INFLUENCE OF PREVIOUS BACTERIAL GROWTH ON THE BIOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF BEEF EXTRACT MEDIUM

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INTRODUCTION: Pure cultures on heat-sterilized meat media have generally been employed to understand how spoilage flora develops in fresh meat. Infusion of meat, peptone, sterile meat tissue and beef extract has been used (Bender et al., 1958; Bridson and Brecker, 1970; Hone et al.,1975; Buckley et al.,1976). However, these approaches are not totally appropriate because of previous microbial growth, heat and unevenly distributed tissue within a muscle (Chiambalero et al., 1959; Hamm and Deatherage, 1960; Nanninga, 1962; Bendall, 1964; Hamm and Hofmann, 1965). Lerke et al. (1963) developed sterile press juice but there has been no studies on sterile beef extract. The objective of this study was to compare as a growth medium for *Pseudomonas* sterile beef extract medium (SBE) with beef extract medium (IBE) which was inoculated with "normal flora" and allowed to grow until it was halted by autoclaving.

MATERIALS AND METHODS

Experimental Design: This experiment studied the effect of "normal flora" and various incubation times on the changes of 4 spoilage variables in uninoculated and *Ps.* inoculated media (Fig. 1). Sterile beef was ground, divided into 2 lots, one (SBE) was not inoculated, incubated for 48 hr. at 5 °C, homogenized with 10 times its weight of distilled water, autoclaved at 121 °C for 15 min, and centrifuged at 2,500 rpm (1,020 G) for 10 min at 4 °C in order to adjust the dry matter to that of commercial beef extract (ca. 0.4-0.45%). The other lot (IBE) was inoculated with "normal flora" (ca. 10^5 cells/g), incubated for 48 hr. at 5 °C and was prepared according to the same procedure. After SBE and IBE substrates were prepared, each was divided into two subunits. One was inoculated with *Ps. fluorescens* (ca. 10^2-10^3 cells/ml) and the other was maintained as a control sterile medium. All were incubated aerobically at 25 °C for 48 hr. and were analyzed at 0, 6, 12, 24 and 48 hr. and replicated 7 times.

Materials

A. Sterile Muscle Tissue: Prior to sticking the beef animal, the skin of the neck region was saturated and rubbed with 70% alcohol. A sterile knife was used to cut the skin and another sterile knife was used to stick the ventral cervical area. Carcasses were chilled for 48 hr. at 1±2 °C and L. dorsi from the 6th through 12th rib section was removed. A metal cylindrical (38 x 610 mm) coring device was made and sterile muscle tissue was collected by the aseptic technique described by Hone et al. (1975). Both of the exposed ends of the L. dorsi and the subcutaneous fat covering the central portion of the rib were cauterized. Chunked meat excluding the cauterized tissue was ground aseptically through a 5 mm plate into a sterile stomacher bag (Tekmar Co., Cincinnati, OH). One half of the muscle was inoculated with "normal flora" and the rest was uninoculated and both were stored at 5°C for 48 hr.

B. Culture of "Normal flora": "Normal flora" was obtained from beef ribs and grown to ca. 10^8 cell/ml in tryptic soy broth at 5 °C for 7 days. Approximately 10^5 cells per g were inoculated into the sterile beef.

C. Sterile Meat Extract: After 48 hr incubation at 5 °C uninoculated and inoculated samples were blended with ten times its weight of sterile distilled water (Lab-Blender stomacher 400; Tekmar Co., Cincinnati, OH) and both were autoclaved.

E. Culture of *Ps. fluorescens*: *Ps.* was obtained from the Culture Collection of OSU and maintained on a trypticase soy agar (BBL, Beckton Dickinson Microb. Syst., Cockeysville, MD) slant and grown to approximately 10^9 cells/ml in tryptic soy broth (Difco Lab. Detroit, MI) for 24 hr at 25 °C. Decimal dilutions were prepared in saline (0.85 %) resulting in a level of ca. 2.5 x 10^2 cells/ml.

Analytical Methods

A. The pH measurement: The pH was measured without dilution by using a Corning pH meter model 7 (Corning Medical and Glass Works, Medfield, MA) equipped with a Polymer Body Calomel Combination Fisher Electrode (Cat.# 13-639-272, Fisher Sci. Co., Pittsburgh, PA).

B. *Pseudomonas* count: Serial dilutions were prepared and poured with plate count agar (Difco, Detroit, MI). Plates were incubated aerobically for 2 days at 25 °C (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 the macro-distillation technique described by Pearson (1968b).

