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# The Impact of Household Refrigeration Storage Conditions on the Shelf Life of Fruits and Vegetables

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# The impact of household refrigerator storage conditions on the shelf life of fruits and vegetables

by

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# ABSTRACT

Approximately 20% of fruit and vegetable production is lost annually due to post-harvest spoilage. Technologies exist that reduce the spoilage during transport and storage, such as ethylene-permeable bags and controlled environment crispers. Although methods exist for controlling environmental conditions to a closer tolerance, these are currently cost-prohibitive. This research focused on the direct relationship between produce spoilage and tightly controlled humidity and temperature settings, with the goal of generating new information capable of driving innovation in the field. The objectives of this research were to store a model fresh fruit (strawberries) and a vegetable (romaine lettuce) in an environmental chamber capable of maintaining and reporting specified humidity and temperature conditions, and quantifying spoilage effects at various temperature and humidity levels. Ultimately, the goal is to provide the industry with a better understanding of the intersection between controllable food storage conditions, microbial spoilage, and food safety. Nine spoilage tests were completed at 7°C, 5°C and 3°C at relative humidities of 95%, 75% and 50% (see Figure 1). The overall results can be seen in Table 2 and the summary is as follows:

Romaine lettuce heads lasted the longest, and stayed the freshest at high humidity (~95%), with temperature having a lesser impact. If humidity control is not possible, aseptically separating leaves and storing them individually in sterile, sealable bags will enhance organoleptic quality at low temperatures (~3°C), although this is probably not possible in practice.

Strawberries stored at low temperature and humidity (~3°C and ~50%) resisted molding the longest, and lost firmness after 1-2 weeks. To balance the preservation of freshness and firmness, storage at low temperature and high humidity (~3°C and ~95%) was found to be ideal. Initial microbial count had important impacts on mold growth rate. Either high mold

inoculum or metabiotic interactions between spoilage bacteria and molds may be responsible for these effects.

# CHAPTER 1. OVERVIEW

## Introduction

Little information can be found in open literature regarding the relationships between key storage parameters such as temperature or relative humidity and produce storage life and safety. Additionally, the current state-of-the-art household refrigerator design is not optimized for control of humidity migration from special-purpose compartments intended to maintain a high-humidity environment (e.g. vegetable crisper).

Most current designs also rely on vapor-compression systems that are turned on or off at high and low set points, resulting in temperature fluctuations that exceed the recommended variation of ± 5°C. Although refrigerators incorporating technologies for more precise temperature and humidity control are available on the market (e.g. those with variable-speed compressors, isolated compartments, etc.), little information on the relationship between these parameters and food shelf life is available to drive the rationale for designing truly improved humidity and temperature systems for household refrigerators.

Apart from spoilage concerns, the microbiological quality of produce, including lettuce and strawberries, also has an important food safety component. Pathogenic bacteria, including *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas* spp. have been identified as the causative agents in disease outbreaks involving minimally-processed salads (Sivapalasingam et al., 2004). In fruits, mold growth is generally associated with organoleptic concerns, such as breakdown in fruit texture and the generation of off flavors. However, some species of fruit spoilage molds are also known to produce potentially harmful metabolites such as patulin, byssotoxin A and related natural toxins (Beuchat and

Pitt, 2001), again underlying the importance of proper refrigeration conditions to both food spoilage and safety.

The objectives of the study were to:

• Utilize a test stand capable of data collection and maintaining specified humidity and temperature conditions to quantify the effects of low and high storage humidity and low and high storage temperatures on the shelf life of one model vegetable (romaine lettuce) and one model fruit (strawberries).

• Disseminate the knowledge gained from this work to ASHRAE and both the engineering and food science communities. Access to the data generated in this work will help provide a rationale for design of more advanced home refrigeration systems, as well as heighten the public's understanding of relationships between food storage conditions and microbial spoilage and, possibly, food safety.

The research addresses ASHRAE's stated mission to serve humanity and promote a sustainable world by advancing the arts and science of refrigeration (ASHRAE). This work is aligned with the "Security, Safety and Health" theme described in ASHRAE's strategic plan for research and provides much needed data on the relationship between relative humidity and produce spoilage. Ultimately, this work may lead to improvements in refrigeration technology that may find applications in preventing economic losses due to spoilage and ensuring the safety and wholesomeness of produce. Most directly affected by this research are refrigeration system designers, who may be able to use the results to justify design and production of more precise and adjustable storage products intended to reduce current produce losses due to microbial spoilage.

## Test Conditions

The test stand consisted of an environmental control system and a test chamber whose design and construction was based on the requirement that specified conditions be achievable within certain accuracies ("dial-in" operation), in order to ensure reproducible test conditions. Achieving these conditions required not only that the control system supply these conditions accurately, but also that the sensors used to report the measurements were capable of operating within the same limits of accuracy.

The test stand used for this study was able to achieve the required relative humidities over a 50% to 95% range within a specified accuracy of better than ± 5%. These relative humidities were achievable over a dry bulb temperature range of 3°C to 7°C within a specified accuracy of ± 0.3ºC. The airflow capacity of the test stand was based on a maximum velocity in the test chamber (i.e. flowing over the produce specimen) of 1. 0 ft/min. The spoilage tests were performed using an existing humidity generator environmental chamber capable of achieving the required conditions.

Tests were performed, using the required test conditions and specifications described earlier, on a model vegetable (romaine lettuce), and a model fruit (strawberries). In conjunction with the ASHRAE committee for over the 4-year duration of this study, a suitable operational definition for freshness and a set of tests used to quantify produce freshness were developed and are reported here. Specified test conditions for this research project (dry bulb temperature and relative humidity %) are shown below in Table 1.

| Relative Humidity (%) | 3°C(dry bulb) | 5°C(dry bulb) | 7°C(dry bulb) |
|---|---|---|---|
| 100% | Test Complete | Test Complete | Test Complete |

| | | | |
|---|---|---|---|
| 75% | Test Complete | Test Complete (2 Replications Completed) | Test Complete |
| 50% | Test Complete | Test Complete | Test Complete |

**Table 1. Matrix of tests required to complete research**

Nine different environmental conditions were used for the tests: Relative humidity levels of 50%, 75% and 95%, at dry bulb temperatures of 3°C, 5°C and 7°C including two extra tests at 5°C 75%RH (to show repeatability). Produce spoilage evaluation (as described elsewhere) was performed at intervals of 48 hours, starting with the initiation of a test run, and continued for 14 days or until the freshness threshold was determined to have been reached. At 48-hour intervals, samples were removed for visual/organoleptic and microbiological evaluation. In each case, multiple samples of the same food type (i.e. lettuce or strawberries) were removed at the same time to improve the statistical robustness of the data obtained.

## Environmental Control System

**Figure 1. Thunder Scientific Series 2500 Bench top two-pressure humidity generator**

The humidity sensor calibration system worked as follows: a water/glycol jacket surrounded the chamber (Series 2500). This water/glycol was circulated through a chiller/heater system providing a constant temperature. A compressor lowered the moisture content of the room air. The pressurized air was then throttled at a specific rate (in this case 10 liters per minute) into the test chamber, resulting in achievement of the specified relative humidity. The following is a description of how the relative humidity test chamber operated. An elemental schematic of the generator is shown in Figure 2.

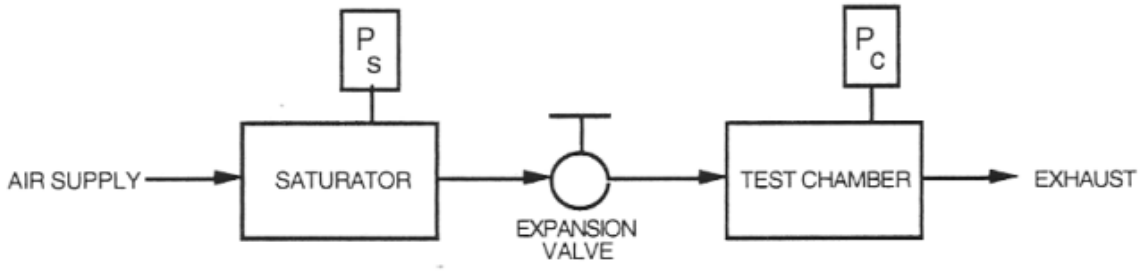


**Figure 2. Elemental schematic of the humidity generator test chamber**

Operation of the humidity generator is based on the two-pressure method of producing known atmospheres of relative humidity and assumes that the water vapor pressure remains a fraction of the total pressure, known as Dalton's Law of Partial Pressure. Dalton's Law states that the pressure exerted by a mixture of gases in a given volume at some temperature is equal to the sum of the pressures that would be exerted by each individual gas if it alone occupied the volume at the same temperature.

The two pressure method involves saturating air with water vapor at a given pressure and temperature. The saturated gas then flows through an expansion valve where it is isothermally reduced to chamber pressure. If the temperature of the gas is held constant during pressure reduction, the humidity, at chamber pressure, may then be approximated as the ratio of two absolute pressures.

$$\%RH \approx \frac{P_{chamber}}{P_{saturator}} \times 100$$

**Equation 1.** RH Formula for Ideal Gas

Humidity produced in the test chamber of this system does not depend on devices such as psychrometers, dewpoint hygrometers, or solid state sensors for the measurement of water vapor content. Humidity that is produced is solely dependent on the measurement of absolute pressures and on the maintenance of isothermal conditions. Precision humidity generation is determined by the accuracy of these pressure measurements and uniformity of temperature throughout the generating system.

The relative humidity formula (Equation 1) is a correct relationship between pressures and relative humidity when dealing with perfectly isothermal conditions and perfectly ideal gases. However, under dynamic conditions where some slight temperature differences do exist and since gases do not behave ideally; any expectation of this equation to accurately

represent the actual relative humidity is overly optimistic. In its strictest form, relative humidity is defined in terms of mole fractions and is given as

$$\%RH = \frac{X_V}{X_W}\Big|_{P,T} \times 100$$

**Equation 2.**

where $X_V$ = the mole fraction of water vapor in a sample of moist air at a specific pressure, P, and temperature, T, and

$X_W$ = the mole fraction of water vapor which would exist in a sample of air if it were saturated with water vapor at the same pressure, P, and temperature, T, as the unsaturated sample Xv.

