

PACKAGING FRESH AND CURED MEAT

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THE SURVIVAL OF POTENTIAL FOOD POISONING BACTERIA IN
VACUUM-PACKAGED MEAT

J. T. PATTERSON & P. A. GIBBS

Department of Agriculture for Northern Ireland and the Queen's
University of Belfast,
Agriculture & Food Science Centre,
Newforge Lane,
Belfast BT9, 5PX.

Inoculation experiments have shown that strains of Staphylococcus aureus, Clostridium welchii and Salmonella can survive in vacuum-packaged meat for at least 6 weeks. There is no evidence to show that multiplication occurs when the meat is stored at 0-2°C in vacuo nor when the meat is subsequently stored aerobically at 15°C for 3 days.

LA SURVIVANCE DES BACTERIA-QUI POURRAIENT BIEN CONTAMINER DES
ALIMENTS-DANS LA VIANDE EMBALLÉE SOUS VIDE

J. T. PATTERSON & P. A. GIBBS

Le Département de l'Agriculture de l'Irlande du Nord et de
l'Université Queen's à Belfast,
Le Centre de la Science Agronomique et de la Science de
l'Alimentation,
Newforge Lane,
Belfast BT9, 5PX

Les expériences d'inoculation ont indiqué que les races de Staphylococcus aureus, Clostridium welchii et Salmonella peuvent survivre dans la viande emballée sous vide au moins pendant 6 semaines. Rien ne marque que la multiplication aie lieu quand on emmagasine la viande à une température de 0-2°C in vacuo, ni quand on emmagasine la viande d'une façon aérobie à une température de 15°C pendant 3 jours.

Выживаемость в мясе упакованном в вакууме бактерий способных вызвать отравление пищеварительной системы

Дж.Т.Паттерсон и П.А.Гиббс

Центр для изучения сельского хозяйства и науки питания при Отделе сельского хозяйства по Северной Ирландии и Королевском университете, Белфаст

DAS ÜBERLEBEN LATENTER NAHRUNGSMITTELVERGIFTUNGSBAKTERIEN IN
VAKUUMVERPACKTEM FLEISCH

J. T. PATTERSON & P. A. GIBBS

Das nordirlandische Landwirtschaftsministerium und die
Universität Queens in der Hauptstadt Belfast,
die Landwirtschafts- und Ernährungswissenschaftsanstalt,
Newforge Lane,
Belfast BT9, 5PX

Verimpfungsversuche haben das folgende als wahr erweist:
Stämme von Staphylococcus aureus, Clostridium welchii, und Salmonella können zum mindesten sechs Wochen in vakuumverpacktem Fleisch noch leben. Es gibt keinen Beweis dafür, dass Vermehrung der Bakterien sich ereignet, wenn das Fleisch bei einer Temperatur von 0-2°C in vacuo gelagert wird; bei einer aerobischen Nachlagerung, die drei Tage dauert, und eine Temperatur von 15°C erfordert, gibt es auch keine Vermehrung der Bakterien nach Ermittlungsergebnissen.

Конспект

Опыты с применением инокуляции показали, что штаммы Clostridium welchii, Staphylococcus aureus и Salmonella могут переживать по крайней мере 6 недель в упакованном в вакууме мясе. Нет никаких сведений позволяющих заключать, что размножение происходит либо тогда, когда мясо хранится в вакууме при температуре 0-2° по Цельсию, либо тогда, когда оно впоследствии хранится три дня аэробным способом при температуре 15° по Цельсию.

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J. T. PATTERSON and P. A. GIBBS

Department of Agriculture and The Queen's University of Belfast,
Newforge Lane, Belfast BT9 5FX, Northern Ireland

A PREVIOUS REPORT (Patterson & Sutherland, 1973) dealt with the microbial changes which take place on vacuum-packaged beef at 0-2°, and the development of the microbial flora on slices of meat cut from packaged meat and stored under aerobic conditions at 4-6°. In addition some data were presented to show that 2 strains of *Staphylococcus aureus* could survive on packaged meat for at least 4 weeks, and 1 strain for 8 weeks, though with declining numbers. There was little evidence that this organism could multiply quickly after the meat was removed from the vacuum-package and held at 15°, probably due to the more rapid growth of competing organisms. However it was felt that further work was necessary, and this report describes inoculation experiments to study the survival of *Staph. aureus*, *Salmonella* and *Clostridium* on such meat.

