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MICROBIAL SPOILAGE AND SUBSTRATE CONCENTRATION OF NORMAL AND DFD-BEEF

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SUMMARY

Only during the first stage of aerobic storage the growth rate of total psychrotrophic bacteria, pseudomonas and B. thermosphacta was obviously higher for DFD-meat than for normal meat. After this initial phase, bacterial growth rates in both normal and high pH meat were similar.
In normal pH ground beef stored in air, both substrates, glucose and lactic acid were at first and simultaneously used to meet the energy requirements of the bacterial flora present.
As those substrates were depleted (10^8-10^9 psychrotrophes/gram) the amount of total volatile N-compounds increased.
An inverse relationship was found between pH and glucose.
The fresh DFD-samples contained little or no glucose. Consequently a depletion of this substrate occurred while bacterial counts were still low ($< 10^7$ psychrotrophes/gram) and the amount of total volatile N-compounds started to increase early during storage.

INTRODUCTION

Meat stored at chill temperatures spoils as a result of growth and metabolic activity of psychrotrophic microorganisms.
Considerable research has been made over the years on enumerating and classifying of microorganisms and developing of identification schemes for these organisms.
On the other hand rather little attention has been paid to the metabolic activities of these organisms despite the fact that these are the major causes of the appearance of undesirable properties such as off-odour and off-flavour (2).
It is the purpose of this paper to compare in a preliminary investigation the course of spoilage in normal and DFD ground beef including some chemical changes (glucose, lactic acid, volatile N-compounds) in association with bacterial growth during aerobic storage (+2°C).

MATERIALS AND METHODS

At 24h. post mortem samples (± 1kg) of the LD-muscles adjacent to the 8th-10th vertebra were cut from 3 DFD beef carcasses ($\overline{pH_{24}}$LD = 6.56), from 2 carcasses with intermediate pH ($\overline{pH_{24}}$LD = 6.05) as well as from 3 normal beef carcasses ($\overline{pH_{24}}$ = 5.63).
After 1 day cold storage at the laboratory the samples were ground using a meat grinder with a 10 mm size hole plate and wrapped in polyethylene foil with high oxygen permeability.
Following aerobic storage (+2°C) for 2, 8, 13 and 16 days respectively portions of the samples were examined for counts of :

- total aerobic bacteria (PCA, OXOID CM325, 3-5 days at 22°C)
- pseudomonas (Pseudomonas Agar Base, OXOID CM 559, 3-5 days at 22°C)
- Brochotrix thermosphacta (STAA according Gardner, 3-5 days at 22°C)
- enterobacteriaceae (VRBG, OXOID CM323, 24h. at 30°C)

Simultaneously an additional sample was taken and frozen for the following analyses :
- glucose, mg/100gram, using an enzymatic kit Glucose Enzymatic Color, Biotrol A 02460
- lactic acid, mg/100gram, using an enzymatic kit, L-Lactic Acid UV-method, Boehringer 139084
- total volatile N-compounds, mg/100gram, using a chemical method from Lücke and Geidel (3) modified by Antonacopoulos (1)

Eventually a pH measurement and an evaluation of meat flavour and appearance were made for each sample at the different storage times.

RESULTS AND DISCUSSION

Figure 1 presents the mean growth curves of psychrotrophic bacteria, pseudomonas and B. thermosphacta for both normal (n=3) and high pH meat (n=3) during aerobic storage (+2°C).
The initial contamination (2 days p.m.) with psychrotrophic bacteria for normal ($\overline{pH_{24}}$LD = 5.63) and DFD-meat samples ($\overline{pH_{24}}$LD = 6.56) was low and amounted to 3.53 and 3.77 respectively (logN/gram).
During the entire storage period (16 days) the mean counts of psychrotrophic organisms, pseudomonas and B. thermosphacta in the DFD ground beef were higher as compared with normal beef.
Only during the first stage of storage (8days) the growth rate of the above mentioned bacteria was obviously higher for DFD-meat than for normal meat. After this initial phase, bacterial growth rates in both normal and high pH meat were similar which has been demonstrated earlier by Newton and Gill (4). These authors found that the growth rates of most meat spoilage bacteria (except Acinetobacter) were unaffected by pH-values between 5.50 and 7.00.
The number of enterobacteriaceae was below or slightly above the limite of detection for the different storage times. This may be explained by the very low initial contamination of all meat samples with these bacteria ($< 10^2$/gram).

