COMBAT RATION NETWORK

FOR

TECHNOLOGY IMPLEMENTATION

Defining the growth/no-growth boundary for *Listeria monocytogenes* in Shelf Stable Pocket Sandwiches

Final Technical Report STP#2023

Results and Accomplishments (July 2006 – December 2008)

Report No: FTR 209

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December 2008

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DEFENSE LOGISTICS AGENCY
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Fort Belvoir, VA 22060-6221

Contractor:
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Dr. Donald Schaffner
Mr. Henderikus B. Bruins

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14. ABSTRACT

Honey Barbeque Beef and Bacon Cheddar pocket sandwiches were formulated such that their pH values and water activity values were much higher than allowable under the product specification, to create an environment that might support the growth of L. monocytogenes. . They were inoculated with a seven strain L. monocytogenes cocktail and repackaged The product was incubated at 25 C and tested for the presence of viable L. monocytogenes over a 12 month period.

It was concluded that L. monocytogenes does not grow in pocket sandwiches, including pocket sandwiches that are grossly mis-formulated. Listeria monocytogenes growth is, therefore, not a “hazard reasonably likely to occur” in these products and there is no need to include L. monocytogenes as an hazard in the HACCP plan. Even if the organism does somehow contaminate the sandwiches, it presents no risk to consumers of this product as long as the product is produced within the specification limits.

15. SUBJECT TERMS

Shelf Stable Sandwich, Pocket Sandwich, Listeria, L. monocytogenes, MRE, HAACP

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Abstract:

Honey Barbeque Beef and Bacon Cheddar pocket sandwiches were formulated such that their pH values and water activity values were much higher than allowable under the product specification, to create an environment that might support the growth of *L. monocytogenes*. The worst case BBQ beef sample had a target pH = 5.16 and water activity = 0.94. The worst case Bacon Cheddar product had a target pH = 5.75 and water activity = 0.92.

The Honey Barbeque Beef and Bacon Cheddar pocket sandwiches were inoculated with a seven strain *L. monocytogenes* cocktail. The samples were repackaged after inoculation in new packing material, an oxygen scavenger was added and the packages were heat sealed. The product was then incubated at 25 C and sampled at various time intervals. The product was tested for the presence of viable *L. monocytogenes* over a 12 month period.

It was determined that *L. monocytogenes* does not grow in pocket sandwiches, including pocket sandwiches that are grossly mis-formulated. For example, if a Honey Barbeque Beef sandwich is manufactured with meat that contains no glycerol and no rice syrup, and a sauce that contains 50% of the usual glycerol amount, 50% of the usual tomato paste amount, no vinegar, no brown sugar and bread that contains no glycerol and no sorbic acid, the product will still not support the growth of *L. monocytogenes*. Likewise, if a Bacon Cheddar sandwich is manufactured with 50% of the usual amount of cheddar flakes, no glycerol, no sorbic acid, no GDL, and no butter flavor, this product will also not support the growth of *L. monocytogenes*.

In short, *Listeria monocytogenes* growth is not a “hazard reasonably likely to occur” in these products. Based on the results of this study and summarized above, there is no need to include *L. monocytogenes* as a hazard in the HACCP plan. Even if the organism does somehow contaminate the sandwiches, it presents no risk to consumers of this product.

Index

1 Results and Accomplishments

1.1 Introduction and Background

Mobility-enhancing ration components, such as sandwiches filled with meat have been developed by The US Army Natick Soldier Center. These “Pocket Sandwiches” are not sterile, but formulated, baked and packed to prevent growth of pathogenic and spoilage bacteria, yeast and molds, while maintaining organoleptic acceptable quality, using hurdle technology to control several microbiological growth parameters. Shelf stability was accomplished primarily by reducing the water activity and the pH of the product and sometimes also by using antimicrobial agents. The product is baked and then packaged in high barrier packaging material with oxygen scavenger to create an anaerobic environment.

Because there is no defined "kill step" in the processing of these shelf stable, ready-to-eat products, the US Army Natick Soldier Center has performed microbiological challenge studies on these type sandwiches to ensure that the products were safe and to provide guidelines to the manufacturers by determining Aw and pH factors that prevent or influence the growth of *Staphylococcus aureus*. Challenge studies were not conducted with *Listeria monocytogenes*, another pathogen of concern for ready-to-eat meat products, because it was not considered a hazard reasonably likely to occur in the sandwiches. These products are considered stable with respect to growth of *L. monocytogenes* because the water activity is either less than 0.90, or the water activity is less than 0.95 and the pH is less than 5.5.

Despite this fact, there is interest in determining the margin of safety provided by the current formulation with respect to *L. monocytogenes*. A challenge study of *Listeria monocytogenes*, in these pocket sandwiches, will serve to assist both producers and regulatory agencies in the formulation and validation of effective HACCP plans.

The Center of Advanced Food Technology, Rutgers Food Science and the CORANET Demo facility conducted a microbiological challenge studies in respect to *Listeria monocytogenes* in partnership with Bridgford Foods for the production of the sandwiches using their manufacturing technology under the challenge conditions determined based on product specification limits and process capability data.

Rutgers partnered with the Natick Soldier Center to develop the protocols for challenge study to validate growth inhibition of *Listeria monocytogenes*, and with Bridgford Foods to determine process capability data of their sandwich production line.

In cooperation with Bridgford Foods and the Natick Soldier Center, challenge product samples were produced and packed at the Bridgford facility in individual high barrier pouches and then sent to Rutgers for the challenge study. The samples were inoculated at Rutgers University with *L. monocytogenes*, and resealed following similar protocols as developed and used by the Natick Soldier Center for the challenge studies of *Staphylococcus aureus.* Products were stored at 25 C for twelve month while samples were taken at regular time intervals for determination of *L. monocytogenes* growth and/or survival.

1.2 Objectives

Defining the growth/no-growth boundary for *Listeria monocytogenes* in Shelf Stable Pocket Sandwiches, using challenge manufacturing conditions

1.3 Results and Conclusions

Two products were used for the challenge study:

- Honey Barbeque Beef, which specification is based on MIL-DTL-32141: 28 September 2006 and PKG & QAP MIL-DTL-32141: 28 September 2006

- Bacon Cheddar which specification is based on MIL-DTL-32223: 31 October 2006 and PKG & QAP MIL-DTL-Draft: 12 September 2005

A total of 5 Honey Barbeque and 4 Bacon Cheddar variants, with different PH and water activity levels, were made, including control for each. All product variants were inoculated at an inoculum level of 10^5 CFU/g with a *seven strain* of *L. monocytogenes* cocktail. The seven strains represented the strains that were involved in various outbreaks and were selected based on those likely to occur in the components found in these type products. Samples were then incubated at 25°C for 360 days. Microbial testing was performed at regular time intervals during this period. *Listeria monocytogenes* appears to gradually die off in all formulations of BBQ Beef and Bacon Cheddar sandwiches. A decline from 5 log CFU to 3 log CFU was observed over a 6 month period or about 0.33 log CFU per month. Therefore, *L. monocytogenes* growth in pocket sandwiches (even if incorrectly formulated) appears very unlikely.

2 Program Management

The project was awarded on September 18, 2006, under SPO103-02-D-0024, delivery order 0013 with a full obligation of $146,715. Performance period for this delivery order was set at 12 months from September 18 2006 through September 17, 2007. The objective of the project was as follows: "In cooperation with Bridgford Foods and the Natick Soldier Center, challenge product samples will be produced and packed at Bridgford facility in individual high barrier pouches and sent to Rutgers for the challenge study. The samples will be inoculated at Rutgers University with *L. monocytogenes* and resealed following protocols developed and used by the Natick Soldier Center for the challenge studies of *Staphylococcus aureus*. Products will be stored at ambient or elevated temperature for six month while samples will be taken at regular time intervals for determination of L. monocytogenes growth and/or survival."

The following modifications were issued:

- May 15, 2007 0013/01 Approval of Rutger's request for a budget allocation at no additional cost to the project per correspondence dated 3 April 2007
- Sept 13, 2007 0013/02 No cost extension of the performance period from September 17, 2007 to December 31, 2007
- Dec 13, 2007 0013/03 No cost extension of the performance period through June 30, 2008 at no additional cost to the Government
- June 19, 2008 0013/04 No cost extension of the performance period through December 30, 2008 at no additional cost to the Government

Rutgers issued a sub-contract to Bridgford Foods for them to participate in meetings to establish challenge conditions and manufacture challenge products for the study. A copy of the subcontract is attached as Appendix 4.1

3 Short Term Project Activities

3.1 Literature Review

A comprehensive literature search was performed. Published data show those environments with pH values less than 4.4 or a_w less than 0.90 do not support the growth of *Listeria monocytogenes* at various temperatures. Growth could occur above these limits. A microbial model for the limits to *L. monocytogenes* growth in suspension culture and on solid surfaces has been performed by Koutsoumanis *et. al.* (2004), and a key figure from this study is shown as Figure 2 below. This study was used as a guide to determining appropriate product formulations for subsequent study.

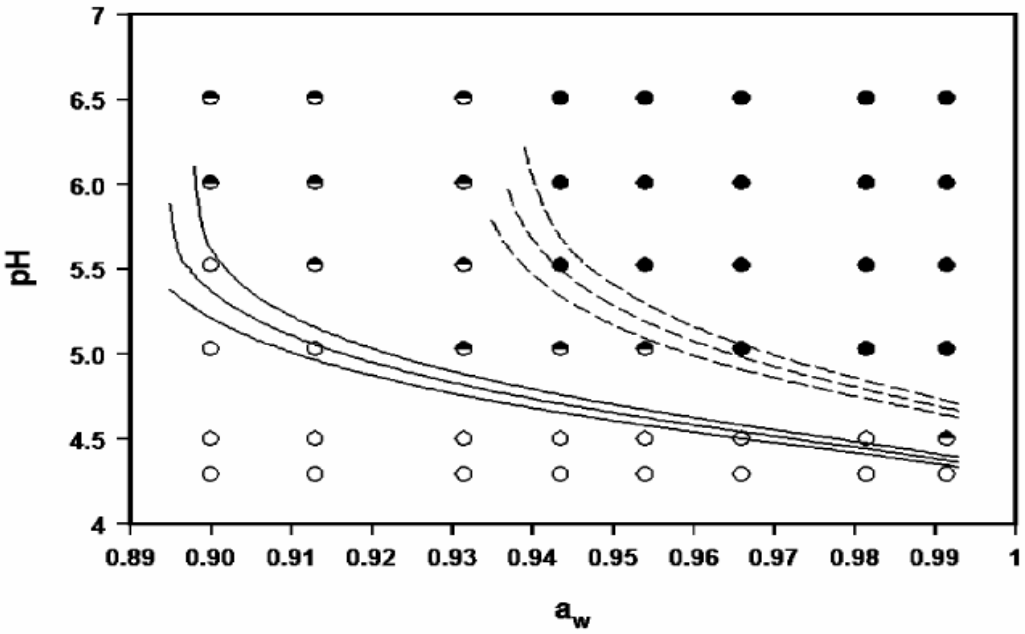


Fig. 2. Growth/no growth interface of *Listeria monocytogenes* in broth (solid line) and agar (dotted line) medium at 25°C with respect to pH and a_w predicted by the model at probabilities 0.5 (middle line), 0.1 (upper line) and 0.9 (lower line), compared to the data used to generate the model (●: growth in both broth and agar medium; ○: no growth in both broth and agar medium; semisolid circles: growth only in broth medium).

References:

Koutsoumanis, K.P., P.A. Kendall, and J.N. Sofos. 2004. A comparative study on growth limits of Listeria monocytogenes as affected by temperature, pH and a_w when grown in suspension or on a solid surface. Food Microbiol. 21:415-422.

3.2 Development Test Protocols/ Challenge Conditions

The shelf stability of the pocket sandwich is controlled by several critical factors. These factors need to be controlled during the production process to ensure that no outgrowth occurs of micro organism that can cause illness and/or spoil the product. The main critical factors are Acidity (pH) and Water Activity (Aw). In addition anti-microbial ingredients can be used.