MATERIALS AND METHODS

Inoculation, packaging and storage of meat

Slices, c 1 cm thick, of hindquarter beef (topside or rump) were obtained from a retail butcher and in the laboratory were cut into pieces 4 x 4 cm (c 25 g). Dilutions of cultures of *Staphylococcus aureus* (3 strains, including one capable of producing enterotoxin A, strain 23), *Salmonella muenster*, *S. dublin* and *Clostridium welchii*, the latter containing c 50% spores, were spread on the surface of the pieces of meat as previously described (Patterson & Sutherland, 1973). Two levels of inoculum were used for each organism as detailed in Tables 1-3, and the inoculated and control (uninoculated) pieces of meat were vacuum-packaged in nylon/polythene film and stored at 0-2° for 8 weeks (Patterson & Sutherland, 1973).

Recovery of inoculated organisms after storage

One package of meat for each of the two inoculum levels of an organism and an uninoculated control, were withdrawn from storage each week and opened aseptically. In some experiments pieces of meat were placed in new polystyrene trays overwrapped with an oxygen-permeable film (Patterson & Sutherland, 1973) and stored for 72 h at 15° before sampling (see Tables 1-3). Sterile diluent (0.5% peptone water) was added to the meat which was then homogenized in an AtoMix blender (MSE Ltd.) for 18,000 rev. in the experiments with *Staph. aureus*, or recovered using the Colworth Stomacher 400 (for 2½ min) in experiments with *Salmonella* spp., and *Cl. welchii*. *Staph. aureus* was enumerated by the MPN technique of Giolitti & Cantoni (1965) and confirmed by the coagulase test. *Salmonella* spp. were recovered

directly by plating on brilliant green agar (Ebel & Kampelmacher, 1969) or Wilson & Blair bismuth sulphite agar as modified by McCoy (1969), and confirmed by slide agglutination tests. *Clostridium welchii* was enumerated by the MPN 'total' count technique in the differential reinforced clostridial medium (DRCM) of Gibbs & Fream (1965) as modified by Gibbs (1973), and confirmed by streaking presumptive positive cultures on the lactose-egg yolk-milk agar of Willis & Hobbs (1959) and incubating anaerobically.

RESULTS AND DISCUSSION

The results of the inoculation experiments are given in Tables 1-3. Those in Table 1 show that the 3 strains of *Staph. aureus* used survived for 4 weeks, and in the case of 2 strains for 8 weeks, when initial numbers were relatively high. The numbers recoverable by the technique used however, decreased progressively and there was little indication that the staphylococci could multiply rapidly in competition with the other organisms present when the package was opened and the meat stored at 15°. Such inhibition by competing organisms under aerobic conditions is well known (Troller & Frazier, 1963), and certain lactobacilli and streptococci also have this ability (Sabine, 1963; Haines & Harmon 1973a, b). We have isolated many strains of *Es. fluorescens* which markedly inhibit *Staph. aureus* in plate culture. The one instance where multiplication at 15° was probable (MP 136, week 0) may have been due to fewer competing organisms, outgrowth of the strain of *Staph. aureus* already present on the meat. Since numbers of the order 10⁷/g of this organism are required to produce a food poisoning dose of enterotoxin, by an enterotoxin producing strain (Gilbert, Wieneke, Lamer & Simkovičová, 1972), there appears to be little danger from this source unless the meat was very heavily infected, or stored at higher temperatures. The position with *Salmonella* (Table 2) may however be somewhat different in that, although the numbers inoculated declined rapidly, some cells survived until at least 6 weeks, and probably longer since the technique used did not give a resuscitation period, nor detect less than 10⁶/g. Since even a few cells of certain strains may cause infection in the consumer, vacuum-packaging cannot be regarded as giving a safer product than when the meat is sold unpackaged. There was however no sign of rapid multiplication of the salmonellae when the meat was stored in air at 15°, again possibly because of competition from other organisms, and the relatively low storage temperature; *Pseudomonas* strains from poultry have been found which inhibit the growth of salmonellae when grown together (Oblinger & Kraft, 1970).