In Figures 2 to 6 the evolution of the bacterial contamination during storage as well as the concentration of glucose, lactic acid and total volatile N-compounds for LD-samples with different pH_{24}-values are given :

Figure 2	LD-sample 1	pH_{24} = 5.53
Figure 3	LD-sample 2	pH_{24} = 5.53
Figure 4	LD-sample 3	pH_{24} = 6.19
Figure 5	LD-sample 4	pH_{24} = 6.43
Figure 6	LD-sample 5	pH_{24} = 6.93

LD-samples 1 and 2 presented both a normal pH_{24}-value but their initial contamination varied considerably i.e. 3.37 and 4.57 respectively (logN/gram).
The initial amounts of glucose and lactic acid in these LD-samples were approximately 80mg/100gram and 800mg/100gram respectively.

For sample 1 the glucose remained at its initial level during 13 days by which time the bacterial count reached 10^7/gram. Then the glucose content decreased rapidly to 20mg/100gram at the end of the storage period. The initial amount of volatile N-compounds in sample 1 did not increase during the experimental storage time and no off-odours were perceptible.
For sample 2 a total aerobic count of 10^8/g was already reached after 8 days cold storage. and subsequently the corresponding fall in the glucose concentration occurred.
Both substrates glucose and lactic acid were simultaneously used to meet the energy requirements of the bacterial flora present. As glucose and lactic acid were depleted (10^8-10^9 psychrotrophes/gram) the initial amount of total volatile N-compounds in sample 2 (23mg N/100gram) increased up to 29mg N and 43mg N/100gram and the pH-values amounted to 6.40 and 6.80 after 13 and 16 days of cold storage respectively. From that time spoilage odours were clearly detectable.
These results confirm the findings of Newton and Gill (4) who demonstrated under aerobic conditions the lack of degradation of amino acids until glucose had been consumed by which time the count was $10^8/cm^2$.

The pH_{24}-values of LD-samples 3, 4 and 5 were 6.19, 6.43 and 6.93 respectively and their initial contamination was low and amounted to 4.04, 3.73 and 3.94 respectively (logN/gram). The initial content of the mentioned substrates in LD-samples 4 and 5 (DFD) was much lower as compared with LD-samples 1 and 2 and amounted to 21mg and 4mg/100gram respectively for glucose and to 533mg and 262mg/100gram respectively for lactic acid.
In these samples little or no glucose was available for the contaminating bacteria and consequently a depletion of this substrate occurred early in the storage period (< 8days) while bacterial counts were still low (< 10^7 psychrotrophes/gram).
Their high initial amount of total volatile N-compounds, 28mg N and 26mg N/100gram respectively, raised to 30mg N and 32mg N/100gram after 8 days while off-odours were hardly perceptible at that time. However a normal meat flavour was completely absent even in the fresh DFD-samples.
These results were in accordance with the findings of Newton and Gill (4) who reported that in meat devoid of glucose the spoilage flora proceeds immediately to degrade amino acids to satisfy their energetic requirements with concomitantly earlier production of NH_3 and other odoriferous by-products.

Sample 3 (pH_{24}LD = 6.19) which was classified as intermediate still contained 33mg glucose and 710mg lactic acid /100gram.
The changes in the substrate contents and in the amounts of volatile N-compounds of this sample during the storage period could be compared with those of sample 2 with a normal pH_{24}-value.
However both meat samples, 2 and 3, presented a high initial number of pseudomonas (≥ 10^4/gram) which was responsible for a rapid glucose and lactic acid depletion and subsequent amino acid degradation resulting in the production of NH_3 and other odoriferous by-products.

