Pseudomonas Growth as Influenced by Autoclaving of Sterile Beef Extract

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INTRODUCTION: It is necessary to use an appropriate medium to understand how spoilage flora develops in fresh meat and heat-sterilized meat media have generally been employed. Meat infusion with peptone and beef extracts have been utilized (Wood and Bender, 1957; Bridson and Brecker, 1970). Unknown amount of heat utilized in these media causes many chemical (Wierbicki et al., 1957; Bendall, 1964; Hamm and Hofmann, 1965) and enzymatical (Chiambalero et al., 1959; Nanninga, 1962) alterations. Sterile meat has been suggested (Hone et al., 1975; Buckley et al., 1976) but suffers from lack of uniformity of muscle. Lerke et al. (1963) evaluated sterile press juice as a medium. Utilization of sterile extract as a microbial medium has not been evaluated. The objective is to evaluate undenatured sterile extract as a suitable medium for *Ps.* , and to observe the influence of autoclaving on this medium.

MATERIALS AND METHODS

Experimental Design: Sterile beef was homogenized with 10 times its weight of distilled water, divided into 2 lots, and one, not autoclaved, was centrifuged at 4,500 rpm (3,300 G) for 10 min at 4 °C (SBE, Fig. 1). The other was autoclaved at 121 °C and 15 psi for 15 min and centrifuged at 2,500 rpm (1,020 G) for 10 min at 4 °C (SBEA, Fig. 1). G force and time of centrifugation was varied between treatments to adjust dry matter of both experimental extracts to that of commercial beef extract (ca. 0.4-0.45%). After the 2 substrates were prepared (SBE and SBEA) they were divided into 2 subunits. One was inoculated with *Ps. fluorescens* (ca. 10^2 - 10^3 cells/ml) and the other was a sterile control. Both were incubated aerobically at 25 °C for 48 hr. and analyzed at 0, 6, 12, 24 and 48 hr. The experiment was replicated 7 times.

Materials

A. Sterile Muscle and Beef Extract: Sterile beef tissue was collected by the aseptic coring technique and sterile beef extract was obtained by an extract procedure as described by Ockerman and Kim (1992).
B. Culture of *Pseudomonas fluorescens*: Culture of *Ps. fluorescens* was obtained by the Ockerman and Kim (1992) procedure.

Analytical Methods

A. The pH measurement: The pH was measured without dilution by using a pH meter (model 7; Corning Medical and Glass Works, Medfield, MA).
B. *Pseudomonas* count: Plate count agar (Difco, Detroit, MI) was used for *Ps.* count and incubated aerobically for 2 days at 25 °C and reported as Log CFU/ml (U.S. FDA, 1984).
C. Glucose assay: Glucose was assayed by the method of Salomon and Johnson (1959).
D. Total volatile nitrogen (TVN): TVN was measured by macro-distillation technique (Pearson, 1968b).

Data Analysis: A three way SAS analysis of variance (ANOVA) was performed and mean effects and interaction between treatments, conditions and incubation time were estimated by least significance differences (Steel and Torrie, 1960). Correlation coefficients (SAS, 1985) were determined between the variables in each treatment which was inoculated with *Ps.* The pH and *Ps.* count had a significant ($P<0.01$) three way interaction. There was no significant difference in uninoculated media, therefore the effect of condition was removed from the analyses. The differences between the inoculated and uninoculated samples were calculated and the data reanalyzed. Two way analysis for the pH differences and log of *Ps.* count difference were analyzed by SAS (SAS, Cary, NC, 1984, 1988).

RESULTS: There was no growth of *Ps.* in uninoculated media. *Ps.* showed consistent significant growth in non-autoclaved sterile extract medium (SBE) over 48 hr., however, in the case of autoclaved sterile extract medium (SBEA) a significant decrease of *Ps.* growth was observed at 6 hr. compared to 0 hr. (Fig. 2). With this exception it seemed that *Ps.* in both media showed a normal growth curve. The *Ps.* increase of SBE were significantly higher than SBEA from zero to 12 hr., however, the difference of *Ps.* growth tended to disappear after 24 hr. (Fig. 2).

There was no significant increase of pH value due to inoculation of *Ps.* in SBE and SBEA for 12 hr. (Fig. 3). The pH value obtained from these two media increased significantly different at 24 hr. and 48 hr. The pH increase of SBE was significantly higher at 48 hr. than SBEA.

