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Electrical stimulation of beef carcasses (n=18) did not cause a significant reduction in microbial population on three different sampling positions immediately following slaughter. In constrast, significant microbial reduction ($P > 0.05$) was found at position 2 (muscle above aitch bone), but not at position 1 (inside of neck) or position 3 (fat on outside of round) after 72 h. of chilling storage. Nine bacterial species from eight different genera were inoculated on three different agar media which varied in electrical conductivity. Electrical stimulation of these media caused a reduction in recoveries of microorganisms under various voltage and time treatments. Spore-forming bacteria were the most resistant to the electrical treatment. Among the non-spore-formers, gram negative bacteria were more resistant to electrical treatment than gram positive bacteria. Also, microorganisms inoculated on the lower resistance medium A revealed greater reduction in the recoveries than that of the other media with higher resistance. A five log number reduction (99.999%) in E. coli, P. putrifaciens, and

P. fragi was found in 0.85% saline and phosphate buffered saline after a 30 V, 5 min. treatment, but little change in count was detected in o.1% aqueous peptone or 0.25 M sucrose solution.

The Effect of Electrical Current on Bacteria on Beef Carcasses, on Agar Media and in Suspensions

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INTRODUCTION

General appearance, color, tenderness, juiciness, and flavor are important sensory quality attributes of meat for consumer acceptability. Electrical stimulation of pre-rigor muscle has been found to improve tenderness (Bendall et al., 1976; Bouton et al., 1978; 1979; Calkins et al., 1980; Callow, 1936; Cross, 1979; Cross et al., 1979; Deatherage, 1980a; 1980b; Gilbert & Davey, 1976; Grusby et al., 1976; Hall et al., 1980; MacKeith et al., 1979; Raccach & Henrickson, 1979; Savell et al; 1977; 1979a; 1979b; Smith et al., 1979), muscle color and maturity (Hall et al., 1980; MacKeith et al., 1980; Savell et al., 1978b; Smith et al., 1977), marbling (Savell et al., 1978b; 1978c; 1978d), flavor and palatability (Bouton et al., 1980; Savell et al., 1977), time required for aging (Savell et al., 1978c), and drip loss (Savell et al., 1980). In addition, electrical stimulation increases post-mortem glycolysis and hastens the onset of rigormortis (Bendall, 1976; Bouton et al., 1973; Carse, 1973; Chrystall & Hagyard, 1976; Chrystall & Devine, 1978; Chrystall et al., 1980; McCollum & Henrickson, 1977; Shaw & Walker, 1977; Taylor & Marshall, 1980; Taylor et al., 1981), reduces the toughening effects of cold-shortening and thaw rigor (Bouton et al., 1973; Chrystall & Hagyard, 1976; Davey et al., 1976), accelerates the release of lysozymal enzyme which increases proteolysis (Dutson et al., 1980a; Gilbert & Davey, 1976; Parrish, 1977; Sorinmade et al., 1978), causes the physical disruption of muscle fiber and increases the sarcomere length (Demeyer & Vandendriessche, 1980; MacKeith et al., 1980; Nicholes & Cross, 1980; Savell et al., 1978a; Swatland, 1977; Will et al., 1979;

1980).

Bacteriological condition is also an important quality attribute of meat. However, only a few studies concerning the effect of electrical stimulation on the bacteriological quality of meat have been made (Gill 1980; Kotula & Emswiler-Rose, 1981; Maigadat et al., 1980; Raccach & Henrickson, 1978). Also, a controversy regarding the effect of electrical stimulation of meat carcasses on the microbial flora has recently developed (Gill, 1980; Raccach & Henrickson, 1978; 1979).

The objectives of the present study were: a) to determine the effect of electrical stimulation on the number of naturally-occurring microorganisms on the surface of meat carcasses slaughtered under standard packing plant procedures, b) to determine the effect of electrical current on a number of specific organisms in different types of media.

LITERATURE REVIEW

Meat Microflora

After animals are slaughtered, microorganisms are transferred from the slaughter instruments, animal hide, and viscera to the underlaying tissue in the stages of skinning, eviscerating, and cutting. These microorganisms rapidly utilize and metabolize low molecular weight compounds such as amino acids, dipeptides, lactic acid, and sugars present in meat and give off mixtures of spoilage nucleotides, cadeverine, putrescine, organic acids, carbon dioxide, hydrogen sulfide, and ammonia. During the storage, a rapid increase in microbial population may occur in meat if the physico-chemical factors are appropriate to the growth of microorganisms. These factors are temperature, water activity, osmotic pressure, and pH. The most important controllable factor is temperature. In general, the higher the temperature of the substrate, the faster the rate of growth of the microorganisms, and the rapidity with which spoilage occurs. Under different storage temperatures, the dominated microflora in meat vary in number and species. The aerobic spoilage flora of fresh meat stored at chill temperatures is usually dominated by species of Pseudomonas and Acromobacter, although other bacteria such as Acinetobacter, Enterobacter sp, Microbacterium thermosphatum, Flavorbacterium, Micrococcus may be present (Barlow & Kitchell, 1966; Roth & Clark, 1972; Ingram & Dainty, 1971; McMeekin, 1975; and Jay et al., 1972).

At intermediate temperatures (15-25°C) and warm temperatures (25-40°C), the Salmonella typhimurium and Escherichia coli are likely to be the main hazards on meat of normal pH because of their largely uninhibited growth at these temperatures under either aerobic or anaerobic conditions. In addition, Staphyloccus aureus and Clostridium perfringens are

both sensitive to pH in the range found on normal meat (WHO Technical Report No. 598, 1976) at 20°C but could grow on meat at 30°C. Bacillus cereus and Yersinia enterocolitica are somewhat sensitive to pH below 6.0 although they can grow on meat at 20°C (Carpenter et al., 1975; Gill & Newton, 1979). The slaughter of tired, stressed, and hungry animals which would have low glycogen levels would produce carcasses having high final pH values that could present a considerable health hazard for Staph. aureus, Y. enterocolitica, and B. cereus. Fresh meats have water activity values (aw) of 0.99 and are readily infected by several food poisoning strains of S. aureus which have optimum aw values of about 0.995. Lactobacillus and H. thermosphactum could grow at lower aw levels (0.93-0.94) than Psuedomonas (0.95-0.96). The Achromobacter strain grew on meat at an aw level of 0.98.

Microbial contamination is the result of processes used in the slaughter and dressing of meat animals. Growth of organisms on meat is one of the main causes of discoloration and spoilage that results in the loss of quality attributes of meat. For quality control, it is necessary to efficiently clean and sanitize carcasses following slaughter to reduce microbial contamination. During washing, the removal of microbes from meat is affected by physical factors such as volume of solution, angle of impact, line pressures, force of spray, and speed of meat movement through the water sprays as reported by Anderson et al., (1975). Sanitizing carcasses with chemical sprays to reduce bacteria has also been reported (Biemuller et al., 1973), using hydrogen peroxide, stannous chloride, acetic acid, and steam. These reagents were effective in eliminating salmonella and reducing total bacterial populations, but not all treatments were acceptable from the standpoint of carcass appearance.

Reynolds & Carpenter (1974) found that treatment with 1.5 M acetic:propionic acid 60:40, w/w (pH 2.3 resulted in two log cycle reduction in total numbers with no apparent detrimental effect on the carcass. Emswiler et al., (1976) recommended spraying hog carcasses with acetic acid solution at pH_2 for control of surface bacteria. Kotula et al., (1974) found that spraying beef forequarters with water chlorinated at 200 ppm reduced total aerobic bacteria on the surface of the forequarters by two to three logs. Their study also showed that pressure was more important than pH or temperature of the wash water. Patterson (1968a, 1970, 1972) also reported that chlorination of carcass wash water could reduce the bacterial counts by one log.

Chlorinated water significantly reduced the large bacterial number associated with poultry processing as reported by Patterson (1968b) and Ranken et al., (1965). Anderson et al., (1977a) using strips of plate meat sprayed with acetate sodium hypochlorite or tap water found that reduction in counts exceeded 99.9% when samples washed with 25.4 liters/min were sanitized with 3% acetate sodium hypochlorite 200 to 250 mg/liters and tap water reduced counts about 90%. They concluded that acetate has a much greater residual effect on viable bacteria than did hypochlorite. Marshall et al., (1977) also reported that sanitized beef with sodium hypochlorite could reduce microbial numbers. The bacterial numbers on meat were reduced most when the highest pressure 14.0 kg/cm^2, the highest flow rate, 6.8 liters/min, and the longest time of spraying, 15 sec, were used. Anderson (1977b) used three sanitizers (chlorine, 200-250 ppm, acetic acid, 4.0%, and quaternary ammonium, 3.78 g/liters) to reduce the microbial numbers on the surface of beef. All of these research reports showed that various sanitizers and appropriate spraying systems can

reduce the microbial numbers and increase the shelf life of meat.