CONCLUSIONS

In normal pH ground beef stored in air, both substrates glucose and lactic acid were at first and simultaneously used to meet the energy requirements of the bacterial flora present.
As these substrates were depleted (10^8-10^9 psychrotrophes/gram) the amount of total volatile N-compounds increased.
An inverse relationship was found between pH_{24} and glucose.
The fresh DFD-samples contained little or no glucose. Consequently a depletion of this substrate occurred while bacterial counts were still low (< 10^7 psychrotrophes/gram) and the amount of total volatile N-compounds started to increase early during storage.

The data presented were in accordance with the conclusions of Dainty (2) stating that chemical changes associated with the initial glucolytic phase of growth should be amongst the earliest indicators of the extent of microbial growth and spoilage development.
Rapid and simple methods for determination of glucose and/or products linked with glucose metabolism are of great value in the study of the evolution of spoilage in fresh meat. These methods, however, are not applicable to DFD-muscles due to the lack of glucose.

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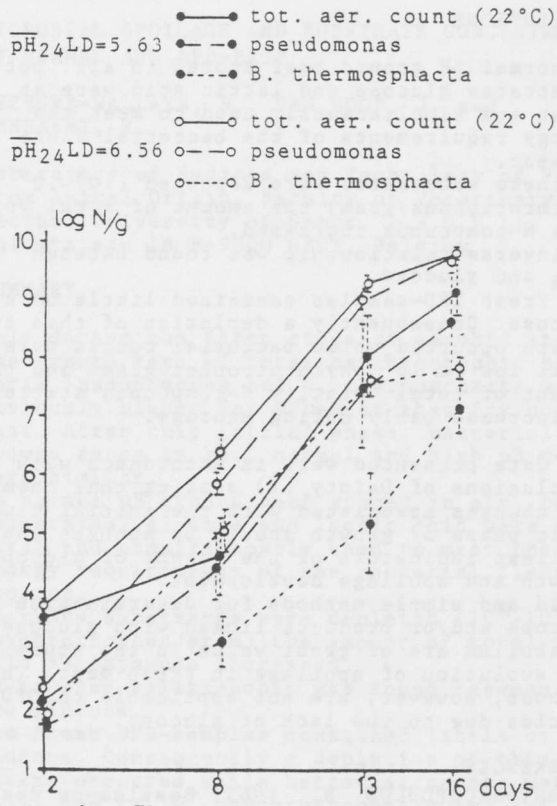

Figure 1 : The mean growth curves of psychrotrophic bacteria, pseudomonas and B. thermosphacta for both normal (n=3) and high pH meat (n=3) during aerobic storage (+2°C)

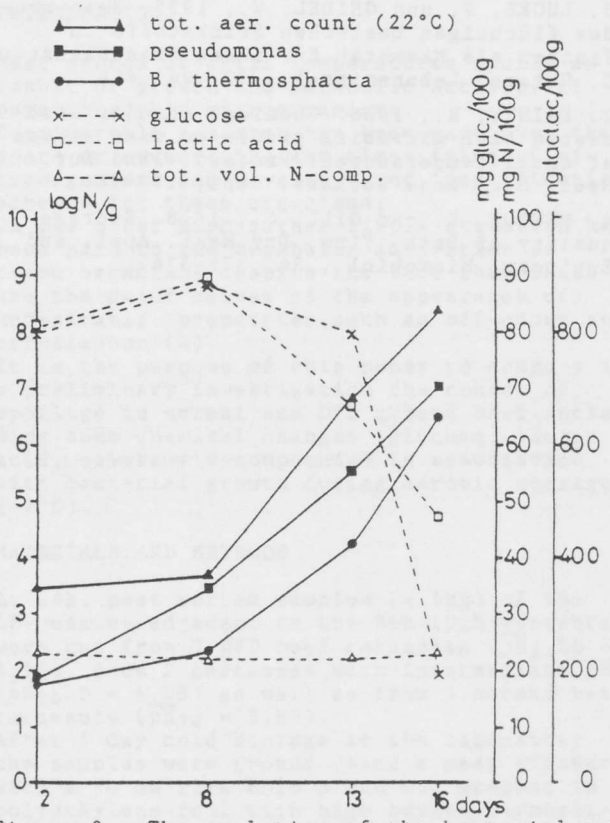

Figure 2 : The evolution of the bacterial contamination during aerobic storage (+2°C) and the concentration of glucose, lactic acid and total volatile N-compounds for LD-sample 1 with pH_{24} = 5.53.