Two products were selected for the challenge studies that are representative for the products that are being produced for the Military as "Pocket Sandwiches".
The first product is Honey Barbeque Beef Sandwich. The formulation, preparation, baking and packaging procedures are described in two technical documents:

- MIL-DTL-32141: 5 April 2004 (Draft 16 June 2006)
- PKG & QAP MIL-DTL-32141: 16 June 2006

The specification limits for the critical factors are: pH<4.8, Aw<0.89 and Oxygen content in the finished product pouch <0.30%

The second product is a Bacon Cheddar Sandwich in which a precooked bacon product is enrobed into bread. The formulation, preparation, baking and packaging procedures are described in two technical documents:

- MIL-DTL-Draft: 14 September 2005
- PKG & QAP MIL-DTL-Draft: 12 September 2005

The specification limits for the critical factors are: pH<5.4, Aw<0.88 & >0.85 and Oxygen content in the finished product pouch <0.30%

The Quality Assurance specification limits are established to assure that the product is of high quality, has excellent shelf life and safe to consume. From a Hazard Analysis Critical Control Point (HACCP), it is also important to know the critical limits at which growth/no growth of micro organisms of concern occurs and to assure that the occurrence of this is very unlikely to happen under the controls that have been implemented in the process.

There are two factors that need to be considered in a challenge study that produces worst case product samples. First, accidental formulation errors can occur in which critical ingredients that control water activity, pH and/or microbial inhibitors are omitted. The second factor to consider is the variability of the process. The batch might be non homogeneous mixed, the ratio of filling and bread can vary, the baking conditions can vary, etc. This process variability can cause variation in the critical factors and might produce limited product that is outside the quality assurance specifications. By measuring the variability in critical factors of the finished product one can determine the process capability of the process. If we can prove that the no growth of *Listeria* occurs in products that are +/- 6 σ from the production target values, then it is very unlikely that *Listeria* growth will occur using the regular production procedures and controls implemented. In the case where two hurdles (pH and Aw) are used, the probability of both critical factors simultaneous be at this level is $<10^{-12}$.

The process variability of Honey Barbeque Beef and Bacon Cheddar processes were studied and the following production targets and standard deviations in critical factors determined:

- Honey Barbeque Beef
  - Production Capability Data
    - pH: avg: 4.80 and std: 0.029, hence +6σ: 4.974
    - Aw: avg: 0.87 and std: 0.010, hence +6σ: 0.93
- Bacon Cheddar
  - Production Capability Data
    - pH: avg: 5.02 and std: 0.071, hence +6σ: 5.446
    - Aw: avg: 0.84 and std: 0.015, hence +6σ: 0.93

Following the six sigma concept, the challenge study aimed to include critical factor conditions for Honey Barbeque Beef: pH: 5.0 & Aw: 0.93 and for Bacon Cheddar: pH: 5.4 & Aw: 0.93. If it can be proven that no growth occurs under these conditions then it is very unlikely that growth will occur in any of the product under normal production conditions.

To manipulate the pH and Aw for the challenge study, it was proposed to omit certain ingredients from the formulation, as well as varying the bread to filling ratio and reduce the baking time/temperature. The target was to produce four variants per product, each with a different pH and/or Aw value.

3.3 Manufacturing Challenge Samples

Before manufacturing of the challenge samples, Bridgford Foods Partner, RDI Foods, produced several small batches of BBQ Beef Filling and Bread without various acidulants and moisture-control ingredients. The results of these tests were used to determine the formulations and manufacturing procedures for each variant that could be used to establish the growth/no growth boundary line for *Listeria*. The proposed protocol for manufacturing was submitted by Bridgford Foods and approved. In addition to the ingredient omissions, Bridgford Foods also proposed to increase the filling to bread ratio (~10%) in the direction that

would increase water activity (this will be based on water activity measurements on the products prior to filling), reduce the baking temperature to barely meet 185 F. and reduce the baking time with about 10% from normal conditions. These process changes would further increase the water activity of the product.

Bridgford Foods/RDI Foods manufactured the challenge product on February 15 and 16, 2007. At least 45 samples of each formulation were sent to Rutgers, for a total of approximately 550 samples. The tables below list the product variants and the ingredient omissions that had been induced.

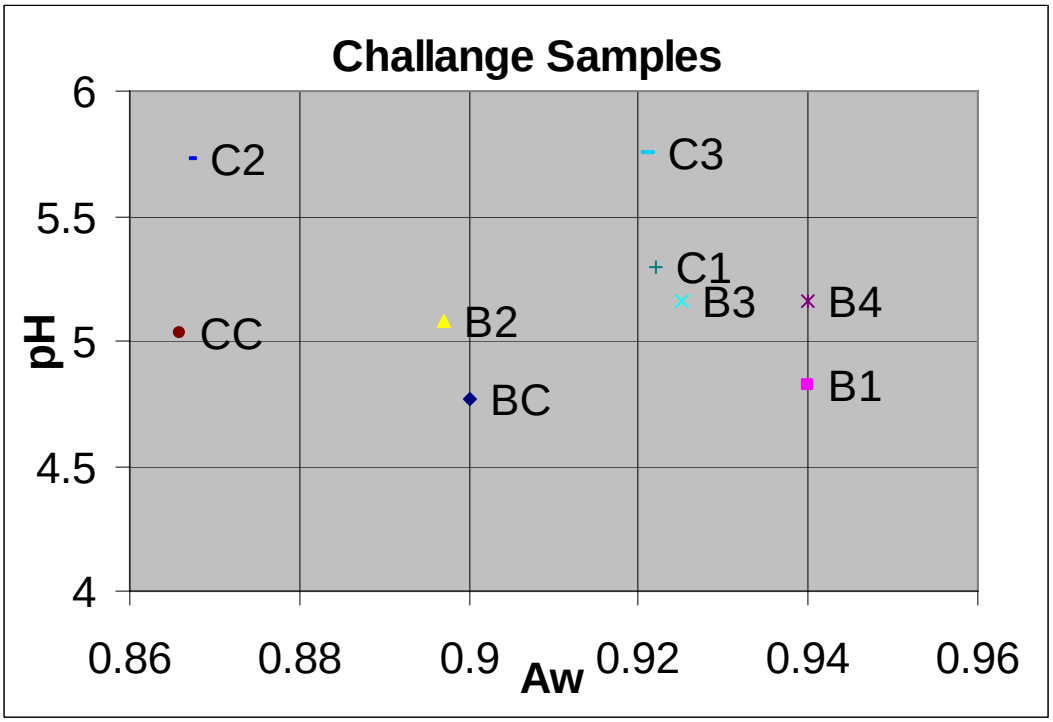
For the Honey Barbeque Beef Sandwich, the ingredients of the meat filling, the sauce and the bread were altered, leading to a wide variation in pH and Aw. In the table below lists the finished product pH and Aw.

Code	Honey Barbeque Beef Sandwich		pH	Aw
BC	Control	No Omissions	4.77	0.90
B1	Meat Filling	No Glycerol, No Rice Syrup	4.82	0.94
	Sauce	No Glycerol		
	Bread	No Glycerol		
B2	Meat Filling	No Omissions	5.08	0.90
	Sauce	50% Tomato Paste, No Vinegar		
	Bread	No Sorbic Acid		
B3	Meat Filling	No Glycerol, No Rice Syrup	5.16	0.92
	Sauce	50% Glycerol, 50% Tomato paste, No Vinegar		
	Bread	No Glycerol, No Sorbic Acid		
B4	Meat Filling	No Glycerol, No Rice Syrup	5.16	0.94
	Sauce	50% Glycerol, 50% Tomato Paste, No Vinegar, No Brown Sugar		
	Bread	No Glycerol, No Sorbic Acid		

For the Bacon Cheddar Sandwich, only the ingredients of the bread could be altered, as the cooked bacon is a purchased ingredient. The table below lists the finished product pH and Aw.

Code			pH	Aw
CC	Control	No Omissions	5.03	0.87
C1	Bread	50% Cheddar Flake & No Glycerol	5.3	0.92
C2	Bread	50% Cheddar Flakes, No Sorbic Acid, No GDL, No Butter Flavor	5.73	0.87
C3	Bread	50% Cheddar Flakes, No Glycerol, No Sorbic Acid, No GDL, No Butter Flavor	5.75	0.92

It should be noted that their were clear visual indications, that the product formulation was “out-of-control”, and that the likelihood of occurrence of these gross misformulations would easily be detected and therefore not likely to occur.
It should also be noted pH and Aw values reported in the tables above are based on random samples pulled from the production batches and analyzed by an outside certified Lab.



3.4 Inoculation

All formulation variants of two products (Bacon Cheddar and Honey BBQ Beef) were inoculated on February 27 and 28, 2007 with a *L. monocytogenes* seven strain cocktail.

The seven strain cocktail consisted of:

Five meat associated strains:

- FSL J1-177, ribotype DUP-1051D; lineage I; serotype 1/2b; isolated from human sporadic case (1997);
- FSL C1-056; ribotype DUP-1030A; lineage II; serotype 1/2a; isolated from human sporadic case, New York state (1998);
- FSL N3-013 (previous ID#s: TS45/L.3350/1050); ribotype DUP-1042B; lineage I; serotype 4b; isolated from food, linked to pate listeriosis outbreak in UK in 1988-1990;
- FSL R2-499 (previous ID#: J0161); ribotype DUP-1053A; lineage II; serotype 1/2a; isolated from human case linked to 'sliced turkey' outbreak, US, 2000;
- FSL N1-227 (previous ID#: H7738); ribotype DUP-1044A; lineage I; serotype 4b; isolated from food linked to 1998-99 listeriosis outbreak in US (aka the 'Sara Lee outbreak').

and two other diary associated strains:

- J1-110 (previous ID#: TS29/F2365/DD6306); ribotype DUP-1038B; isolated from Jalisco Soft Cheese in Los Angeles, USA 1985,
- R2-500 (previous ID#: J0144); Ribotype DUP-1042B; isolated from Mexican Cheese in North Carolina, USA 2000

The following method of inoculation was used:

1. Transfer one loop of each strain into 10 ml Tryptic Soy Broth (TSB) and incubate at 37°C for ~18 hours to reach a concentration of ~10^8 cells/ml.
2. Make a multi-strain cocktail by mixing 1ml of the growth media from each of the seven strains
3. Inoculate each sample [using a pipetteman (Gilson)] from the back side, at the filling/bread interface, with 10µl of the cocktail to reach a final concentration of 10^4 cells/g.
4. Repack the inoculated sandwiches with two Oxygen Scavengers in a new pouch and seal pouch using the hot seal sealer.
5. Incubate sandwiches at 25°C (Fisher Isotemp) for appropriate time periods for bacteria recovery.

3.5 Microbiological Testing

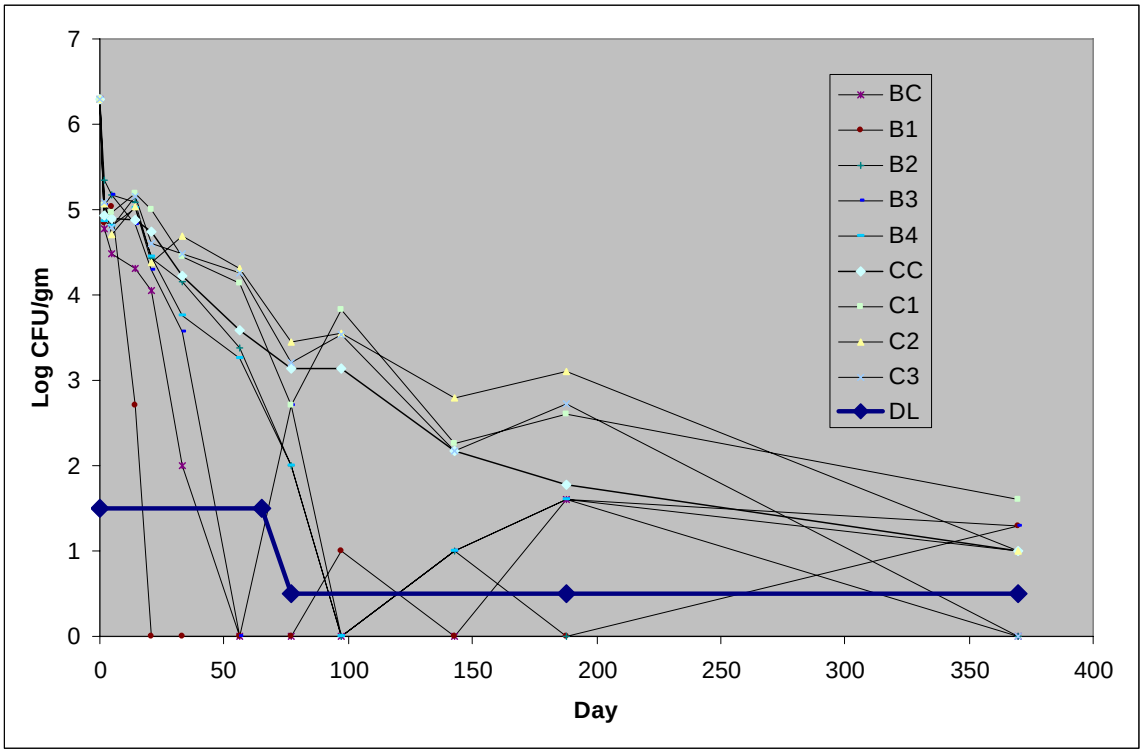
Although the optimum growth temperature of *L. monocytogenes* is typically assumed to between 30-37ºC, the growth/no-growth boundary for the organism is relatively unchanged for a given pH value for temperature above 20ºC. Since the growth-no growth boundary is unchanged at ambient or higher temperatures; incubation of samples was done at 25ºC, which is easily attained and controlled in the laboratory.