Electrical Treatment and Microorganisms

Fedotkin & Zharik (1978) studied the stabilizing effect of an electric current (applied for 1 h. at 0.05-0.1 A/cm and 20 V) on low concentration sugar juice and found the electric current did possess a bactericidal effect. They explained that this effect was due to the formation of atomic oxygen and ozone. Glushchenko et al., (1977) reported taht the effect of the electric field discharge on growth rate and content of nucleic acids is greatly affected by the electric field and ionic flow. The method can be used advantageously to foster growth, propagation and development of microorganisms. Gvozdyak et al., (1977) discussed the effect of increased electrical field tension on the flow rate and retention of macromolecules which could separate microorganisms and macromolecular substances. Zhuravleva (1977) developed a system for using an electric current to improve the treatment of water supplied for drinking purposes. He found that the effect of a direct current on E. coli, S. albus, and B. anthracoids in water containing various concentrations of Al_{3+} and Fe_{3+} cations had destructive effects which were 99.99, 99.84, and 45.7% respectively. Rudenko & Bretosh (1975) used an electric spark discharge method for destruction of bacteria in effluents from meat processing plants and slaughter houses. Wenzel (1971) found that a wide variety of foods may be preserved by using a direct current course producing an electrostatic field. The product is placed for a predetermined period between two electrodes generating a acurrent with certain intensity, the connection being arranged so that the product constitutes the negative pole. The microorganisms can be carried away from the product by the flow of the

current toward the positive pole. Urusov et al., (1971) investigated the efficiency of disinfecting waste meat processing water by spark discharges in an electric field and found the electric current causes plasmolysis of the cells. This is due to electrolysis of certain components of the medium and disruption of the cell wall following the shock wave. The generated radicals, H and OH, accelerate disintegration of the proteins, and also depolarize the nucleic acids, inactivate the enzymes and separate the purine substances. Kietzmann & Rakow (1970) reported that cod fillets salted on board and stored in an electrostatic field of 10 V/cm density have 1:2 reduction of airborne microorganisms by comparing to the conventional storage. Doskoch et al., (1974) found that electrical current could stimulate the microbial spore germination. Zhuk (1977) reported the disinfecting properties of pulsed electrical charges on bacterial suspension. Destructive effects of bacteria in milk were also reported (Anderson & Finkelstein, 1919; Beattle & Lewis, 1925; Prescott, 1927; and Sandorf, 1938).

Electrical Stimulation and Meat Quality Attributes

Restraint, suspension, delayed chilling, ultra hydrostatic pressurization, aging, and electrical treatment methods have been used to improve the tenderness of meat. Recently, electrical stimulation has been proven to be the most accepted method by the industry. Numerous papers have been written, showing that electrical treatment caused improvement of the quality attributes of meat, i.e., tenderness (Carse, 1973; Chrystall & Hagyard, 1976; Davey et al., 1976; Dutson et al., 1980; Gilbert & Davey, 1976;Grusby et al., 1976; Savell et al., 1977; 1978b; Smith et al., 1977; Sorinmade et al., 1978; Will et al., 1980), flavor and palatability, (Davey et al., 1976; Savell et al., 1977; 1978a; 1979; 1980), color and maturity, (Hall et al., 1980; Savell, et al., 1979; 1980), and retail appearance (Hall et al., 1980; Riley et al., 1980b; Savell et al., 1979; 1980; Tang & Henrickson, 1980). Although many papers have reported that quality attributes of meat were imporved by electrical stimulation, the real mechanisms by which electrical stimulation improves these qualities has not been well understood. There are three theories of the mechanisms described: a) reduction of "cold shortening", b) increased activity of acid proteases (Dutson et al., 1980; Judge et al., 1980; Savell et al., 1977; Sorinmade et al., 1978; Will et al., 1980), c) physical disruption of myofibrills (Savell et al., 1978a; Will et al., 1980).

Dutson et al., (1980b) indicated that a greater amount of enzymes have been released from the lysosomes of electrically stimulated samples than those from the control samples, and enzymes were free in the cytoplasma. The total activity of both β-glucuronidase and Cathepsin C was significantly lower in the stimulated sample. This indicated that the extent of autolysis was greater in the stimulated muscle. Sorinmade

(1978) found that the non-stressed, stimulated carcasses has the least amount of free activity of β-glucuronidase and the lowest pH.

Dutson et al., (1980b) and Moeller et al., (1977) also concluded that low pH and high temperature conditions in the electrically stimulated samples disrupted the lysosomal membrane, freeing lysosomal enzymes into cytoplasma. Harsham (1951) attributed the effects to the release of catheptic enzyme during the vigorous muscle contraction that was electrically stimulated. Deatherage (1980) concluded that lysosomal enzymes responsible for aged meat tenderness affected the tenderness of electrical stimulation by self-digention or autolysis.

The physiological level of ATP and pH are permissive factors for cold-shortening since it occurs only in pre-rigor muscle and reversibility of cold-shortening based on the response of releasing calcium ions from SR membranes and mitochondria to change in temperatures. Electrical stimulation post-mortem could increase the rate of the pH decline and the development of rigor by accelerated rate of glycolysis, which could prevent cold-shortening.

Savell et al., (1978a) suggested that physical disruption of muscle fibers resulting from massive contractions during electrical stimulation could result in tenderization. In electron micrographs, the electrically stimulated samples showed a less well defined l-band and z-line through the contracture bands, and sarcomeres on either side of the contracture bands seemed to be stretched or broken. Will et al., (1980) reported that electrical stimulation caused specific structural changes in the muscles, i.e., swollen sarcoplasmic reticulum, mitochondria and T-tubes.

Chrystall et al., (1980) suggested that the nervous stimulation will

give smaller ΔpH value, and the muscles will require slightly longer to reach pH 6.0 than direct stimulation of muscle using a high voltage. In addition, stimulation as soon as possible after slaughter with a voltage sufficiently high to stimulate muscle directly, and also to elicit nervous responses in the remote muscles, should give the most uniform effect in all muscles. Similar conclusions were reported by Deatherage (1980) and Swatland (1980). Muscle will increase impedence due to a drop in temperature.

Various voltages have been used in electrical stimulation to tenderize the meat. Harsham and Deatherage (1951), used forty to fifty volts, Davey et al., (1976), and Gilbert & Davey (1976), used 1600 volts, Shaw and Walker (1977), and bouton et al., (1978), used 110 volts, Demayer and Vandendriessche (1980), used 165 volts, Taylor (1980), used thirty-two volts. Houlier (1980) studied the different effects on muscle with various electrical field and duration of stimulation. Ruderous (1980) tested the effect of (0.5, 15, and 160 Hz) on the pH drop. Deatherage (1980) reported that frequency of forty to sixty cycles per second is satisfactory.

After extended post-mortem time, the muscles will lose the excitability and the nerves will lose their capacity to trigger the muscles to contract (Swatland, 1980).

All of these reports concerned the various voltages, frequency, resistance, current, or electrical field with the electrical stimulation that would give different effects on the quality attributes of the meat. However, only a few authors reported the effect of electrical stimulation on meat spoilage flora and its effect on meat storage shelf-life. Also, the results of the effect of electrical stimulation on meat

microflora were controversial. Gill (1980) reported that there was no differences in lag phase, growth rate, or maximum cell density of the bacteria between electrically stimulated or control samples of sheep carcasses. Raccach & Henrickson (1978) reported that electrical stimulation of beef carcasses prolonged the lag phase of the psychotrophic bacterial population by two days, but enhanced the growth rate during the logarithmic phase of growth. Shelf-life of ground beef from electrically stimulated carcasses was prolonged by three days as compared to the control samples. Maigadat et al., (1980) reported that electrical treatment of rabbit muscles caused a reduction in the count of Pseudomonas putrifaciens and of a Lactobacillus sp. when inoculated muscles were held for forty-five minutes after electrical stimulation. Electrical stimulation of pork carcasses did not affect the aerobic plate count (APC) of the skin surface. APC of cutaneous trunci from electrically stimulated sides of beef and lamb carcasses were similar to those of muscles from unstimulated sides or carcasses. APC of ground beef and blade steaks fabricated pre-rigor from electrically stimulated sides were often numerically lower after three days of storage than those of corresponding samples from unstimulated sides. However, electrical stimulation did not cause any consistent substantial changes in microbial types on ground beef, blade steaks, t-bone steaks, or rib steaks. Hall et al., (1980) found no significant differences in bacterial counts between electrically stimulated and control samples, either initially or at the termination of the display period for either steak or ground beef samples.

