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TO MARIE AND GORDON

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MICROBIAL SPOILAGE

OF

POTATO TOP PIES

BY

FIONA MASTERS

A THESIS SUBMITTED IN PARTIAL  
FULFILMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF MASTER OF SCIENCE

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S U M M A R Y

The microbial spoilage of Potato Top pies was investigated to try to provide a view of the events taking place during the spoilage process which renders these pies unsuitable for public consumption. Pies used in this study were obtained from a commercial pie manufacturer and were stored at 4°C, 25°C and 37°C, and the effects of storage at these temperatures studied. During the examination biochemical techniques were used in conjunction with microbiological methods.

Aspects gained from this study can be summarised as below:

- . Major flora of all pie component parts (meat filling, pastry surround, potato topping) of freshly cooked pies and of pies stored at the three temperatures consisted primarily of gram positive rods and cocci.
- . The origins of these organisms could be traced directly back to various stages of manufacture.
- . Within 24-36 hours of storage at 25°C and 37°C numbers of gram positive organisms could reach above  $10^7$ /gram of pie component.
- . No obvious organoleptic spoilage took place.
- . Lack of off odours ( $\text{NH}_3$ ;  $\text{H}_2\text{S}$ ); the presence of amylase producing *Bacillaceae*; pH decreases in spoiled pies - suggested that the utilisation of low molecular weight compounds (such as glucose) resulted in saccharolytic spoilage of the pies.
- . Bacterial numbers in the pies stored at 4°C did not rise above  $10^5$ /gram within 28 days of storage.
- . Isolated dominant bacterial species were identified as *Bacillus*, *Streptococcus*, *Staphylococcus* and *Micrococcus*, with the *Bacillus* species and *Streptococcus* species inhibiting the growth of the other organisms.

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I N T R O D U C T I O N

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Studies of cooked meat products, such as that of Bell and Gill (1982), on chubs, have resulted in some knowledge of the spoilage process which renders cooked meat unacceptable - however, there is a lack of information on cooked meat pies. Because of the popularity and accessibility of cooked meat pies, it seems appropriate to investigate various spoilage aspects of pies stored at a variety of temperatures.

Such a study should include an examination of the origin and development of spoilage flora which develop for a range of storage temperatures, the metabolic processes leading to spoilage and a study of inter-relationships between bacterial species found in spoiled pies.

This thesis, therefore, examines aspects of the spoilage process which takes place within cooked individual Potato Top pies stored at 4<sup>0</sup>C, 25<sup>0</sup>C and 37<sup>0</sup>C. Biochemical and microbiological techniques were used to gain an understanding of the process which leaves the pies unacceptable for consumption.

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L I T E R A T U R E   R E V I E W

## INTRODUCTION

Although there has been a number of studies carried out on the spoilage of meats (Gill and Newton 1977, 1980; Gill, 1979; Ingram and Dainty, 1971) and meat products (Bell and Gill, 1982; Hall and Angelotti, 1965) there has been a marked lack of information on the spoilage of cooked meat pies. Information, therefore, that will give some idea of the processes occurring during the spoilage of meat pies can only be obtained indirectly, by applying knowledge in parts from the studies done on meat and other products.

This review will, therefore, examine various aspects of contamination of meats, and meat spoilage; as well as aspects of food-borne illness in relation to these products.

Topics to be discussed will include:

- (1) Spoilage flora of meat
- (2) Spoilage flora of meat products
- (3) Biochemical changes associated with the growth of micro-organisms in meat.
- (4) A quick note on the spoilage microflora of potatoes and pastry
- (5) Public Health aspects of spoiling meats.

### 2.1 Spoilage of Meat

When heavy consumption of meat (and meat products) occurs, knowledge of both biochemical and microbiological aspects of this spoilage is important. This knowledge can prevent mass food-borne illness due to the consumption of incorrectly prepared and stored meats - and foods in general.

Bacteria found associated with meats are present as either deep tissue contaminants or as surface associated contaminants. The surface

associated contaminants are present as a result of post-slaughter contamination of meat from external sources (Ingram, 1949). Gill (1979) examined deep tissue bacteria and found that the most likely type of organism present in the tissue of healthy normal animals were small numbers of the genus *Clostridia* (these organisms commonly occur as soil organisms and are also commonly found in the animal gut). Gill concluded that further work was required to determine the nature, incidence and spoilage potential of deep tissue contaminants.