The mole fraction of water vapor in a sample of gas is given by

$$X = \frac{P_V}{P}$$

**Equation 3.**

where $P_V$ = the partial pressure of the gas which is exerted by the water vapor constituent alone, and

P = the absolute (or total) pressure of the gas, which is also equal to the sum of the partial pressures exerted by the water vapor and dry air constituents.

When a gas is fully saturated with water vapor, the partial pressure, $P_V$, exerted by the water vapor constituent is a known quantity, $e_W(T)$, and is termed “the saturation vapor pressure of air with respect to water”. Since, at saturation, $P_V = e_W(T)$, the mole fraction equation of a saturated gas may be written as

$$X = \frac{e_W(T)}{P}$$

**Equation 4.**

where $e_W(T)$ = the saturation vapor pressure of air with respect to water (at temperature T), and is the partial pressure exerted by the water vapor constituent, and P = the absolute (or total) pressure of the gas.

The mole fraction of water vapor which would exist in a saturated gas sample at the chamber pressure, P, and chamber temperature, $T_C$, would be the quantity, $X_W$, which is needed to calculate the relative humidity relationship previously discussed. Here, the mole fraction, under saturated conditions, may be expressed by

$$X_W = \frac{e_W(T_C)}{P_C}$$

**Equation 5.**

where $e_W(T_C)$ = the saturation vapor pressure of air with respect to water at the chamber temperature, T, and $P_C$ = the measured absolute pressure in the chamber expressed in the same units as $e_W(T_C)$.

The other quantity, $X_V$, required for the calculation of relative humidity, is that mole fraction of water vapor which actually exists in the air sample within the chamber at pressure $P_C$, and temperature $T_C$. If the chamber pressure, $P_C$, were used in the calculation of the mole fraction $X_V$, the expression would be

$$X_V = \frac{P_V}{P_C}$$

**Equation 6.**

which would require direct measurement of the water vapor content. However, this requirement is eliminated by using the relationship

$$\frac{e_W(T_S)}{P_S} = \frac{P_V}{P_C}$$

**Equation 7.**

where $e_W(T_S)$= the saturation vapor pressure of air with respect to water at the temperature of saturation, $T_S$, (the saturation temperature), and $P_S$ = the measured absolute (or total) pressure at which the sample is saturated (the saturation pressure).

The basis for this relationship lies in the fact that the number of molecules of the constituents within a sample of gas remains constant regardless of the pressure or temperature, provided that the temperature or pressure applied does not cause a change in phase (i.e., gas to liquid).

Since the saturation vapor pressure, $e_W(T)$, is a well-known function of the temperature alone, the total pressure at saturation, $P_S$, may be adjusted to any reasonable value to achieve the desired mole fraction of water vapor. Relying on this relationship, the mole fraction of water vapor entering the chamber (and at chamber temperature) may be written as that mole fraction of water vapor existing in the saturator at the saturation pressure and temperature. Thus,

$$X_V = \frac{e_W(T_S)}{P_S}$$

**Equation 8.**

The relative humidity may now be expressed in terms of these other quantities by returning to the original definition and substituting the appropriate expressions.

$$\%RH = \frac{X_V}{X_W}\Big|_{P,T} \times 100 = \frac{\left(\frac{e_W(T_S)}{P_S}\right)}{\left(\frac{e_W(T_C)}{P_C}\right)} \times 100$$

After rearrangement of terms, the relative humidity formula for ideal gases may then be expressed as:

$$\%RH = \frac{e_W(T_S)}{e_W(T_C)} \times \frac{P_C}{P_S} \times 100$$

**Equation 9.**

where $e_W(T_S)$ = the saturation vapor pressure at the saturation temperature, $T_S$, $e_W(T_C)$ = the saturation vapor pressure at the chamber temperature, $T_C$, $P_C$ = the absolute pressure in the chamber, and $P_S$ = the absolute pressure in the saturator.

Air, a mixture of gases with varying compressibilities, exhibits non-ideal properties, which affect the saturation vapor pressure, $e_W(T)$. The saturation vapor pressures, $e_W(T_S)$ and $e_W(T_C)$, in the relative humidity formula (above) must be replaced by their "effective" saturation vapor pressures which are related to the "ideal" saturation vapor pressure by

$$e'_W(P,T) = f_W(P,T)e_W(T)$$

**Equation 10.**

where $e'_W(P,T)$ = the "effective saturation vapor pressure of air with respect to water" at absolute pressure, P, and temperature, T, and $f_W(P,T)$ = the "enhancement factor for moist air" at pressure, P, and temperature, T.

The relative humidity formula for air, based on the effective saturation vapor pressures, is then written as

$$\%RH = \frac{e'_W(P_S, T_S)}{e'_W(P_C, T_C)} \times \frac{P_C}{P_S} \times 100$$

and, after making the appropriate substitutions, is expressed by

$$\%RH = \frac{f_W(P_S, T_S)}{f_W(P_C, T_C)} \times \frac{e_W(T_S)}{e_W(T_C)} \times \frac{P_C}{P_S} \times 100$$

**Equation 11.**

It can now be seen by inspection of the relative humidity formula, expressed in its final form, that known relative humidities may be accurately generated, using air, through measurement and control of pressure and temperature alone.

The term $\frac{P_C}{P_S}$ is simply the ratio of the chamber pressure to the saturator pressure. This is the "idealistic" portion of the relative humidity formula which ignores minor temperature differences between the saturator and chamber. It also assumes that moist air behaves as an ideal gas. This ratio closely approximates the actual relative humidity, and is often used alone to express the humidity when ease of calculation outweighs the need for the additional accuracy provided by the temperature and pressure corrections. The pressures $P_C$ and $P_S$ are measured directly through the use of high accuracy absolute pressure transducers.

The equations for "effective degree of saturation" and "enhancement factor ratio" (not shown) explain how relative humidity accuracy and resolution can be further enhanced. The relevant specifications for this humidity generator are:

Relative Humidity Range 10% to 95%

Relative Humidity Resolution 0.02%

Relative Humidity Accuracy ±0.5% @ PcTc

Chamber Temperature Range 0°C to 70°C

Chamber Temperature Resolution ±0.02°C

Chamber Temperature Uniformity ±0.1°C *

Chamber Temperature Accuracy ±0.06°C

* When operating at a test temperature that is within ± 10°C of the ambient room temperature.

Test runs of this instrument have found that low temperatures can be achieved with the specified required tolerance of ± 0.3°C. As for the relative humidity, the environmental chamber is much more accurate than the required ±5%. Temperature and humidity data can be seen later in the Results section of this proposal.

# CHAPTER 2. BIOLOGICAL METHOD OF TEST USED FOR SPOILAGE EVALUATION

## Introduction

This biological method of test was intended to provide all procedures and background needed for testing everything outside of the mechanical system. Instructions start with the procurement of the produce and sample preparation prior to placement within the environmental chamber, data collection procedures used throughout the experiments are specified and a checklist for visually identifying physical attributes of "spoilage" and "freshness" are developed. Procedures for counting of microorganisms and data collection are explained step-by-step.

Apart from spoilage concerns, the microbiological quality of produce, including lettuce and strawberries, also has an important food safety component. Pathogenic bacteria, including *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas* spp. have been identified as the causative agents in disease outbreaks involving minimally-processed salads (Sivapalasingam et al., 2004). In fruits, mold growth is generally associated with organoleptic concerns, such as breakdown in fruit texture and the generation of off flavors. However, some species of fruit spoilage molds are also known to produce potentially harmful metabolites such as patulin, byssotoxin A and related natural toxins (Beuchat and Pitt, 2001), again underlying the importance of proper refrigeration conditions to both food spoilage and safety.

With this method of test, research may be conducted and that will ascertain the progression of spoilage in produce. Because of the wide variety of produce available, one model vegetable (romaine lettuce) and one model fruit (strawberries) were chosen for these tests. This method of test has been written specifically for these model systems, and

is therefore not fully applicable to every other fruit or vegetable. It is up to the researcher to develop suitable handling procedures and freshness criterion for their specific needs. The freshness threshold is somewhat subjective as the concept of "spoilage" varies relative to the end users of the produce.

The method of test starts with materials and procedures needed to procure and prepare produce samples. The sampling and plating process will be described, along with a definition of freshness threshold for both romaine lettuce and strawberries. Additional spoilage measurement procedures are given for detecting water loss, off colors and other changes in organoleptic quality.

## Materials and Methods for Spoilage Testing

Test produce (romaine lettuce and strawberries) was procured from a large Midwest grocery chain. Arrangements were made to ensure that the sample produce was not stocked or shelved. This ensured the use of the freshest produce possible. Before inserting the produce samples into the test stand, some level of sample homogeneity was ensured among the two types of food. The microorganisms present, being naturally occurring spoilage microflora on lettuce and strawberries, were likely to be distributed heterogeneously from batch to batch. In order to ensure an even distribution of these endogenous spoilage organisms on produce surfaces, samples of either lettuce or strawberries were placed in a large, sterile Whirl-Pak bag and mixed gently, before being placed in the test stand for incubation. This allowed for a more even distribution of naturally occurring microbial flora on the surfaces of each sample type and controlled for the potentially confounding factor of uneven distribution in microbial load or species composition of the initial spoilage inoculum. Although there was potential for natural variation in species composition or overall microbial load among the samples as a function of seasonal variation or region of production, it was expected, (from experience and from previous literature reports), that the microbial flora of these produce types remained fairly similar, with the largest uncontrollable variable being overall microbial load. Because

strawberries are intrinsically fragile (e.g. prone to bruising and other physical damage) and have a more limited shelf life than lettuce, they were purchased with the goal of obtaining the freshest berries possible.

Romaine leaf lettuce (standard PLU: 4640) was purchased for use in approximately 14-day test sets. Four heads of average size were used. Two heads, placed side-by-side on an aluminum tray, were used for weight loss calculations, and documentation of leaf color, turgor and overall organoleptic quality. The other two heads of romaine were aseptically separated into loose leaves, mixed gently then sealed in a large sterile Whirl-Pak bag (184 oz, part number B01447WA, Nasco, Inc., Fort Atkinson, WI).

Strawberries were treated in much the same way as the romaine lettuce. Four quarts of berries (about 2kg) were procured before being stocked/shelved. The strawberries were examined visually and the worst 5% (discolored, physically damaged, visually molded) discarded; then the berries were mixed lightly in a plastic Whirl-Pak bag. Two quarts of berries placed on an open sterile aluminum container were used for weight loss calculations, and documentation of berry color, turgor and overall organoleptic quality. The other two quarts were placed onto an open sterile aluminum container and used as samples for microbial counts.