Potential commercial advantage of the elevtrical stimulation techniques makes hot-deboning somewhat easier because the muscles become firmed as they go quickly into rigor. When hot-deboning is combined

with electrical stimulation, it can result in a considerable reduction in time, space, and refrigeration capacity required to produce beef of good eating quality. However, microbiological quality of hot-boned beef must be controlled. Stern (1980) found that standard plate counts and psychotrophic plate counts were consistently higher for hot-boned lamb chops than for cold-boned lamb chops. (Kotula & Emswiler-Rose (1981) reported that mesophilic and psychotrophic bacteria numbers were higher on primal cuts from hot-boned beef sides than from cold-boned sides before storage. Fung et al., (1980) indicated that hot-boned meat had higher mesophilic and psychotrophic counts than conventionally processed meat.

Emswiler & Kotula (1979) reported that the microbial quality and shelf-life of ground beef from hot-boned carcasses were equal to or better than those properties of ground beef from chilled carcasses. Contreras et al., (1981) reported that electrically stimulated hot-boned (ESHB) samples had less than one log difference in microbial counts per gram of meat at 32°, 25°, and 5°C than did the conventionally chilled beef sides. Also, pH of ESHB samples was lower than that for conventionally chilled samples. In the future, developing methods to reduce the initial microbial load on carcasses that are hot-deboned will become important to the meat industry.

MATERIALS AND METHODS

Design

Eighteen cattle (460-540 kg) were slaughtered at Clark Meat Science Laboratory of Oregon State University. Each carcass was split longitudinally into two sides, and the right sides were electrically stimulated with 380 V, 4.4 amp and 60 hz, for 2 min. Stimulation was carried out within twenty min. of exsanguination. Stimulation was applied using three electrodes. One electrode was inserted at the distal end of the junction of the biceps fermoris muscle and the semitendinousus muscle. The other was inserted into the brachiocephalicus muscle, and the third into the triceps brachii. Both sides of each carcass was placed in a cooler at 1°C for one week after the process was completed.

Sampling Procedure

For each sample, a sterile cotton swab was moistened in a test tube (kept in 4°C ice bath) containing 6 ml of 0.1% peptone water. Three positions on each side of the carcass, one electrically treated and one untreated, were sampled: The inside of the neck (position 1), the exposed gracilis muscle above the aitch bone (position 2), and the fat on the outside of the round (position 3). A stirile stainless steel template (40 sq cm) was used to define each sample area. Aliquots (1 ml) from serial dilutions of each of the sample tubes were transferred to petri dishes, and standard plate count agar at 40°C was added, followed by incubation at 28±2°C for 72 h. The six positions were each swabbed four times on each carcass. This sampling was repeated twice for each position on each side of the carcass. Samples were taken immediately after slaughtering and again after the carcass had been chilled for 72 h. The method of counting the colonies on the plates and

recording of these results followed the method described by Speck (1977). Colonies were counted by using a colony counter (Model C-100, New Brunswick Scientific Co.).

Microorganisms and Agar Media

Strains of Escherichia coli ATCC 25922, Bacillus cereus, Pseudomonas fragi, Pseudomonas putrifaciens, Microbacterium lacticum, Lactobacillus arabinosus, Alcaligenes lacticum, Micrococcus roseus, and Streptococcus lactis were used. All microorganisms were from the culture collection maintained in the Department of Microbiology, Oregon State University. Medium A contained sodium chloride, 4 g; dipotassium phosphate, 4 g; Tryptone, 5 g; yeast extract, 2.5 g; glucose, 1.0 g; agar, 15.0 g; and distilled water, 1.0 l. Medium B contained dipotassium phosphate, 8 g; magnesium sulfate, 0.5 g; Tryptone, 5 g; yeast extract, 2.5 g; glucose, 1.0 g; agar, 15.0 g; and distilled water, 1.0 l g. Medium C contained dipotassium phosphate, 8 g; Tryptone, 5.0 g; yeast extract, 0.5 g; glucose, 1.0 g; agar, 15.0 g; and distilled water, 1.0 l. Media were adjusted to pH 7.1±0.1 with sulfuric acid and were autoclaved at 121°C for 15 min.

Cultures were held at 4°C on standard plate count agar slants between subculturing transfers for maintenance. For experiments, cultures were routinely obtained by inoculating cells from slant cultures into trypticase glucose yeast broth with incubation at 30°C on a shaker rotating at 180 rpm for 18 h. The microorganisms were appropriately diluted to approximately $1.5\text{-}2.5 \times 10^2$/ml and were spread on media A, B, and C.

Electrical Treatment on Media

Electrical treatment of media was applied from a transformer (Model 3-IMB, Dressen-Barnes corp., Pasadena, CA) for 5, 10, and 20 min. with

voltages of 20, 30, 40, and 50 for each time period. The aluminum electrodes were 6x1 cm^2 and the power supply was arranged as shown in Fig. 1.

Control media were inoculated with the same number of microorganisms, however, they were without electrical stimulation. Results are shown as percent recovery of the different species of microorganisms present. Each treatment was repeated three times under the same experimental conditions.

Electrical Treatment of Cell Suspensions

E. coli, P. fragi, P. putrifaciens were grown in trypticase glucose yeast broth overnight at 30°C on a rotating shaker operating at 180 rpm. The microorganisms were serially diluted in 0.1% peptone water, 0.25 M sucrose, 0.85% saline or phosphate buffered saline. Forty ml of each suspension were taken from the stock solutions and put into a 50 ml test tube. A 30 V electrical stimulation was applied for 5 min. or 15 min. to these tubes through a carbon electrode 0.5 cm in diameter and 10 cm in length. The instruments were arranged as shown in Fig. 2. Control groups were handled in the same way except that electrical stimulation was omitted.

Spread plate procedures were used to determine microbial growth. Each determination was repeated three times under the same experimental conditions.

Statistical Analysis

The statistical paired difference t-test was used to analyze the data and to determine the significant difference between treatments.

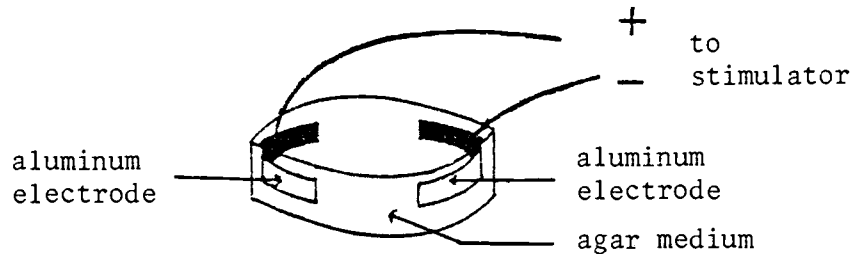

Figure 1. Stimulating electrode on agar medium.

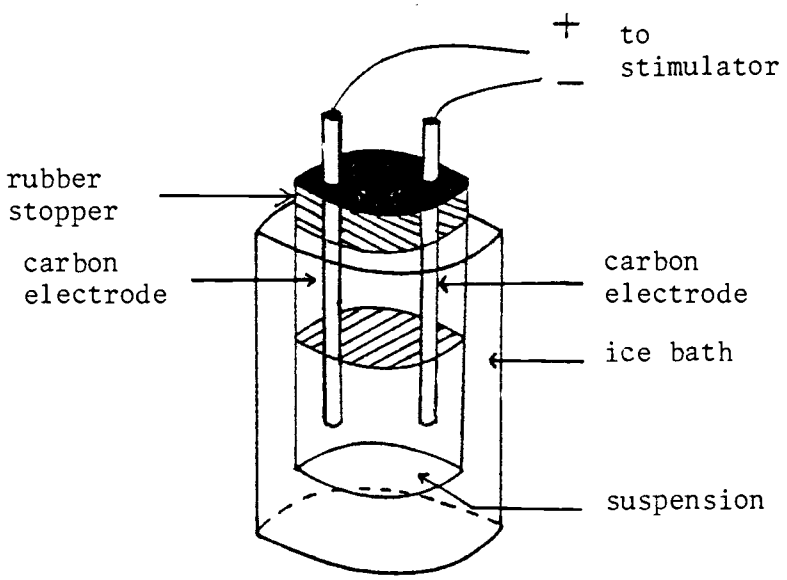

Figure 2. Stimulating electrode in suspension

RESULTS AND DISCUSSION

Electrical stimulation (ES) did not have a statistically significant effect on the microbial population of beef carcasses immediately following slaughter (Table 1). However, there was a significant difference ($P < 0.05$) in bacterial count between control and ES samples at position 2 (muscle above aitch bone) after 72 h. of chilling storage. This may be due to the position of the muscle above the aitch bone having higher conductive characteristics for electrical current causing greater chemical reaction in the muscle than that of the other two positions containing fat and bone (Raccach, 1980; Swatland, 1977; 1980a; 1980b). Electrical stimulation could enable the pre-rigor muscle to release lysosomal enzyme (Dutson et al., 1980a; 1980b; Sorinmade et al., 1978), causing increased activity of acidic protease (Parrish, 1977; Sorinmade et al., 1978), changing the ionic concentration of the muscle (Joseph et al., 1980; Lee et al., 1965), increasing the rate of pH drop (Devine et al., 1979; Halland and Bendall, 1965), and increasing the temperature on post-mortem muscles (Gilbert and Davey, 1976). These factors may affect the viability of the microorganisms on the meat. In addition, the microorganisms could be injured by the presence of free radicals or the flow of current, and the injurious effect on these microorganisms could be magnified by the chilling or freezing process.