L. monocytogenes were recovered from the inoculated samples by diluting it 1:10 in Butterfield's Phosphate Buffer, masticating for 2 min using a stomacher, and diluting the homogenate 1:10 in Butterfield's Phosphate Buffer. These dilutions were spread-plated on Oxford Agar, incubated 20-24 hours, and enumerated. Representative colonies were confirmed by appropriate methods to ensure that the test organisms are being recovered.

The microbial inoculation protocol was executed by the Rutgers Food Micro Lab. The monitoring phase of *Listeria* growth started on 2/28/07. Triplicate samples were taken at thirteen time points during a period of twelve month. The microbial inoculation protocol requires testing of the inoculated samples at day 0, 1, 2, 3 and week 1, 2, 3 and month 1, 2, 3, 4, 5, 6 and 12 month, with a completion date of February 28, 2008. All samples showed graduate decline in *L. monocytogenes* concentration over time.

Some pouches developed gas during the incubation period. Some of these pouches were sent to Natick for headspace gas analysis. Results of the Natick analysis indicated that high levels of Carbon Dioxide were present in the samples as well as detectable levels of ethyl alcohol. These findings are consistent with yeast spoilage of these products.

Water activity and pH were measured starting during week 2. Because water activity and pH measured by the Food Science Microbiology group differed significantly from the numbers recorded by Bridgford, a certified lab was contracted and triplicate samples of each variable were submitted. Water activity and pH values measured by the certified lab were slightly lower than measurements taken by Rutgers but higher than valued reported by Bridgford. Water activity and pH used in this report were based on the measurements by the certified lab.



3.6 Meetings and Correspondence

The project kick-off meeting was held on October 24, 2006 at the CORANET Demo Facility. The presentation overheads are include in the Appendix 4.2

An end of phase II review was held by e-mail exchange.

A final IPR meeting was held on November 27, 2007 at Myrtle Beach during the CORANET #20 workshop. The project results were reviewed, including the manufacturing and test protocols. The overheads for this presentation are included in Appendix 4.4

On April 27, 2008, Dr Schaffner from Rutgers University summarized the results of this research study in a letter to Bridgfords Foods. A copy of this letter is included as Appendix 4.5

4 Appendix:

4.1 Sub-Contract Bridgford Foods

4.2 Project Kick-Off Meeting

4.3 Final Project Meeting

4.4 Letter from Don Schaffner

Sub-Contract Bridgford Foods

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

SUBCONTRACT AGREEMENT #3033
IN AGREEMENT WITH
RUTGERS, THE STATE UNIVERSITY
and
BRIDGFORD FOODS CORPORATION

Address:	**1707 S. Good-Latimer Expressway** **Dallas, TX 75226**
For:	Performance of certain work and services in connection with Rutgers account number: **4-25921** and organizational code **10391**
Project Sponsor:	**US Department of Defense**
CFDA #:	
Sponsor Award #:	**SP0103-02-D-0024/0013**
Project Title:	**Defining the Growth/No-growth Boundary for L*isteria monocytogenes* in Shelf Stable Pocket**
Rutgers DUNS #:	**00-191-2864**
Rutgers EIN#:	**1-226001086-A1**
Rutgers Project Director/ Principal Investigator:	**Dr. Henderikus B. Bruins**
Department:	**Center for Advanced Food Technology**
Type of Contract:	Cost Reimbursement
Period of Performance:	**September 17, 2006 through June 30, 2007**
Maximum Allowable Price:	**$6,750**
Issued by:	Rutgers, The State University Office of Research and Sponsored Programs ASB III – 3 Rutgers Plaza New Brunswick, New Jersey 08901
Invoice to:	Rutgers, The State University Disbursement Control Administrative Services Building 65 Davidson Road, Room 302 Piscataway, NJ 08854

This Agreement is entered into by and between Rutgers, the State University of New Jersey, with principal offices in New Brunswick, New Jersey (hereinafter called "RUTGERS"), and Bridgford Foods Corporation (herein after called "SUBCONTRACTOR"), and constituting a subcontract under Grant/Contract No.SP0103-02-D*0024/0013 from the US Department of Defense issued to Rutgers, The State University. The US Department of Defense shall hereinafter be referred to as Sponsor.

WITNESSETH THAT:

SUBCONTRACTOR agrees to perform the work and services in accordance with the terms and conditions set forth in this Agreement for the consideration stated herein. Therefore, it is agreed as follows:

ARTICLE 1. SCOPE OF WORK

a) SUBCONTRACTOR shall provide the necessary personnel, equipment, facilities, and supplies to perform the work described in the Statement of Work, which is attached hereto as Exhibit A.

b) Unless specifically stated elsewhere in this Agreement, the quality of all services rendered hereunder shall conform to the highest standards in the relevant profession, trade, or field of endeavor. All services shall be rendered by or supervised directly by individuals fully qualified in the relevant professions, trade, or field, and holding any licenses required by law.

ARTICLE 2. KEY PERSONNEL

SUBCONTRACTOR shall designate **John Simmons** as its Project Director and **Dr. Richard R. Hawkins** Principal Investigator. **John Simmons and Richard R. Hawkins** shall not be removed or replaced without the prior written approval of RUTGERS.

RUTGERS hereby designates **Henderikus Bruins** as its Project Director/Principal Investigator for this work.

For official correspondence and communication the following contacts are listed below:

Technical Matters

For Subcontractor:	PI Name	**Dr. Richard R. Hawkins**
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	Fax:	**732-932-0162**
	Email:	obrien@orsp.rutgers.edu

ARTICLE 3. PERIOD OF PERFORMANCE

The period of performance under this Agreement shall begin on **September 17, 2006** and shall end on **June 30, 2007**, unless extended by mutual written agreement, or terminated in accordance with the terms of this Agreement.

ARTICLE 4. COMPENSATION AND METHOD OF PAYMENT

a) The total amount available to SUBCONTRACTOR for performance hereunder is **$6,750** as specified in the budget, Exhibit B hereunto, which shall not be exceeded unless changed by written amendment to this Agreement.

b) SUBCONTRACTOR may transfer funds within approved budgeted categories in accordance with Office of Management and Budget (OMB) Circular A-110, "Uniform Administrative Requirement for Grants and Agreements with Institutions of Higher Education, Hospitals, and Other Non-Profit Organizations," as in effect on the date of this Agreement and in accordance with the additional requirements of the sponsor in Exhibit C.

c) The allowable cost of performing the work under this Agreement shall be the cost actually incurred by SUBCONTRACTOR, both direct and indirect, if applicable. The allowable direct cost, including acceptability of cost allocation methods, shall be determined by RUTGERS in accordance with:

(1) OMB Circular A-21, "Cost Principles for Educational Institutions," as in effect on the date of this Agreement.
(2) Allowable facilities and administrative costs shall be in the amount provided by the sponsor in accordance with SUBCONTRACTOR'S current Negotiated Facilities and Administrative Rate Agreement. Otherwise, facilities and administrative costs shall be specified and agreed to in accordance with Exhibit B, Budget.

d) SUBCONTRACTOR shall, at monthly intervals following commencement of work, submit invoices to RUTGERS for payment of costs incurred during the preceding month. Each original invoice will present, by approved budget line item, costs for the current period being billed along with cumulative amounts billed to date according to Exhibit D. These invoices shall contain all costs incurred during the billing period and shall be sufficiently detailed to allow RUTGERS personnel to make the required fiscal reports to the sponsor. Invoices shall be submitted to Rutgers, The State University,

Disbursement Control, Administrative Services Building, 65 Davidson Road, Room 302, Piscataway, NJ 08854 referencing the RUTGERS' Account No. **4-45921**, organizational code **10391** and the purchase order number. Payment of final invoice shall be withheld pending receipt and acceptance of all closeout documents, including final cost-sharing certification. Rutgers must receive the final invoice within 45 days of project termination date. Invoices received after this date may not be paid if the prime sponsor deobligated the funding.

Each invoice must include the following statement and be certified by a business official:

> I certify that the above charges accurately represent actual expenditures incurred during the period listed, that any prior approvals required for these items under the terms and conditions of the subaward have been obtained, and all claimed costs are allowable under the terms and conditions of the subaward. I further certify that payment for the costs claimed above has not been received.

ARTICLE 5. MATCHING AND COST SHARING REQUIREMENTS

The subgrantee shall be required to account to the satisfaction of RUTGERS and the sponsor for matching and cost-sharing requirements of this subcontract if specified in the Request for Proposal or Budget, Attachment B.

ARTICLE 6. REPORTING REQUIREMENTS

a) SUBCONTRACTOR shall submit such technical reports to the RUTGERS Project Director/Principal Investigator as required by RUTGERS to meet the technical report requirements of the prime agreement. Each report shall be submitted sufficiently in advance of the report deadline to allow review and comment by the RUTGERS Project Director/Principal Investigator prior to transmittal to the funding agency. Reports are due within 45 days of termination of grant/contract period.

b) All required technical/financial reports and project-related records will be maintained and made available by SUBCONTRACTOR in accordance with FAR 52.215.2, "Examination of Records by Controller General," for a period of not less than three (3) years following the submission and acceptance of the final reports.

ARTICLE 7. AUDIT

a) SUBCONTRACTOR shall maintain appropriate accounting records sufficient to properly document costs claimed as incurred in the performance of this Agreement, and shall make such records available, upon request, to authorized RUTGERS or sponsor personnel for audit purposes pursuant to FAR 52.215.2, ALT 2 "Audit Negotiation." Said records shall be retained and kept available by SUBCONTRACTOR for a period of not less than three (3) years after final payment by the University, or if notified of an audit and notification by RUTGERS of resolution of any exceptions resulting there from, whichever occurs first.

b) If any amount paid hereunder by RUTGERS is subsequently disapproved or disallowed by the sponsor or another authorized agency, SUBCONTRACTOR shall upon demand and without litigation, promptly repay RUTGERS said disapproved or disallowed amount.

ARTICLE 8. EQUIPMENT

a) Title to equipment acquired with subcontract funds shall be vested in SUBCONTRACTOR, unless otherwise stated in Article 10, and subject to the rights of the Government, if applicable. However, unless so provided in SUBCONTRACTOR's budget, SUBCONTRACTOR shall not acquire any items of equipment with subcontract funds unless prior written approval has been obtained from RUTGERS.

b) SUBCONTRACTOR shall be responsible for maintaining equipment and associated materials, including inventory, accountability, and disposition of equipment in accordance with the SPONSORS policy.