The survival of relatively low numbers of *Cl. welchii* (Table 3) was rather better than of the other organisms tested, and after 8 weeks in the vacuum package there was evidence for multiplication of the test organism at 15° provided the meat was kept in the evacuated bag. Since certain strains of pathogenic clostridia, notably *Cl. botulinum* type E, have the ability to grow at temperatures down to 3° (Schmidt, Lechowicz & Folinazzo, 1961) and whose growth will be enhanced by vacuum-packaging, it is essential to maintain the storage temperature close to 0° in order to minimize the risk from this source. The possible effects of the microflora developing on

vacuum-packaged meat on growth and toxin production by clostridia are not known and further work is indicated. While adequate cooking will destroy any botulin toxin, it may not be sufficient to kill all clostridial spores and the normal precautions concerning the handling of cooked meat must be observed.

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TABLE 1
SURVIVAL OF *STAPH. AUREUS* ON VACUUM-PACKAGED BEEF STORED AT 2°

| Strain | Inoculum/g | MPN/g recovered after (weeks) | | 29 | 72 | 72 h at 15° |
|--------|---------------------|-------------------------------|-----|-----|-----|-------------|
| | | 0 | 1 | | | |
| MP 1 | 9 (8-10) | 2 | 2 | 0 | 0 | 0 |
| | | 6 | 0 | 0 | 0 | 0 |
| MP 1 | 915 (742-1043) | 13 | 13 | 13 | 13 | 13 |
| | | 128 | 128 | 128 | 128 | 128 |
| 23 | 13 (11-15) | 2 | 2 | 2 | 2 | 2 |
| | | 2 | 2 | 2 | 2 | 2 |
| 23 | 1530 (1207-2089) | 184 | 164 | 79 | 19 | 19 |
| | | 325 | 325 | 325 | 325 | 325 |
| MP 136 | † | 9 | 171 | 0 | 0 | 0 |
| MP 136 | 3 (2-5) | 28 | 13 | 7 | 3 | 3 |
| MP 136 | 326 (227-452) | 335 | 80 | 118 | 97 | 27 |

† The meat was found to be already contaminated with a different phage-type of *Staph. aureus*.

TABLE 2

SURVIVAL OF SALMONELLA ON VACUUM-PACKAGED BEEF STORED AT 2°

| Serotype | Inoculum/g | Recovery/g after (weeks) | | | | | | |
|--------------------|------------|--------------------------|-------|-----|-----------------|-----|-----|-----------------|
| | | 0 | 2 | 4 | (+ 72 h at 15°) | 6 | 8 | (+ 72 h at 15°) |
| <u>S. muenster</u> | 360 | 320 | 80 | 40 | 40 | 40 | <40 | <40 |
| <u>S. muenster</u> | 3,600 | 3,600 | 500 | 80 | 40 | 40 | <40 | <40 |
| <u>S. dublin</u> | 420 | 260 | 400 | <40 | <40 | 120 | <40 | <40 |
| <u>S. dublin</u> | 3,350 | 2,400 | 1,080 | 80 | <40 | 200 | 120 | 40 |

TABLE 3

SURVIVAL OF CL. WELCHII ON VACUUM-PACKAGED BEEF STORED AT 2°

| Inoculum/g | MPN/g recovered after (weeks) | | | | | | | | | | | |
|------------|-------------------------------|-----|---|-----|----|-------------------------|-----|---|-----|-----|-------------------------|-------------------------|
| | 0 | 1 | 2 | 3 | 4 | (+ 72 h at 15°) open | 5 | 6 | 7 | 8 | (+ 72 h at 15°) open | (+ 72 h at 15°) vac. |
| 84 | 12 | 88 | 1 | 19 | 37 | (19) | 37 | 0 | 31 | 88 | (4) | (88) |
| 840 | 8 | 880 | 4 | 880 | 37 | (880) | 370 | 1 | 192 | 232 | (19) | (192) |