Data Analysis: Analysis of variance (ANOVA) was performed on the data set by using SAS. The mean effects and their interaction between treatments (SBE and IBE), conditions (uninoculated and inoculated) and incubation time period (0, 6, 12, 24, 48 hr.) were determined (Steel and Torrie, 1960). Correlation coefficients (SAS, 1985) were determined between the variables (log of *Ps.*, pH, glucose and TVN) in each treatment which was inoculated with *Ps. fluorescens*.

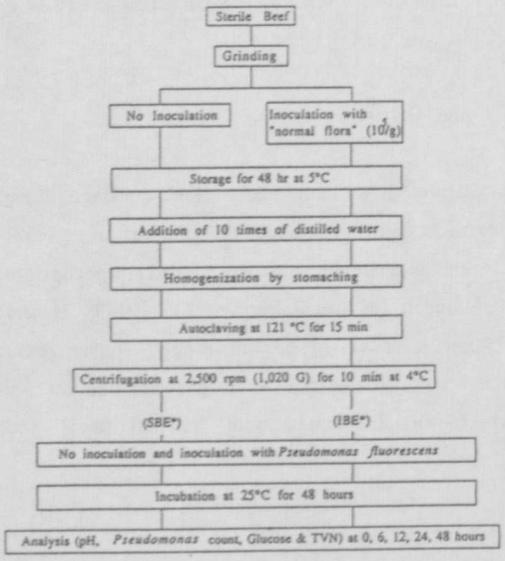
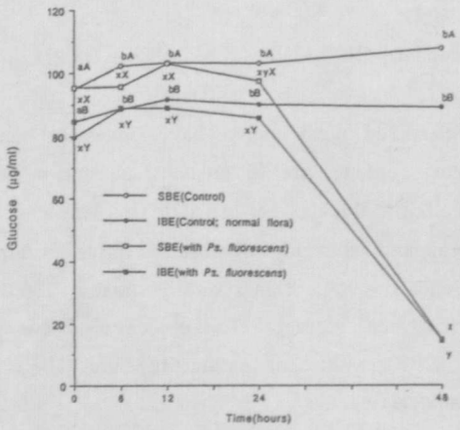


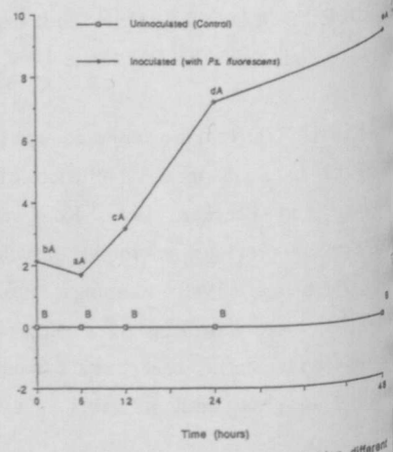
Figure 1. Experimental flow chart.

*SBE; Uninoculated sterile beef extract; IBE; Inoculated beef extract



Points among incubation time (a and b, x, y, and z) at same treatment, bearing different small letters are significantly different (P<0.05). Points between treatments (A and B, X and Y) at same incubation time, bearing different capital letters are significantly different (P<0.01).

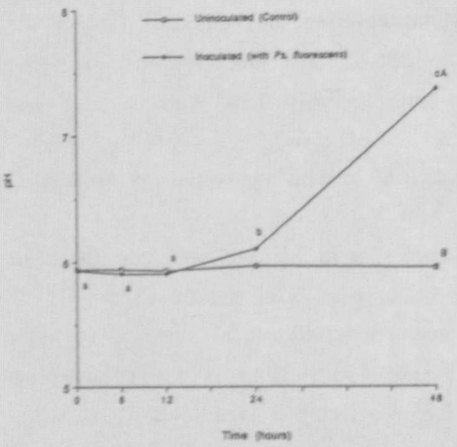
Figure 2. Comparison of glucose content in uninoculated sterile beef extract (SBE) and inoculated beef extract (IBE), that were not and were inoculated with *Ps. fluorescens*, during 48 hr. of incubation (Analysis in Fig. 1).



Points among incubation time at same condition, bearing different small letters are significantly different (P<0.01). Points between conditions at same incubation time, bearing different capital letters are significantly different (P<0.001).