## Sampling Process

From test day 1, and every other day following, samples were collected and examined visually for organoleptic quality (brown spots, soft spots, wilting/loss of turgor, visible indicators of microbial growth, etc.). Each test day, the exposed trays of lettuce and strawberries were taken down and sampled. The produce was weighed, organoleptic quality noted and photos taken for visual documentation of produce condition. This created an easy-to-follow visual dataset for each test condition for use in subsequent correlation with microbial data. The photo background was a simple piece of white canvas cloth. As

noted elsewhere, camera settings and backlighting was standardized for all tests and a "live" color comparison legend (green for lettuce and red for strawberries) was included in each photo as an internal color standard.

Once the open samples were photographed, they were set back into the environmental chamber, and samples were prepared for microbial enumeration (Whirl-Pak bag of romaine lettuce and separate open aluminum tray of strawberries).

## Plating Process

Sampling time, appropriate sampling techniques and sufficient replication are important factors when performing this type of spoilage experiment. The following are instructions for the sampling of both romaine lettuce and strawberries from day '0' until day '14.'

Day Zero: Two samples of romaine lettuce were selected at random from within the Whirl-Pak storage bag. Each sample was inoculated with a dilution of 0.1% peptone water in at least a 1:1 dilution, and stomached (see discussion below) in separate bags for 60 seconds at 230 RPM. Approximately 100 grams of strawberries were selected from random locations on the tray, separated into two sterile stomacher bags, and stomached using the same method as for lettuce. Macerated strawberry slurries were then diluted and plated (The goal being to obtain both yeast/mold (strawberries) and bacterial (lettuce) counts from each sample = 8 counts total).

Typically, plating inoculum involves dilution of food (1:1 to 1:10) in an appropriate growth medium and maceration or comminution in a "stomacher." The stomacher is a mechanical device designed to disrupt foods and ensure even mixing prior to plating. The technique used in this experiment for microbial enumeration was called "track plating", or the "track dilution method", described by Jett et al. (1997). Track plating is an abbreviated form of the traditional plating technique seen in most microbiology textbooks and explained in

Compendium of Methods for the Microbial Analysis of Foods (2001). According to the authors (Jett et al.,) this method yields colony counts that are statistically comparable to those achieved with traditional plating, but the method significantly reduces demands on labor and materials, with the information gained from one track plate being equal to that of six traditional plates. Because track plating can be carried out using any existing medium, approaches were adapted for enumeration of spoilage microflora that have been previously described and validated in the literature. Specifically, the approach used by Magnuson et al., (1990) for characterizing the microflora of processed lettuce was utilized. Plate Count Agar (PCA) for total microbial counts and Oxytetracycline Glucose Yeast Extract (OGYE) agar for selective identification of yeasts and molds were used after initial experimentation with various other media (data not shown). In an initial evaluation, both of these agars performed well for track plate-based enumeration of bacteria (for romaine lettuce), and yeasts & molds (for strawberries). Media selection was critical to the success of the microbial enumeration, and varied greatly depending on the nature of the desired test and selected produce type. Figure 3 provides an example of track plating onto PCA for the enumeration of bacteria. Figure 4 illustrates the results of sample maceration via stomacher. Figure 5 shows microbial spoilage trends for strawberries and lettuce plated onto OGYE agar and PCA agar respectively.

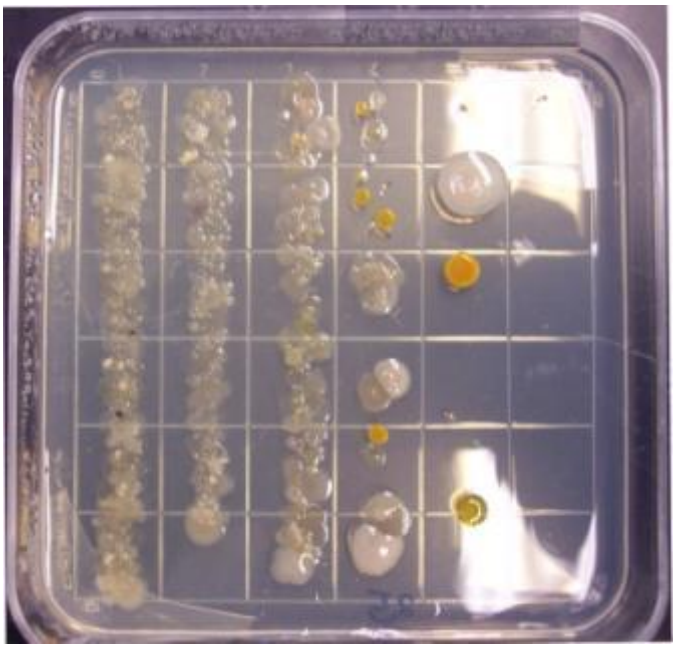

**Figure 3. Photo of a plate count agar (PCA) plate showing ten-fold dilutions of microbial colonies**

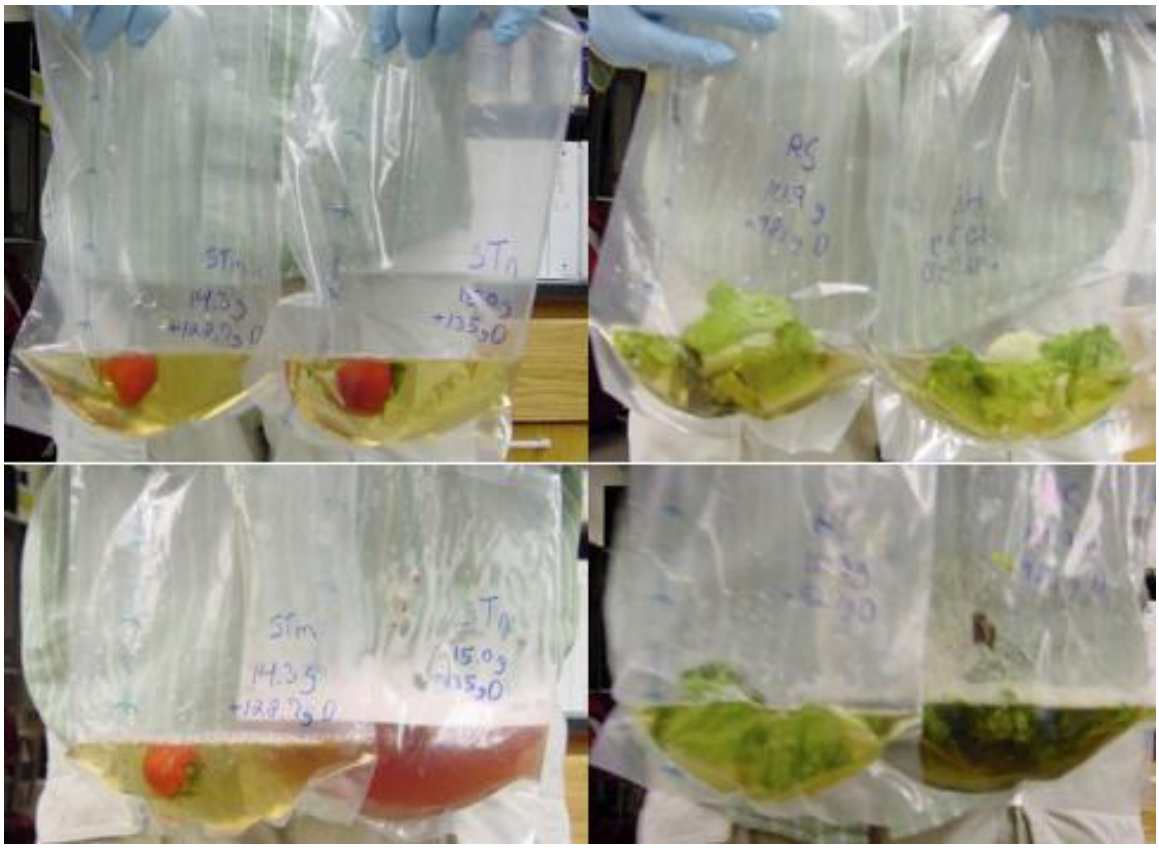

**Figure 4. Maceration (blending) of strawberry and lettuce samples**

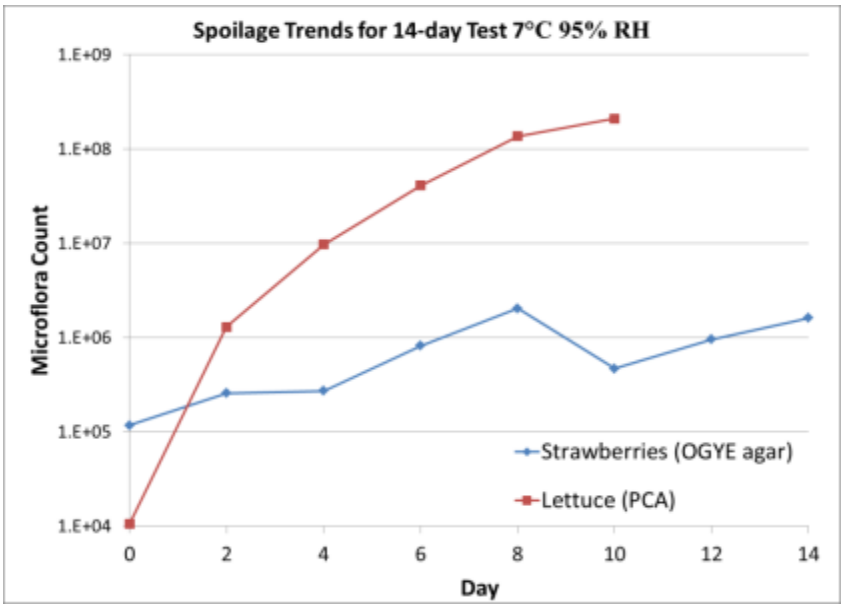


**Figure 5. Microbial spoilage trends for strawberries and lettuce plated onto OGYE (yeast and mold specific) agar and PCA (Plate Count Agar) respectively**

## Freshness Testing

Days 0, 2, 4, 6 8, 10, 12 and 14:

Tests were conducted in the same method as Day 0. Taking samples from two random places on the sample containers instead of one allowed an average to be built. Bacterial spoilage count readings were taken from the PCA (lettuce) 24 hours after the plating process. Mold & yeast count readings (OGYE agar: strawberries) were taken 48 hours after plating process. Tests for organoleptic quality (visible defects, weight, turgor etc.) were conducted using the non-bagged and non-separated lettuce and strawberries (respectively). Photographs were taken on each of these test days. When possible, the experimenter watched for turning points (from "not spoiled" to "spoiled") and conducted these tests daily once the produce had reached or neared the "freshness threshold", defined below.

