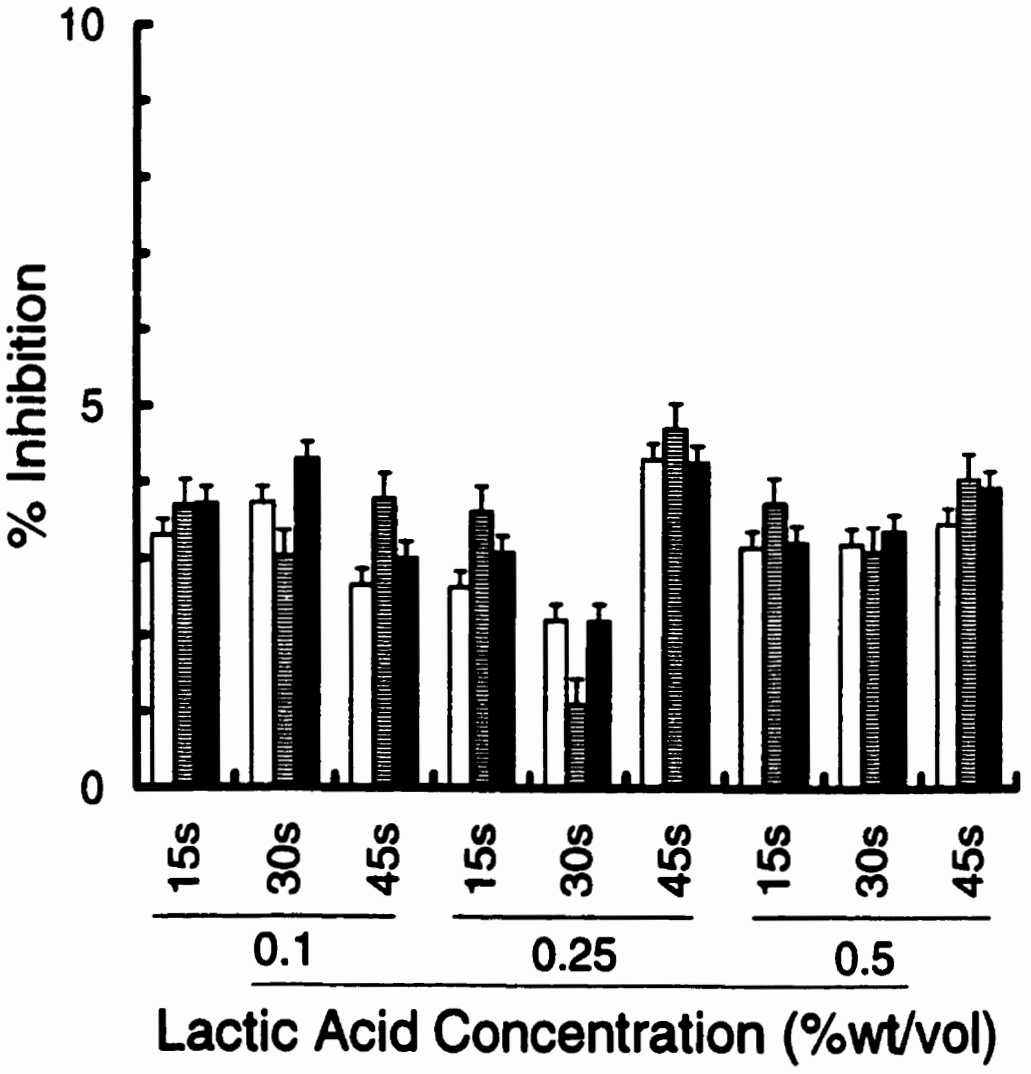

Figure 15. Effects of lactic acid concentration and rinse times on microcolony formation of *Pseudomonas fluorescens* on agar surfaces. Incubation times: □ 1 h,■2 h,■ 3 h.

