5:6 Effects of packaging, processing and formulation on spoilage of pasteurized meat products

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Introduction

Chub-packed luncheon meat, a pasteurized product shielded from post-cooking contamination, is a convenient model system in which to study the effects of product, processing and storage variables on microbiological stability. Only those microorganisms in the raw emulsion that survive the cooking process and then develop under the subsequent prevailing storage conditions can cause product spoilage. The present study was undertaken to determine the spoilage characteristics of chub-packed luncheon meat subjected to gross storage-temperature abuse and then to evaluate how microbiological stability is influenced by product formulation and by processing and storage conditions.

Materials and Methods

Microbiological examination of chubs

The surface and core of individual 250 g luncheon meat chubs were aseptically sampled by means of the cork-borer technique of Bell and Gill (1982). Samples (10 g) were homogenized in 90 ml of 0.1% peptone supplemented with 0.2% tergitol by the use of a Colworth Stomacher, and serial dilutions prepared from this homogenate in 0.1% peptone. Spread plates of appropriate dilutions were made in triplicate on plate count agar. The colonies that developed after 72 h incubation at 25°C were counted. Representative isolates were subcultured and identified to genus level (Bell & Gill, 1982). The pH of the initial homogenates was determined with a glass electrode.

Effect of thermal processing

Raw commercially prepared chubs were placed into a water bath cooker maintained at 68.5°C. Groups of chubs were removed when they had received a predetermined amount of thermal processing calculated as a pasteurization value for reference temperature 70°C (P_{70}) (Shapton et al., 1971). The P_{70}-values were calculated as described by Bell (1983). After removal from the cooker the chubs were immediately placed into crushed ice, cooled for 30 min and then dipped for 10 seconds into 95°C water to shrink the casing onto the meat. The chubs were either sampled immediately to determine the surviving microflora or stored at 25°C and then sampled at intervals to observe spoilage microflora development.

Effect of oxygen availability

Commercially prepared chubs vacuum mixed and filled into either conventional plastic casings or permeable fibrous salami casings were obtained from a manufacturer. Chubs with air trapped in the emulsion were prepared by filling plastic casings with commercial emulsion by means of a horizontal piston-type sausage stuffer and then cooking as described above to a P_{70}-value of approximately 125. All chubs were stored at 25°C in air; in addition, some vacuum filled, plastic-wrapped chubs were stored at 25°C under hydrogen (Bell & De Lacy, 1982). Five chubs from each group were removed from storage at intervals and examined microbiologically.

Heat injury and recovery of Streptococcus faecium

The ability of a Streptococcus faecium strain isolated from soured luncheon meat [Bell & De Lacy, 1983(a)] to grow aerobically and anaerobically in Brain Heart Infusion (BHI) broth containing 0.5-12% NaCl at pH 7.4; 0.02 to 1% $NaNO_2$ at pH 6.8; or at different pH values (4.0 to 6.8) within 7 days at 25°C was determined. The aerobic and anaerobic sensitivity to NaCl and $NaNO_2$ of S. faecium cells was determined by heating cells at 70°C for 10 min in BHI, BHI (2% NaCl), BHI (0.02% $NaNO_2$), BHI (2% NaCl, 0.02% $NaNO_2$) and in luncheon meat emulsion. Heated cells were plated onto BHI, BHI (2% NaCl) and BHI (2% NaCl, 0.02% $NaNO_2$), and the colonies that developed after 72 h incubation at 25°C were counted. The ability of cells heated in the four BHI-based media to recover and grow in these heating media was determined by storing heated cell suspensions at 25°C and daily removing samples for plate count enumeration on BHI and BHI (2% NaCl) agar [Bell & De Lacy, 1984(a)]. The survival and subsequent development of naturally occurring streptococci in luncheon meat chubs cooked to a P_{70}-value of 40 was also determined.