For the purposes of these tests, "freshness threshold" was defined as follows:

Romaine Lettuce:

-Bacterial counts of $10^7$ or above OR

-Wilting of 1/3 to 1/2 of the total leaf surface area OR

-Slime seen on at least 5% of the leaf surface area OR

-Loss of 20% of original water content

Strawberries:

-Yeast/Mold counts of $10^6$ or above OR

-Loss of firmness in 1/3 of the berries OR

-Visible mold on 1/4 of the berries OR

-Loss of 20% of original water content

The freshness threshold is an endpoint beyond which the produce is likely to be of reduced value to consumers and therefore not salable. Once the produce was determined to have exceeded its freshness threshold, as described above, the test run was terminated. These thresholds vary greatly depending on the type of produce and application.

## Additional Measurements

**Molecular Testing:** During testing of various environmental conditions, the diversity of the microbial flora present in the produce samples was also examined using molecular methods, both initially and after the products had reached a spoilage endpoint. This was

done for both strawberries and lettuce, and provided an indication of how prevailing environmental conditions affected the composition of microbial flora. Briefly, typical colonies from bacterial or yeast and mold plates were sub-cultured and cellular morphology characterized via microscopy. Figure 6, Figure 7 and Figure 8 show preliminary microscopic results. Total genomic DNA from pure cultures of each representative organism were isolated using the PrepMan Ultra sample preparation kit (Life Technologies Corporation, Carlsbad, CA) and variable regions of the ribosomal DNA were amplified via the polymerase chain reaction (PCR) using previously published primer sets (Boye et al., 1999; Fell et al., 2000). PCR products were then sequenced at the Iowa State University's Office of Biotechnology DNA Sequencing Facility and the resulting sequences compared against published sequence data to obtain the molecular identities of each isolate. This enabled confirmation, on a molecular level, the identities of the predominant microorganisms present in the initial inoculum and after spoilage had occurred. Microbial growth data were also complimented with visual characterization of produce samples (i.e. moldy, slimy etc.).

In addition to microbial counts, other parameters providing information on degree of spoilage or overall organoleptic acceptability of the test produce were collected and examined. These included water loss as a function of storage time, and changes in color or texture, as described below.

**Water Loss:** Because the test stand was passing air (albeit at a low rate) over the produce, it was considered that there would be a possibility that this could result in net moisture loss, which could affect both overall organoleptic quality of the produce, as well as microbial spoilage (by modulating available water values – a critical requirement for microbial growth). Moisture loss was examined using a simple weight measurement of the produce. Samples were weighed prior to being introduced into the test stand, and again at sampling

time. Weight measurements were carried out using a standard +/- 0.01 g accuracy scale. The water loss was calculated on a percentage basis and displayed on an excel chart.

**Photo Documentation:** As a complement to microbial spoilage and physical (e.g. water loss) data, accurate documentation of the physical impact various storage conditions have on the test produce were made via photographic means. At each sampling point, test produce was digitally photographed, controlling for lighting and background (a piece of white canvas cloth served as a standard background, allowing for high-contrast images of test produce). As an internal visual standard, green and red color-cards approximating the colors of lettuce and strawberries were photographed with each sample of test produce. Additionally, whiteness correction was employed to further enhance the comparability of the photographs.

**Color changes:** Color changes are commonly associated with vegetable tissue senescence and decay and therefore represent an important indicator of overall produce quality that can be examined during the course of the shelf life studies. Color-cards were used, as described above, to provide an internal standard for comparison of photos made at each time point.

**Texture:** A subjective determination of produce texture was made at each testing interval. Firmness of each sample was determined with a simple physical examination. Lettuce leaves were monitored and reported as crisp, slimy, or leathery. Strawberries was monitored and reported as firm, soft or pulpous.

**Data tabulation and summary:** Data for each test was tabulated and summarized in spreadsheet form and photographs for each sample were stored via the web to a database containing the photographs for these samples. Access to these full-sized digital photographs allows future examiners of this research to obtain finer visual detail. Smaller, less detailed pictures are shown for each test day in this report.

## Summary

The approach described here provides standard methods for sampling and pre-analytical preparation for lettuce and strawberries, enabling consistent and programmatic collection of data associated with organoleptic and microbial spoilage of these produce items as a function of temperature and relative humidity.

# CHAPTER 3. RESULTS

## Microscopy Tests

As mentioned in the biological method of test, the following three figures show photographic results of microscopy confirming colony types. Visual confirmation of the general types of organisms present on each of the model food types helped determine the most appropriate type of agar growth media to use for microbial evaluation of strawberries and romaine lettuce.

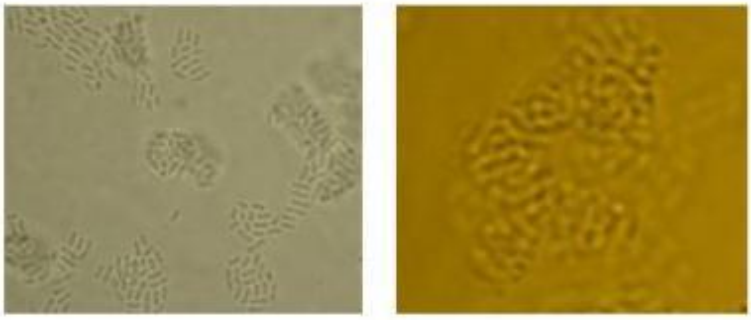

**Figure 6. Bacteria from PCA – romaine lettuce at 5°C and 95% relative humidity (bacilli)**

**Figure 7. Fungi from DRBC agar- strawberries at 5°C and 95% relative humidity (fungal hyphae)**

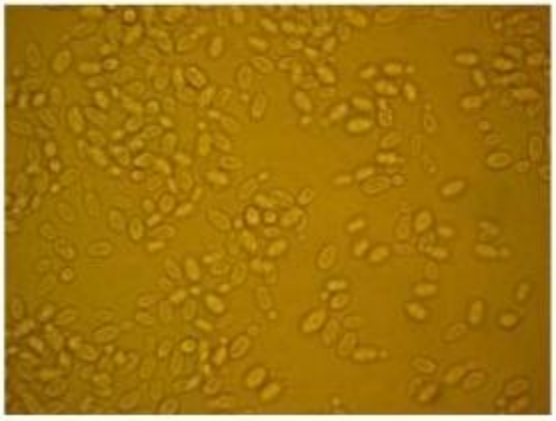

**Figure 8. Yeast from DRBC agar- strawberries at 5°C and 95% relative humidity**

## Molecular Identification of Spoilage Flora

Typical spoilage microflora were isolated onto plate count (PCA) or dichloran rose bengal chloramphenicol (DRBC) agars for bacteria associated with romaine lettuce or yeast and molds associated with strawberries, respectively. Representative colonies were selected from each of these agars and restreaked onto fresh media to obtain pure cultures of each isolate. Total nucleic acids were isolated from individual colonies using the PrepMan Ultra sample preparation reagent (Life Technologies Corporation, Carlsbad, CA) and the polymerase chain reaction (PCR) was used to generate amplicons suitable for sequencing. For bacteria on lettuce, the DA71/DA72 primer pair described by Boye et al., 1999 was used. For yeasts and molds on strawberries, the ITS1/ITS4 primer pair described by Diaz and Fell, 2004 was used. Briefly, PCR was carried out essentially as described by Boye et al., or Diaz and Fell and the forward primer was used for fluorescent cycle sequencing of the resulting amplicons. Sequencing was performed at Iowa State University's Office DNA Sequencing Facility and sequences were compared against the GenBank database using the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the closest-match identification of each isolate. Prior to choosing romaine lettuce as the model test vegetable, initial experiments were conducted to determine if Dole Iceberg head lettuce would be a suitable candidate. Representative DNA sequencing results for this sample, as well as for romaine lettuce and

strawberries are provided below. After conducting tests on the two types of lettuce (Iceberg and romaine) it was clear that romaine spoiled more rapidly and was thus more desirable as a test subject in this experiment.

Sample Description: dole iceberg lettuce

Colony Description: taken directly from DRBC – small, orange smooth colony

Sequencing result: *Rhodosporidium lusitaniae*

Sample Description: romaine lettuce

Colony Description: medium sized, white rough colony

Sequencing result (Ribosomal Database II): *Bacillus* species

Sample Description: romaine lettuce

Colony Description: medium sized, yellow and smooth colony

Sequencing result (Ribosomal Database II): *Pantoea* species

Colony samples taken from strawberries were initially grown on DRBC agar for about 3 days, and then isolated onto PDA (potato dextrose agar). The sequencing results can be seen below.

Sample Description: strawberries

Colony Description: white and smooth

Sequencing result: *Cryptococcus magnus*

Sample Description: strawberries

Colony Description: mold, medium sized, white and rough

Sequencing result: *Cladosporium* species

By confirming the types of spoilage-causing microflora present on the produce, a better idea of what type of handling procedures and required growth media was formed. Because strawberries are acidic, yeast and mold, which are capable of growing under acidic conditions, were the chief microflora found on the surfaces of this model fruit. Therefore,

Oxytetracycline Glucose Yeast Extract (OGYE) agar was used for selective recovery of yeasts and molds from strawberries. The oxytetracycline in this agar is toxic to any bacteria that may be present on the fruit surface. Although bacteria are present on strawberry surfaces in lower numbers than yeasts and molds, they are faster growing than the fungi, potentially allowing relatively low bacterial inocula that may be present to rapidly outgrow fungi on plates not treated with a selective bacterial inhibitor. For romaine lettuce samples, plate count agar, which is able to support the growth of a wide variety of bacteria, was used; as bacteria were identified as the main spoilage microflora on this model vegetable.

A series of initial spoilage experiments were conducted to determine the parameters needed to adequately test for microbial spoilage of strawberries and romaine lettuce. Once these tests runs were completed, the humidity generating environmental chamber set up, and a suitable biological method of test approved, data were collected. Eleven spoilage tests were performed according to the matrix (Table 1). The results from these tests will be discussed in descending order of highest temperature then humidity (e.g. 7°C with 95% RH, then 7°C with 75% etc.).