ARTICLE 9. RIGHTS IN DATA AND COPYRIGHTS

a) Unless otherwise specified herein, any data developed by SUBCONTRACTOR in the performance of this Agreement shall be and remain the sole property of SUBCONTRACTOR.

b) SUBCONTRACTOR is free to copyright material developed under or in connection with this Agreement, and shall give notice to RUTGERS of any material so copyrighted.

c) RUTGERS and sponsor shall have a royalty-free, nonexclusive, world-wide and irrevocable right to reproduce, publish, or otherwise use, and to authorize others to use, such data and material.

d) The Subcontractor shall be free to publish results of the Work provided that advance copies of material intended for publication are submitted to the RUTGERS Authorized Representative for Technical Matters for review prior to publication. The Subcontractor agrees to give reviewers' comments serious consideration prior to publishing and to include the following statement in any publication resulting from the Work: "This publication was supported by a subcontract from Rutgers University, **Unit Name**, under **Prime Agreement Award Number** from the **Prime Sponsor**." All materials, except scientific articles or papers published in scientific journals, must also contain the following disclaimer: "Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of Rutgers University or those of the **Name of Prime Sponsor.**

ARTICLE 10. INTELLECTUAL PROPERTY

a) "Intellectual Property" shall mean patents, patent applications, and know-how.

b) Unless otherwise provided herein, all Intellectual Property relating to inventions conceived and reduced to practice solely by SUBCONTRACTOR in the performance of this Agreement shall be and remain the sole property of SUBCONTRACTOR. Intellectual Property relating to inventions conceived and reduced to practice solely by RUTGERS in the performance of this Agreement shall be and remain the sole property of RUTGERS. Intellectual Property relating to inventions conceived and reduced to practice jointly by RUTGERS and SUBCONTRACTOR in the performance of this Agreement shall be jointly owned.

c) Unless otherwise provided herein, RUTGERS shall have a royalty-free, nonexclusive, worldwide, and irrevocable right to use SUBCONTRACTOR'S Intellectual Property for research and educational purposes, and to satisfy the requirements of the Sponsor.

d) In the event that commercially useful developments are made from SUBCONTRACTOR'S rights to Intellectual Property originating under or derived from this Agreement, SUBCONTRACTOR, in consideration for RUTGERS funding hereunder, shall provide RUTGERS reasonable compensation which shall be mutually determined by the parties at the time these developments are reasonably identified.

e) Certain patent and invention rights and other rights of RUTGERS, SUBCONTRACTOR, and the U.S. Government relating to inventions hereunder are specified in and governed by 48CFR227 and 252, as amended, and 37CFR401.14 of July 1, 1987, which provisions are incorporated herein by reference

ARTICLE 11. TERMINATION

a) RUTGERS or SUBCONTRACTOR may terminate this Agreement with or without cause at any time by giving thirty (30) days written notice. SUBCONTRACTOR shall, upon receipt of notice of termination from RUTGERS, refrain from incurring any further costs under this Agreement and shall use its best efforts to cancel any commitments made by it prior to receipt of such notice. Such termination shall, however, not affect any commitments which, in the judgment of RUTGERS, have properly become legally binding prior to the effective date of termination and which could not reasonably have been rescinded by SUBCONTRACTOR. Any prepaid but unearned funds shall be returned to RUTGERS.

b) It is understood and agreed, however, that in the event that SUBCONTRACTOR is in default upon any of its obligations hereunder at the time of termination, RUTGERS reserves the right to pursue, in addition to termination, any other rights or remedies which RUTGERS may have against SUBCONTRACTOR, and RUTGERS may withhold any payments to SUBCONTRACTOR for the purpose of set-off until such time as the exact amount of damages may be determined.

ARTICLE 12. PROVISIONS OF PRIME AGREEMENT

All provisions contained in Exhibit C shall be binding upon the SUBCONTRACTOR and SUBCONTRACTOR hereby agrees with same.

ARTICLE 13. PUBLICITY

Neither the SUBCONTRACTOR nor RUTGERS shall use the other's name or that of any member of the other's staff for publicity or advertising purposes without prior written approval of the other party. This restriction shall not include internal documents or those available to the public that identify the existence of this agreement.

ARTICLE 14. DISPUTES

Any disagreements arising out of this Agreement, or from a breach thereof, shall be submitted to arbitration, and the judgment upon the award rendered by the arbitrators may be entered in any court having jurisdiction thereof. The arbitration shall be held under the procedures and rules of the American Arbitration Association. Any arbitration shall be held in Newark, New Jersey, unless mutually agreed otherwise.

ARTICLE 15. PHS ATTESTATION

If this Agreement is funded as a result of an award to RUTGERS from US Public Health Service, SUBCONTRACTOR attests that it has filed all the proper assurances/certifications in compliance with PHS Form 398. In the event that SUBCONTRACTOR cannot make such an attestation, then it agrees to be subject to the policies of RUTGERS with respect to the research being supported, and RUTGERS will send a copy of its policies to SUBCONTRACTOR upon request.

ARTICLE 16. PROTECTION OF HUMAN SUBJECTS

Funds awarded for research involving human subjects may be used only if the SUBCONTRACTOR has an approved assurance of compliance on file with the Office of Human Research Protection. The SUBCONTRACTOR shall **submit to Rutgers an assurance, reviewed and approved by an appropriate institutional committee, that the rights and welfare** of any human subjects involved in this project are adequately protected in accordance with DHHS Regulations, (45 CFR, Part 46). The Assurance, whether general or Special, must be submitted to Rutgers prior to the expenditure of any funds provided under this agreement. Further information on the requirements of 45 CFR 46 may be obtained from the Office for Protection from Research Risk, National Institutes of Health, Building 31, Room 4B09, Bethesda, Maryland 20892.

All personnel engaged in research involving human subjects are required to provide documentation of education in the protection of human subjects. The SUBCONTRACTOR shall **submit to Rutgers written evidence of successful completion of an appropriate education program for each individual engaged in human subjects research supported by this Subcontract.** Or, personnel may complete the Rutgers education program, accessible via the World Wide Web. Access to the Rutgers education program may be arranged with Ms. Karen Janes, Sponsored Programs Administrator, at 732-932-0150.

ARTICLE 17. CARE AND TREATMENT OF LABORATORY ANIMALS

The SUBCONTRACTOR, if using warm-blooded animals in agreement-supported project, shall comply with applicable portions of the Animal Welfare Act (P.L. 89-544 as amended (P.L. 91-579 and 94-279) U.S.C. 2131 et. Seq.) and will follow guidelines prescribed in DHHS Publications No. 86-23, Rev. 1985 or succeeding revisions (NIH), "Guide for the Care and Use of Laboratory Animals". If using animals, as specified in NIH GUIDE, Vol. 14, No. 8, June 25, 1985. SUBCONTRACTOR should comply with regulations cited therein and **provide the University an assurance, reviewed and approved by an appropriate institutional committee,** that the policy requirements are being met.

ARTICLE 18. DEBARMENT AND SUSPENSION

a) In accepting this Agreement, SUBCONTRACTOR certifies that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in the transaction by any Federal department or agency. Any change in the debarred or suspended status of SUBCONTRACTOR during the life of this Agreement must be reported immediately to RUTGERS. SUBCONTRACTOR agrees to incorporate the Debarment and Suspension Certification into any subcontract that they may enter into as a part of this Agreement.

b) If SUBCONTRACTOR is unable to certify to any of the statements in this certification, SUBCONTRACTOR shall attach an explanation to this Agreement.

c) This certification is required by the regulations implementing Executive Order 12549, Debarment and Suspension, 34 CFR Part 85, Section 85.510, Participant's responsibilities. The regulations were published as Part VII of the May 26, 1988 Federal Register, pages 19160-19211. Copies of the regulations may be obtained by contacting the authorizing official of RUTGERS.

ARTICLE 19. EQUAL OPPORTUNITY/AFFIRMATIVE ACTION

a) This Agreement is subject to the requirements of Executive Order 11246 and 11375 and the rules and regulations of the Secretary of Labor (41 CFR Chapter 60) in promoting Equal Employment Opportunities.

b) SUBCONTRACTOR hereby certifies that is does not and will not maintain any facilities it provides for its employees in a segregated manner, or permit its employees to perform their services at any location under its control, where segregated facilities are maintained; and it will obtain a similar

certification prior to award of any non-exempt subcontract approved hereunder.

ARTICLE 20. INDEMNIFICATION

a) All persons rendering services covered by this Agreement on behalf of SUBCONTRACTOR, including faculty, staff, students, or other agents shall be considered to be employees of SUBCONTRACTOR for the purpose of any state workers' compensation laws or federal workers' compensation statutes. SUBCONTRACTOR hereby agrees to indemnify RUTGERS against all claims or awards under such workers' compensation laws arising out of this Agreement.

b) SUBCONTRACTOR further agrees to hold RUTGERS and its employees and agents harmless, and to defend and indemnify them against all claims, actions, liability, damage, loss, and expenses (including reasonable attorney's fees) by reason of injury, illness or death to any person or persons or damaged property arising or alleged to have arisen from any negligent or willful act or omission of the SUBCONTRACTOR, or its employees or agents, arising out of SUBCONTRACTOR'S performance under this Agreement

ARTICLE 21. ASSIGNMENT

This Agreement shall not be assigned in whole or in part without the prior written consent of RUTGERS.

ARTICLE 22. ENTIRE AGREEMENT

This Agreement and any/all attachments constitute the entire agreement between RUTGERS and SUBCONTRACTOR with respect to the subject matter hereof, and supersedes and replaces any other arrangements, oral or written, between the parties hereto pertaining to this subcontract. No waiver, modification, or amendment of any of the terms and conditions hereof shall be effective unless set forth in writing duly signed by RUTGERS and SUBCONTRACTOR. Any non-material changes to this agreement will be executed via a unilateral modification. Changes of a material nature will require execution by the authorized official of Rutgers and Subcontractor.

ARTICLE 23. SITUS

Regardless of place of physical execution or performance, this Agreement shall be construed according to the laws of, and be deemed to have been executed in, the state of New Jersey.

IN WITNESS WHEREOF, the respective parties have executed this Agreement on the dates indicated below.

BRIDGFORD MARKETING COMPANY DUNS #: 00850-6768 EIN/IRS ID #: 95-2312874	**RUTGERS, THE STATE UNIVERSITY**
_____ Authorized Institutional Official	_____ Maryellen O'Brien. Acting Director
_____ Date	_____ Date

Revised 11/29/06

EXHIBIT A

Statement of Work/Sole Source Justification

STP#2023 (ORSP Log # 06122611), requires the manufacturing of pocket sandwiches that have specific acidity (pH) and water activity (a_w) to define the growth/no-growth boundary for *Listeria monocytogenes* in these sandwiches.

In order to obtain these "challenge samples", we are asking the current and only manufacturer of these sandwiches, "Bridgford Foods" and its partner "RDI" to change their current formulation and/or processing procedures and manufacture these samples in their lab. Because Bridgefords Foods is the only producer of these sandwiches for the military, a sole source justification is defensible

The manufacturing will include two products: Bacon Cheddar and Honey BBQ Beef. We anticipate 5 variants of each product. Each variant will consist of 55 sandwiches, for a total of 550 sandwiches. Bridgford Foods quoted us an approximate cost of $6,750 for this effort.

Note: *After the first project meeting, during which we discussed in much greater details the parameters under which the challenge samples need to be manufactured, Bridgford Foods has re-estimated their cost for sample manufacturing to $8,000 (Materials $2,000 and Labor $6,000). In addition, they are being requested to attend a total of three meetings at Rutgers University to review the progress of the project. They are requesting to be reimbursed for their travel expenses and their labor cost: $4,500 (Travel: $2,100 and Labor: $2,400). To cover these extra expenses, a request for incremental funding has been submitted to DLA and award is pending.*

EXHIBIT B

RUTGERS THE STATE UNIVERSITY OF NEW JERSEY
CENTER FOR ADVANCED FOOD TECHNOLOGY - FMT FACILITY
SUBCONTRACT WITH BRIDGFORD FOODS CORP.
FOR THE PERIOD 9/17/06 AND 6/30/07

Meeting Expenses		
Labor Costs	800	
Travel Costs	700	1,500
Manufacturing Costs		
Labor Costs	4,000	
Materials	1,250	5,250
TOTAL COSTS		**6,750**

NOTE: It is anticipated that total costs will be $12,500. Additional funds will be subcontracted to Bridgford when available.

EXHIBIT C

Expires Jun 30, 1997

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for revi ...aring and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0187), Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO EITHER OF THESE ADDRESSES.
SEND YOUR COMPLETED FORM TO THE PROCUREMENT OFFICIAL IDENTIFIED IN ITEM 6.

1. CONTRACT/PURCH ORDER NO.	2. DELIVERY ORDER NO.	3. DATE OF ORDER (YYMMMDD)	4. REQUISITION/PURCH REQUEST NO.	5. PRIORITY
SP0103-02-D-0024	0013	06-09-18	BAA 01-0004	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than 6)	CODE	8. DELIVERY FOB
Defense Supply Center Philadelphia 700 Robbins Avenue Philadelphia, PA 19111-5096 DSCP-PBA-A.Bonanno/215-737-7339	SP0103	Office of Naval Research Boston Regional Office 495 Summer Street Boston, MA 02210-2109	N62879	☐ DEST ☐ OTHER (See Schedule if other)

9. CONTRACTOR	CODE	FACILITY CODE	10. DELIVER TO FOB POINT BY (Date) (YYMMMDD)	11. MARK IF BUSINESS IS
NAME AND ADDRESS Rutgers, The State University of New Jersey Division of Grant and Contract Accounting 3 Rutgers Plaza New Brunswick, NJ 08901-8559	4B883		12. DISCOUNT TERMS	☐ SMALL ☐ SMALL DISAD-VANTAGED ☐ WOMEN-OWNED
			13. MAIL INVOICES TO See page 2	

14. SHIP TO	CODE	15. PAYMENT WILL BE MADE BY	CODE	MARK ALL PACKAGES AND PAPERS WITH CONTRACT OR ORDER NUMBER
		DFAS-Columbus Center DFAS-CO-TLCO P.O. Box 369016 Columbus, OH 43236-9016	HQ0131	

16. TYPE OF ORDER — DELIVERY ☒ This delivery order is issued on another Government agency or in accordance with and subject to terms and conditions of above numbered contract.
Reference your ... furnish the following on terms specified herein.
PURCHASE ☐ ACCEPTANCE. THE CONTRACTOR HEREBY ACCEPTS THE OFFER REPRESENTED BY THE NUMBERED PURCHASE ORDER AS IT MAY PREVIOUSLY HAVE BEEN OR IS NOW MODIFIED, SUBJECT TO ALL OF THE TERMS AND CONDITIONS SET FORTH, AND AGREES TO PERFORM THE SAME.