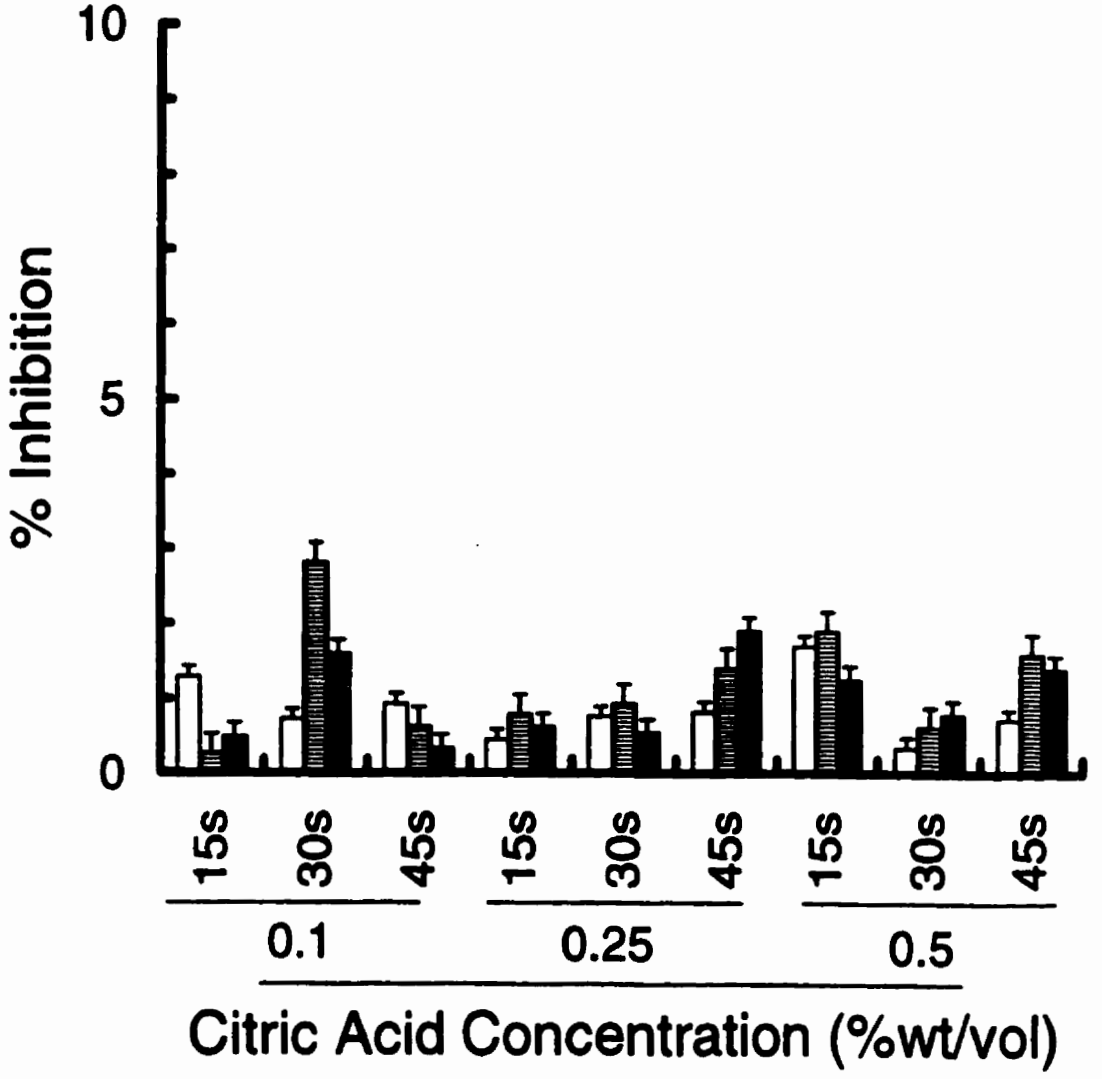

Figure 16. Effects of citric acid concentration and rinse times on microcolony formation of *Pseudomonas fluorescens* on agar surfaces. Incubation times: □ 1 h, ▤ 2 h, ■ 3 h.