Surface associated microflora of meats, however, vary because the numbers and types of bacteria present are dependent upon environmental conditions - nutritional value of the meat, storage temperature and conditions of storage. Several authors (Carse and Locker, 1974; Dainty, 1982; Gill and Newton, 1977, 1978; Grau, 1981; Ingram and Dainty, 1971; McMeekin, 1981; Newton and Gill, 1978) have looked at various aspects of meat stored at chill temperatures. The aerobic spoilage flora on meat stored at chill temperatures consists primarily of pseudomonads (Gill and Newton, 1978; Ingram and Dainty, 1971) with *Acinetobacter* spp, *Enterobacter* spp and *Microbacterium thermosphactum* involved to a lesser extent (Gill and Newton, 1977). These species preferentially utilise glucose (Gill and Newton, 1977, 1978, Ingram and Dainty, 1971) as their nutrient source, subsequently utilising amino acids, lactic acid and possibly nucleotides (Dainty, 1982), when the growth of these bacteria exhausts the glucose supply. Utilisation of these nitrogen and sulphur containing substrates soon leads to the production of malodorous end-products such as ammonia and hydrogen sulphide, with a subsequent increase in pH because of the release of ammonia (Gill, 1983). Under anaerobic conditions (usually when meats are vacuum packaged), however, lactobacilli are the main spoilage flora when the storage temperature is 0-15°C; as well as *Microbacterium thermosphactum* and Enterobacteriaceae (Grau, 1978, 1981; Newton and Gill, 1978).

As with the aerobic spoilage flora, these organisms preferentially utilise glucose as a nutrient source (Newton and Gill, 1978), however spoilage does not occur as rapidly as it does under aerobic conditions, and is detectable only after maximum cell density has been attained (Sutherland *et al*, 1976). When lactobacilli dominate the anaerobic spoilage flora, it is the accumulation of short chain fatty acids that

is indicative of spoilage occurring (Gill, 1983). Because all of the above species utilised glucose, competition for this substrate arose under the glucose limiting conditions at the surface of the meat, leading to *Enterobacter* limiting the growth of *M. thermosphactum*, and lactobacilli limiting the growth of both, apparently by producing an inhibiting substance (Grau, 1981; Newton and Gill, 1978). Growth of these bacteria soon ceases, however, because the diffusion of fermentable substrates to the surface is not rapid enough to support further growth (Gill and Newton, 1978).

At the higher storage temperatures (above 10<sup>0</sup>C) pseudomonads are once again the dominant spoilage flora under aerobic conditions (Gardener, 1965), but lose this dominance to *Acinetobacter*spp and the Enterobacteriaceae at temperatures near 30<sup>0</sup>C (Gill and Newton, 1980). It is assumed that once again glucose is preferentially utilised, and amino acids used only once this nutrient source is depleted (Gill, 1983). This would once again lead to the production of malodourous end-products and an ultimate increase in pH.

Under anaerobic conditions, lactobacilli and Enterobacteriaceae are the dominant spoilage flora up to temperatures of 20<sup>0</sup>C, but Enterobacteriaceae decrease, and species of *Clostridium* increase when temperatures rise above 20<sup>0</sup>C. Further data is required as to which substrates are utilised under anaerobic conditions at these higher temperatures, but presumably glucose is again preferentially utilised as it is under chill temperatures.

In an experiment carried out by Gill and Newton (1980), it appeared that when meat was artificially inoculated with pathogens, the only species not inhibited by normal microflora were *Escherichia coli* and *Salmonella typhimurium*. Their growth was only inhibited at 20<sup>0</sup>C under anaerobic conditions. It would therefore seem important to store meats at refrigeration temperatures, because the growth of these pathogens is limited at chill temperatures, so do not therefore present a food-bourne hazard.

It is important to note that spoilage of meats is largely dependant upon the microflora initially present on the meat, and that the growth of these organisms depends upon substrate utilisation and storage temperature.



## 2.2 Spoilage of Meat Products

Meat products show different patterns of spoilage to that of meat, which is largely due to differences in processing and the inclusion of additives. The total count of freshly made meat products therefore reflects the overall quality of the ingredients, handling, and storage (Jay, 1978). This includes the handling the raw meat undergoes to form the meat product. Often, as in the case of luncheon meat, the meat is cooked, so the flora present in the final product will include those heat resistant bacteria and spore-forming bacteria that have survived the cooking process.