Glucose content tended to increase significantly at 12 hr. compared to 0 hr. in the uninoculated media (Fig. 4). After 24 hr. *Ps.* inoculated media showed a significant decrease of glucose. Highly significant difference of glucose content was

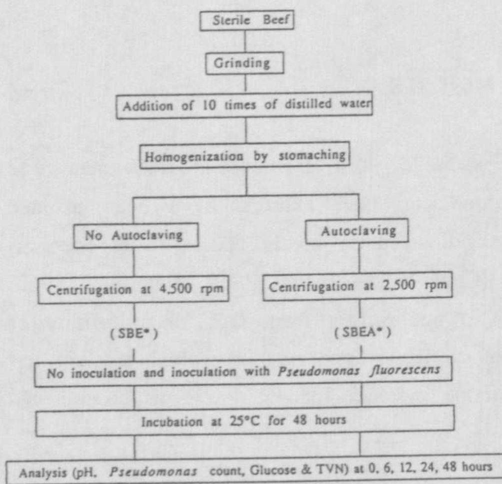
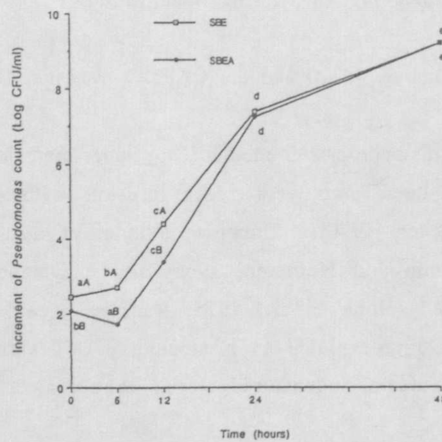


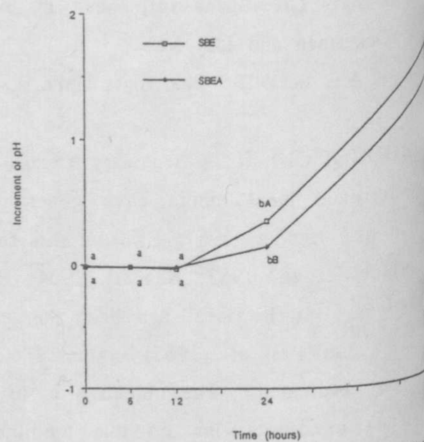
Figure 1. Experimental flow chart.

* SBE; Non-autoclaved sterile beef extract; SBEA; Autoclaved sterile beef extract



Points among incubation time at same treatment, bearing different small letters are significantly different (P<0.05). Points between treatments at same incubation time, bearing different capital letters are significantly different (P<0.01). Increment of *Ps.* count = Log of *Ps.* count of inoculated sample - Log of *Ps.* count of uninoculated sample

Figure 2. Comparison of the increments of *Pseudomonas* count in non-autoclaved (SBE) and autoclaved sterile beef extract (SBEA) that during 48 hr. of incubation (Analysis in Fig. 1).

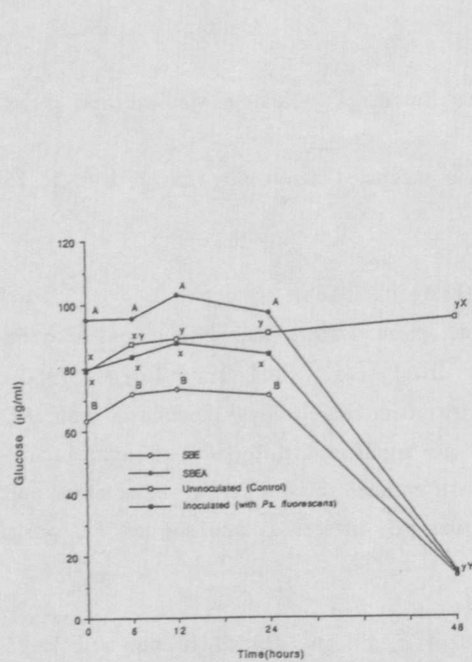


Points among incubation time at same treatment, bearing differ small letters are significantly different (P<0.05). Points betw treatments at same incubation time, bearing different capi letters are significantly different (P<0.01). Increment of pH value pH value of inoculated sample - pH value of uninoculated sample

Figure 3. Comparison of the increments of pH value in no autoclaved (SBE) and autoclaved sterile beef extract (SBEA) th were caused by inoculation with *Ps. fluorescens* during 48 hr. incubation (Analysis in Fig. 1).

observed between uninoculated and inoculated media at 48 hr. Non-autoclaved medium showed significantly less glucos content than autoclaved medium from zero up to 24 hr. but this disappeared at 48 hr.