NAME OF CONTRACTOR	SIGNATURE	TYPED NAME AND TITLE	DATE SIGNED (YYMMMDD)

☐ If this box is marked, supplier must sign Acceptance and return the following number of copies:

17. ACCOUNTING AND APPROPRIATION DATA/LOCAL USE

CA 97 6 0400 5107 006 P79198 CR 2527 0602D00240013 S33181 APC: ERR 6 - Net increase: $146,715.00

18. ITEM NO.	19. SCHEDULE OF SUPPLIES/SERVICE	20. QUANTITY ORDERED/ ACCEPTED*	21. UNIT	22. UNIT PRICE	23. AMOUNT
0008	Short Term Project (STP) #2023 - "Defining the growth/no growth for Listeria Monocytogenes in Shelf Stable Pocket Sandwiches" This order obligates the total estimated dollar value of this project.	NOA Rec'd			

* If quantity accepted by the Government is same as quantity ordered, indicate by X. If different, enter actual quantity accepted below quantity ordered and encircle.

24. UNITED STATES OF AMERICA
BY: ASSUNTA BONANNO CONTRACTING/ORDERING OFFICER

25. TOTAL	$146,715.00
29. DIFFERENCES	

26. QUANTITY IN COLUMN 20 HAS BEEN
☐ INSPECTED ☐ RECEIVED ☐ ACCEPTED, AND CONFORMS TO THE CONTRACT EXCEPT AS NOTED

DATE ... SIGNATURE OF AUTHORIZED GOVERNMENT REPRESENTATIVE

36. I certify this account is correct and proper for payment.
DATE ... SIGNATURE AND TITLE OF CERTIFYING OFFICER

27. SHIP. NO. ☐ PARTIAL ☐ FINAL
31. PAYMENT ☐ COMPLETE ☐ PARTIAL ☐ FINAL

28. D.O. VOUCHER NO.
32. PAID BY

30. INITIALS
33. AMOUNT VERIFIED CORRECT FOR
34. CHECK NUMBER
35. BILL OF LADING NO.

37. RECEIVED AT	38. RECEIVED BY (Print)	39. DATE RECEIVED (YYMMMDD)	40. TOTAL CONTAINERS	41. S/R ACCOUNT NUMBER	42. S/R VOUCHER NO.

DD FORM 1155, JUN 94 (EG) PREVIOUS EDITION MAY BE USED. Designed using Perform Pro, WHS/DIOR, Jun 94

1. This contract action is issued to initiate performance and to provide funding in the amount of $146,715.00 for Rutgers' University in the performance of Short Term Project (STP) #2023 entitled "Defining the growth/no-growth boundary for *Listeria monocytogenes* in Shelf Stable Pocket Sandwiches".

2. The objective of this project is as follows:

In cooperation with Bridgeford Foods and the Natick Soldier Center, challenge product samples will be produced and packed at the Bridgeford facility in individual high barrier pouches and then sent to Rutgers for the challenge study. The samples will be inoculated at Rutgers University with *L. monocytogenes*, and resealed following protocols developed and used by the Natick Soldier Center for the challenge studies of *Staphylococcus aureus*. Product will be stored at ambient or elevated temperature for six month while samples will be taken at regular time intervals for determination of *L. monocytogenes* growth and/or survival.

3. The scope of this project is composed of three Phases:

Phase I – Literature Search
Phase II – Develop Test Protocol
Phase III – Microbiological Challenge Studies

The project is further defined into the following major task areas:

1) Conduct a literature search on *L. monocytogenes* growth guideline in intermediate moisture products, using USDA's ComBase database and other relevant databases
2) Review and assess the relevance of the challenge study protocols used for*Staphylococcus aureus* developed by the Natick Soldier Center
3) Review the product specification and process capability data collected by theNatick Soldier Center
4) Develop/update challenge protocols for *L. monocytogenes*
5) Manufacture challenge samples using "worst case conditions"
6) Conduct microbiological challenge studies for six month according to the protocols developed under task #2
7) Write final report

These tasks and associated costs are further described in detail in the contractor's technical and cost proposals dated June 8, 2006 incorporated herein by reference.

4. All deliverables identified in the technical proposal and those stated in the basic contract are required.

5. In performing this project, the contractor is not authorized to expend funds exceeding $146,715.00 without written approval from the Contracting Officer. The contractor should notify the Government in writing when costs incurred reach 75% of the allotted amount of this delivery order and/or it is anticipated that additional funds are needed.

6. Payments shall be in accordance with section G-1 of the contract. Vouchers should be submitted directly to the cognizant ACO at the Office of Naval Research with copies

forwarded to the Contracting Officer, Ms. Sue Bonanno and the Program Manager, Mr. Jesse Burns.

8. The estimated period of performance is 12 months from the effective date of this order. At the completion of Phase I, an In-Process Review will be conducted and authorization to proceed to the next Phase(s) must be provided in writing by the Contracting Officer.

9. Changes/Additions/Deletions to this STP shall be provided through the issuance of modifications.

Exhibit D Sample Invoice

Subcontractor Name	**Invoice#:**
Address	**Rutgers Account#:**
	Grant Period:
	Reporting Period:
	Rutgers Subcontract #:
	Rutgers Organizational ID #:
	Rutgers Purchase Order #:

Total Amount of Award	**$0.00**
Total Amount Received to Date	**$0.00**

EXPENDITURES	**CURRENT Subcontractor**	**CUMULATIVE Subcontractor**	**CURRENT Cost Sharing***	**CUMULATIVE Cost Sharing***
Salaries & Wages	**0.00**	**0.00**	**0.00**	**0.00**
Fringe Benefits	**0.00**	**0.00**	**0.00**	**0.00**
Supplies	**0.00**	**0.00**	**0.00**	**0.00**
Travel	**0.00**	**0.00**	**0.00**	**0.00**
Other Services	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	**0.00**	**0.00**	**0.00**
MTDC	**0.00**	**0.00**	**0.00**	**0.00**
Equipment	**0.00**	**0.00**	**0.00**	**0.00**
Other Expenses	**0.00**	**0.00**	**0.00**	**0.00**
TOTAL DIRECT EXPENDITURES	**0.00**	**0.00**	**0.00**	**0.00**
Facilities and Administrative Cost %=_____ of MTDC	**0.00**	**0.00**	**0.00**	**0.00**
TOTAL COSTS	**$0.00**	**0.00**	**$0.00**	**$0.00**
Less Previously Invoiced		**0.00**		
PAYMENT REQUESTED THIS REPORT		**$0.00**		

***NOTE:** Cost Sharing information must be provided if cost sharing is required per the subcontract documents.

" I hereby certify, to the best of my knowledge and belief, that the above properly summarizes the grant expenditures and are supported by adequate documentation."

Authorized Institutional Fiscal Official **Date**

Invoices to:
Rutgers, The State University
Disbursement Control
Administrative Services Building
65 Davidson Road, Room 302
Piscataway, NJ 08854

Project Kick-Off Meeting

Pocket Sandwich Project Kick Off Meeting

CORANET Demo Facility

October 24, 2006

Agenda

- Objective (Bruins)
- Project Outline (Bruins)
- Product and Specifications (Bruins)
- Literature Review (Schaffner/Liu)
- Test Protocol (Schaffner/Liu/Bruins)
  - Critical Factors
  - Process Capability Data
- Manufacturing Challenge Samples (All)
  - Manufacturing Capability
  - Number of Samples
- Microbial Test Protocol (Schaffner/Liu)
  - Inoculation Protocol/Repacking
  - Microbial Testing

Project Objective

- Defining the growth/no-growth boundary for *Listeria monocytogenes* in Shelf Stable Pocket Sandwiches

Project Outline

- Literature Search (Phase I)
- Develop Test Protocol (Phase II)
  - Determination of Challenge Conditions
  - Determination of Challenge Protocols for *Listeria monocytogenes*
- Microbiological Challenge Samples (Phase III)
  - Manufacture Challenge Samples
  - Inoculate with *Listeria monocytogenes*
  - Store and Conduct Microbiological Testing for 6 month

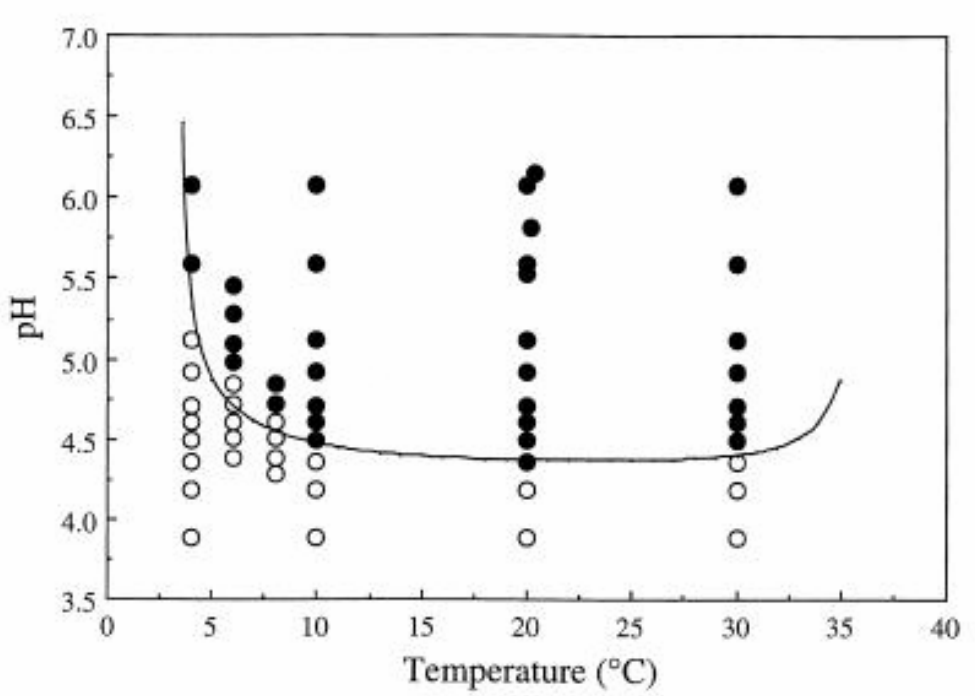
Challenge Products

- Honey Barbeque Beef
  - Specification:
    - MIL-DTL-32141: 5 April 2004 (Draft 16 June 2006)
    - PKG & QAP MIL-DTL-32141: 16 June 2006
- Bacon Cheddar
  - Specification:
    - MIL-DTL-Draft: 14 September 2005
    - PKG & QAP MIL-DTL-Draft: 12 September 2005

Literature Review

- *Listeria monocytogenes*
  - Gram positive rod (1µm x 2 µm)
  - Growth temperature (1-45 ºC)
  - Optimal growth temperature 30-37°C
  - Acid and salt tolerant
  - CDC estimates 2,500 cases per year; Mortality 20-28%

Growth/No Growth Interface



- Growth (●) or no growth (o) within 90 days at Aw = 0.993
- S. Tienungoon, AEM, 2000 November; 66(11): 4979–4987

