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COMPARISON OF LAB-MADE STERILE BEEF EXTRACT MEDIUM AND COMMERCIAL BEEF EXTRACT MEDIUM

H.W. OCKERMAN and J.G. KIM

Department of Animal Science, The Ohio State University, Columbus, OH, United States

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INTRODUCTION

Numerous culture media have been devised for evaluating spoilage flora in fresh meat. The use of sterile meat tissue and extracts as experimental substrates have been developed (Ockerman *et al.*, 1969; Buckley *et al.*, 1976; Gill, 1976; Ockerman and Kim, 1992). The objectives of this study were to compare microbial growth in lab-made beef extract medium, prepared from meat that carried a natural flora, with growth in commercial beef extract medium.

MATERIALS AND METHODS

Experimental Design

Commercial beef extract (DCBE; Difco, Detroit, MI) was prepared as a 0.5%, w/v and solution which was sterilized by heating at 121°C for 15 minutes. Sterile samples of beef *longissimus dorsi* were collected by the aseptic coring technique of Hone *et al.*, (1975) as modified by Kim (1991). A spoilage flora inoculum was obtained by swabbing the surfaces of beef ribs and growing the flora so obtained in tryptic soy broth at 5°C for seven days. This culture, which contained approximately 10^8 cells/ml, was diluted to ca. 10^6 cells per gram of sterile meat for the inoculation. The inoculated ground was incubated for 48 hours at 5°C. It was homogenized with 10 times its weight of distilled water, sterilized by heating at 121°C for 15 minutes, then centrifuged at 2,500rpm for 10 minutes in order to adjust the dry matter (ca. 0.4-0.45%) to that of the commercial beef extract.

A culture of *Pseudomonas fluorescens* was prepared. One plash of Laboratory Beef Extract (LBE) was inoculated with *Ps.fluorescens* (ca. 10^2-10^3 cells/ml) and the other was used as a control. They were analyzed at 0, 6, 12, 24 and 48 hours. The experiment was replicated seven times. Culture of *Ps.fluorescens* was also prepared (Ockerman and Kim, 1992).

Analytical Methods

The pH of experimental media were measured using a Corning model 7 pH meter. Plate count agar was used for *pseudomonas* enumeration. 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 the macro-distillation technique described by Pearson (1968).

Data Analysis

A three way analysis of variance (ANOVA) was performed using SAS (Cary, NC, 1984; 1988). The means and the interaction between treatments and incubation times were estimated by calculating the least significance differences (LSD; Steel and Torrie, 1960). There was no significant difference of pH, *pseudomonas* count, glucose content and TVN over incubation time periods in all uninoculated media; therefore, a two way analysis of the pH difference, log of *pseudomonas* count difference and TVN increase and glucose decrease were analyzed. Pearson correlation coefficients (SAS, 1985) were determined in each treatment which was inoculated with *Ps.fluorescens*.

RESULTS AND DISCUSSION

Pseudomonas showed consistent significant growth ($P<0.01$) in CBE and LBE over the incubation time, except that a significant ($P<0.01$) decrease was found in LBE from zero to 6 hours (Figure 1). Significant differences in *pseudomonas* growth tended to disappear after 24 hours of incubation. A significant increase of pH due to the inoculation of *Ps.fluorescens* into CBE or LBE occurred after 12 hours (Figure 2).

Significant difference of pH between CBE and LBE was found after 24 hours of incubation. There was no significant change of glucose in CBE over the incubation time (Figure 3). The LBE showed a significant ($P<0.001$) decrease in glucose at 48 hours and there was a significant difference of decrement of glucose between CBE and LBE. A TVN increment was observed in both media at 48 hours of incubation (Figure 4).

The TVN increment obtained from CBE was significantly ($P<0.001$) greater than that obtained from LBE at 48 hours. A high correlation ($P<0.01$) between *pseudomonas* count, pH and TVN value was observed in CBE (Table 1). LBE also showed a very highly significant ($P<0.001$) correlation between *pseudomonas* count and pH and glucose content (Table 2). However, *pseudomonas* count was not significantly correlated with TVN value in contrast to the CBE medium.

The *pseudomonas* count increased significantly in CBE and LBE media at each incubation period and showed a normal growth curve since substantial amounts of low molecular weight compounds and an appropriate pH were provided (Whitaker, 1959; Gill and Newton, 1977). The decrease of *pseudomonas* growth in LBE at 6 hours of incubation might be caused by the loss of enzymatic activity and nutritive value of the meat components (Giri *et al.*, 1953; Chiambalero *et al.*, 1959). The pH increased significantly after 12 hours of incubation in both media. Although CBE initially contained lower amount of glucose than LBE, *pseudomonas* growth was not affected. *Pseudomonas* can grow readily on a wide range of substrates (Stanier *et al.*, 1966) and even though this species utilized glucose preferentially in liquid media it did not affect the growth of *pseudomonas* spp. on meat (Gill, 1976). The TVN development of the two media significantly increased after 24 hours of incubation. CBE increased significantly higher than LBE at 48 hours. It is generally accepted that the psychrophilic bacteria causing spoilage in beef are mostly *pseudomonas*, which cause the production of ammonia (McMeekin, 1975). There was very highly significant correlation between increment of *pseudomonas* count and pH value in CBE and LBE media (Rogers and McCleskey, 1961). *Psedomonas* count was also significantly correlated with TVN in CBE but not in LBE. Pearson (1968) has shown that TVN estimations correlate well with spoilage.

CONCLUSIONS

Four spoilage variables changed in significantly different patterns between CBE and LBE from 24 to 48 hours. These media showed a normal growth curve and rise in pH over 48 hours of incubation. Due to little glucose in CBE, glucose did not decrease but a rather drastic decrease was noted in LBE. A typical increase was noted for TVN with CBE, however, a less drastic change occurred in LBE. Significant correlation between *pseudomonas* count and pH was observed in CBE and LBE.

It is concluded that *pseudomonas* reacts differently to these two media and since the lab-made medium (LBE) is closer to refrigerated beef, it would be a more logical choice for evaluating the spoilage development in fresh meat .

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Table 1. Correlation coefficients (r) among spoilage variables of commercial beef extract (CBE) that was inoculated with *Ps.fluorescens*.

Variable[a]	Variables pH	Glucose	TVN
PC	0.805***	-0.198	0.475***
pH		-0.077	0.744**
Glucose			-0.060

Table 2. Correlation coefficients (r) among spoilage variables of lab-made sterile beef extract (SBE) that was inoculated with *Ps.fluorescens*.

Variable[a]	Variables pH	Glucose	TVN
PC	0.799***	-0.521***	0.276
pH		-0.736***	0.541***
Glucose			-0.248

[a] pH=pH of media; PC=Pseudomonas count (log CFU/ml); Glucose=Glucose content (μm/l); TVN=total volatile nitrogen content (mg N/100ml).
** $P<0.01$; *** $P<0.001$.