Germination and growth of Bacillus licheniformis

The ability of heat activated spores of a Bacillus licheniformis strain isolated from spoiled luncheon meat [Bell & De Lacy, 1983(a)] to grow aerobically or anaerobically in pH 6.8 TYG broth (liquid equivalent of plate count agar) containing either 0 to 12% NaCl; 0 to 1% $NaNO_2$ or in TYG broth at pH values ranging from 4.8 to 6.8 within 7 days at 25°C was determined. In tests involving anaerobic incubation, freshly prepared media were pre-reduced under hydrogen in an anaerobic jar at 15°C for 24 h [Bell & De Lacy, 1984(b)]. To determine the effect of curing salts on thermal resistance spores of B. licheniformis were suspended in TYG, TYG (4% NaCl), TYG (0.02% $NaNO_2$) and TYG (4% NaCl, 0.02% NaCl), all at pH 6.8, and then heated at 70 and 80°C. Samples were removed at intervals, dilutions prepared and plated onto plate count agar. Plates were incubated aerobically at 37°C and the colonies that developed within 24 h counted. Loss of heat resistance, the earliest event in spore germination (Dring & Gould, 1971), provides a convenient means of monitoring the germination process. The aerobic and anaerobic germination of B. licheniformis spores in the four TYG-based media was determined by comparing plate counts of incubated spore suspensions obtained before and after the suspension had been held at 80°C for 10 min [Bell & De Lacy, 1984(b)].

Results

Effect of thermal processing

Increasing the lethality of the cooking process from a P_{70}-value of 40 to one of 150 caused the surviving population to decrease tenfold and to change from a Brochothrix/Lactobacillus/Micrococcus codominant microflora to a more thermoduric Bacillus/Micrococcus microflora (Table 1) typical of the commercial product (Bell & Gill, 1982).

Upon abusive storage (25°C) those chubs that were cooked to a P_{70}-value of 90 or less showed typical Streptococcus spoilage (Fig. 1) producing neither surface softening nor gas (Bell & De Lacy, 1983(b)). A two-stage Bacillus/Streptococcus spoilage sequence (Fig. 2) with accompanying surface softening and gas production (Bell & De Lacy, 1982) developed in chubs cooked to a P_{70}-value between 90 and 120. Those chubs that received heat treatments of P_{70}-values exceeding 120 spoiled by the gas-producing surface-softening single-stage Bacillus sequence, Fig. 3 (Bell & De Lacy, 1982).

Table 1. Microflora surviving in luncheon meat chubs cooked at 68.5°C to process P_{70}-values from 40 to 150.

Process P_{70}-value	Log_{10} bacteria surviving cooking (aerobic plate count/g)				
	Brochothrix	Lactobacillus	Micrococcus	Bacillus	Total
40	2.78	2.78	2.32	-	3.15
60	2.32	2.26	2.30	-	2.78
75	-	-	-	-	-
90	-	-	-	2.30	2.30
105	-	-	1.54	2.15	2.24
120	-	-	1.82	1.53	2.00
135	-	-	2.00	2.00	2.30
150	-	-	1.52	1.99	2.11

-, not detected (limit of detection 1.52)

Microbial growth occurred throughout the chubs that spoiled by the Streptococcus sequence but was restricted to the surface in the other sequences with spoilage most advanced under the side seam and end seals. The dominant spoilage bacteria were identified as Streptococcus faecium and Bacillus licheniformis [Bell & De Lacy, 1983(a)].

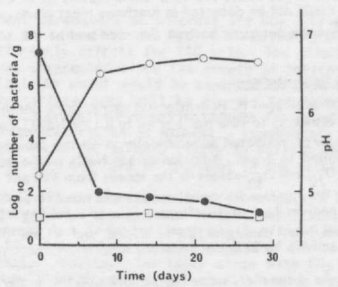

Fig.1. Development of the Streptococcus spoilage sequence on the surface of chubs stored at 25°C. ○ Streptococcus, □ Bacillus and ● pH.

Effect of oxygen availability

After 3 days storage in air at 25°C, chubs packed in salami casings developed a confluent bacterial growth on the outside of the casing. This encrustation appeared to be composed of a single bacterial species subsequently identified as B. licheniformis. By day 7 the meat was deep red, semi-fluid and had a putrid odour.

Air trapped within the emulsion of chubs in plastic casings allowed the spoilage microflora, Bacillus/-Streptococcus spoilage sequence to develop throughout the product. Product spoilage (gas production and surface softening) was evident by day 14, some 7 days earlier than occurred in vacuum filled chubs. Storage under hydrogen, on the other hand, markedly extended shelf-life. Not only did the chubs show no obvious signs of spoilage after 28 days storage at 25°C, but there was no detectable growth of the spoilage microflora.

Heat injury and recovery of Streptococcus faecium

Unheated S. faecium had the same aerobic and anaerobic tolerance to NaCl and acidity with growth being inhibited at 8% NaCl and at pH 4.4. At pH 6.8, 1.1% $NaNO_2$ inhibited anaerobic growth and 1.5% inhibited aerobic growth.