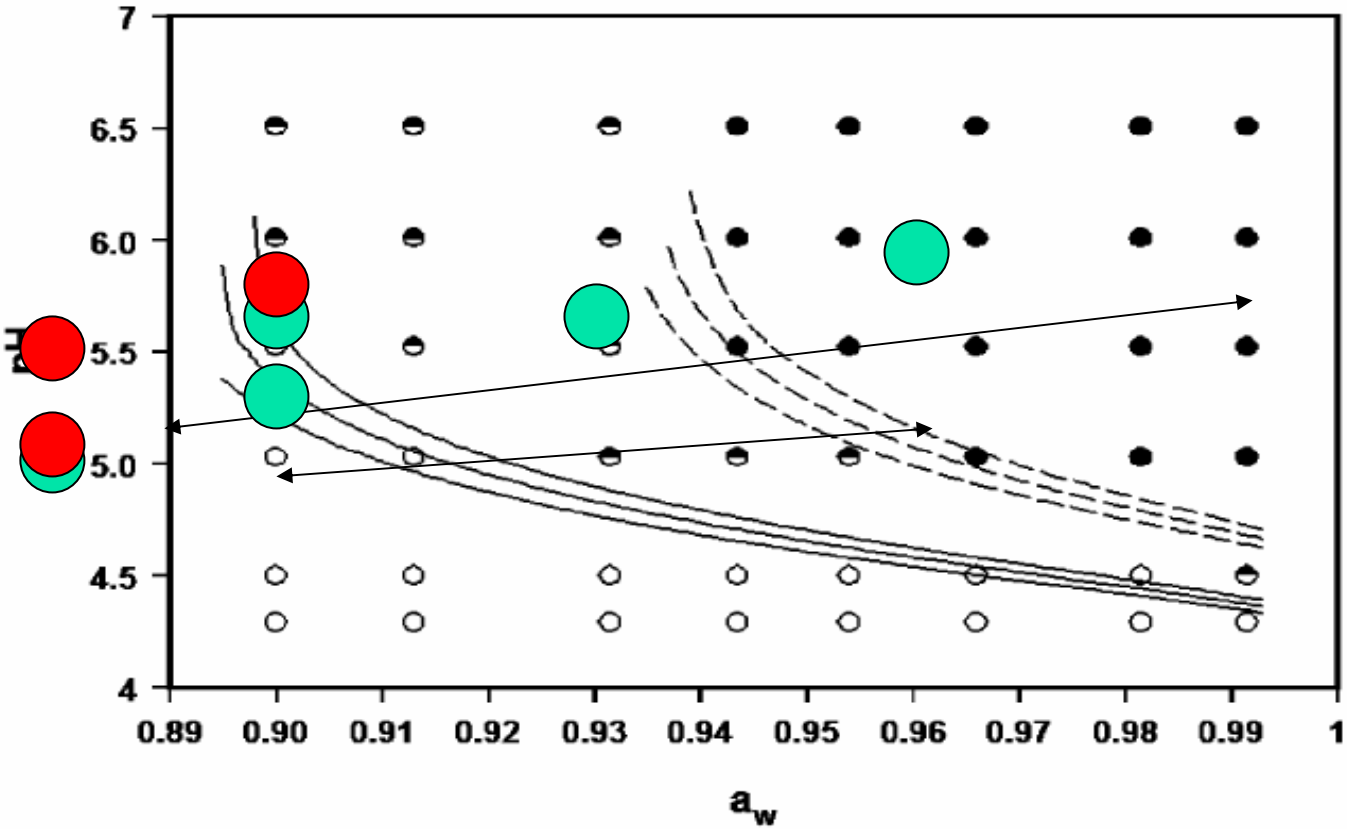


Fig. 2. Growth/no growth interface of *Listeria monocytogenes* in broth (solid line) and agar (dotted line) medium at 25°C with respect to pH and a_w predicted by the model at probabilities 0.5 (middle line), 0.1 (upper line) and 0.9 (lower line), compared to the data used to generate the model (●: growth in both broth and agar medium; ○: no growth in both broth and agar medium; semisolid circles: growth only in broth medium).

Preservatives

- Sorbic acid
- Glucono-delta-lactone
- Sodium tripolyphosphate

- All have been shown to have anti-listerial activity, depending upon concentration and subtrate

Test Protocols

- Critical factors
  - pH
  - Aw
  - Anti-microbial ingredients
- Process Capability
  - Specification Limits
  - Manufacturing Capability
  - +/- 6 sigma concept

Process Capability Data (1)

- **Honey Barbeque Beef**
  - **Specification**
    - **pH < 4.8**
    - **aw < 0.89 (?16 June Draft Spec?)**
    - **O2< 0.30%**
  - **Production Capability Data**
    - **pH: avg: 4.80 and std: 0.029**
    - **Aw: avg: 0.87 and std: 0.010**

Process Capability Data (2)

- **Bacon Cheddar**
  - **Specification**
    - **pH < 5.4**
    - **aw < 0.88, >0.85**
    - **O2< 0.30%**
  - **Production Capability Data**
    - **pH: avg: 5.02 and std: 0.071**
    - **Aw: avg: 0.84 and std: 0.015**

Microbial Testing Summary

- *Listeria* Strains
- Inoculation Protocol
- Repackaging
- Microbial Testing

Challenge Samples

- pH and Aw primary means for adjustment will be formulation changes

- Honey Barbeque
  - pH (3σ=0.09) : 5.0 5.3 5.6 5.6 5.9
  - Aw (3σ=0.03) : 0.87 0.90 0.90 0.93 0.96

- Bacon:
  - pH (3σ=0.2): 5.0 5.2 5.2 5.5 5.8
  - Aw(3σ=0.05): 0.85 0.85 0.88 0.88 0.91

Materials

- Product Samples/Variant
- Pouches
- Oxygen Scavengers

Microbial Testing Details

- Inoculation
  - 45 samples of each formulation will be tested
  - Five strain *L.m.* cocktail inoculated in product in suitable location(s)
  - A final inoculum level of 10^4 CFU/g
- Samples incubated at 25°C for six months
  - Thirteen (13) time points in triplicate
    - Day 0,1,2,3; Week 1,2,3; Month 1,2,3,4,5,6
    - Six samples serve as control and/or reserve samples
- Microbial testing
  - *L.m.* growth at 37°C for 24 hours on Oxford agar
    - TPC, yeast and molds will also be enumerated
    - pH, Aw will also be measured

Final Project Meeting

RUTGERS

School of Environmental and Biological Sciences

Pocket Sandwich Project
Final Interim Project Review

Myrtle Beach

November 27, 2007

Project Participants

- PI's: Donald Schaffner and Rieks Bruins, Rutgers University
- Coranet Liason: Steve Moody, Natick Lab
- Sub-Contractor: Bridgford Foods
  - John Simmons
  - Rody Hawkins
  - Larry Chandler

Agenda

- Objective (Bruins)
- Project Outline (Bruins)
- Product and Specifications (Bruins)
- Test Protocol (Bruins)
  - Critical Factors
  - HACCP
  - Process Capability Data
- Manufacturing Challenge Samples (Bruins)

- Literature Review (Schaffner)
- Microbial Test Protocol (Schaffner)
  - Inoculation Protocol/Repacking
  - Microbial Testing
- Microbial Test Results (Schaffner)

Project Objective

- Defining the growth/no-growth boundary for *Listeria monocytogenes* in Shelf Stable Pocket Sandwiches

Project Outline

- √ Literature Search (Phase I)
- √ Develop Test Protocol (Phase II)
  - √ Determination of Challenge Conditions
  - √ Determination of Challenge Protocols for *Listeria monocytogenes*
- √ Microbiological Challenge Samples (Phase III)
  - √ Manufacture Challenge Samples
  - √ Inoculate with *Listeria monocytogenes*
  - √ Store and Conduct Microbiological Testing for 6 month

Challenge Products

- Honey Barbeque Beef
  - Specification:
    - MIL-DTL-32141: 28 September 2006
    - PKG & QAP MIL-DTL-32141: 28 September 2006

- Bacon Cheddar
  - Specification:
    - MIL-DTL-32223: 31 October 2006
    - PKG & QAP MIL-DTL-Draft: 12 September 2005

Product Formulation and Specification (1)

- **Honey Barbeque Beef**
  - **Beef Filling + Sauce + Bread**
  - **Specification**
    - **pH < 4.8**
    - **aw < 0.89**
    - **O_2< 0.30%**

Bread	
Flour	50.22%
Water	28.76%
Shortening	8.61%
Glycerol	6.30%
Yeast	2.24%
Salt	1.28%
Sucrose Ester	0.99%
Control S (ADM)	0.50%
Gum arabic	0.50%
Calcium sulfate	0.25%
Xanthan gum	0.25%
Glucono delata lactone	0.00%
Sorbic acid	0.10%

Sauce	
Tomato Paste	35.94%
Brown sugar	14.87%
Yellow Mustard	11.70%
Honey	10.42%
Glycerol	7.71%
Molasses	5.94%
Ground Mustard	3.65%
Vegetable Oil	3.13%
Salt	2.03%
Worchestershire sauce	1.98%
Onions (dehyd)	1.54%
Smoke (liquid)	0.55%
Garlic powder	0.42%
Red Pepper	0.06%
Black Pepper	0.06%

Processed Meat	
Beef	86.13%
Beef Broth	7.71%
Rice Syrup	3.65%
Glycerol	**3.65%**
Salt	1.32%
Sodium Tripolyphosphate	0.36%
Black Pepper	0.18%

Filling	
Barbeque Sauce	54.15%
Marinated Beef	44.30%
Encap Vinegar powder	1.55%

Sandwich	
Bread	2.6 oz
Filling	1.3 oz

Product Formulation and Specification (2)

- **Bacon Cheddar**
  - **Precooked Bacon + Bread**
  - **Specification**
    - **pH < 5.4**
    - **aw < 0.88, >0.85**
    - **O_2 < 0.30%**

Bread	
Flour	48.07%
Water	26.60%
Cheddar Flakes	7.50%
Glycerol	6.00%
Shortening	5.55%
Yeast	2.00%
Salt	1.10%
Sucrose Ester	1.00%
Dough Conditioner	0.50%
Gum arabic	0.45%
Butter Flavor	0.35%
Glucono delata lactone	0.28%
Calcium sulfate	0.25%
Xanthan gum	0.25%
Sorbic acid	0.10%

Sandwich	
Dough	2.7 oz
Bacon	0.6 oz

Challenge Conditions

- Critical factors
  - pH
  - Aw
  - Anti-microbial ingredients
- HACCP approach
  - Likely causes for deviations in critical factors to occur?
  - Could these deviations result in Hazards?
  - Implement process controls to prevent Hazards
- Engineering perspective: Process Capability
  - Normal variations occur in batching, filling and baking process steps: Average and Standard Deviation

HACCP

- Batching
  - Batching errors could result in higher pH and/or Aw
  - Controls: Batching Sheet and measurements of pH and Aw
- Filling
  - Incorrect filling can change ratio of bread and filling and result in higher pH and/or Aw
  - Controls: Fill weight depositor monitored
- Baking
  - Oven malfunctioning (Time/Temperature) could raise water activity
  - Controls: Oven Time/Temperature monitored Packaging
- Packaging
  - Omission of O_2 scavenger could cause O_2 content > 0.30% and change environment
  - Controls: count of scavengers used
- End Item Exam:
  - O_2 content, pH and Aw measurements

Manufacturing

- Manufacturing Steps
  - Batching
  - Filling
  - Baking
  - Packaging
- Process Capability
  - Specification Limits
  - Manufacturing Capability
  - Robust process: no defective product produced if we can tolerate +/- 6 sigma. Probability: 10^{-9}

Process Capability Data

- **Honey Barbeque Beef**
  - **Production Capability Data**
    - **pH: avg: 4.80 and std: 0.029**
      - **+6 Sigma: 5.00**
    - **Aw: avg: 0.87 and std: 0.010**
      - **+ 6 Sigma: 0.92**

Process Capability Data

- **Bacon Cheddar**
  - **Production Capability Data**
    - **pH: avg: 5.02 and std: 0.071**
      - **+ 6 sigma: 5.44**
    - **Aw: avg: 0.84 and std: 0.015**
      - **+ 6 sigma: 0.93**

