S6P10.WP

THE EFFECT OF CENTRIFUGATION OF STERILE BEEF HOMOGENATE MEDIUM ON *PSEUDOMONAS* GROWTH

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INTRODUCTION

Numerous culture media have been devised for the purpose of evaluating the development of spoilage flora in fresh meat at refrigeration temperature. The objective of this study to observe the effect of centrifugation on Pseudomonas growth in sterile beef homogenate medium and to study its effectiveness as a growth medium for *pseudomonas*.

MATERIALS AND METHODS

Experimental Design

Sterile ground beef homogenate was divided into two lots and one was not centrifuged (SBH). The other was centrifuged at 4,500rpm (3,300G) for 10 minutes at 4°C (SBE) to obtain the same dry matter as a typical commercial beef extract (ca. 0.4-0.45%). One subunit was inoculated with *pseudomonas fluorescens* (ca. 10^2-10^3 cells/ml) and the other was maintained as a control and they were analyzed at 0, 6, 12, 24 and 48 hours. The experiment was replicated seven times.

Materials

Sterile tissue was collected by the aseptic coring technique of Hone *et al.*, (1975) as modifications by Kim (1991). The homogenate was obtained by an extract procedure (Ockerman and Kim, 1992). A culture of *Ps.fluorescens* was obtained by the Ockerman and Kim procedure (1992).

Analytical Methods

The pH was measured using a Corning pH meter model 7. Plate count agar was used for *Pseudomonas* counts. The plates were incubated aerobically for two days at 25°C and reported as Log CFU/ml (U.S. FDA, 1984). Glucose was assayed by the method of Salomon and Johnson (1959). Total volatile nitrogen (TVN) was measured by macro-distillation technique described by Pearson (1968b).

Data Analysis

A three way analysis of variance (ANOVA) was performed and mean effects and the interaction between treatments, conditions and incubation time were estimated by calculating least significance differences (LSD; Steel and Torrie, 1960). There was no significant ($P>0.05$) difference of pH and TVN in uninoculated media, therefore, the differences

between the inoculated and uninoculated samples was calculated and two way analysis was performed (SAS, Cary, NC, 1984, 1988). Pearson correlation coefficients (SAS, 1985) were determined in each treatment which was inoculated with *Ps.fluorescens*.

RESULTS AND DISCUSSION

Pseudomonas showed consistent significant growth ($P<0.001$) in inoculated SBH and SBE and normal growth curves over 48 hours of incubation.

Glucose content of non-inoculated samples increased significantly ($P<0.05$) during the incubation time and SBH showed significantly higher ($P<0.05$) increasing rate than SBE after 12 hours (Figure 1). Inoculated media showed a significant increase of glucose content in the early stages of incubation (Figure 2), however, glucose content decreased after 24 hours of incubation ($P<0.05$).

Significant increases of pH values, due to inoculation with *Ps.fluorescens* were observed in both SBH and SBE when compared with the uninoculated samples after 12 hours of incubation (Figure 3) and significant difference of pH between SBH and SBE disappeared when both of these media were inoculated with *Ps.fluorescens*.

Total volatile nitrogen development increased significantly ($P<0.05$) in inoculated SBH and SBE after 24 hours (Figure 4). Significant ($P<0.01$) differences in TVN between SBH and SBE were observed throughout the 48 hours, as a result of the initial difference.

A very high correlation ($P<0.001$) between *pseudomonas* growth and pH values was found. There was also a significant negative correlation between the *pseudomonas* count and glucose level and a positive correlation between *pseudomonas* count and TVN values. Significant correlations among pH, glucose and TVN were also observed.

When media were inoculated with *Ps.fluorescens* significant differences of *pseudomonas* count, glucose and TVN were observed between SBH and SBE.

The *pseudomonas* counts increased significantly in SBE as well as SBH in spite of the exclusion of higher dense meat particles removed by centrifugation (Bate-Smith, 1948; Whitaker, 1959).

The glucose contents of uninoculated SBH and SBE showed significant increases during incubation. SBE contained less glucose than SBH. It is apparent that meat tissue which contained glucose or glycogen was removed during centrifugation. There was a significant decrease of glucose between 24 hours and 48 hours of incubation in inoculated SBH and SBE. Gill (1976) also indicated that a limited availability of glucose did not affect the growth of *pseudomonas* spp. on meat.

The pH increased significantly after 12 hours of incubation in both media despite centrifugation. Ockerman *et al.* (1969) reported that higher level inoculated samples increased in pH late in the storage period.

TVN development of the two inoculated media significantly increased after 24 hours of incubation. It is generally accepted that the psychrophilic bacteria growing on and causing spoilage in beef are mostly *pseudomonas*, which frequently cause the production of ammonia by deamination of amino acids under aerobic conditions (Ayres, 1960; McMeekin, 1975). There was a significant difference between SBH and SBE media over 48 hours caused by more nonprotein substances, nucleotides and nitrogen containing compounds, resulting in the increase of ammonia. Pearson (1967; 1968a; 1968b) has shown that TVN estimations, in the case of meat stored at low temperatures, are essentially estimations of ammonia produced.

CONCLUSIONS

Centrifugation did not influence the patterns of all spoilage variables in SBH over incubation time even though *pseudomonas* count, glucose and TVN were significantly different because of the different initial values due to the centrifugation procedures. The two media showed a normal growth curve shape and the same decrease of glucose and increase of TVN over incubation time. There was significant correlation between *pseudomonas* growth and pH, glucose and TVN in SBH and SBE with a level of $P<0.001$, $P<0.001$ and $P<0.05$, respectively.

It is concluded that SBE, which was produced by centrifugation of SBH, be used as a more appropriate microbial growth medium for the study of microbiological change and other changes in fresh meat system since it simulates the product being tested and gives an uniform distribution and concentration of bacterial cells.

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