Bell and Gill (1982) found the initial flora of luncheon meats to be mainly *Bacillus* and *Micrococcus* spp. When "chubs" were stored at 10°C, bacterial numbers changed little. However, when the chubs were stored at 25°C, surface growth of *Streptococcus* spp and *Bacillus* spp occurred, with the streptococci replacing the *Bacillus* spp after 14 days storage. Streptococci were able to grow then because of the de-nitrifying capabilities of the *Bacillus* spp. This surface growth was accompanied by a fall in pH, and an increase in lactic acid. The glucose concentration varied with the changes in the *Bacillus* population, indicating that glucose converted to lactic acid was largely replaced by hydrolysis of the starch portion of the luncheon meat, mediated by the amylases produced by the *Bacillus*. Under the more anaerobic conditions in the centre of the chub, little change occurred when the chub was stored at 25°C and 10°C, where *Enterobacter* spp dominated the flora. No evidence of spoilage occurred under these anaerobic conditions, but at the surface when the chub was stored at 25°C, spoilage was evident by day 14 when the casings were distended by gas production, surface softening and discolouration was evident and the meat had a distinctly sour smell.

Grau (1978) found that the predominant organisms developing during storage of vacuum-packaged (and therefore essentially anaerobic), cured cooked products are lactic acid bacteria, which cause slime production and sourness of the product, as spoilage indicators. There is, however, debate on the relationship of the bacterial count of the product to spoilage (Grau, 1978). Hamburgers are essentially minced beef, which spoils in the same manner as does whole muscle tissue (Gill, 1983), but often commercially made hamburger patties contain additives, which therefore increases the variability of the microflora present. In Tamminga *et al.*'s (1982) study of hamburgers, the experiments

showed that the raw minced meat used for hamburgers contained large numbers of a wide range of micro-organisms, and these included potential pathogens such as *Salmonella* spp. Cooked samples differed little in their microbial load and also contained salmonella, and this was due to the prior cooking time these hamburgers received - often the centre of the pattie was uncooked. It therefore seems appropriate to set correct cooking standards for uncooked meat products, in order to decrease the microbial load and therefore reduce the presence of hazardous bacterial types. Just as important is the storage temperature.

Hall and Angelotti (1965) found that proliferation of *Clostridium perfringens* in meat and meat products did not occur at temperatures of 5-15<sup>0</sup>C, but rapid growth occurred at higher temperatures, especially around 45<sup>0</sup>C. Since this seems to be the case in most examples quoted, it seems appropriate to store meat products, as well as meats, at chill temperatures.

### 2.3 Biochemical changes associated with the growth of micro-organisms in meat

As Ingram and Dainty (1971) have already noted, there is a scarcity of information regarding the nature of biochemical changes that occur with meat during spoilage. The most obvious change that can occur in spoiling meats and meat products is the production of malodourous gases such as NH<sub>3</sub> and H<sub>2</sub>S. These form from the utilisation of amino acids and other N & S containing compounds (Doelle, 1969). However, although these are obvious indicators of spoilage, other biochemical changes occur to the meats which are also indicative of bacterial spoilage, even though they may all occur after microbial proliferation (Ingram and Dainty, 1971).

Changes in levels of glucose and related compounds; end products of glycolysis such as lactic acid; and end products of amino acid utilisation such as NH<sub>3</sub>, have been measured by various authors (Bell and Gill, 1982; Gill, 1976; Gill and Newton, 1977, 1978) as well as changes in pH. Because these changes are often easier and quicker

to measure than bacterial growth, it has been suggested that these be used to assess bacterial spoilage (Ingram and Dainty, 1971). Thus, a high pH could be indicative of bacterial spoilage, due to the production of  $\text{NH}_3$ . However, there has been some dispute as to the validity of this (Gardner, 1965; Turner, 1960) because the pH does not necessarily rise with bacterial proliferation - Bell and Gill's (1982) study on chubs, for example, where the pH decreased with bacterial proliferation.

As a quick summary of the data from authors quoted in sections 1 and 2, the biochemical changes occurring in spoiled meats and meat products; are as follows:

Under aerobic conditions of storage, the bacteria present preferentially utilise glucose as their main nutrient source. Unless this source is quickly depleted (when the cell density rises above  $10^8/\text{cm}^3$  - Gill, 1983) the bacteria continue utilising this, resulting in an accumulation in lactic acid and subsequent pH decrease, with no obvious outward changes (Bell and Gill, 1982). Once this glucose is depleted, however, amino acids and lactic acid (as well as other low molecular weight compounds) are used, resulting in the production of malodourous compounds such as  $\text{NH}_3$  and  $\text{H}_2\text{S}$  and a rise in pH. Distention of the meat can also result (because of gas formation). These are obvious organoleptic signs that spoilage is taking place. Breakdown of protein and lipids occurs only in the very late stages of spoilage, by which time the meat/meat product is unsightly (Dainty, 1982).