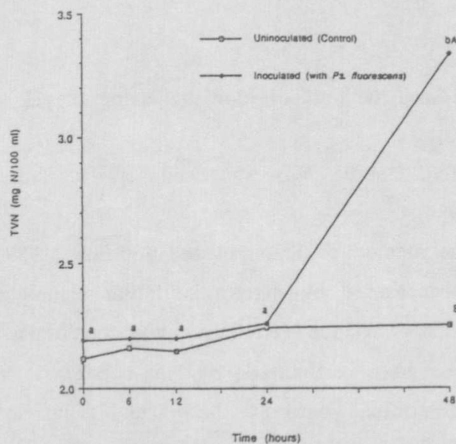
TVN development was very stable in uninoculated media (Fig. 5). Inoculated media did not show a significant increase o TVN up to 24 hr. but at 48 hr. a significant increase of TVN was found. A significant difference of TVN values between SB and SBEA was also observed from 0 to 24 hr. (Fig. 6). SBE tended to have significantly higher TVN value than SBEA at a periods except 48 hr.



Non-autoclaved (SBE) and autoclaved (SBEA) samples were analyzed and the 3 way interaction (Autoclaving x Time x Only for *Ps.* inoculated sample) statistical results are shown (A and B) in the graph and also inoculated and uninoculated (autoclaving absorbed) samples were analyzed and the 2 way interaction (Time x inoculation) statistical results are shown (x, X, y and Y).

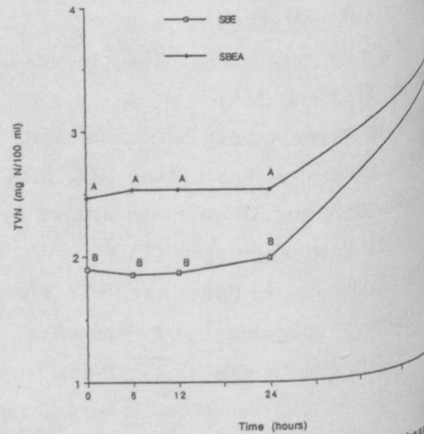
Points between treatments (A and B, X and Y) at the same incubation time bearing different letters are significantly different (P<0.01). Points among incubation time at the same condition, bearing different samil letters (x and y) are significantly different (P<0.05).

Figure 4 Comparison of glucose content between uninoculated (average of SBE and SBEA) and inoculated (average of SBE and SBEA) and between SBE and SBEA that were inoculated with *Ps.* during 48 hr. of incubation (Analysis in Fig. 1)



Points among incubation time at same condition, bearing different small letters are significantly different (P<0.05). Points between conditions at same incubation time, bearing different capital letters are significantly different (P<0.05).

Figure 5. Comparison of total volatile nitrogen in uninoculated (average of non-autoclaved and autoclaved) media and media inoculated with *Ps. fluorescens* (average of non-autoclaved and autoclaved) during 48 hr. of incubation (Analysis in Fig. 1).



Points between treatments at same incubation time, bear different letters are significantly different (P<0.05).

Figure 6. Comparison of total volatile nitrogen in non-autocla (SBE) and autoclaved sterile beef extract (SBEA) media that w inoculated with *Ps. fluorescens* during 48 hr. of incubation (Analy in Fig. 1).

Table 1. Correlation coefficients (r) among spoilage variables of non-autoclaved sterile beef extract (SBE) that was inoculated with *Ps. fluorescens* (Analysis in Fig. 1).

	Variables [a]		
Variables [a]	pH	Glucose	TVN
PC	0.837***	-0.576***	0.351*
pH		-0.806***	0.556***
Glucose			-0.392*

[a] pH = pH of media; PC = *Pseudomonas* count (Log CFU/ml); Glucose = Glucose content (μg /ml); TVN = Total volatile nitrogen content (mg N/100 ml).
* P<0.05, *** P<0.001

Table 2. Correlation coefficients (r) among spoilage variables autoclaved sterile beef extract (SBEA) that was inoculated with *fluorescens* (Analysis in Fig.1).

	Variables [a]		
Variables [a]	pH	Glucose	TVN
PC	0.794***	-0.604***	0.246
pH		-0.834***	0.480***
Glucose			-0.300

[a] pH = pH of media; PC = *Pseudomonas* count (Log CFU/ml); Glucose Glucose content (μg /ml); TVN = Total volatile nitrogen cont (mg N/100 ml).
*** P<0.001

Table 1 shows the correlation from the inoculated SBE medium. *Ps.* count was highly correlated with pH and glucose and correalted with TVN at the $P<0.05$ level. It was found that pH, glucose and TVN were significantly correlated with each other. Correlation coefficients of SBEA medium are shown in Table 2. As with non-autoclaved medium there was a very high correlation between *Ps.* count and pH and glucose. However, the correlation between *Ps.* count and TVN was not significant.