Raccach and Henrickson (1978) reported a significant reduction in aerobic plate counts on ground beef from electrically stimulated hot-boned carcasses and Riley et al., (1980a; 1980b) found a significant reduction in bacterial counts on the lean surface of retail cuts from electrically stimulated lamb carcasses. Maigadat et al., (1980) reported that electrical stimulation of rabbit muscles and supraspinatus of beef

caused a reduction in count of P. putrifaciens and Lactobacillis sp. when inoculated muscles were held for forty-five min. (Maigadat et al., 1980). These studies and our results tend to support increased storage stability and bacteriological quality of meat resulting from electrical stimulation.

One of the most important quality control procedures in meat plants is to avoid high initial contamination of microorganisms during the meat slaughtering process. Moreover, high initial microbial contamination of meat will cause discoloration and spoilage, reducing the shelf-life (Bala et al., 1977; Dahl et al., 1978; Tarrant et al., 1973; Thomas et al., 1977) and making sanitizing more difficult. Position 1 and 2 had higher microbial count than position 3 (Table 1). This suggests higher contamination of these positions during the slaughter process. Sanitizing carcasses could be accomplished by spraying them with a mixture of 1.5 M acetate and propionate (Reynolds and Carpenter, 1974) with no more than 220 ppm chlorine water or with 3% acetate (Anderson et al., 1977a; Empey and Scott, 1939a; 1939b).

Since the major components of biological material (protein, lipid, polysaccarides and nucleic acid) have quite different charge behaviors, the biological activity of living cells could be affected by the electrical field and current flow. Numerous reports have shown the effects of an electrical field on living cells, i.e., lethal effects on a number of species of bacteria and yeasts in the vegetative state (Sale and Hamilton, 1967). Also, direct current pulse treatment has disrupted the limiting membrane of erythrocytes and bacterial protoplast (Hamilton & Sale, 1967), inactivated enzymes (Gilliland & Speck, 1967a) and changed the nucleic acid content of yeasts, molds, and bacteria (Glushchenko et

Table 1. Comparison of Aerobic Plate Counts (APC) from control (CON) and electrically stimulated (ES) samples from three positions on beef carcasses.

Positions	Treatment	Sampling time (days)	APC $\log_{10}/in^2$ (6.45 cm^2)	Significance of t - value df	
1. Inside of neck	CON	0	3.56	17	n.s.[a]
	ES		3.32		
	CON	3	3.29	17	n.s.
	ES		2.83		
2. Gracilis muscle above aitch bone	CON	0	3.65	17	n.s.
	ES		3.23		
	CON	3	3.79	17	s.[b]
	ES		2.75		
3. Outside of round	CON	0	2.83	17	n.s.
	ES		2.98		
	CON	3	2.74	17	n.s
	ES		2.65		

[a] n.s. = not significant

[b] s. = significant ($P < 0.05$)

al., 1977). While bacteriophages are more resistant to heat and chemical bactericides than bacteria, they are the most susceptible microorganisms to electric treatment (Gilliland and Speck, 1967b).

Results presented in Table 2 show that the total number of E. coli, P. fragi, and P. putrifaciens in 0.1% peptone water and 0.25 M sucrose solution were not affected by the electrical stimulation under our experimental conditions. However, reduction in microorganism counts was observed in 0.85% saline and phosphate buffer solutions under the 30 V - 5 min. treatment. This reduction could result from increased electrical flow in saline and phosphate solutions and/or from chlorine produced by electrolysis of sodium chloride.

Microorganisms can be affected by changing electrical fields in media. The recovery of different microorganisms decreased with increasing voltage and treatment time, Figures 3 through 23. The mechanisms by which electrical stimulation decreased the number of microorganisms is still not well understood. There are four possible mechanisms according to R. P. Quellette: 1) reduction in number of viable microorganisms by absorption on the electrodes, 2) electrochemical oxidation of microbial components at the anode, 3) destruction of the microorganisms by production of biocidal chemical species, and 4) destruction by electric field effects which is caused by changing electromotive forces resulting from the impressed alternative current (Quelette and Farah, 1978). Decreasing recovery may also be due to the heat resulting from current flow, free radicals produced by chemical reaction, or mechanical action (Allen and Koike, 1966; Brandt et al., 1962; Foner, 1964; Gilliland and Speck, 1967a; Zhuk, 1977). However, the heating effect in our experiment was not a significant factor in decreasing the bacterial

Table 2. The effects of electrical treatment (30 volts) of different solutions on the development of *E. coli*, *P. putrifaciens*, and *P. fragi*.

Solutions	Treatment Time (minutes)	E. coli (log_{10}/ml)		P. putrifaciens (log_{10}/ml)		P. fragi (log_{10}/ml)	
		control	treatment	control	treatment	control	treatment
0.1% peptone water	0	4.08±0.05	4.08±0.05	4.58±0.12	4.58±0.12	4.93±0.05	4.93±0.05
	15	4.12±0.06	3.98±0.03	4.52±0.18	4.47±0.10	4.76±0.11	4.58±0.09
0.25 M sucrose solution	0	4.61±0.06	4.61±0.06	4.81±0.11	4.81±0.11	5.49±0.07	5.49±0.07
	15	4.64±0.19	4.64±0.18	4.81±0.16	4.51±0.16	5.51±0.16	5.56±0.09
0.85% saline	0	4.57±0.12	4.57±0.12	5.67±0.08	5.67±0.08	5.59±0.12	5.59±0.12
	5	4.46±0.14	0	5.65±0.05	0	5.63±0.04	0
Phosphate buffer saline	0	4.65±0.01	4.65±0.01	5.72±0.13	5.72±0.13	4.53±0.09	4.53±0.09
	5	4.68±0.02	0	5.71±0.14	0	4.57±0.14	0

number, since temperatures higher than 37°C on the media were not observed. Electrical current could also affect the growth of microorganisms by: stimulating germination (Doskoch et al., 1974), apparent cooperativity of amino acid transport as for example in H. halobium (Lanyi, 1978) and electric potential retention (Gvozdyak et al., 1977).

The recovery of microorganisms during the present study did not reach 1% optimal treatment for 40 V - 5 min. of three media. Although the decimal reduction of microorganisms is necessary for the food industry as a significant killing effect, if we increase the electrical field and treatment times, this may be achieved.

Of the bacteria examined, B. cereus was the most resistant to electrical stimulation, with L. arabinosus being the least resistant (Table 3). This may be due to the fact the B. cereus is a spore-forming bacterium with greater resistance to chemical and physical effects resulting from the electrical stimulation. In general, the gram positive bacteria seemed to have less resistance to electrical stimulation than the gram negative bacteria. This might be due to the lipid content of the latter which gives higher resistance to the current flow. Cell walls of gram negative organisms are higher in lipid content than those of gram positive organisms. S. lactis, which has a higher germicidal resistance to the electrical treatment than these bacteria. (Figures 3, 4, 5, 6, 14, 15).

We also observed that microorganisms could not grow in media near the anode or cathode after electrical treatment. Also, the morphology of colonies near the probes was different from that of normal colonies, being smaller. Colonies of M. lacticum near the probes on medium A treated with electrical stimulation were replicated onto minimal salts agar revealing that colonies near the cathode failed to grow, but that those

Table 3. The effects of electrical treatment of media A[a] on the recoveries of microorganisms.