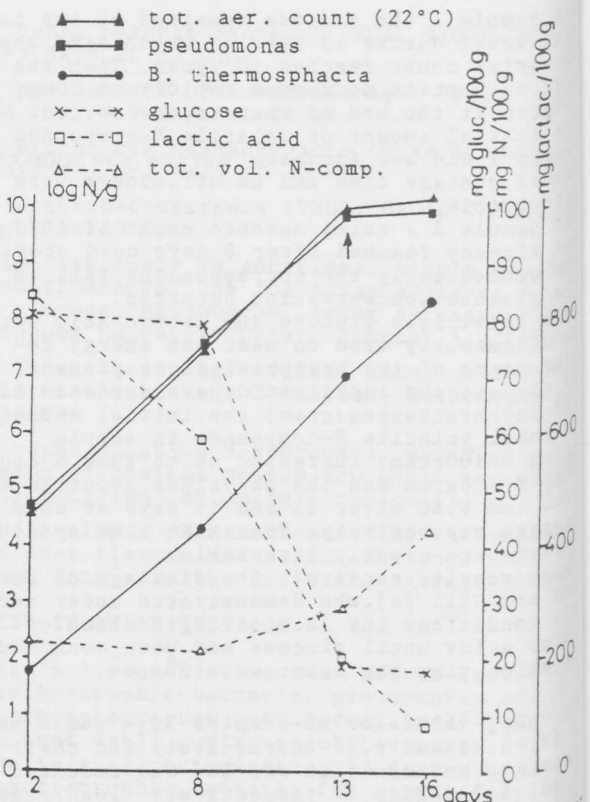

Figure 3 : The evolution of the bacterial contamination during aerobic storage (+2°C) and the concentration of glucose, lactic acid and total volatile N-compounds for LD-sample 2 with pH_{24} = 5.53 (↑ spoilage odours detected)

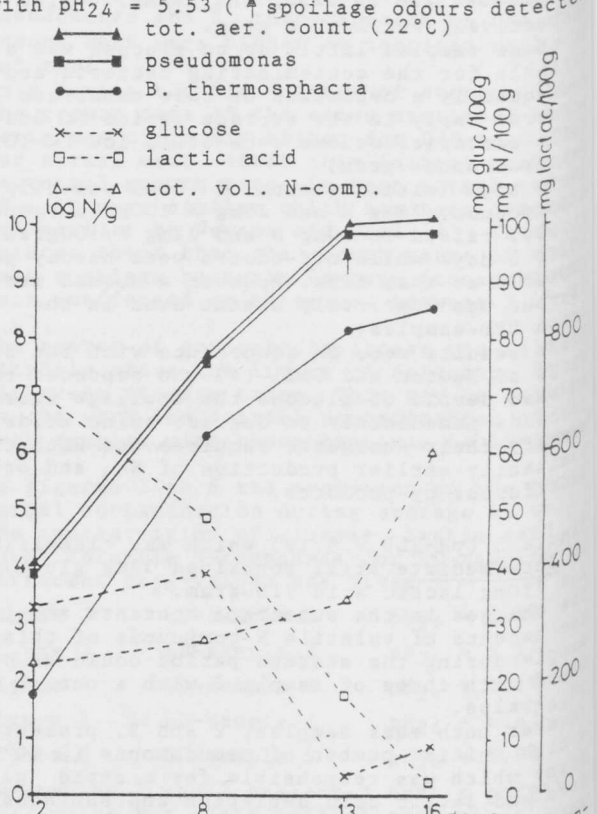

Figure 4 : The evolution of the bacterial contamination during aerobic storage (+2°C) and the concentration of glucose, lactic acid and total volatile N-compounds for LD-sample 3 with pH_{24} = 6.19 (↑ spoilage odours detected)