Under anaerobic conditions, a similar situation applies, but often the bacteria cannot survive when the diffusion of fermentable substrates to the surface is not rapid enough to support further growth (Gill and Newton, 1978).

Usually by the time malodourous end-products are produced, the bacterial population has reached numbers above  $10^8/\text{cm}^3$  (Gill, 1983), so if pathogenic species are present, food poisoning may result from the consumption of the product. Although biochemical changes do occur with changes in the bacterial load of the food, there has not been proven to be a direct link between the two parameters, such that measurement of one parameter can give information about the microbiological quality of the meat, especially since inherent decay occurs in the food (Ingram and Dainty, 1971).

## 2.4 The spoilage microflora of potatoes and pastry

Because potato top pies are made up of potato and pastry, it is appropriate to review spoilage aspects of these products.

Some authors (Duran *et al*, 1982; Notermans *et al*, 1985; Snyder *et al*, 1983; Surkiewicz *et al*, 1967) have looked at various aspects of bacterial contamination of potatoes and have concluded that because of the sheer mechanics of producing potato products, the sanitary conditions under which these products are produced are poor. Build up of potato slices and juice on the machines provides ideal conditions for bacterial proliferation, which leads to the contamination of the end product. However, the final cooking process (for example, deep fried chips, dehydrated potato) the product goes through effectively sterilises the product (Duran *et al*, 1982; Surkiewicz *et al*, 1967). However, *Clostridium botulinum* has been isolated from reconstituted dehydrated potato (Notermans *et al*, 1985), the growth of which can be effectively stopped when the product is stored at chill temperatures - thereby stopping the production of the fatal toxin. *Bacillus cereus* has been implicated as the causative agent of food poisoning due to the consumption of incorrectly held whipped potatoes (*in* Snyder *et al*, 1985) due to improper temperature of storage. It seems, therefore, that potato products must be treated correctly in order to eliminate bacteria, and must be stored at the correct temperatures to prevent bacterial proliferation. Little information is available on the spoilage process that occurs when bacteria proliferate on potato.

There is a distinct lack of information regarding the microbiological quality of pastry itself, although pastry products have been investigated. (Hyatt and Guy, 1981 a, b) In these investigations *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* were isolated from pumpkin pies, but it was not stated whether the pastry itself contained these bacteria, and other investigations give similar non-specific results. Flour (Silliker *et al*, 1980) does contain bacteria, but these are not specified.

## 2.5 Public Health aspects of spoiling meats

From a Public Health point of view, it is important to have some knowledge of food handling and food storage conditions, in order to prevent food-borne illness occurring as a result of mishandling of foods. Data, with respect to these points, has been previously well documented (Hobbs and Gilbert, 1978 for example). However, food-borne illnesses do occur, and although details have not previously been well documented, attempts are now being made to collate incidences and the types of organisms and foods involved (Hobbs and Christian, 1973; Hobbs and Gilbert, 1978). Food-borne illnesses resulting from the consumption of meat, and meat pies specifically, have not been very well reported, although some cases have been documented. Hutchinson and Taplin (1977) found *Bacillus cereus* in meat loaf and meat dishes, and salmonella (Hobbs and Gilbert, 1978), *Staphylococcus*, *Clostridium welchii* (Hobbs and Christian, 1973), streptococci (Cory *et al*, 1938) and *Bacillus cereus* (Lechowich, 1978) have all been implicated in food poisoning incidences directly attributable to meat products. The illness due to food poisoning varies with the bacteria involved, these have been outlined by several authors (e.g. Archer and Kvenberg, 1985; Bryan, 1978; Lechowich, 1978; Todd, 1978) - it is important to note the different types of bacterial food poisoning that can occur, because these affect the way foods can be handled (Bryan, 1978).

Because meats do not always show obvious organoleptic changes as they spoil (based on their microbiological load) there is a danger present to the public, because consumption of contaminated meats (and foods in general) can occur, and if pathogens are present, under the correct conditions, food poisoning may result.

The growth of micro-organisms in meat products (with meat pies in mind) is distinctly possible, and, under elevated temperatures of storage, organisms present in the products can multiply to levels high enough to cause food poisoning. In the case of a product that is handled a lot (such as a meat pie) the possibility of potential food poisoning organisms such as staphylococci contaminating the product is high, so hygiene is very important to prevent this from occurring.

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Failure to cook the product correctly (thereby eliminating the bacteria present) and cooling it quickly (preventing elevated temperatures, and therefore ideal growth temperature) will lead to the danger of these potential food poisoning organisms growing to such numbers as to cause poisoning.