5:22 Limiting conditions for yeast growth on frozen meat

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Introduction

Yeasts and moulds are usually thought to play merely a minor role in the spoilage of red meats, both because they constitute only a small portion of the initial microflora and because they grow slowly compared with most bacteria. Without doubt red meats stored at chill temperatures spoil predominantly by bacterial activities, spoilage characteristically being manifest as off-odours produced by the developing flora.

The slower-growing moulds can spoil meat by forming visible colonies, but this occurs only when storage conditions selectively inhibit bacterial development. Recent studies have shown that, the moderately xerotolerant meat spoilage moulds usually develop on meat at temperatures approximating 0°C when bacterial growth is inhibited by surface desiccation (Gill & Lowry, 1982; Lowry & Gill, 1984a). Although most mould spoilage occurs on meat that is either completely or partially thawed, some of the causal species are notably psychrotolerant, being capable of growth down to -5°C albeit at a very slow rate. Therefore, mould spoilage will also develop after prolonged storage at this temperature as bacterial growth would be prevented by both the low temperature and the reduced water activity (a_w) of the substrate (Scott, 1957).

Like moulds, most yeasts are significantly more xerotolerant than the spoilage bacteria and psychrotrophic species could reasonably be expected to develop on frozen red meats. However, little consideration has been given to this possibility despite a report 50 years ago (Haines, 1931) that frozen lamb carcasses stored at -5°C for prolonged periods developed extensive yeast microfloras on exposed muscle surfaces. The present study was therefore undertaken to determine the storage conditions under which yeasts might contribute significantly to the microflora of frozen red meats.

Materials and Methods

Frozen-storage trial

Short loins cut from six lamb carcasses frozen to -35°C were shrinkwrapped in gas-permeable moisture-impermeable plastic film (T-film, Trigon Plastics, New Zealand) before storage at either -5° ± 1°C or -10° ± 1°C. Tissue samples (5 cm^2) for microbiological analysis were excised with sterile cork-borer and scalpel from the dorsal surface fat layers of each loin immediately before freezing, after 5 weeks of storage, at subsequent 5-week intervals up to 25 weeks and finally at 40 weeks. Storage temperatures were monitored continuously throughout the trial (Lowry & Gill, 1984b).

Sample treatment

Tissue samples were homogenized individually with an appropriate volume of chilled 0.1% (w/v) peptone using a Colworth Stomacher 400 (Seward & Co., London). Duplicate spread plates of each of the following media were prepared from appropriate dilutions for estimation of total viable counts, enumeration of yeasts and recovery of moulds: nutrient agar (Difco), potato dextrose agar (Difco) supplemented with chlorotetracycline (10 µg/ml) and dichloran-rose bengal chlorotetracycline agar (King et al., 1979). Plates were sealed in plastic bags and incubated at 20°C for 7 days.

Seasonal distribution of yeasts on carcasses

Twenty surface tissue samples (total area 100 cm^2) were collected from each of three carcasses of freshly slaughtered lambs on four separate occasions; twice during winter and twice during summer. Samples were homogenized with an equal volume of diluent and examined for total viable counts and yeasts as above.

Identification of microorganisms

With each medium, representatives of all colonial forms present were selected for identification. The number selected approximated the square root (up to a maximum of ten) of the count. Bacterial isolates were identified to genus level from colony characteristics and the biochemical reactions described by Newton et al. (1978) in conjunction with the keys of Cowan and Steel (1974). Yeast isolates were identified to species level using the classification criteria of Lodder (1971).

Measurement of xerotolerance

The effect of a_w on yeast growth at 15°C was determined. Plates of nutrient agar (Difco) containing 0.1% added glucose and supplemented with glycerol to desired a_w values were placed on a gridded template that divided the plate into 50 sectors. A 1 µl inoculum of yeast culture in nutrient broth (10^6 cells/ml) was placed in the centre of each sector by means of a calibrated loop. The plates were then stored in desiccators, each desiccator holding a sulphuric acid solution of the same a_w as the plates it contained. For sampling, five replicate sectors were excised with a sterile scalpel. Each sector was placed into separate shake bottles containing 5 ml of 0.1% peptone and glass beads, shaken for 30 sec and diluted appropriately where necessary. Yeasts were enumerated on potato dextrose agar, incubated at 20°C for 5 days.

Measurement of growth rates

The gridded plate technique described above was used to measure the growth rates of yeast isolates over a range of supra- and sub-freezing temperatures. The a_w of the medium was adjusted, by addition of glycerol, to that of pure ice for each sub-freezing incubation temperature (Lowry & Gill, 1984a).