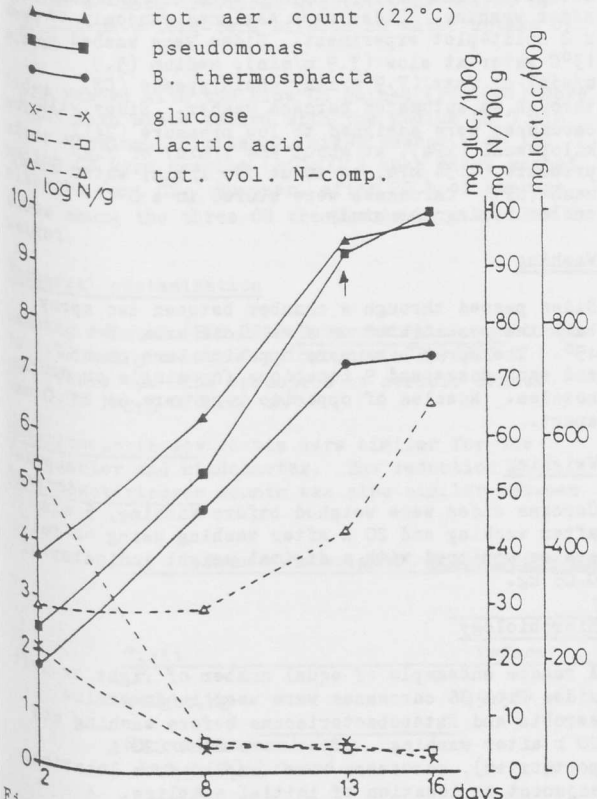

Figure 5 : The evolution of the bacterial contamination during aerobic storage (+2°C) and the concentration of glucose, lactic acid and total volatile N-compounds for LD-sample 4 with pH_{24} = 6.43 (↑ spoilage odours detected)

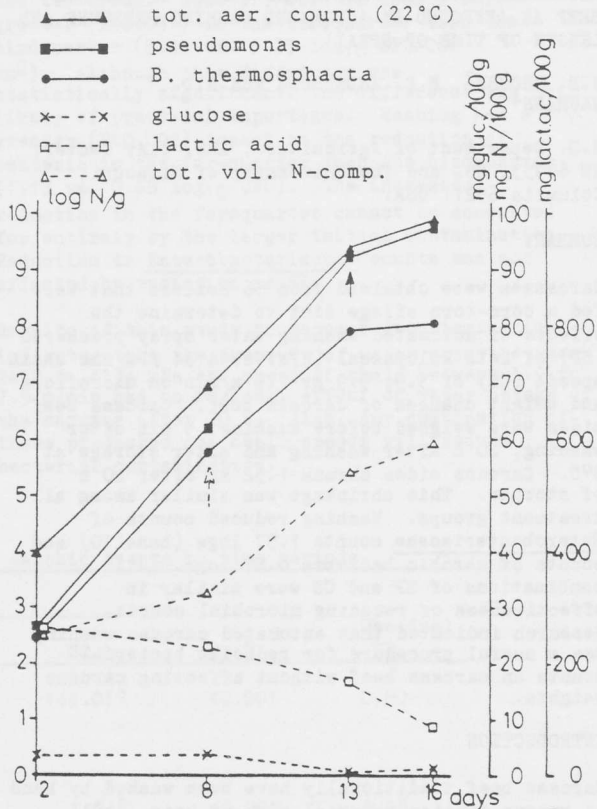

Figure 6 : The evolution of the bacterial contamination during aerobic storage (+2°C) and the concentration of glucose, lactic acid and total volatile N-compounds for LD-sample 5 with pH_{24} = 6.93 (↑ spoilage odours detected)