Challenge Samples BBQ

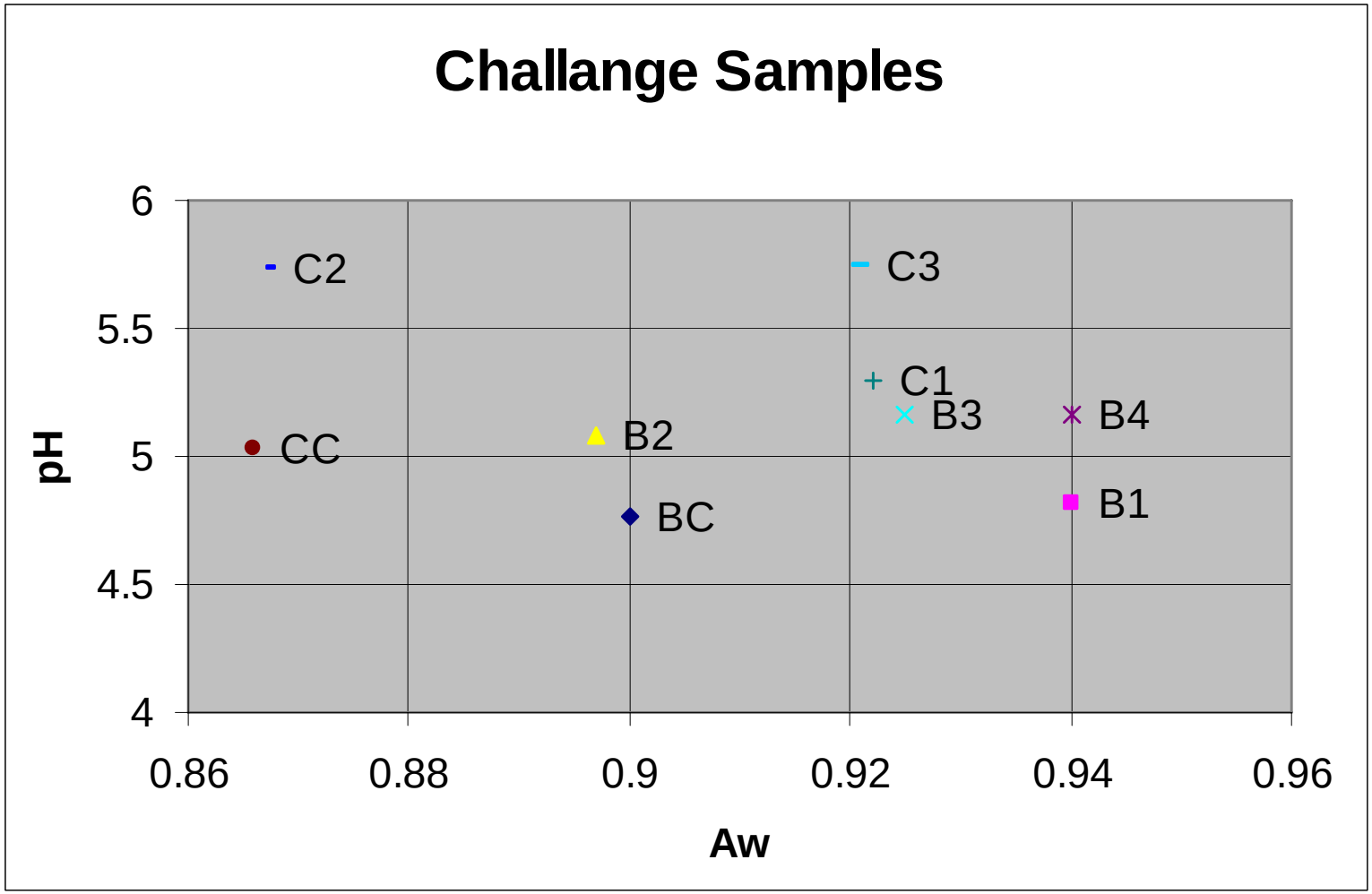
- BC
  - Control
  - pH=4.77 & Aw=0.90
- B1
  - Meat Filling: No Glycerol, No Rice Syrup,
  - Sauce: No Glycerol,
  - Bread: No Glycerol
  - pH=4.82 & Aw=0.94
- B2
  - Sauce: 50% Tomato Paste, No Vinegar
  - Bread: No Sorbic Acid
  - pH=5.08 & Aw=0.90

Challenge Samples BBQ

- B3
  - Meat: No Glycerol, No Rice Syrup,
  - Sauce: 50% Glycerol, 50% Tomato paste, No Vinegar,
  - Bread: No Glycerol, No Sorbic Acid
  - pH=5.16 & Aw=0.92
- B4
  - Meat: No Glycerol, No Rice Syrup
  - Sauce: 50% Glycerol, 50% Tomato Paste, No Vinegar, No Brown Sugar,
  - Bread: No Glycerol, No Sorbic Acid
  - pH=5.16 & Aw=0.94

Challenge Samples Bacon Cheddar

- CC:
  - Control
  - pH=5.03 & Aw=0.87
- C1
  - 50% Cheddar Flake & No Glycerol
  - pH=5.30 & Aw=0.92
- C2
  - 50% Cheddar Flakes, No Sorbic Acid, No GDL, No Butter Flavor
  - pH=5.73 & Aw=0.87
- C3
  - 50% Cheddar Flakes, No Glycerol, No Sorbic Acid, No GDL, No Butter Flavor
  - pH=5.75 & Aw=0.92


Challange Samples
6
5.5
5
4.5
4
pH
0.86
0.88
0.9
0.92
0.94
0.96
Aw
C2
C3
C1
B3
B4
CC
B2
BC
B1

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School of Environmental and Biological Sciences

Pocket Sandwich Project
Final Interim Project Review
Part II

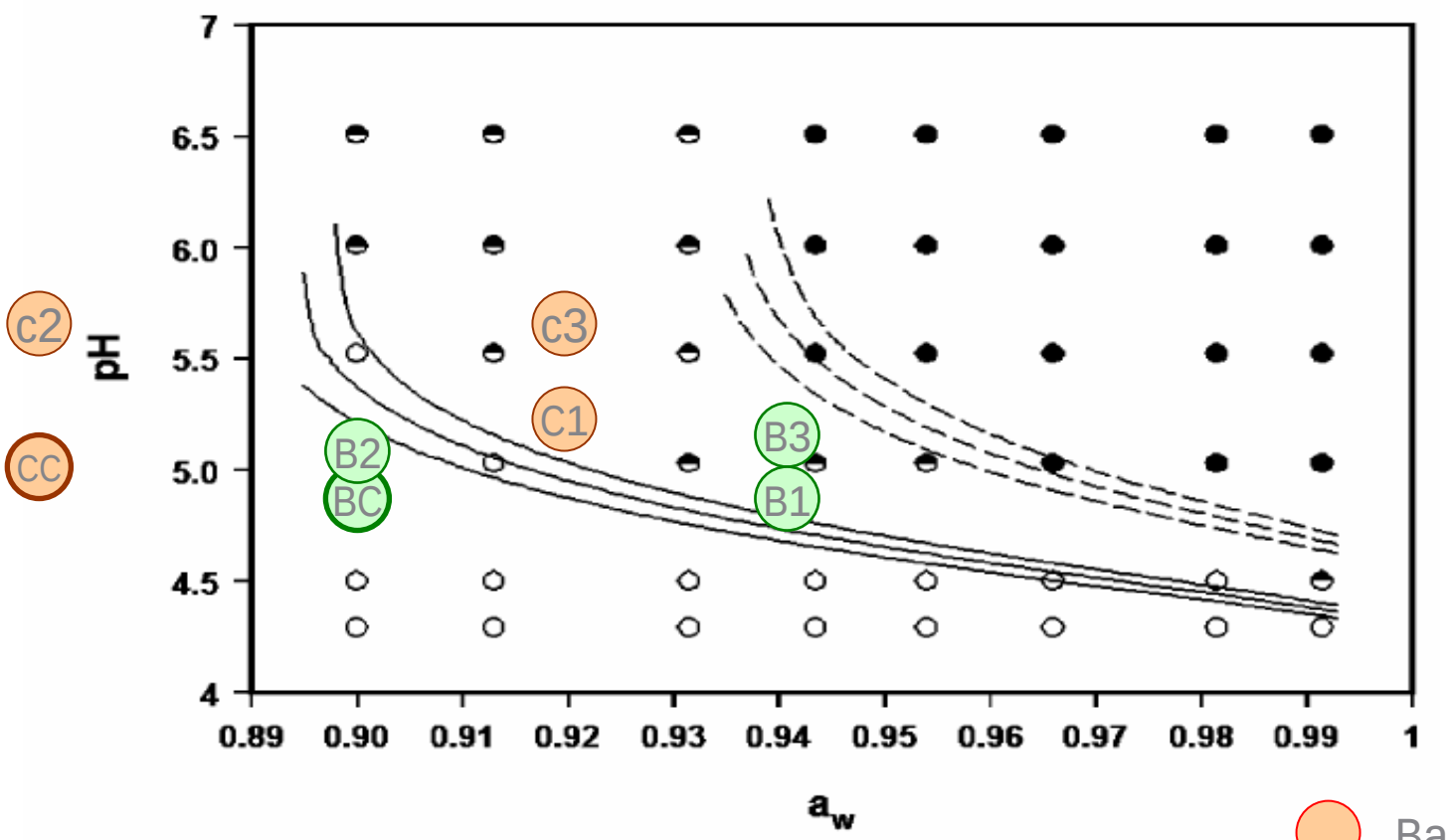
Myrtle Beach

November 27, 2007

Literature Review – why *Listeria?*

- *Listeria monocytogenes:* ubiquitous Gram positive rod
- Common post-process contaminant
- It doesn't cause many reported cases each year (~2,500) but does have a high fatality rate, especially amongst the elderly, the immunocompromised and the fetuses of pregnant women
- USDA-FSIS is very concerned about *L. monocytogenes* presence in RTE meat products
- Data published by Koutsoumanis, et al. (2004) indicate that some formulation errors may pose a risk
  - Limits to growth experiments at pH: 4.24-6.59 and aW: 0.900-0.993 in broth and on agar

Koutsoumanis, et al. 2004



Open circles
No growth

Solid circles
Growth

Half circles
Broth growth only

Bacon Cheddar

Barbeque Beef

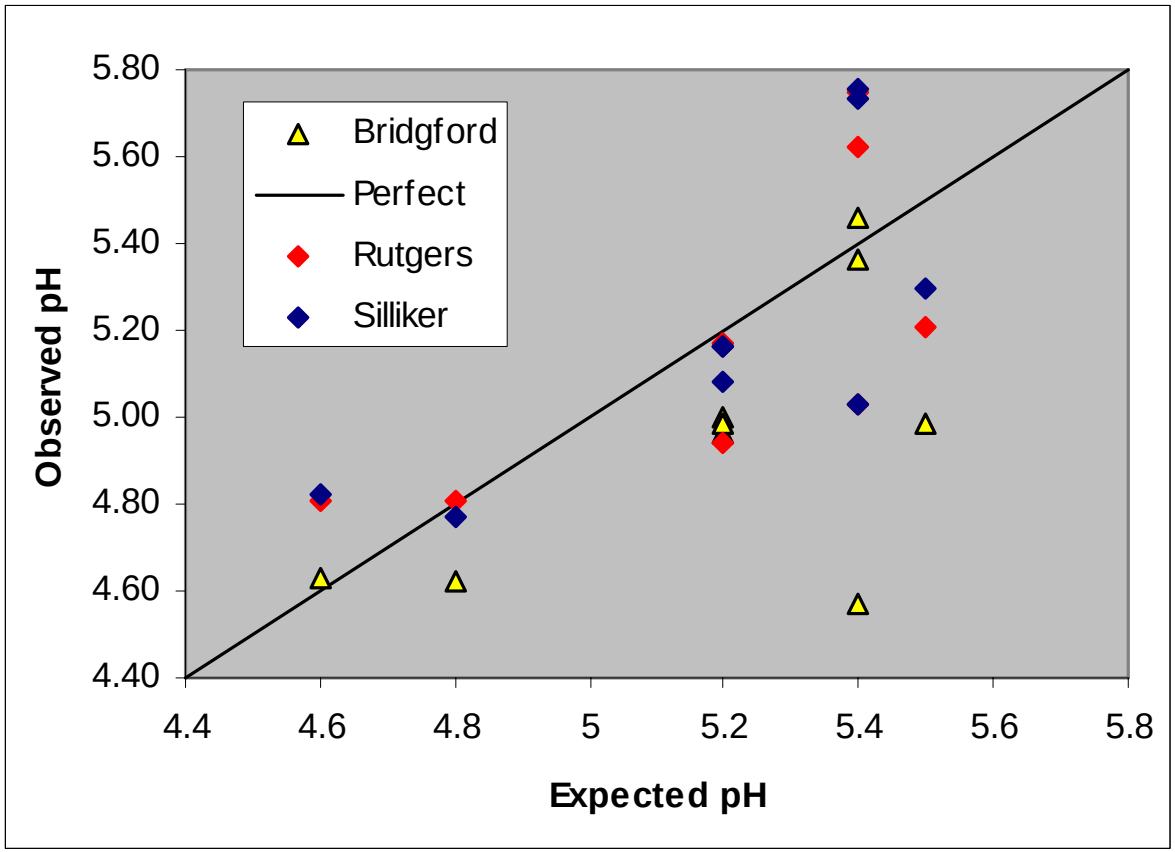
Microbial Testing Details

- Inoculation
  - Seven strain *L. monocytogenes* cocktail inoculated in product (details on next slide)
  - A inoculum level of 10^5 CFU/g was targeted
- Samples incubated at 25°C for 188 days (more than 6 months)
  - 0, 2, 5, 14, 21, 33, 56, 77, 97, 143, 188 days
- Microbial testing
  - *L. monocytogenes* growth at 37°C for 24 hours on Oxford agar
  - TPC, yeast and molds enumerated periodically
  - pH, Aw measured by Bridgford, by our Lab and by Silliker