Results

The initial microflora of the lamb loins, composed predominantly of gram-negative psychrotrophic bacteria, was reduced in number by approximately 5-fold after storage at -5°C for 5 weeks. Pigmented micrococci, minor components of the initial microflora, comprised between 10 and 45% of the surviving bacterial populations. Bacterial numbers remained constant between 5 and 15 weeks, at which time yeast overgrowth of the non-selective agar medium precluded further enumeration. Yeasts, which made up less than 0.1% of the initial microflora, increased exponentially with a mean generation time of about 8 d. Maximum numbers approaching $10^6/cm^2$ were reached after 20 weeks of storage (Fig. 1). Yeast growth was not apparent macroscopically, but microscopic examination revealed discrete pin-head colonies. Four species of yeast were identified: Cryptococcus laurentii var laurentii, Cryptococcus infirmo-miniatus, Trichosporon pullulans and Candida zeylanoides. The relative proportions of the four species did not change significantly during development of the microflora, with Cr. laurentii var laurentii making up 90% or more of the yeast population from any sampling. Mould colonies only became apparent after 40 weeks' storage when an average of six colonies, 1-4 mm in diameter, was present on each loin.

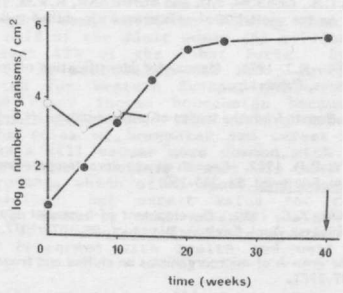

Figure 1. Development of the microflora on frozen lamb stored at -5°C: bacteria (○); yeasts (●). Arrow indicates storage time when mould colonies were first observed. Mean number of mould colonies per loin: 6 (from Lowry & Gill, 1984b).

Seasonal variations in both the number and composition of the yeast component of the initial flora were observed for lamb carcasses. Yeast numbers ranged between 10 and 50 per cm^2 of surface tissue for carcasses processed during winter months and 130-200/cm^2 for carcasses processed over summer. In winter the yeasts present were almost all species of Candida and Cryptococcus, with the former species predominating. Absolute numbers of these two genera were similar over summer, but total yeast numbers were generally greater by an order of magnitude because of the predominance of Rhodotorula species, in particular R. glutinis. For all samplings yeast numbers made up less than 5% of the total microflora.

Table 1. Minimum water activities for growth at 15°C of yeasts isolated from lamb and temperatures at which frozen media would have equivalent water activities.

Yeast	Minimum water activity	Frozen-medium temperature
Cr. laurentii var laurentii	0.92	-9
Cr. infirmo-miniatus	0.92	-9
R. glutinis	0.94	-6
C. zeylainoides	0.90	-11
T. pullulans	0.90	-11

The minimum water activities at which the yeast isolates developed and the temperatures of frozen media at which the minimum water activities for each yeast would be attained are shown in Table 1. T. pullulans exhibited dimorphic growth behaviour and under conditions of increased water stress (a_w < 0.92) developed solely in the filamentous form. At their observed a_w growth minimum, most isolates had a lag phase of growth ranging between one and two weeks. At water activities below their growth minimum, all yeast species steadily declined in number, the half-life for the Cryptococcus and Rhodotorula species approximating two days.

The approximate minimum temperatures for growth of the yeast isolates from meat were: Cr. laurentii var laurentii and Cr. infirmo-miniatus, -7°C; C. zeylanoides, -5°C; and R. glutinis, -3.5°C (Fig. 2). Specific growth rate data for T. pullulans were not recorded, as the yeast assumed a filamentous growth form at temperatures below -2°C. However on the criterion of presence or absence of growth, T. pullulans developed at temperatures of -6.5°C and higher within a time scale similar to that of the fastest growing yeast species, Cr. infirmo-miniatus. None of the isolates initiated growth within 6 months at -8°C or during a similar period at the equivalent a_w (0.92) but higher incubation temperature of -5°C. Absolute numbers of each species declined gradually at temperatures below their minimum for growth.

At sub-zero temperatures the two Cryptococcus species grew three to five times more rapidly than the other markedly psychrotolerant species, C. zeylanoides, (Fig. 2). At -5°C, a temperature near its growth minimum, C. zeylanoides had a lag phase of growth approximating 16 weeks compared with 3 to 4 weeks for the cryptococci; the lag phase of the latter species extending to 8 to 10 weeks at -6.5°C.