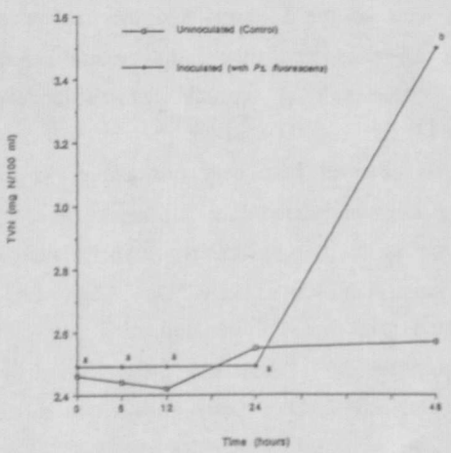
Figure 3. Comparison of *Pseudomonas* count in uninoculated experimental media (average of SBE and IBE) and experimental media (average of SBE and IBE) inoculated with *Ps. fluorescens* during 48 hr. of incubation (Analysis in Fig. 1).

RESULTS: The analysis indicated that glucose had a P<0.05 three way interaction and only it is discussed. However, there was no significant three way interaction in the other dependent variables, *Ps.* count, pH and TVN. Therefore, two way interaction effect between condition and incubation time is discussed. Fig. 2 shows when SBE and IBE were not inoculated with *Ps.*, their glucose content increased significantly at 6 hr. incubation. The former tended to increase from zero up to 48; however, the latter tended to decrease after 12 hr. The former also showed significantly higher glucose content at all time periods than the latter which was inoculated with "normal flora". When SBE and IBE media were inoculated with *Ps.* their glucose content tended to increase from zero up to 12 hr., however, after 24 hr. they showed a significant decrease of glucose content due to growth of *Ps.* A significant difference of glucose content was observed between these media up to 24 hrs.



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<0.001).

Figure 4. Comparison of pH value in uninoculated experimental media (average of SBE and IBE) and experimental media (average of SBE and IBE) inoculated with *Ps. fluorescens* during 48 hr. of incubation (Analysis in Fig. 1).



Points among incubation time at same condition, bearing different letters are significantly different (P<0.05). There was no significant difference between treatments at same incubation time.

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

Table 1. Correlation coefficients (r) among spoilage variables of uninoculated sterile beef extract (SBE), which was inoculated with *Ps. 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 content (mg N/100 ml).
*** P<0.001

Table 2. Correlation coefficients (r) among spoilage variables of inoculated beef extract (IBE), which was inoculated with *Ps. fluorescens* (Analysis in Fig. 1).

Variables [a]	Variables [a]		
	pH	Glucose	TVN
PC	0.779***	-0.521***	0.276
pH		-0.736***	0.541***
Glucose			-0.246

[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<0.001

No microorganisms were detected when media were not inoculated with *Ps.* (Fig. 3) but inoculated media showed consistent significant growth over 48 hr except for 6 hr. when they decreased significantly. It indicated that *Ps.* in both media had a normal shape growth curve.

The pH values of two media which were not inoculated were very stable over 48 hr. (Fig. 4). It was also found that there was no significant increase of pH value due to inoculation of *Ps.* in SBE and IBE from 0 to 12 hr.; however, a significant increase of pH value was observed after 12 hr. in these media. A significant difference in pH values between uninoculated media and media which were inoculated with *Ps. fluorescens* was observed after 12 hr.

TVN development increased significantly in both SBE and IBE media after 24 hours of incubation when they were inoculated with *Ps.* However, no significant development of TVN was observed over 48 hr. in media not inoculated with *Ps.* (Fig. 5). There was also no significant difference between SBE and IBE, and between uninoculation and inoculation with *Ps.* throughout 48 hr.

There was a very high correlation between *Ps.* and pH as well as glucose content in SBE and IBE (Table 1 and 2). However, the correlation between *Ps.* and TVN was not significant. IBE and SBE showed a similar pattern of correlation among the variables.

DISCUSSION: Significant difference of glucose was observed between uninoculated sterile beef extract (SBE) and inoculated beef extract (IBE) and among various incubation time periods. When they were inoculated with *Ps.* significant difference was also noted for the four spoilage variables, *Ps.*, pH, glucose and TVN. Significant increase of glucose content in SBE and IBE media not inoculated with *Ps.* was observed at the later stage of incubation. Apparently heat denaturation causes an unfolding of the peptide chains and thus glycogen was exposed to denaturation conditions and could more easily be hydrolyzed to glucose (Wierbicki et al., 1957; Hamm and Deatherage, 1960). SBE which was not subjected to inoculation with "normal flora" contains significantly more glucose than IBE which was inoculated with "normal flora" due to glucose being used by the "normal flora" during 48 hr., resulting in a different initial glucose content. However, *Ps.* growth was not affected by the low glucose content obtained from this medium. *Ps.* can grow readily on a wide range of substrates (Stanier et al., 1966). Gill (1976) also confirmed this result indicating that a limited availability of glucose did not affect the growth of *Ps.* spp. on meat. There was a significant decrease of glucose between 24 and 48 hr. in both microbial growth media which were inoculated with *Ps.* In this study 15% of the initial glucose content remained after 48 hr. of incubation when the cell density exceeded 10^9 cells/ml. This is different from the observation of Gill (1976) in which the concentration of glucose at the surface decreased when the cell density exceeded 10^7 bacteria/cm^2 and reached zero when the cell density was approximately 3 x 10^8 bacteria/cm^2. Gill and Newton (1977) found that *Ps.* utilized the substrates in the order of glucose, amino acids and lactic acid in the meat juice medium but the growth rates declined when only lactic acid was available.