## Moisture Loss

Although the term “spoilage” is typically used to describe microbial activities, the term may also be used generically to describe non-microbial routes associated with product deterioration. For high-moisture plant-based foods, moisture loss is an important factor impacting product quality and acceptability. Figure 9 through Figure 22 depict weight loss for strawberries and romaine lettuce during the course of each test.

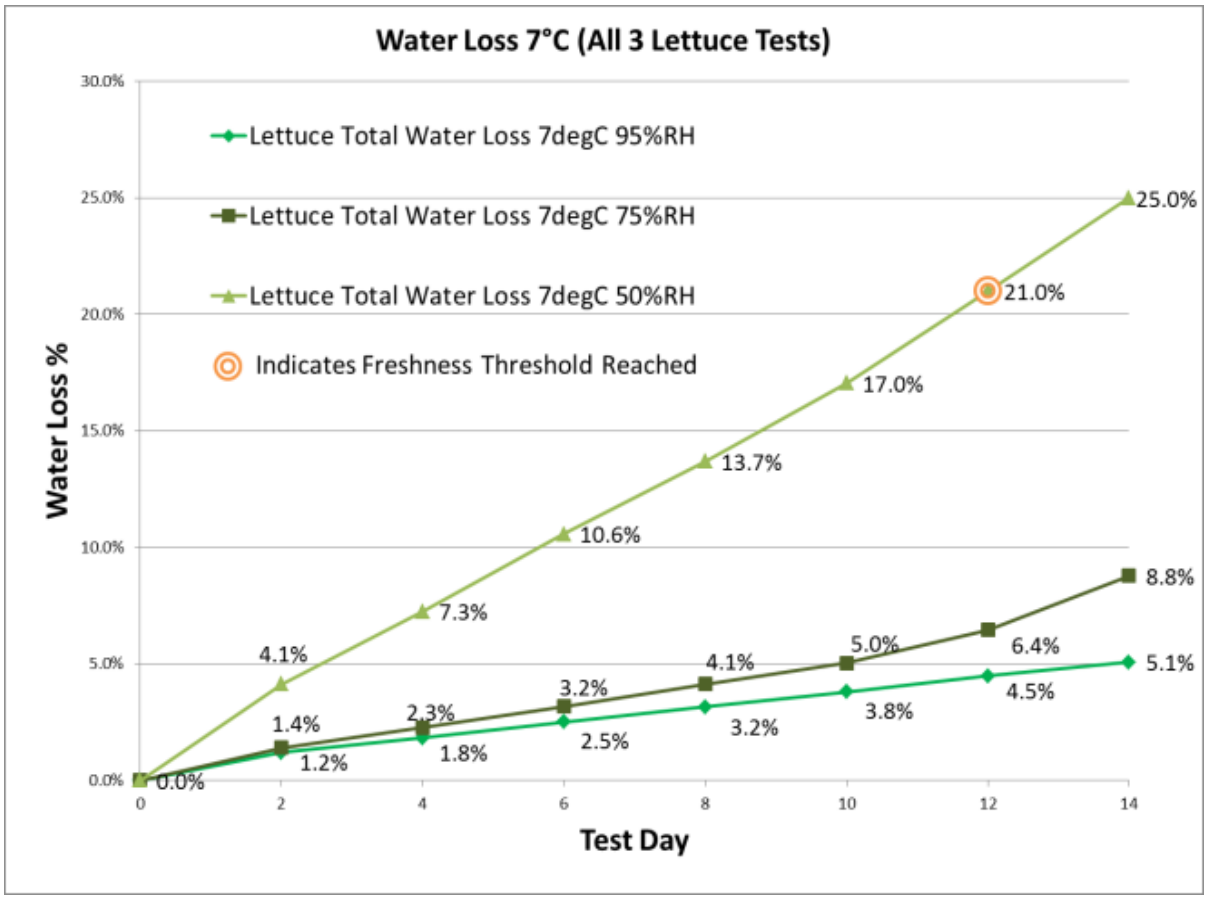


**Figure 9. Water loss data for lettuce (7°C all relative humidities)**

Figure 9 depicts water loss data for lettuce at 7°C and all three relative humidities (95%, 75% and 50%). The legend shows the three tests completed along with a mark indicating where the freshness threshold had been reached. Each data point is shown with a corresponding percentage, indicating the loss of moisture at the time of the test.

For the 7°C tests moisture losses occurred at rates of less than 0.5% to over 2% per day. According to the biological method of test, the established freshness threshold of acceptable moisture losses would be at 20% total weight loss. The only test surpassing this threshold was subjected to the 50% relative humidity setting. For most water loss tests, the second test day (day 2) showed a slightly higher than normal percentage. This effect could be attributable to surface moisture, which may have rapidly evaporated.

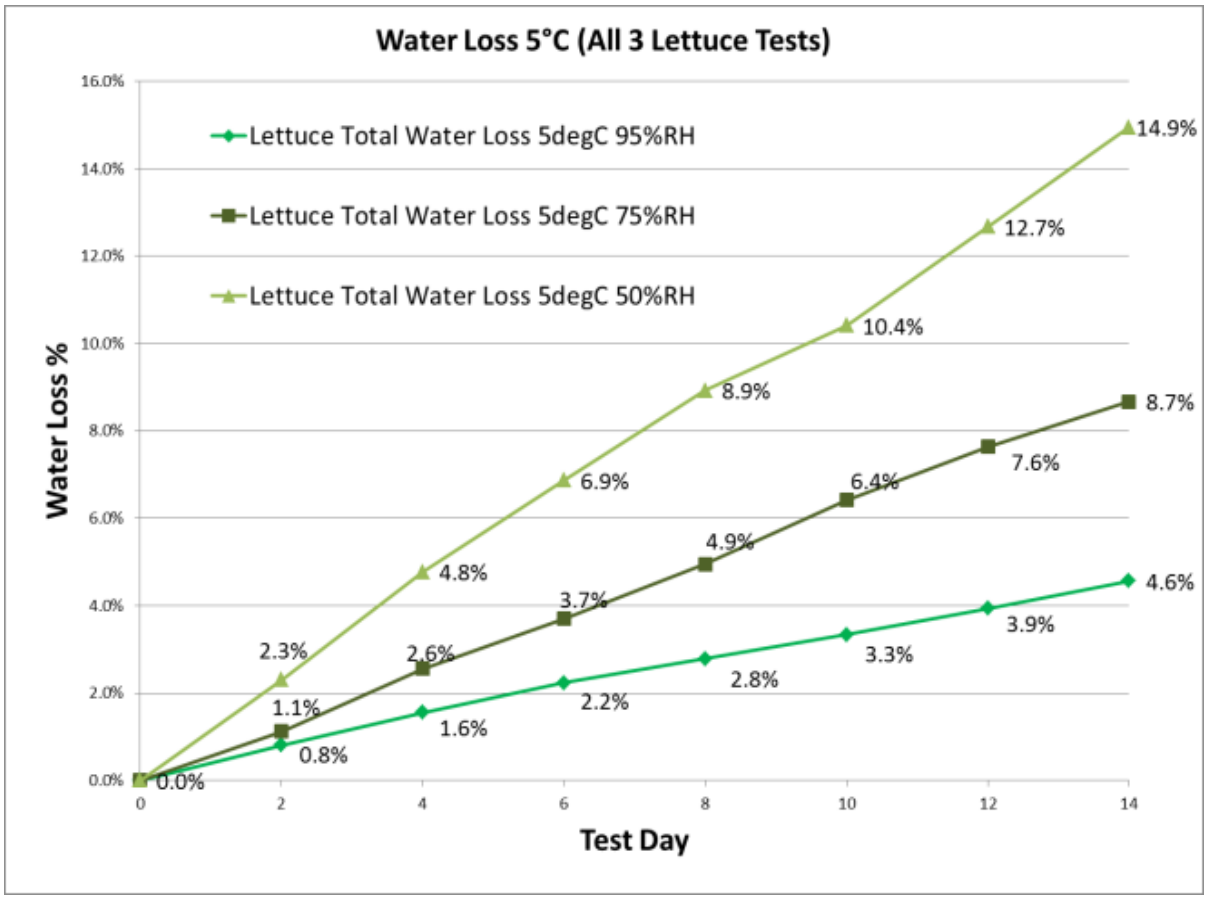


**Figure 10. Water loss data for lettuce (5°C all relative humidities)**

Figure 10 depicts water loss data for lettuce at 5°C and all three relative humidities (95%, 75% and 50%). For these tests no lettuce samples reached the freshness threshold due to moisture loss (20% total weight loss according to the biological method of test). The samples lost moisture in an expected pattern, about 5%, 9%, and 15% at 95%, 75% and 50% relative humidity respectively by day 14. The difference in moisture loss was smaller between the 3°C and 5°C tests than between the 5°C and 7° tests: This pattern was seen in all temperature/moisture loss charts for lettuce.