The presence of 0.02% $NaNO_2$ in the recovery media, whether or not NaCl had been added, had no appreciable effect on the recovery of heat-treated S. faecium compared to recovery in either BHI or BHI (2% NaCl).

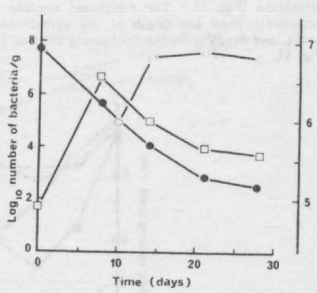

Fig.2. Development of the Bacillus / Streptococcus spoilage sequence on the surface of chubs stored at 25 C. Streptococcus, □ Bacillus and ● pH.

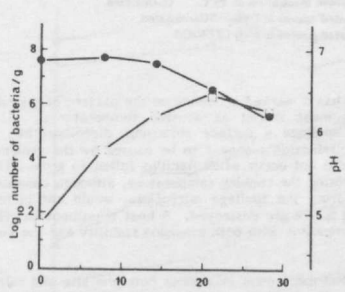

Fig.3. Development of the Bacillus spoilage sequence on the surface of chubs stored at 25 C. Bacillus and ● pH.

For all heating media (Table 2) the Decimal Reduction Times at 70°C, (D_{70}-values) (Katzin & Sandholzer, 1943) for cells recovering on BHI were significantly greater ($P<0.05$ Mann Whitney U-test) than those on BHI (2% NaCl). For cells recovering on BHI the D_{70}-values for cells heated in BHI with 2% NaCl added were significantly greater than those for BHI-based heating media without added NaCl. However, D_{70}-values for cells recovering on BHI (2% NaCl) were similar, regardless of heating medium.

Post-injury development of heated S. faecium cells in broth-based media was influenced as much by the number of surviving undamaged cells (those able to grow in the presence of

Table 2. D_{70}-values of Streptococcus faecium: Influence of heating and plating media. (Each value is the mean of four independent determinations except for luncheon meat values, which are the means of duplicate determinations.)

Heating medium	Plating medium: BHI mean (S.E.)		Plating medium: BHI (2% NaCl) mean (S.E.)	
BHI	1.84[a]	(0.08)	1.39[d]	(0.08)
BHI (2% NaCl)	2.29[b]	(0.09)	1.69[d]	(0.10)
BHI (0.02% $NaNO_2$)	1.82[a]	(0.08)	1.40[d]	(0.09)
BHI (2% NaCl, 0.02% $NaNO_2$)	2.13[b]	(0.08)	1.44[d]	(0.09)
Luncheon meat emulsion	4.00[c]	(0.25)	2.61[e]	(0.03)

a,b,c,d,e - D-values within the same column or row bearing different superscripts and significantly different ($P<0.05$).

2% NaCl) as by the composition of the heating medium. When undamaged cells survived the heat treatment, no substantial proliferation occurred during storage at 25°C for approximately 18 h in BHI and BHI (2% NaCl, 0.02% $NaNO_2$) or within 24 h in BHI (2% NaCl). Once cell growth commenced, the population estimates on BHI and BHI (2% NaCl) plating media were similar. When no surviving undamaged cells were detected immediately after heating, the post-heating lag lasted 48 h in the presence of 2% NaCl but was extended to 72 h in the presence of 4% NaCl or after the more-severe heat treatment of 20 minutes at 70°C [Bell & De Lacy, 1984(a)]. Immediately after cooking to a P_{70}-value of 40, streptococci could not be detected in luncheon meat chubs. After 48 hr storage at 25°C, streptococci were detected both in the core and at the surface of the chubs.

Germination and growth of Bacillus licheniformis

After 7 days incubation at 25°C heat activated B. licheniformis spores grew aerobically in TYG broth at pH 6.8 containing either up to 10% NaCl or up to 0.15% $NaNO_2$; and at pH values down to 5.2. However, spores incubated anaerobically in all the pre-reduced media [Bell & De Lacy, 1984(b)] failed to grow. Addition of 4% NaCl to the heating medium (TYG-broth) increased the D_{70} and D_{80}-values of the spores from 690 and 57 to 890 and 79 respectively, indicating an increased thermal resistance; however, addition of 0.02% $NaNO_2$ to the heating medium had little effect. Spores surviving 10 min exposure to 80°C showed sublethal heat damage: 12.8%, 23.2% and 43.8% of the survivors were sensitive to 2%, 3% and 4% NaCl in the recovery medium, respectively.