DISCUSSION

Several factors are believed to influence the adhesion of microorganisms to meat surfaces: pH, temperature, medium composition, growth conditions (Bouttier *et al.*, 1997; Farber and Idziak, 1984). Adhesion has also been shown to be dependent on bacterial strain and concentration as well as type of meat surface (Piette and Idziak, 1991a; Firstenberg-Eden, 1981). Information is available on adhesion rates and strength of different individual bacteria to meat - the consensus being that microbial adhesion involves several types of interactions leading to reversible and finally irreversible attachment. However, microbial spoilage of meat is influenced not only by the initial adhesion of bacteria to a substrate, but also by subsequent proliferation after adhesion. The objective of this study was to gain a better understanding of the impact of organic acids as well as microcolony formation on bacterial adhesion.

Increased adhesion strength was recorded for *Pseudomonas fluorescens* cells left in contact with meat surfaces for longer periods of time prior to assessing adhesion. This agrees with the findings of Notermans *et al.* (1979) where the adhesion strength of three organisms was evaluated over time. The adhesion strengths of *Escherichia coli* K12, *Klebsiella* sp., and *Pseudomonas* EBT/2/143 were observed to increase with greater storage times of up to 2-3h. However the adhesion strength was shown to decrease after 3h of storage at 20°C (Notermans *et al.*, 1979). One of the proposed mechanisms to account for this change in adhesion strength is the synthesis of exopolymer. Costerton *et al.* (1978) observed thin fibres

of extracellular polymers on meat slices contaminated with *Pseudomonas* EBT/2/143. These fibres were found to increase in numbers and thickness during storage. This secretion of polysaccharides to form a felt-like matrix, termed glycocalyx, would then act to physically strengthen the adhesion of microcolonies to the meat surface. Glycoproteins extending outwards from the surface of the meat would interact with the glycocalyx produced by the bacteria to form even stronger bonds. The link could be provided by lectins that have the ability to bind polysaccharides (Costerton *et al.*, 1978). However there is some evidence that these exopolymers, even though secreted by the microorganisms, do not have the ability to enhance adhesion (McMeekin, 1986).

Our investigation also revealed, that under the same conditions, microcolony formation significantly decreased the adhesion strength of *Moraxella osloensis*, while only slightly decreasing the adhesion strength of *Enterobacter agglomerans*. With growth, the nature of the adhesion interaction changes within the microcolony. The development of microcolonies is such that as bacteria multiply within the microcolony the proportion of new cells in contact with the meat decreases; whereas, the number of daughter cells in contact with each other increases. The recorded decreases in adhesion strength observed in our study could be attributed in large part to the increase in these presumably weaker bacteria-to-bacteria interactions. However, we also observed a decrease in the number of microcolonies after treatment, implying that the bacteria-to-substratum interactions are also weakened but to a lesser extent. A similar conclusion was also reached in the previously discussed study where attachment was seen to decrease after 3h of storage at 20°C (Notermans *et al.*, 1979).

Our observation that a higher percentage of single cells, as compared to microcolonies, remained attached after rinsing (see Figure 10) further supports this conclusion. Moreover, there were instances where we observed a greater number of single cells after the adhesion experiment than was initially present. As the newly formed microcolonies are exposed to the rinse force, some of these cells may separate and become reattached as single cells elsewhere on the surface. This observation could also serve to reinforce the statement that bacteria-to-bacteria interactions represent weaker bonds than bacteria-to-surface interactions. Whether or not the differences in the adherence between single cells and microcolonies of different organisms could also be attributed, in part, to exopolymer synthesis is still to be determined.

In our investigation we employed two different rinsing treatments, i.e., continuous and non continuous. The purpose was to determine the influence of the receding waterline present during the drainage of the chambers. It is our hypothesis that when a non continuous rinse, consisting of flooding the chamber and letting it stand for the proper time period, the only force in effect would be attributed to the receding waterline; whereas, when a continuous rinse was applied to the system under the same condition, the force involved would be that of the combination of the receding waterline and the flow rate of the rinse. As expected the values obtained with the continuous rinse were always slightly higher than those obtained with the non continuous rinse, due to the dual forces acting on the cells on the surface of the meat. As previously reported by Piette and Idziak (1989), the receding waterline was shown to account for a large proportion of the detachment and reduction in bacterial population on the meat surface. The hypothesis being that the receding waterline

represents the most important force, in terms of magnitude, applied to the system, largely surpassing any other force applied to the system. In the design of our adhesion experiments we isolated the effects of the receding waterline in order to establish the effects of microcolony formation and organic acid rinses on the removal of meat spoilage organisms.