Strains	Gram stain	Treatment (recovery %) 30V-10min	30V-20min	40V-10min	40V-20min
S. lactis	+	53	39	31	15
E. coli	-	29	21	21	14
B. cereus	+	48	45	38	35
M. lacticum	+	32	19	27	8
L. arabinosus	+	34	20	16	0
P. fragi	-	34	28	24	20
A. lacticum	-	33	30	31	21
P. putrifaciens	-	42	31	25	12
M. roseus	+	37	15	21	7

[a]Media A contained sodium chloride 4g; dipotassium phosphate 4g; tryptone 5g; yeast extract 2.5g; glucose 1.0g; agar 15.0g; and distilled water 1.0ℓ.

taken from near the anode did grow. These results suggested that electrical treatment was injurious and possibly mutagenic. Also, the pigment producing ability of M. lacticum near the two probes was decreased. Similar results were observed for L. arabinosus and M. roseus.

Lower recoveries of microorganisms during the studies were found in medium A compared to medium B or medium C. By measuring the resistance of these three different media we found the resistance of medium A to be lower than that of medium B or medium C. According to Ohm's Law, the electrical current passing through a conductor increases if the resistance decreases under the same voltage. If the current flow was increased, it could increase the chemical and physical reaction in the material and affect the viability of microorganisms on the material. Such finding have been reported (Gilliland and Speck, 1967a; 1967b).

There are a number of possible applications of electrical stimulation in the food industry, i.e., improving the preservation of food products (Kietzmann and Rakow, 1970; Stersky et al., 1971; Stong, 1957; Wenzel, 1971), destruction of bacteria in waste meat processing, water, drinking water, milk, and low concentration sugar juices (Allen and Koike, 1966; Anderson and Finkelstein, 1919; Beattle and Lewis, 1925; Fedotkin and Zharik, 1978; Gelpi and Pevereaux, 1930; Lundbeck and Skoldberg, 1963; Prescott, 1927; Quelette and Farah, 1978; Rudenko and Bretosh, 1975; Sandorf, 1938; Silverman and Munoz, 1979; Wenzel, 1971; Zhuravleva, 1977), and quantifying microbial content of food by measuring the impedance changes of media (Cady et al, 1978; Hardy et al., 1977; Rowley et al., 1979).

Finally, the possible applications by adding different chemical reagents and treating with higher voltage on agar plates or suspensions

may induce specific mutation for some microorganisms. In addition, increasing the surface conductivity of the carcass by spraying with salt solution, applying multiple electrodes to the carcass surface, and treating with appropriate voltage may reduce the microbial population and increase the storage shelf life of fresh meat.

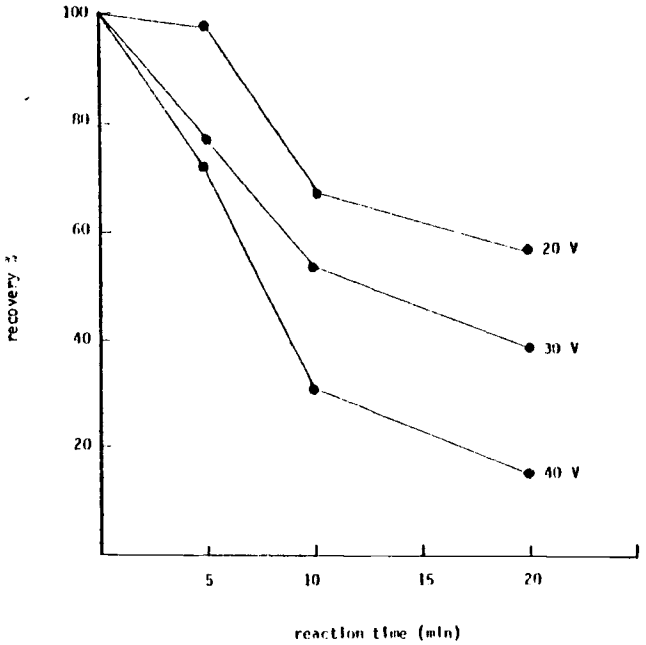

Fig. 3 - The effect of electrical treatment of medium A on the recoveries of S. lactis.

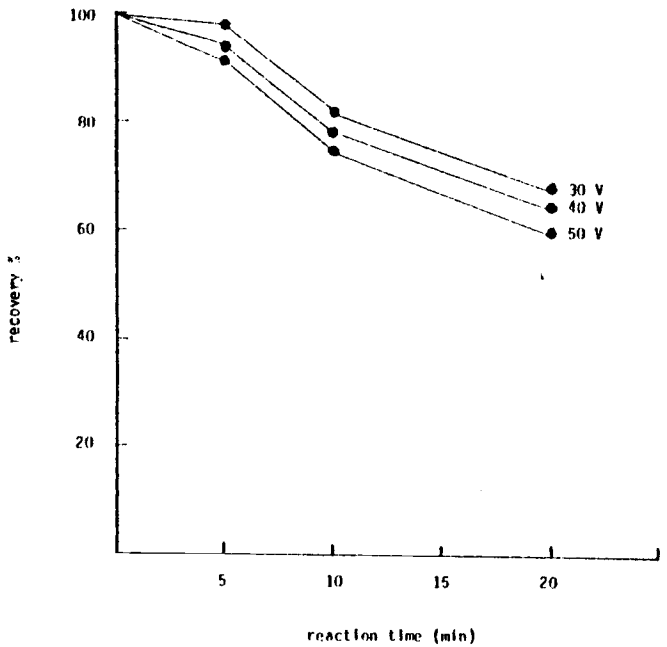

Fig. 4 - The effect of electrical treatment of medium B on the recoveries of S. lactis.

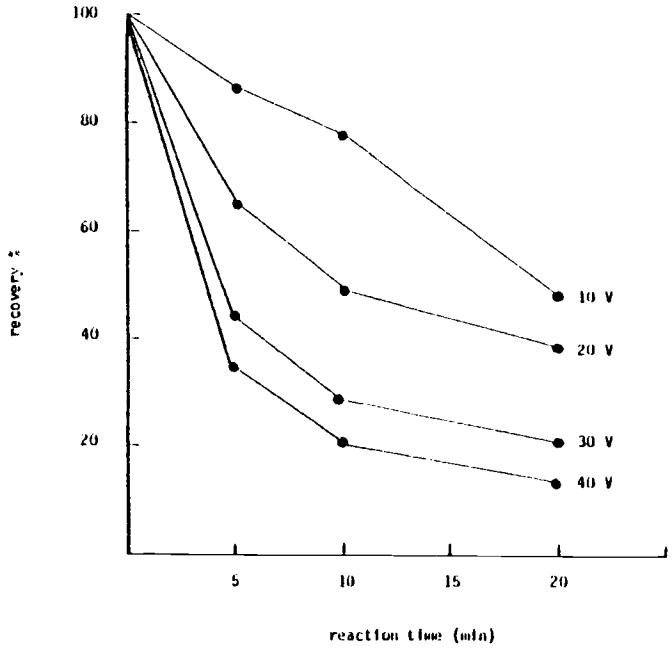

Fig. 5 - The effect of electrical treatment of medium A on the recoveries of E. coli.

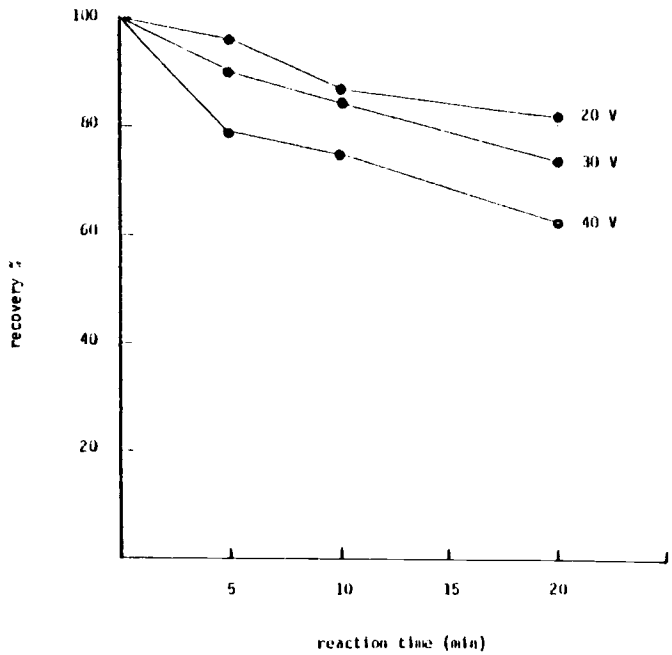

Fig. 6 - The effect of electrical treatment of medium C on the recoveries of E. coli.