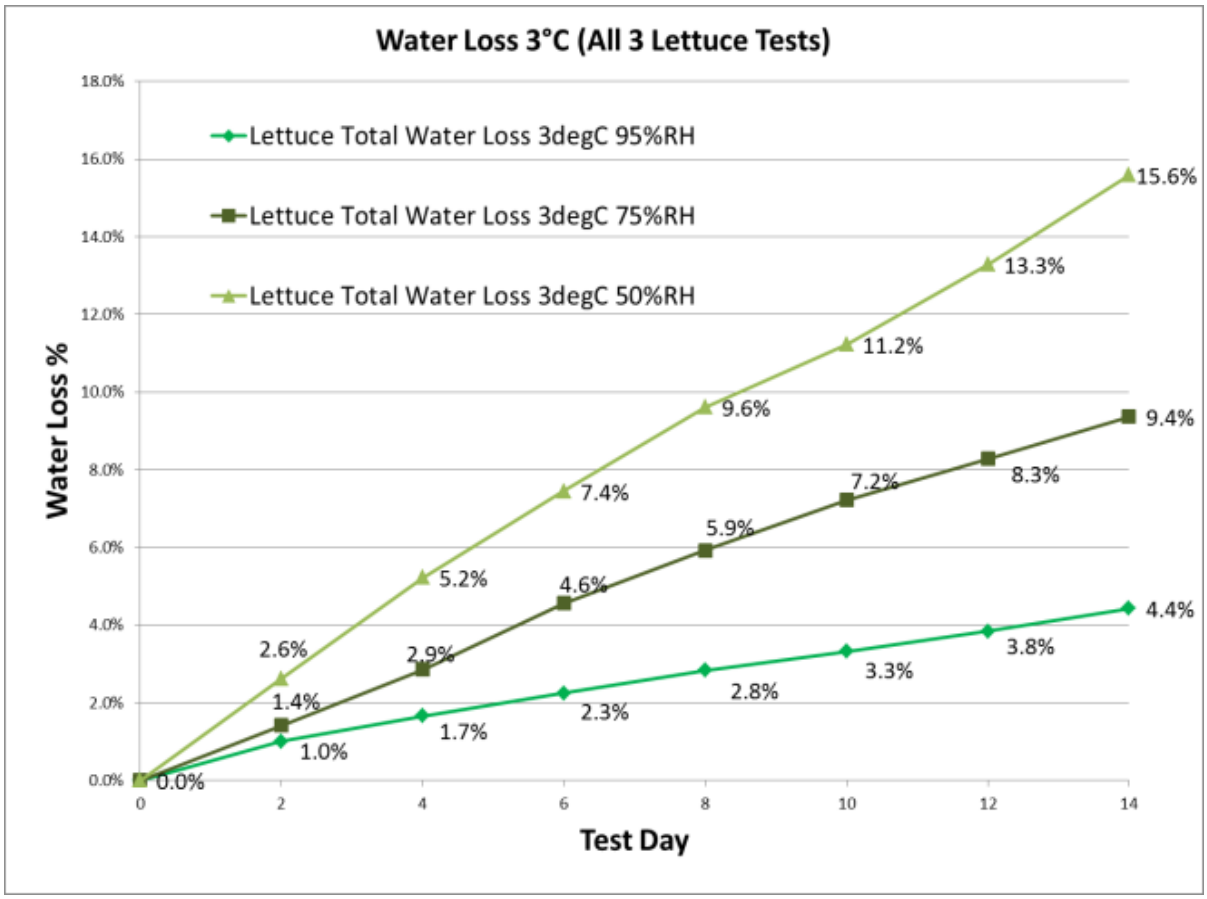


**Figure 11. Water loss data for lettuce (3°C all relative humidities)**

Figure 11 depicts water loss data for lettuce at 3°C and all three relative humidities (95%, 75% and 50%). For these tests no lettuce samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test). These samples lost moisture in an expected pattern, about 4%, 9%, and 16% at 95%, 75% and 50% relative humidity respectively by day 14.

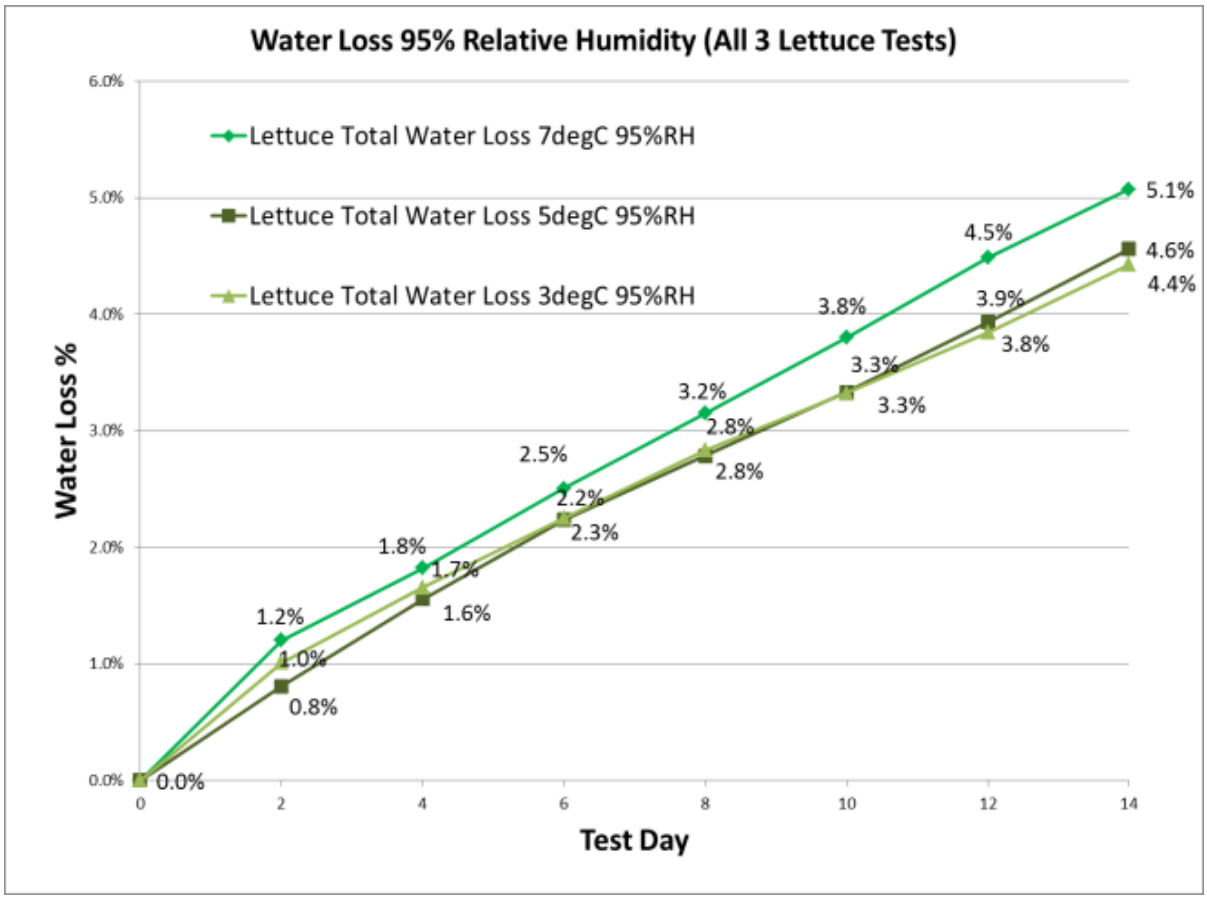


**Figure 12. Water loss data for lettuce (95% relative humidity all temperatures)**

Figure 12 depicts water loss data for lettuce at 95% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests no lettuce samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test). These samples lost moisture at almost exactly the same rate, reaching about 5% total loss within 14 days.

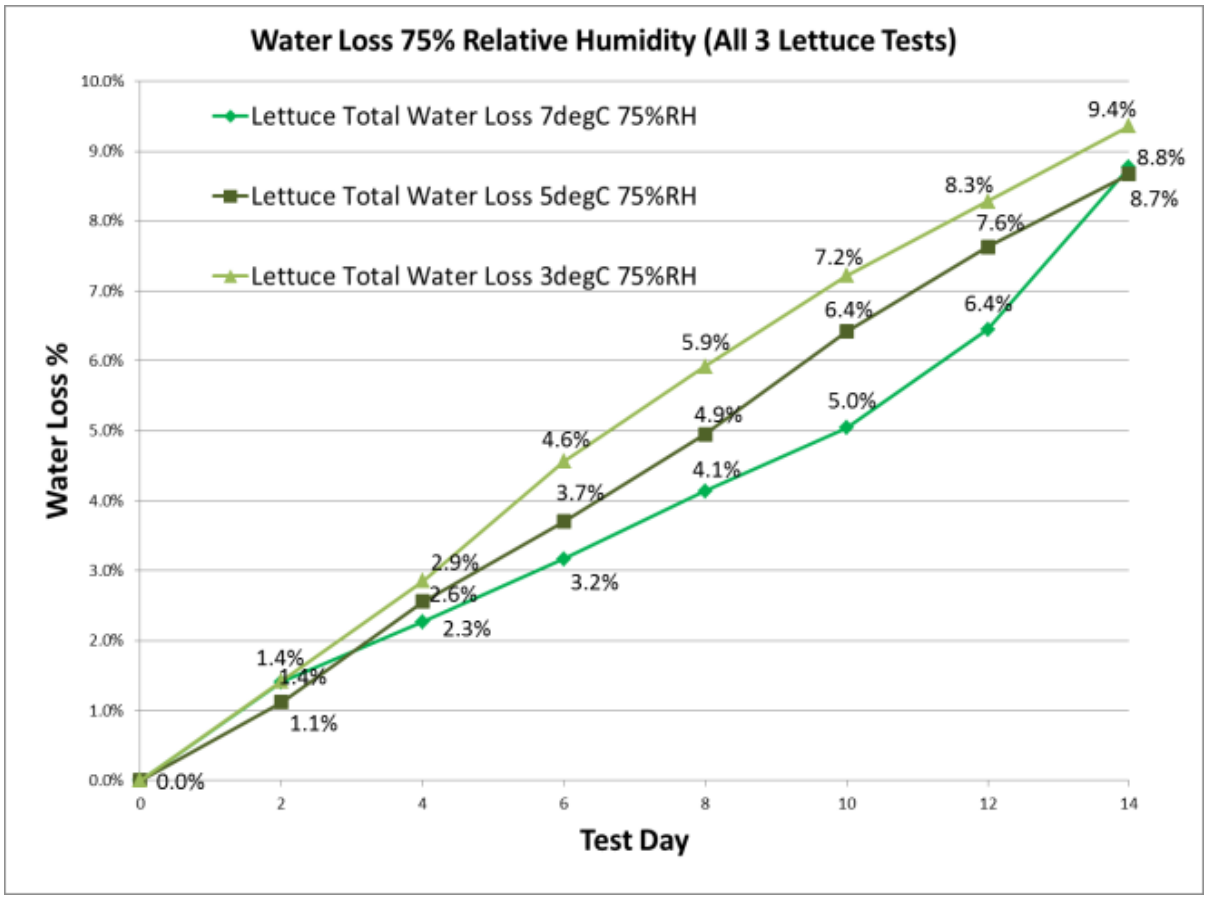


**Figure 13. Water loss data for lettuce (75% relative humidity all temperatures)**

Figure 13 depicts water loss data for lettuce at 75% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests no lettuce samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test). These samples lost moisture at almost exactly the same rate, reaching about 9% total lost at the end of the 14-day test. A possibility for the similar moisture losses it the similar amounts of time the samples spent outside of the environmental chamber for testing every 2 days; the room being much warmer and with generally lower relative humidity. Worth mentioning is the fact that the 7°C test lost less moisture during the 14 days then did the 3°C test, contrary to the 95% and the 75% relative humidity test groups.