Ps. increased significantly in SBE and IBE after 6 hrs. In this study *Ps.* showed a normal shape of the growth curve. This is because meat extract provides an ideal environment for the *Ps.* despite previous inoculation with "normal flora" (Bate-Smith, 1948; Whitaker, 1959). In addition to this *Ps.* spp. was reported to grow at their maximum rates in meat juice medium at pH values between 5.5 and 7.0 (Gill and Newton, 1977). Most species of *Ps.* studied, including parasitic ones, can develop in mineral media with a single organic compound as the source of carbon and energy. An individual strain can use from 60 to more than 80 different carbon sources for its growth (Stanier et al., 1966). The significant decrease of *Ps.* in SBE and IBE at 6 hr of incubation time might be caused by the loss of enzymatic activity and the nutritive value of the meat components. The thermal treatment of muscle causes a loss of its enzymatic activity (Mitchell and Block, 1946; Giri et al., 1953) and the nutritive value of proteins (Chiambalero et al., 1959; Hamm and Deatherage, 1960; Bendall, 1964). There was no significant difference of *Ps.* growth between SBE and IBE media except at 12 hr. *Ps.* can grow readily on a wide range of substrates (Stanier et al., 1966), even though this species utilized glucose preferentially in liquid media (Jacoby, 1964; Gill, 1976).

The initial pH values between the two media was very similar, which suggest that by using glucose the "normal flora" did not grow enough to affect the pH. The pH increased significantly after *Ps.* was inoculated, after 12 hours of incubation in SBE and IBE. Ockerman et al. (1969) reported that higher level inoculated samples increased in pH late in the storage period. *Ps.* grows well at pH 6.0-8.5 and since their metabolic products are strongly alkaline, meat pH shifts to higher levels (Stanier et al., 1966).

The initial TVN values between media was not significantly different. TVN in both media significantly increased after 24 hr. It is generally accepted that the psychrophilic bacteria causing spoilage in beef are mostly *Ps.*, which frequently causes 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 is reported to become detectable when the cell density exceeds 10^8 bacteria/cm^2 (Ingram and Dainty, 1971)

There was very highly significant correlations between *Ps.* and pH and glucose in SBE and IBE. Some authors reported good agreement (Rogers and McCleskey, 1961) but others indicated less agreement (Gardner, 1965). *Ps.* was not significantly correlated with TVN in both media. 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 on meat stored at low temperatures is an estimation of ammonia produced which correlate well with spoilage.

CONCLUSIONS: Uninoculated sterile beef extract medium and beef extract medium inoculated with "normal flora" were incubated for 48 hr at 5 °C. Inoculation with "normal flora" influenced the pattern of glucose, however, its growth did not affect other variables such as pH, *Ps.* and TVN. When SBE and IBE were inoculated with *Ps.*, there was consistent significant growth in both media, resulting in a normal shape growth curve over 48 hrs. Due to autoclaving significant ($P<0.01$)

decrease of microbial growth was observed at 6 hr. in both media. After 24 hr. the two media showed significant decrease of glucose content due to inoculation of *Ps.* A significant increase of pH value due to inoculation with *Ps.* was also observed after 12 hr. Typical increase was also indicated in TVN with both media. *Ps.* was significantly correlated with pH and glucose, but not with TVN. It is obvious that *Ps.* reacts differently to these two media. It would be a more logical choice for evaluating fresh meat product by using a sterile beef extract medium which is not subjected to any contamination.

REFERENCES

Ayres, J.C. 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry, and eggs. J. Appl. Bacteriol. 23:471.

Bate-Smith, E.C. 1948. The physiology and chemistry of rigor mortis, with special reference to the aging of beef. Adv. Food Res. 1:1.

Bendall, J.R. 1964. Meat proteins, p. 225. In Schulz, H.W. and Anglemier, A.F. (eds.), Proteins and Their Reactions. Avi Publishing Co. Inc., Westport, CT.