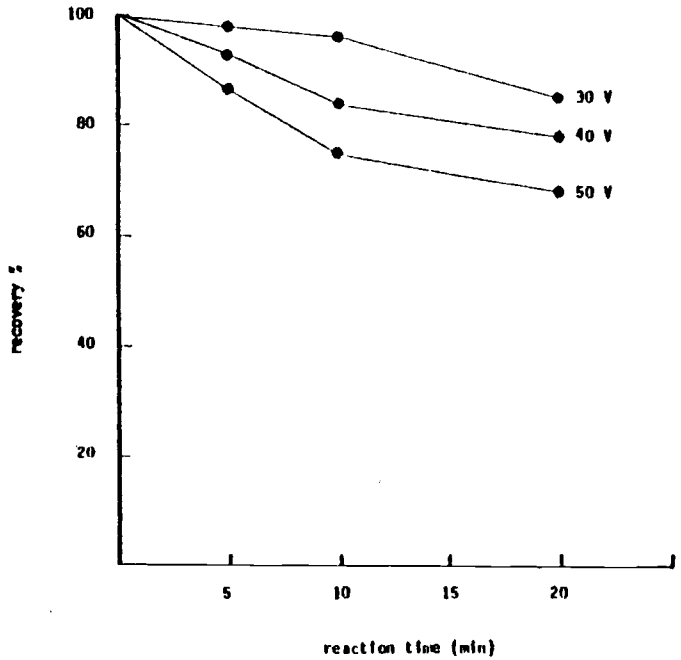

Fig. 7 - The effect of electrical treatment of medium B on the recoveries of E. coli.

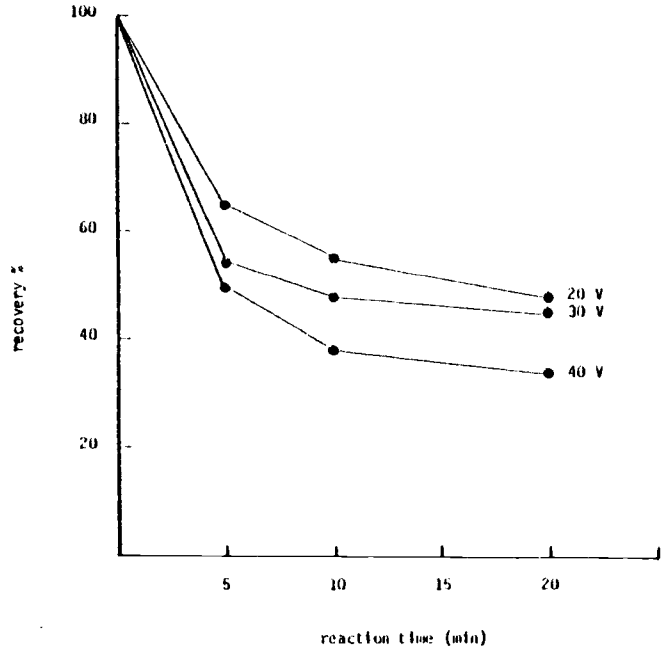

Fig. 8 - The effect of electrical treatment of medium A on the recoveries of B. cereus.

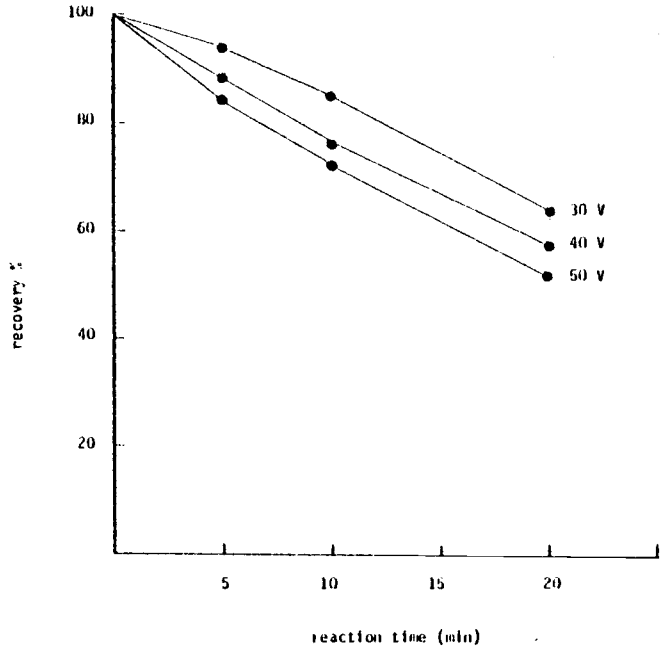

Fig. 9 - The effect of electrical treatment of medium B on the recoveries of B. cereus.

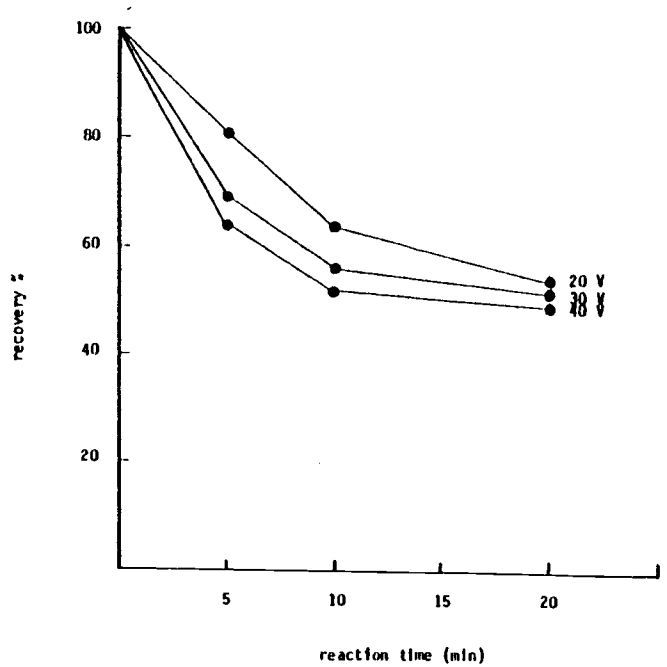

Fig. 10 - The effect of electrical treatment of medium C on the recoveries of B. cereus.

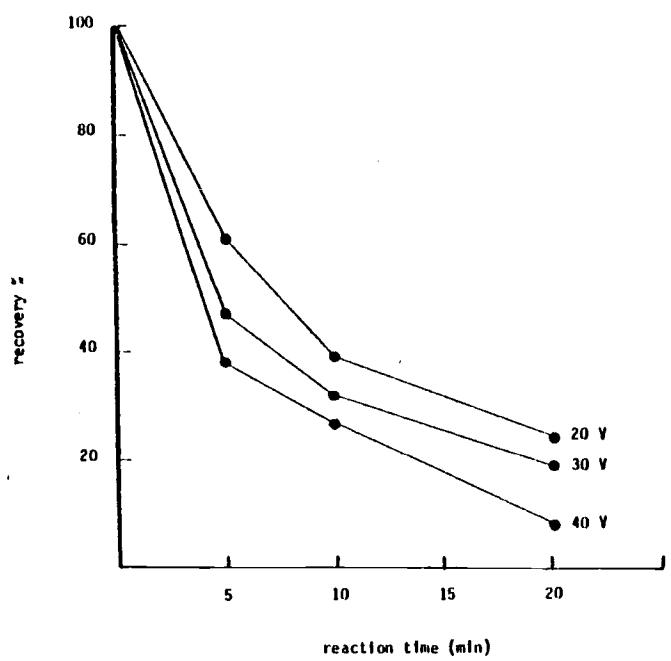

Fig. 11 - The effect of electrical treatment of medium A on the recoveries of M. lacticum.

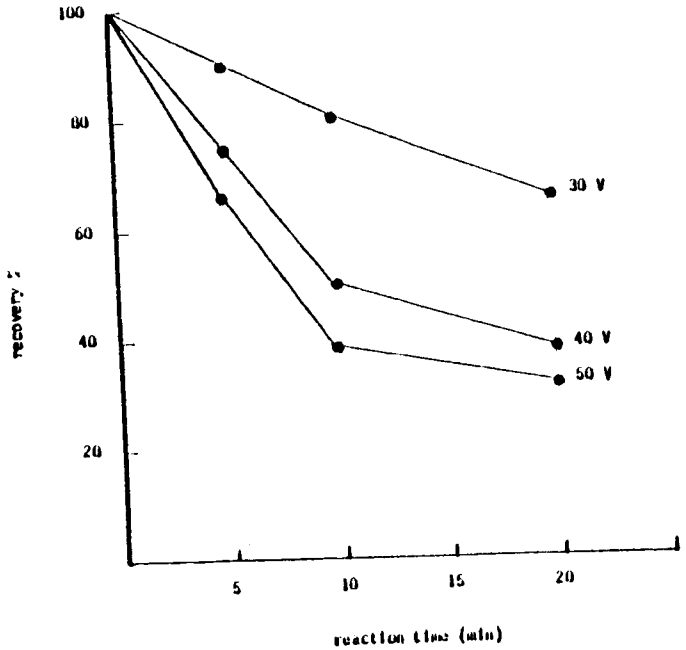

Fig. 12 - The effect of electrical treatment of medium B on the recoveries of N. lacticum.