Factors that may have contributed to this phenomenon may include leaf arrangement or density of the samples, or perhaps uneven initial moisture levels.

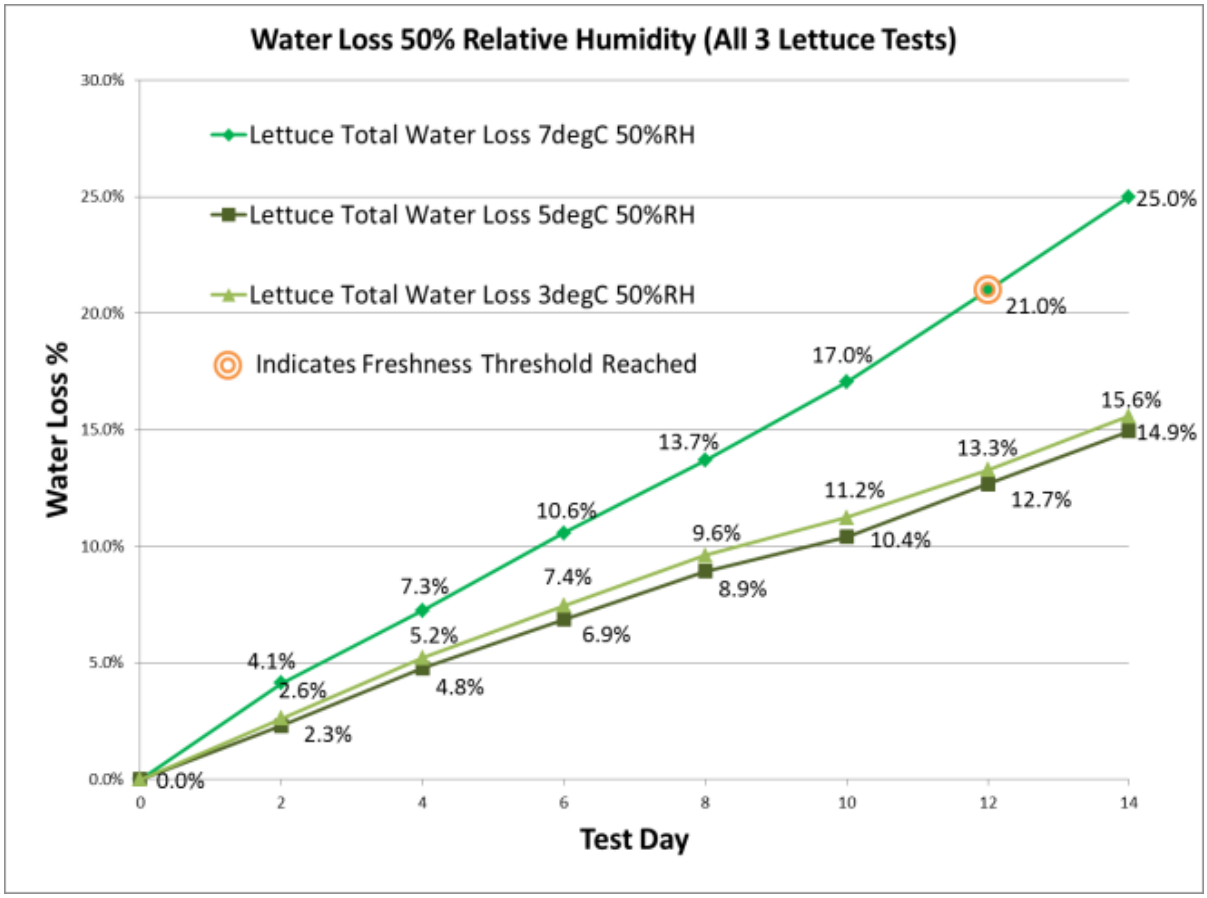


**Figure 14. Water loss data for lettuce (50% relative humidity all temperatures)**

Figure 14 depicts water loss data for lettuce at 50% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests, only the 7°C samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test), arriving at this point sometime between days 10 and 12. The remaining two samples (3°C and 5°C) lost moisture at almost exactly the same rate, reaching about 15% total lost at the end of the 14-day test.

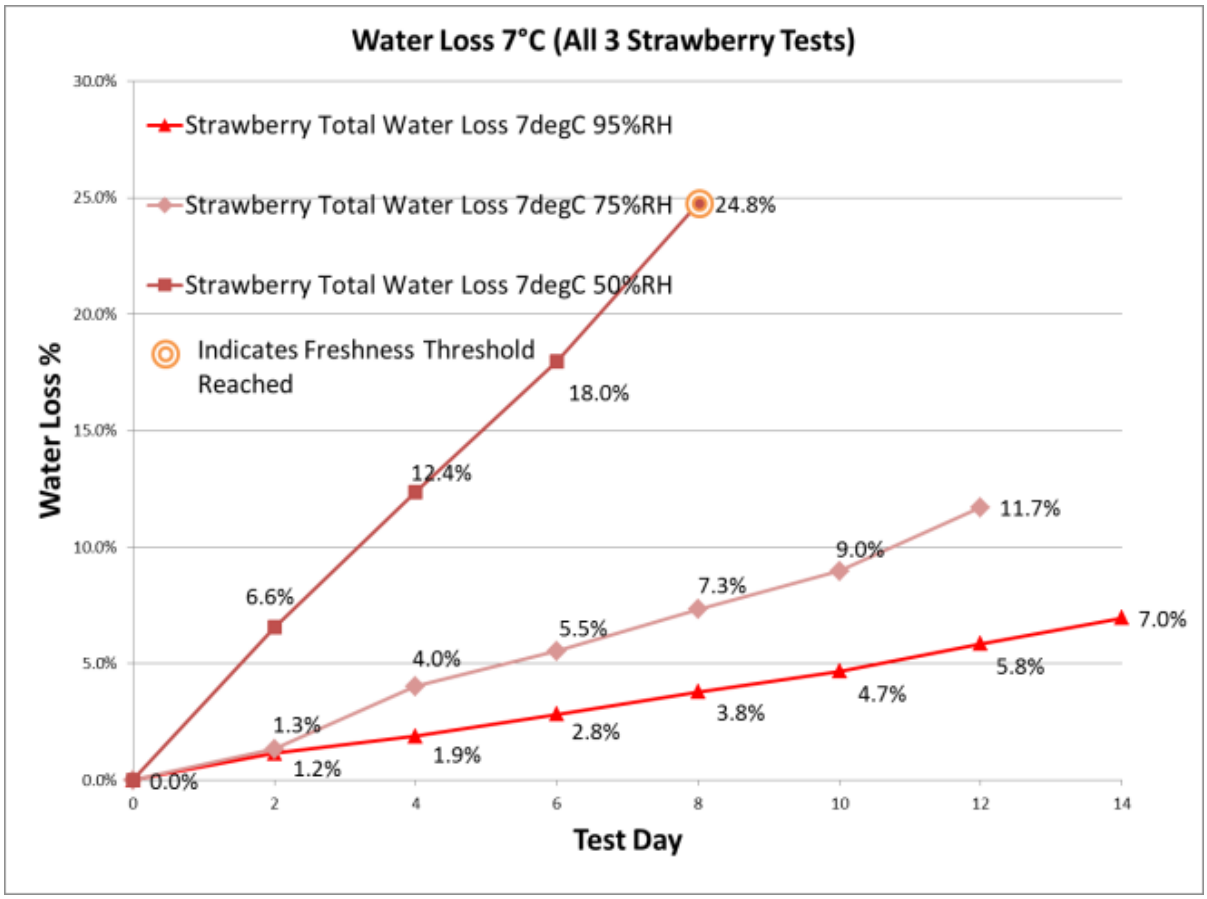


**Figure 15. Water loss data for strawberries (7°C all relative humidities)**

Figure 15 depicts water loss data for strawberries at 7°C and all three relative humidities (95%, 75% and 50%). The legend shows the three tests completed along with a mark indicating where the freshness threshold had been reached. Each data point is shown with a corresponding percentage, indicating the loss of moisture with respect to initial sample mass.

For the 7°C tests, moisture losses occur at rates of about 0.5% to over 3% per day. According to the biological method of test, the freshness threshold of moisture losses occurs at 20% total weight loss. The only test surpassing this threshold was subjected to the 50% relative humidity setting and, reached this level between days 6 and 8. For most water loss tests, the second test day (day 2) showed a slightly higher than normal

percentage. This was most likely due to the fact that surface moisture is present after produce procurement, and evaporates more readily.