Bender, A.E., Wood, T. and Palgrave, J.A. 1958. Analysis of tissue constituents. Extract of fresh ox-muscle. J. Sci. Food Agric. 9:812.

Bridson, E.Y. and Brecker, A. 1970. Design and formulation of microbial culture media. In Methods in Microbiology, vol. 3A. p. 229. Eds. Norris, J.R. and Ribbons, D.W. Academic press, London and New York.

Buckley, J., Morrissey, P.A. and Daly, M. 1976. Aseptic technique for obtaining sterile beef tissue. J. Food Technol. 11:427.

Chiambalero, C.J., Johnson, A. and Drake, M.P. 1959. A time temperature relationship for heat-enzyme inactivation of radiation-sterilized beef and pork. J. Agric. Food Chem. 7:782.

Gardner, G.A. 1965. Microbiological and biochemical changes in fresh meat during storage. Ph.D. thesis, Queen's University of Belfast.

Gill, C.O., 1976. Substrate limitation of bacterial growth at meat surfaces. J. Appl. Bacteriol. 41 : 401.

Gill, C.O. and Newton, K.G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43:189.

Giri, K.V., Rama Rao, P.B. and Rajagopalan, R. 1953. Some studies on the destruction of amino acids in proteins caused by autoclaving in presence of glucose. J. Food Sci. 18:217.

Hamm, R. and Deatherage, F.E. 1960. Changes in hydration, solubility and charges of muscle proteins during heating of meat. Food Res. 25:587.

Hamm, R. and Hofmann, K. 1965. Changes in the sulfhydryl and disulfide groups in beef muscle proteins during heating. Nature 207:1269.

Hone, J.D., Ockerman, H.W., Cahill, V.R., Borton, R.J., and Proctor, G.O. 1975. A rapid method for the aseptic collection of tissue. J. Milk Food Technol. 38 : 664.

Ingram, M. and Dainty, R.H. 1971. Changes caused by microbes in spoilage of meat. J. Appl. Bacteriol. 34:21.

Jacoby, G.A. 1964. The induction and repression of amino acid oxidation in *Ps. fluorescens*. Biochem. J. 92:1.

Lerke, P., Adams, R., and Farber, L. 1963. Bacteriology of spoilage of fish muscle. I. Sterile press juice as a suitable experimental medium. Appl. Microbiol. 11 : 458.

McMeekin, T.A. 1975. A spoilage association of chicken muscle. Appl. Microbiol. 29:44.

Mitchell, H.H. and Bolck, R.J. 1946. Some relationships between the amino acid contents of proteins and their nutritive value for the rat. J. Biol. Chem. 163:599.

Nanninga, L.B. 1962. The effect of heating on the kinetic constants of myosin-adenosin triphosphatase. Nature 194:187.

Ockerman, H.W., Cahill, V.R., Weiser, H.H., Davis, C.E. and Siefker, J.R. 1969. Comparison of sterile and inoculated beef tissue. J. Food Sci. 34:93.

Pearson, D. 1967. Assessing beef acceptability. Food. Mf. 42(11), 42.

Pearson, D. 1968 a. Assessment of meat freshness in quality control employing chemical techniques : A Review. J. Sci. Food Agric. 19:357.

Pearson, D. 1968 b. Application of chemical methods for the assessment of beef quality. II. Methods related to protein breakdown. J. Sci. Food. Agric., 19 : 366.

Rogers, R.E. and McCleskey, C.S. 1961. Objective test for quality of ground beef. Food Technol., 15(4): 210.

Saffle, R.L., May, K.N., Hamid, H.A. and Irby, J.D. 1961. Comparing three rapid methods of detecting spoilage in meat. Food Technol., 15:465.

Salomon, L.L. and Johnson, J.E. 1959. Enzymatic micro-determination of glucose in blood and urine. Anal. Chem. 31 : 453.

SAS, 1985. SAS User's Guide. Statistic version, 5 ed. SAS Institute Inc., Cary, NC, USA.

Stanier, R.Y., Palleroni, N.J. and Doudoroff, M. 1966. The aerobic pseudomonads: a taxonomic study. J. General Microbio. 43:159.

Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistic. McGraw-Hill Book Co., NY, USA.

U.S. FDA. 1984. Bacteriological Analytical Manual: Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration. 6th ed.

Whitaker, J.R. 1959. Chemical changes associated with aging of meat with emphasis on the proteins. Adv. Food Res. 9:1.

Wierbicki, E., Kunkle, L.E. and Deatherage, F.E. 1957. Changes in the water-holding capacity and cationic shifts during heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinking. Food Technol. 11:69.