Whether or not the nature of the meat surface plays a determinant role in the extent of bacterial adhesion remains an issue under debate. Some researchers still believe that bacterial adhesion is significantly affected by the surface to which the bacteria will eventually adhere to, whether it be an inanimate surface (Hood and Zottola, 1997b) or a meat surface (Firstenberg-Eden, 1981). However there is also evidence supporting the opposite view, where no significant differences were observed when adhesion was carried out on different surfaces. (Dickson and Frank, 1993; Chung *et al.*, 1989; Piette and Idziak, 1989). The data that we have collected shows no significant difference in adhesion to agar or meat surfaces, thereby supporting the contention that the nature of the surface does not necessarily influence adhesion of bacterial cells.

This study was also undertaken to ascertain whether the 1 to 3.5 log reductions in bacterial populations previously reported (see Table 1, p.32) with the use of organic acids (ranging in concentration from 1 to 24%) was due to actual removal of microorganisms, or simply due to cell death. Although our studies revealed that organic acids reduced the adhesion strength of bacteria, these reductions were negligible compared to the reductions mentioned above. Similar results were obtained when verifying the bactericidal effect of

organic acids on microcolony formation. These minimal effects are believed to be associated with the very low concentration (0.1, 0.25, and 0.5%) and contact times (15, 30, and 45 s) used throughout this investigation. In fact, greater effects (but nonetheless nonsignificant) on adhesion strength were obtained with both acetic and lactic acid with higher concentrations and longer rinse times. It is our belief that if concentrations comparable to those used in prior experiments were employed (closer to 3% wt/vol), then bacterial reduction would become significant. This is further supported by the knowledge that acid concentration is an important factor in mediating the pH (in a non-buffered system) and hence the proportion of undissociated acid believed to be responsible for the bactericidal activity of the acids (Cutter and Siragusa, 1994a). Therefore increasing the acid concentration in a non-buffered system would have the effect of decreasing the pH of the environment and consequently increasing the proportion of undissociated acid to dissociated acid. However it is important to remember that concentrations should never exceed 3% since off flavours and colours are produced at concentrations higher than this level.

In previous experiments evaluating the efficacy of organic acids in decontaminating meat carcasses (see Table. 1, p.32), the effect of pH was not considered. For the purpose of our investigation the pH was standardised to 5.0. This became necessary because at lower pH and higher acid concentrations, the meat slices swelled up, increasing in thickness generally 100 fold. The now swollen meat slices completely blocked the flow of liquids through the adhesion chambers, thereby making the use of the chambers to measure bacterial adhesion unreliable. This expansion is probably a consequence of a change in surface characteristics

of the meat surface causing the muscle fibres to repel each other resulting perhaps in increased water uptake. If the phenomenon of meat swelling was also observed on meat carcasses, it could become a source of problems. This is due to the fact that meat expansion in such a way would result in an increased number of crevices present on the surface of the carcass. These crevices would then serve as reservoirs for entrapped microorganisms reducing the efficiency of bacterial removal. On the other hand, if organic acids are proven to be bactericidal then this expansion in the meat surface may be deemed beneficial. The crevices would become more accessible to the organic acids which could then act on both adhered and entrapped organisms, while also increasing the weight of the meat.

The ratio of undissociated to dissociated acid present at various pH as well as the amount required to generate an antimicrobial effect has been established in previous studies (Table 2 and 3). Using these tables, it then becomes possible to predict which organic acid under certain conditions will have the greatest influence. The amount of undissociated acid present in the system during our adhesion experiments are represented in Table 4.