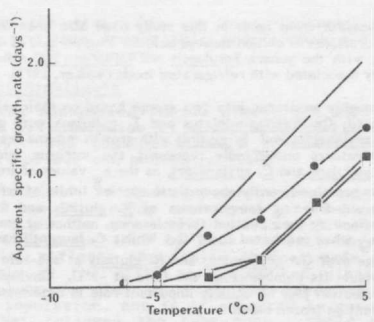

Figure 2. Effect of incubation temperature on the apparent specific growth rates of: Cryptococcus infirmo-miniatus, (○); Cryptococcus laurentii, (●); Candida zeylanoides, (□); and Rhodotorula glutinis, (■). Media used for incubation at sub-zero temperatures contained glycerol to prevent freezing.

Discussion

The comparatively rapid growth of yeasts at -5°C was surprising, as yeasts have not been considered to be significant in the spoilage floras of frozen red meats, although yeast dominance of frozen poultry microfloras at this temperature has been reported (Schmidt-Lorenz & Gutschmidt, 1969). Moulds produced visible spoilage only after 40 weeks storage at -5°C, when they could have contributed little to the total mass of the

yeast-dominated microflora. The development of the yeasts as discrete pin-head colonies would appear to be the result of the reduced a_w of the frozen substrate, Scott (1936) having shown that above 0.98 a_w yeast growth on meat is manifest as slime whilst below this value discrete colonies are formed.

In this study yeast growth on fat surfaces of the lamb loins did not lead to gross colony formation. However it is possible that, under some circumstances, a visible spoilage form could be produced on frozen meat. Spoilage numbers of bacteria are ultimately lower on fat than on muscle tissue because of limited availability of substrates (Gill & Newton, 1980) and yeast numbers on fat surfaces are likely to be similarly restricted. On muscle tissue, substrate limitation would not occur and visible yeast colonies could therefore eventually develop. At higher temperatures, any preformed yeast colonies would rapidly increase in size to give visible spoilage.

The asporogenous yeasts making-up the frozen lamb microfloras are all common species and cosmopolitan in distribution. They are abundant on aerial parts of plants, so it can be assumed that they are transferred to meat from hides during carcass dressing. The observed seasonal variations in yeast contamination of carcasses correlate well with recorded cyclic pasture fluctuations in New Zealand; Cr. laurentii dominating foilage floras in winter and spring and Rhodotorula species predominating in summer and autumn (Di Menna, 1959).

The yeast species isolated from lamb in this study have also been found in similar proportions in the microfloras of chilled turkeys held for long periods at -2°C (Barnes et al., 1978) and, along with the genera Torulopsis and Debaromyces, make up the yeast floras most commonly associated with refrigerated foods (Walker, 1977).

The yeasts can be roughly separated into two groups based on their relative psychrotolerance: Cr. laurentii, Cr. infirmo-miniatus and T. pullulans with growth minima about -7°C, and C. zeylanoides and R. glutinis with growth minima between -3.5° and -5°C. These temperatures undoubtedly represent the intrinsic minimum growth temperatures for T. pullulans and C. zeylanoides, as the a_w values of frozen substrates at these temperatures are significantly above these species' limits of xerotolerance. In contrast, growth at sub-freezing temperatures of R. glutinis and the cryptococci appears to be constrained by their limited xerotolerance, neither of the latter species developing at 0.92 a_w when incubated at -5°C. Whilst C. laurentii has an undoubted growth-rate advantage over C. zeylanoides and R. glutinis at sub-zero temperatures, this alone cannot explain its dominance of the flora at -5°C. Obviously initial flora composition and distribution play an equally important role in determining patterns of yeast flora development on frozen meat.

Clearly yeasts will develop if meat is held at marginal freezing temperatures of -7°C and above. At -5°C the dominant yeast species grow approximately five times more rapidly than Cladosporium herbarum, the fastest-growing black spot mould, and with a lag phase of less than 4 weeks compared with an estimated 16 weeks for Cl. herbarum (Gill & Lowry, 1982). In addition because of their moderate xerotolerance the yeasts, like the moulds, should make a substantial contribution to meat spoilage floras at temperatures above freezing when surface desiccation inhibits bacterial growth. Indeed, dominance of chilled meat microfloras by species of Candida has been reported for shipments of chilled beef when bacterial growth was retarded by control of the chiller humidity (Law & Vere-Jones, 1955). However because they usually form only a minor component of normal chill meat microfloras, high proportions of yeasts in frozen meat spoilage floras would generally provide a strong indication of temperature abuse during frozen storage.

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