Germination, detected as loss of heat resistance, occurred rapidly at 25°C under both aerobic and anaerobic incubation. In both regimes germination was accelerated and enhanced by the presence of NaCl, for example germination rates after 24 h anaerobic incubation in TYG medium containing 0, 1, 2, 3 and 4% NaCl were 95.9, 98.1, 99.4, 99.7 and 99.7% respectively. The presence of $NaNO_2$ in the germination media, either alone or in combination with NaCl, had no significant effect on germination. Vegetative growth of germinated spores commenced after 9 h aerobic incubation but under anaerobic incubation spore outgrowth did not occur and the number of colony forming units progressively declined. No anaerobic growth occurred even after 7 days' incubation (Fig. 4). The continued decline in colony-forming units resulted almost exclusively from the death of the germinated spores, so that by day 5 in NaCl-free media, and day 7 in media containing 4% NaCl, only ungerminated spores were detected (Fig. 4).

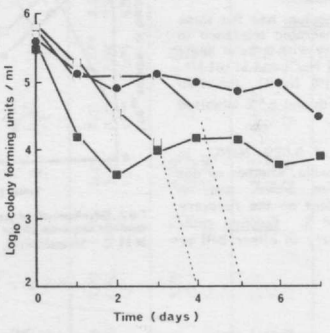

Fig. 4. Germination of Bacillus licheniformis spores during anaerobic incubation at 25°C. ○ Germinated, ● Ungerminated spores in TYG; □ Germinated, ■ Ungerminated spores in TYG (2%NaCl).

Discussion

The severity of thermal processing has a marked influence on the pattern of microbial spoilage of chub-packed luncheon meat stored at abusive temperatures. Those degradative changes regarded as spoilage - surface softening, discolouration, gas production and eventual product liquefaction - appear to be caused by the growth of Bacillus species as these changes did not occur when Bacillus failed to grow. Thus, extending the cooking time or increasing the cooking temperature, although capable of eliminating Streptococcus species from the spoilage microflora, would not increase product shelf-life unless all Bacillus spores are destroyed. A heat treatment to effect such spore destruction appears incompatible with both emulsion stability and the shrink properties of the casing material.

Oxygen availability within a luncheon meat chub influences both the site and rate of growth of surviving spores and hence the shelf-life of the product. Removal of oxygen from the meat emulsion and prevention of oxygen entry into the finished product by packaging in oxygen-impermeable casings appear important if short periods of temperature abuse are to be tolerated.

Luncheon meat contains approximately 2.5% NaCl (4% on a brine basis), a salt concentration quite ineffective in preventing the growth of unheated S. faecium but inhibitory to the growth of heat damaged cells. If heat-injured cells are to develop and cause product spoilage, they must regain viability and then grow in that product. Although NaCl provides some protection from lethal heat damage, it extends the post-heating lag period before growth commences. The presence of $NaNO_2$ in addition to NaCl appears to confound the inhibitory effects of the latter. Both the severity of heat-treatment and the concentration of NaCl increase the post-heating lag period. Therefore the delayed growth of streptococci in temperature abused chubs results from a heat-induced, NaCl prolonged pregrowth recovery phase (time required for repair of sub-lethal heat injury) rather than to any inherent inhibitory property of luncheon meat to the growth of undamaged streptococcal cells.

In luncheon meat curing salts are present in concentrations insufficient to prevent the growth of B. licheniformis. Spores of this organism germinate equally well anaerobically and aerobically but are unable to complete the transformation to vegetative cells without the presence of a yet-undetermined, minimal amount of oxygen. The proportion of the initial spore load that will remain viable, though dormant, after extended anaerobic storage is inversely related to the NaCl concentration of the heating medium. Although NaCl enhances germination this action has lethal consequences where successful outgrowth is impossible. Findings of this study suggest that the NaCl content of "Low Salt" products should not be reduced below a brine concentration of 2% if the germination stimulation mechanism is to remain operative in enhancing product stability.

Unfortunately the rate of oxygen penetration through currently available plastic casings, although low, is still sufficient to allow germinated Bacillus spores to grow. Elimination of viable spores from chub-packed luncheon meat does not appear practical; therefore, refrigeration remains essential for long shelf-life, although the use of oxygen-free storage atmospheres may offer an alternative warranting serious consideration.

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