DISCUSSION: When media were inoculated with *Ps.* significant differences of the four spoilage variables were observed between SBE and SBEA and among various time periods. In this study *Ps.* growth in both media showed a normal growth curve because a desirable environment for *Ps.* growth remained despite autoclaving (Bate-Smith, 1948; Whitaker, 1959). *Ps.* spp. was reported to grow in meat juice at pH's between 5.5 and 7.0 (Gill and Newton, 1977). Most species of *Ps.* studied can develop in mineral media (Stanier et al., 1966). The significant decrease of *Ps.* growth in autoclaved sterile beef extract at 6 hr. might be caused by the loss of enzymatic activity (Mitchell and Block, 1946; Giri et al. 1953) and nutritive value (Chiambalero et al, 1959; Bendall, 1964). This decrease was not observed in non-autoclaved sterile beef extract.

The pH increased significantly after 12 hr. in both media due to *Ps.* growth. Ockerman et al. (1969) reported that higher level inoculated samples increased in pH late in the storage period. Even though the pH of autoclaved medium was significantly higher than that of non-autoclaved medium the increment of pH was significantly lower for autoclaved media for 24 and 48 hr. The initial higher pH obtained from autoclaved medium resulted in lower growth than non-autoclaved medium. Muscle tissue pH increases during heating (Bendall, 1946) and pH is an important factor in bacterial growth (Ingram, 1962). In spite of the differences of pH, non-autoclaved and autoclaved media showed similar growth curves which agrees with Rey et al. (1976). Most *Ps.* grow well at pH 6.0- 8.5 and since their metabolic products are strongly alkaline, meat pH increases (Stanier et al., 1966).

Glucose of media which were not subjected to inoculation tended to significantly increase over time. The hydrolysis of meat protein results in glycogen being more available. Autoclaved medium contains significantly more glucose than non-autoclaved medium. Apparently heat denaturation causes an unfolding of the peptide chains and thus glycogen was hydrolyzed. There was a significant decrease of glucose between 24 hr. and 48 hr. of incubation in both media. In this study 15-20% of the initial glucose content remained after 48 hr. when the cell density exceeded 10^9 cells/ml and this is different from the observation of Gill (1976). Gill and Newton (1977) found that *Ps.* grew at their maximum rate utilizing glucose and amino acids. Although non-autoclaved medium contained lower amount of glucose, *Ps.* growth was not affected. *Ps.* can grow readily on a wide range of substrates (Jacoby, 1964 ; Stanier et al., 1966; Gill, 1976).

TVN development of the inoculated media significantly increased after 24 hr. It is generally accepted that the psychrophilic bacteria growing on and causing spoilage in beef are mostly *Ps.*, which frequently cause the production of ammonia by deamination of amino acids under aerobic conditions (Ayres, 1960; McMeekin, 1975). In this study TVN increased significantly when the cell density exceeded 10^9 cells/ml in both media. Organoleptic spoilage becomes detectable when the cell density exceeds 10^8 bacteria/cm^2 (Ingram and Dainty, 1971). Autoclaved medium showed significantly higher TVN value than non-autoclaved medium at all incubation periods except 48 hr. Beuk et al. (1948), Greenwood et al. (1951) and Hamm (1963) reported that the amino acids of beef, pork and lamb cuts are stable on cooking. However, in this study autoclaving apparently influenced the amino acids content, resulting in more production of TVN.

Highly significant correlations between increment of *Ps.* and pH in non-autoclaved and autoclaved media was obaserved. Some authors reported good agreement (Rogers and McCleskey, 1961) but others indicated less agreement (Gardner, 1965) when comparing these variables. Significant correlation was also observed between *Ps.* and glucose. *Ps.* count was also significantly correlated with TVN in non-autoclaved medium but not in autoclaved medium. Saffle et al. (1961) indicated little correlation between total bacterial numbers and organoleptic properties but Pearson (1967, 1968 a, b) has shown that TVN estimations correlate well with spoilage of beef as judged by odor.

CONCLUSIONS: Non-autoclaved sterile extract and autoclaved sterile beef extract media were inoculated with *Ps. fluorescens* and incubated aerobically for 48 hr. at 25 °C. No microorganisms were found in the uninoculated media. The coring technique (Hone et al., 1975) with some modification is recommended for obtaining sterile muscle. Four spoilage variables changed in significantly different patterns between SBE and SBEA at 0 to 24 hr. Autoclaving increased pH, glucose and TVN. Both media showed normal growth curves, normal rise in pH and TVN values and decrease in glucose over 48 hr. Highly significant correlations between *Ps.* and pH value and glucose content, were observed in SBE and SBEA. The former showed significant correlation between *Ps.* and TVN, however, the latter did not. It is obvious that *Ps.* reacts differently to these two media and since the non-autoclaved sterile beef extract medium is closer to refrigerated beef it would be a more logical choice for evaluating the microbiological state of refrigerated beef.

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