From these data (Tables 2-4) it becomes obvious that sufficient undissociated acid at pH 5.0 to generate a bactericidal effect on the organisms would be realized with the use of 0.25 and 0.5% acetic and lactic acid rinses. Under all other conditions tested, there should not have been enough undissociated acid to cause death of the organisms.

Table 2. Antimicrobial spectra of organic acids used in foods[a].

Organic acid	Concentration of undissociated acid required to inhibit growth of most strains in microbiological media		
	Enterobacteriaceae	Micrococcaceae	Bacillaceae
Acetic	0.05	0.05	0.1
Citric	>0.005	0.001	>0.005
Lactic	>0.01	>0.01	>0.03

[a] Values given as percent in solution. (Microbial Ecology of Foods vol.1)

Table 3. Proportion of total acid undissociated at different pH values[a].

Organic acids	pH values				
	3	4	5	6	7
Acetic	98.5	84.5	34.9	5.1	O.54
Citric	53.0	18.9	0.41	0.006	< 0.001
Lactic	86.6	39.2	6.05	0.64	0.064

[a] Values given as percent in solution. (Microbial Ecology of Foods vol.1)

Table 4. Amount of undissociated acid present during the adhesion experiments[a].

Organic acids	Concentration		
	0.1	0.25	0.5
Acetic	0.035	0.087	0.175
Citric	<0.001	0.001	0.002
Lactic	0.006	0.015	0.030

[a] Values given as percent in solution.

Our initial hypothesis was that bacterial population reductions associated with organic acid rinses were due to cell death and not to actual removal of bacteria. We based this on the fact that pH alone was shown not to affect bacterial adhesion to pork skin of five different bacterial cultures over a pH range of 4.0 to 8.8 (Butler *et al.*, 1979). It was therefore reasonable to assume that the lower pH resulting from the addition of the organic acid would have no direct impact on adhesion strength. The fact that non viable bacteria adhere equally well as viable bacteria added more credit to our hypothesis. Even after reducing the number of viable cells by more than 3.6 $\log_{10}$ CFU·ml^{-1} with the use of lethal treatments (UV, γ rays, or heat) the number of adherent cells were still 1.7 $\log_{10}$ units higher than the number of viable cells present (Piette and Idziak, 1992). Hence the ability of organic acids to kill the adhering microorganism would not affect adhesion strength. Therefore the previous microbial population reduction in viable cells reported in several studies (see Table 1, p.32) must be due to cell death and not removal from the surface.

Our results do not prove unequivalently that the effect of organic acid rinses in reducing viable cell populations on meat surfaces is to destroy rather than remove cells on the surface. Although our evidence points toward cell destruction, observation of increasing inhibitory effect with increasing acid rinse time and concentration, the reduction in numbers is too low to be significant. This is most likely due to the low acid concentrations and rinse times used.

CONCLUSION

Bacterial proliferation and microcolony formation was shown to both increase and weaken adhesion strength of meat spoilage organisms. The nature of the organism known to play a role in initial adhesion was also found to influence bacterial adhesion during subsequent bacterial growth after attachment. The nature of the surface used as a substratum proved to have no impact on the level of bacterial adhesion or on adhesion strength. The composition of the glycocalyx as well as the time required for its production is believed to be responsible for the differences observed in the effect of microcolony formation on adhesion strength. The receding water line, being by far the greatest force applied, was established as having the largest influence in the removal of bacteria from meat surfaces. However the receding water line was observed in all the conditions tested and therefore had no influence on the data collected. Our attempts to determine if the reduction in bacterial population by organic acids observed in previous studies was due to the bactericidal activity or interference with the adhesion process failed to be conclusive. Organic acids exhibited very little impact on the adhesion strength. The influence of organic acid on microcolony formation was also minimal which would be associated with low bactericidal activity. The effect on microcolony proliferation as well as on adhesion strength were observed to increase at higher concentration and during longer rinse times. This would have a tendency of associating the lack of effect demonstrated by the organic acids to the low concentration and short rinse time.

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