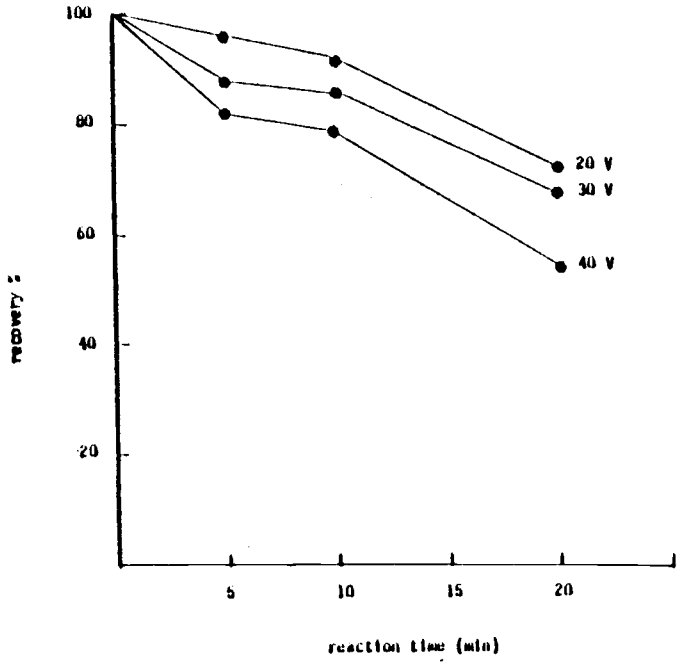

Fig. 13 - The effect of electrical treatment of medium C on the recoveries of N. lacticum.

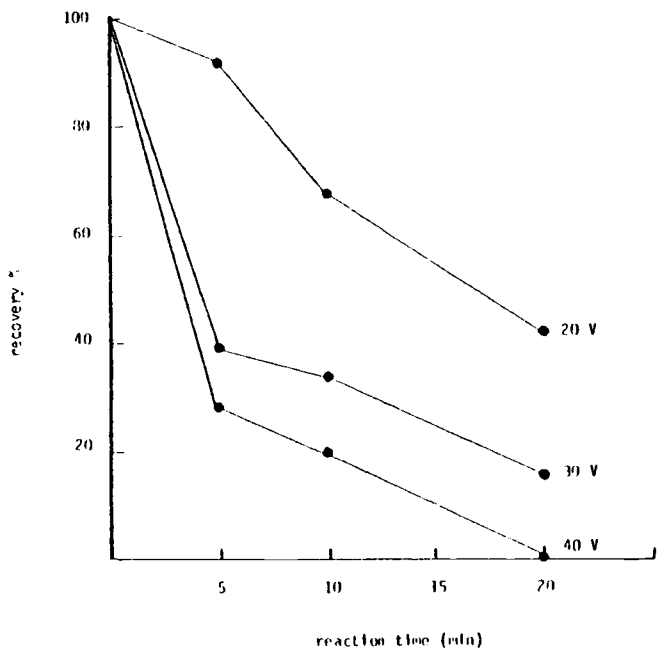

Fig. 14 - The effect of electrical treatment of medium A on the recoveries of L. arabinosus.

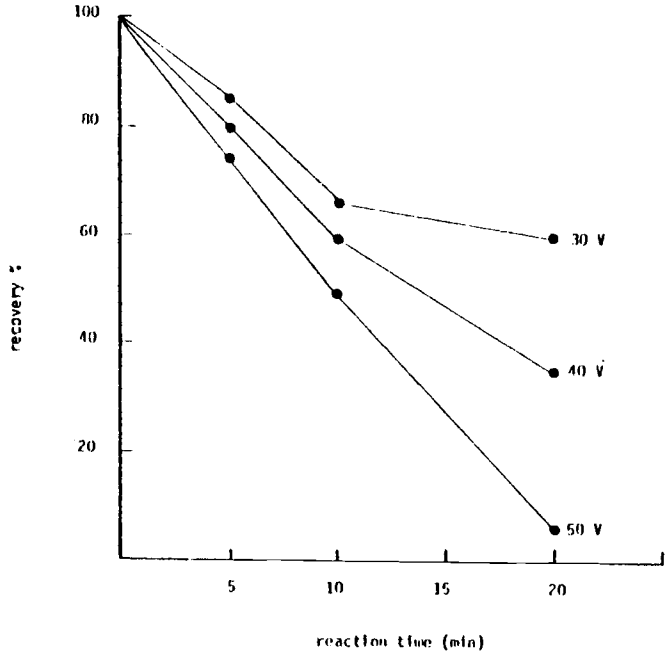

Fig. 15 - The effect of electrical treatment of medium B on the recoveries of L. arabinosus.

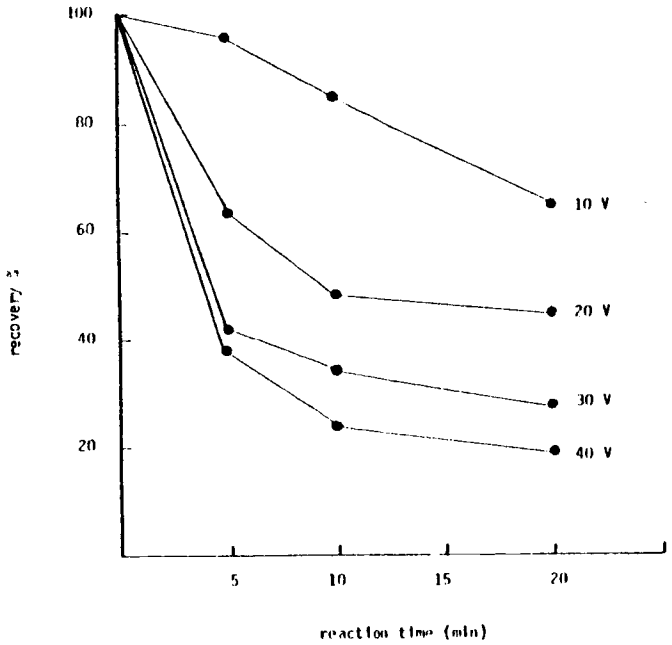

Fig. 16 - The effect of electrical treatment of medium A on the recoveries of P. fragi.

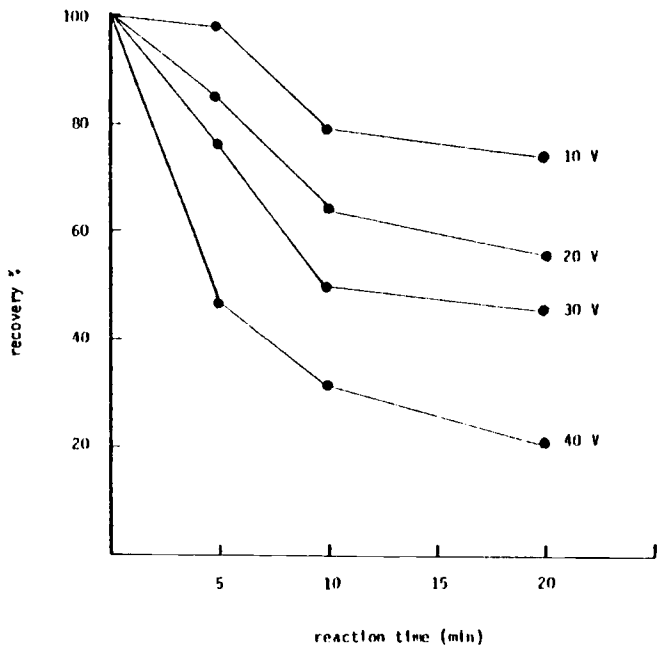

Fig. 17 - The effect of electrical treatment of medium C on the recoveries of P. fragi.