Listeria strains used

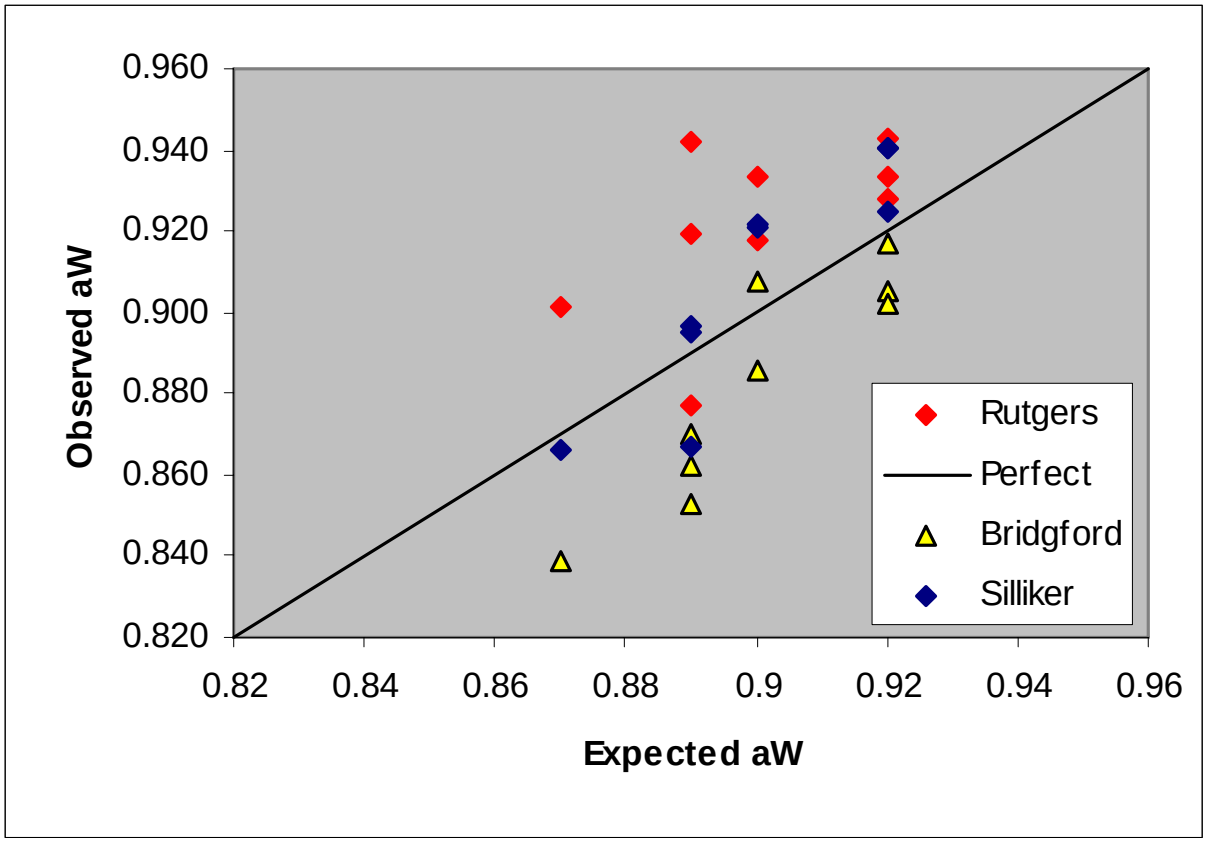
- FSL J1-177: isolated from human sporadic case
- FSL C1-056: isolated from human sporadic case
- FSL N3-013: isolated from food, linked to pate outbreak, UK
- FSL R2-499: isolated from human case linked to sliced turkey outbreak, US
- FSL N1-227: isolated from food linked to deli meat outbreak, US
- FSL J1-110: isolated from Soft Cheese in Los Angeles
- FSL R2-500: isolated from Mexican Cheese in North Carolina

pH results



- Generally good agreement between all three labs
- Slightly higher values reported by RU and Silliker may be due to sample aging

Water activity results

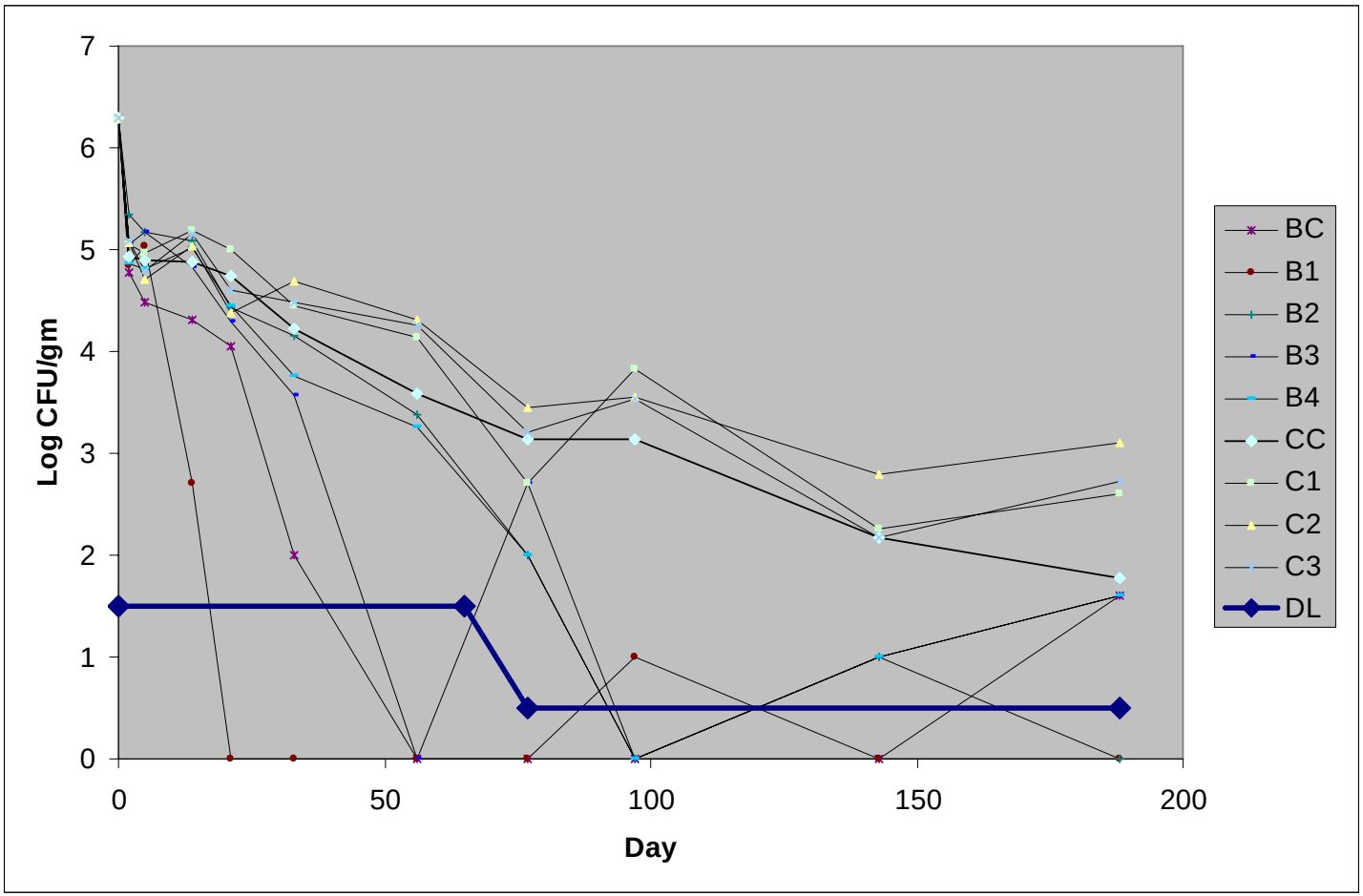


- Generally good agreement between all three labs
- Slightly higher values reported by RU and Silliker may be due to sample aging

Non-*L. monocytogenes* micro results

- TPC, yeast and mold counts were always very low
- Gas blowing of some of the non-spec samples was observed
  - Total aerobic and total anaerobic counts on these samples never showed high counts

L. monocytogenes survival results



L. monocytogenes survival results – key points

- Listeria monocytogenes appears to gradually die off in all formulations of BBQ Beef and Bacon Cheddar sandwiches
  - Decline from 5 log CFU to 3 log CFU in 6 months, or about 0.33 log CFU per month, faster in some samples
- Variation in decline rate is typical for this type of study
- Some samples show low counts at one time point, and then higher counts at a later time point
  - This may be due to sample to sample variations in initial microbial number or product formulation
  - These results are NOT an indication that growth is actually occurring

Summary

- *L. monocytogenes* growth in pocket sandwiches (even if incorrectly formulated) appears very unlikely
- *L. monocytogenes* dies slowly (0.33 log CFU/month) in many formulations
- Data should be useful in convincing USFA-FSIS that *L. monocytogenes* is not a risk in these products

Letter from Don Schaffner

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April 27, 2008

Monty Griffith
Bridgford Foods Processing Corporation
112 progress Place
Statesville, NC 28677

Larry Chandler
RDI Foods
8313 Candlelight Oaks Lane
Raleigh, NC 27603

Dear Gentlemen,

This is in response to your request of a formal letter documenting the *Listeria monocytogenes* risk in pocket sandwiches. As you know we have recently completed a year long challenge study using *L. monocytogenes*.

Although a longer technical report and peer-review publication are currently in development, this letter will lay out the essential aspects of the study, and should be sufficient documentation for your HACCP plan. It should also address any concerns by USDA FSIS inspectors.

First, we worked with you to prepare Honey Barbeque Beef and Bacon Cheddar pocket sandwiches which were improperly formulated such that their pH values and water activity values were as high as practically possible. The worst case BBQ beef sample had a target pH = 5.16 and water activity = 0.94. The worst case Bacon Cheddar product had a target pH = 5.75 and water activity = 0.92.

We inoculated Honey Barbeque Beef and Bacon Cheddar pocket sandwiches with a seven strain *L. monocytogenes* cocktail. The samples were repackaged after inoculation in new packing material, oxygen scavenger was added and the packages were heat sealed. We incubated the product at room temperature and sampled the different formulations at various time intervals. The sampled product was tested for the presence of viable *L. monocytogenes*.

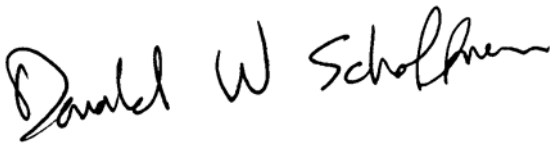
What we learned from this study is the *L. monocytogenes* does not survive in pocket sandwiches, including pocket sandwiches that are grossly mis-formulated. For example, if a Honey Barbeque Beef sandwich is manufactured with meat that contains no glycerol and no rice syrup, and a sauce that contains 50% of the usual glycerol amount, 50% of the usual tomato paste amount, no vinegar, no brown sugar and bread that contains no glycerol and no sorbic acid, the product will still not support the growth of *L. monocytogenes*. Likewise, if a Bacon Cheddar sandwich is manufactured with 50% of the usual amount of cheddar flakes, no glycerol, no sorbic acid, no GDL, and no butter flavor, this product will also not support the growth of *L. monocytogenes*.

In short, *Listeria monocytogenes* growth is not a "hazard reasonably likely to occur" in these products. In my professional opinion, as a practicing food safety microbiologist, with 20 years experience, and based on the results summarized above, there is no need to include *L. monocytogenes* as a hazard in your HACCP plan, since even if the organism does somehow contaminate the sandwiches, it presents no risk to consumers of this product.

Thank you for working with Rutgers University on this project. I think that we have advanced the science of food safety and product formulation significantly with our work.

Please let me know if you have questions or comments.

Sincerely,



Donald W. Schaffner, Ph.D.
Professor