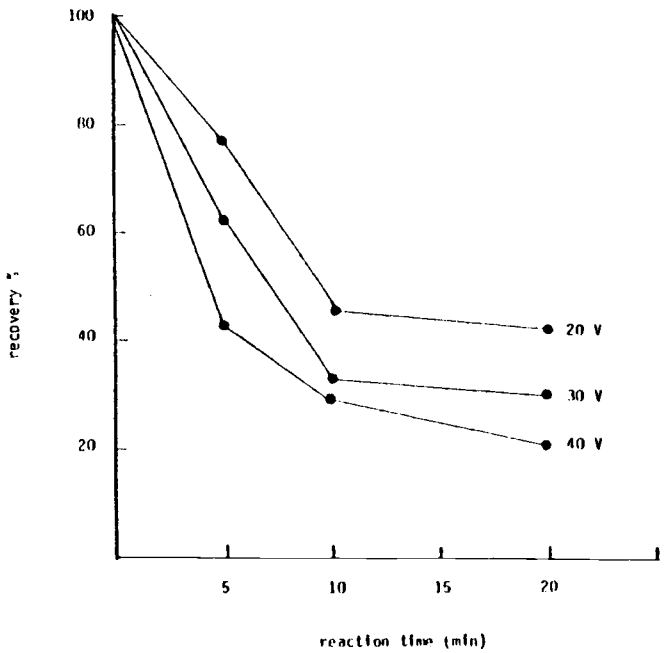

Fig. 18 - The effect of electrical treatment of medium A on the recoveries of A. lacticum.

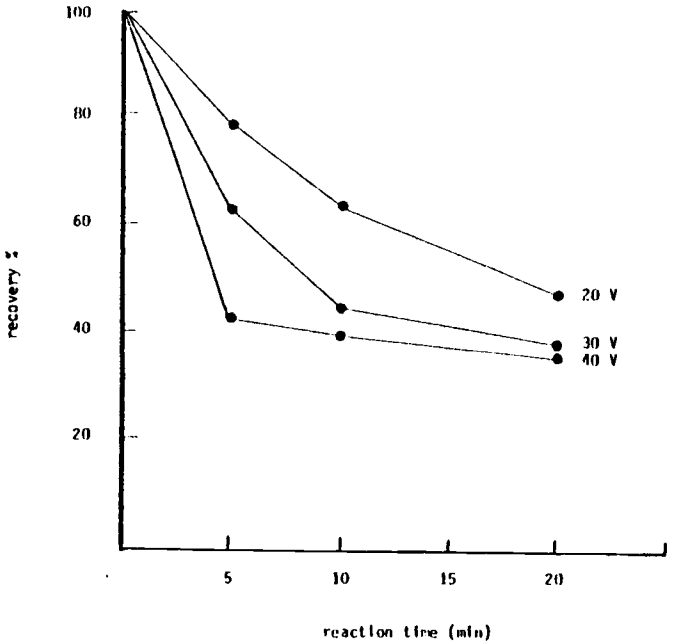

Fig. 19 - The effect of electrical treatment of medium C on the recoveries of A. lacticum.

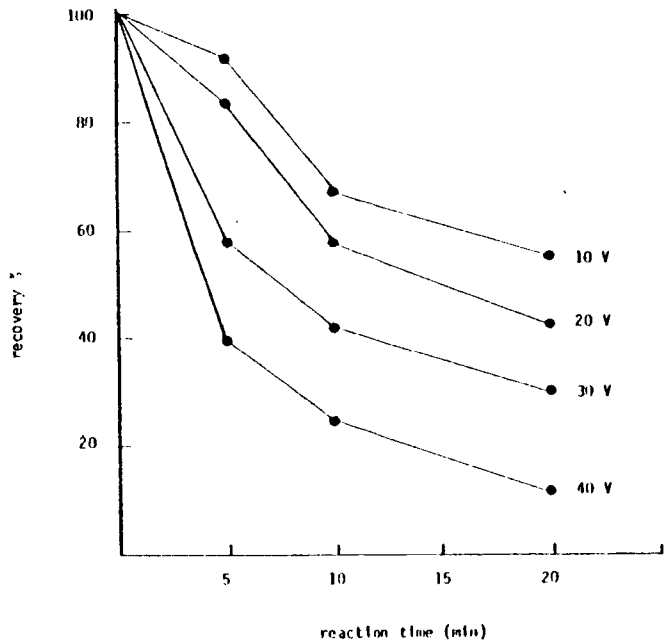

Fig. 20 - The effect of electrical treatment of medium A on the recoveries of P. putrifaciens.

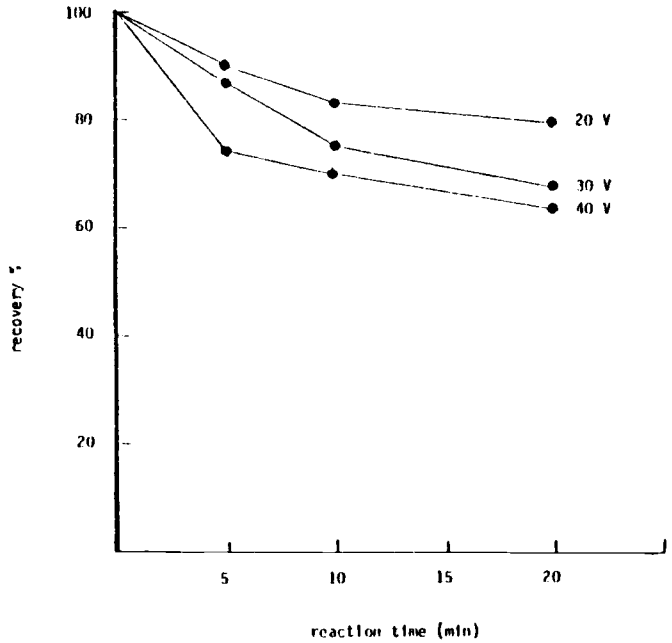

Fig. 21 - The effect of electrical treatment of medium C on the recoveries of P. putrifaciens.

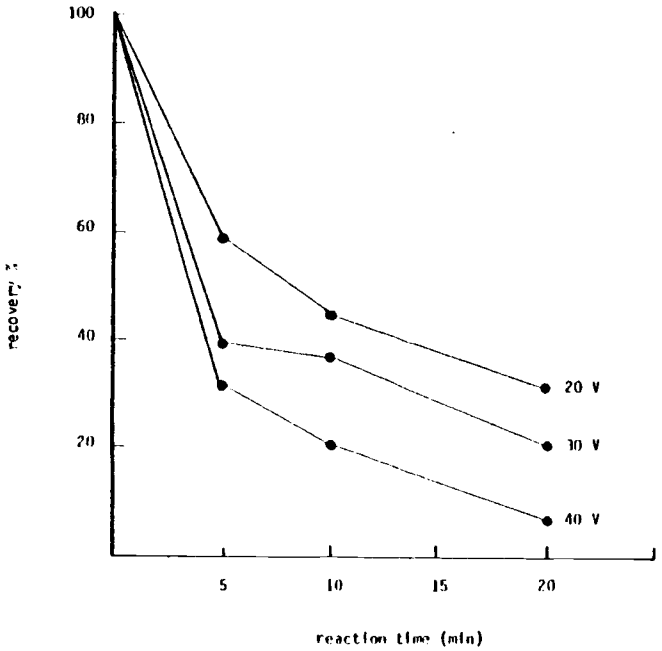

Fig. 22 - The effect of electrical treatment of medium A on the recoveries of N. roseus.

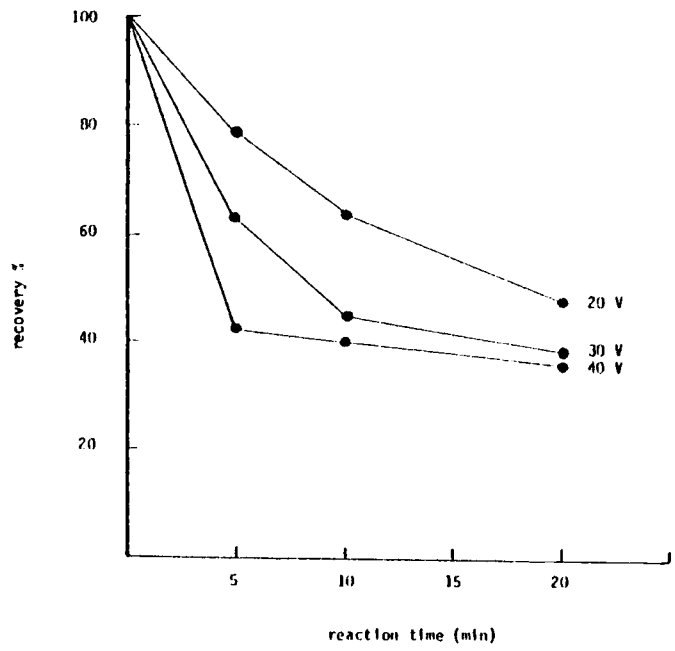

Fig. 23 - The effect of electrical treatment of medium C on the recoveries of N. roseus.

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