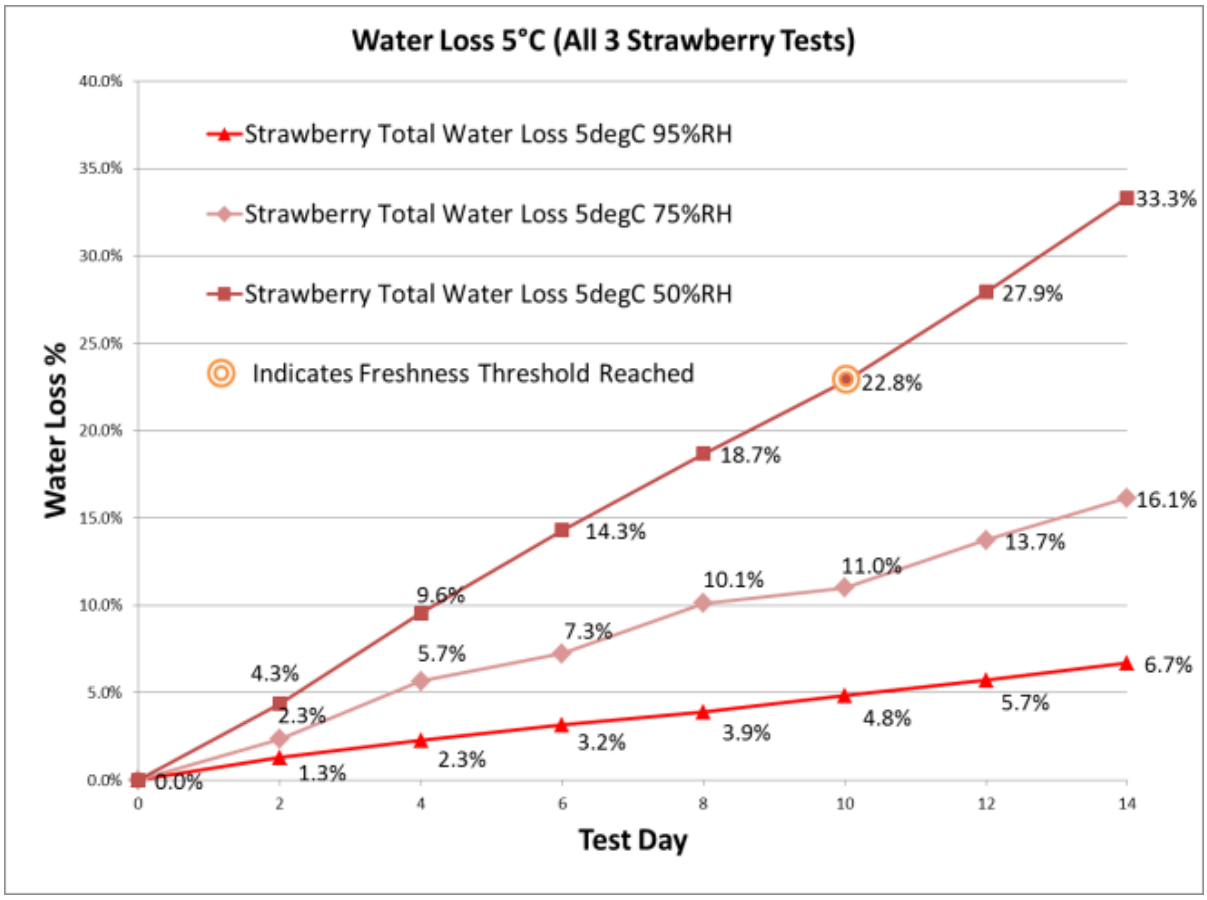


**Figure 16. Water loss data for strawberries (5°C all relative humidities)**

Figure 16 depicts water loss data for strawberries at 5°C and all three relative humidities (95%, 75% and 50%). For these tests, the sample with 50% relative humidity exceeded the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test), reaching this level between days 8 and 10. These samples lost moisture in an expected pattern; in inverse proportion to the percentage relative humidity. The difference in moisture loss is smaller between the 3°C and 5°C tests than between the 5°C and 7° tests: This pattern can be seen in all temperature/moisture loss charts for strawberries.

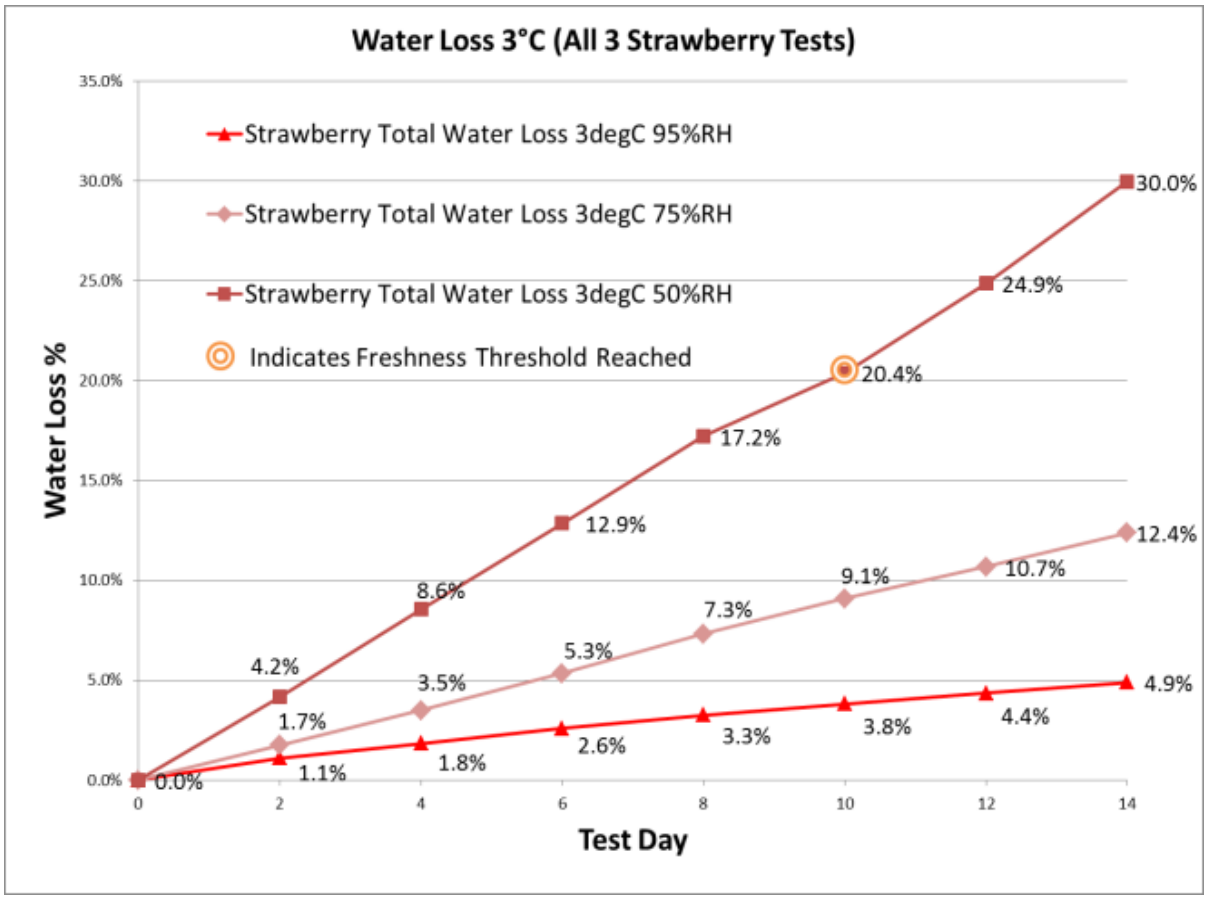


**Figure 17. Water loss data for strawberries (3°C all relative humidities)**

Figure 16 depicts water loss data for strawberries at 3°C and all three relative humidities (95%, 75% and 50%). For these tests, the sample with 50% relative humidity exceeded the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test), reaching this level between days 8 and 10. These samples lost moisture in an expected pattern, in inverse proportion to the percentage relative humidity. The difference in moisture loss was smaller between the 3°C and 5°C tests than between the 5°C and 7° tests: This pattern was seen in all temperature/moisture loss charts for strawberries.

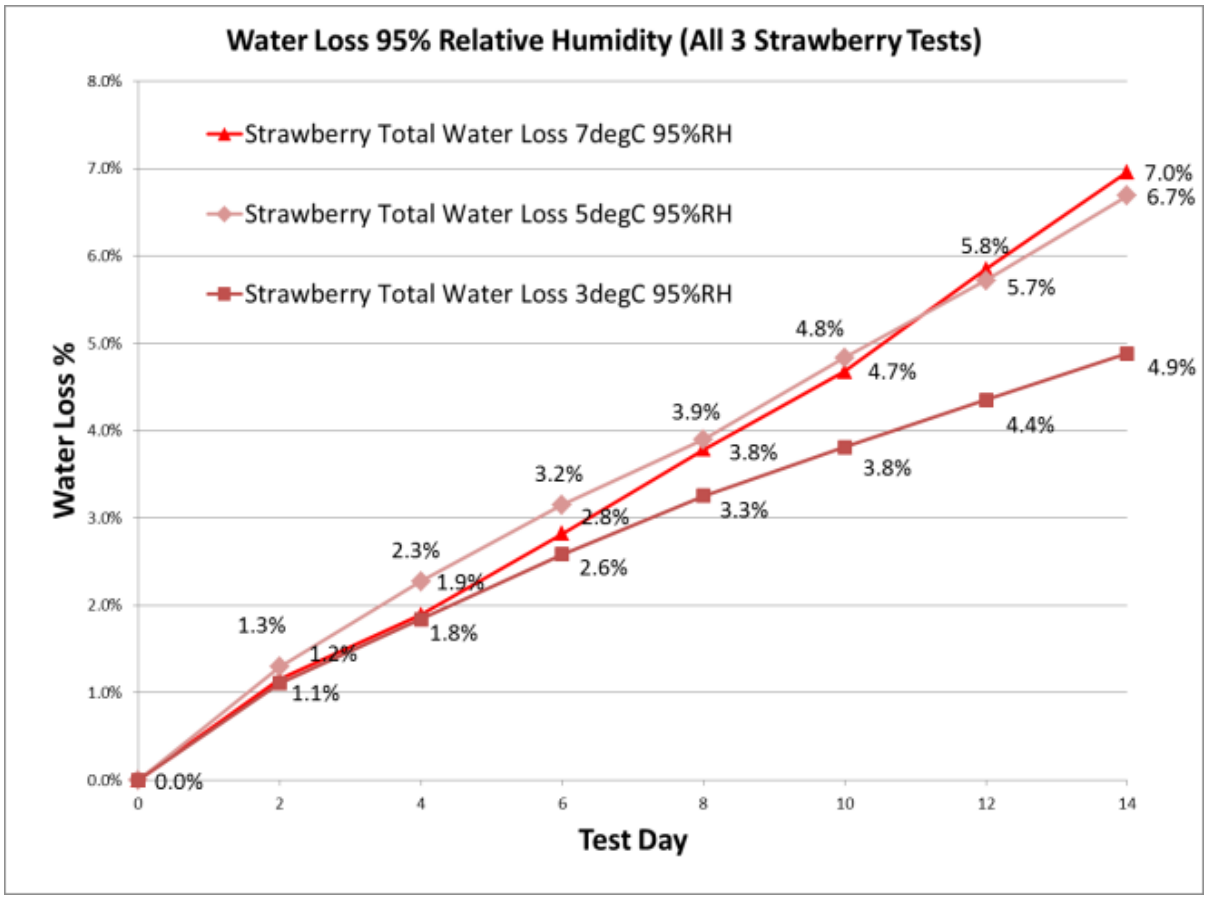


**Figure 18. Water loss data for strawberries (95% relative humidity all temperatures)**

Figure 18 depicts water loss data for strawberries at 95% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests no strawberry samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test). The samples lose moisture at very similar rates, reaching between 5% and 7% total weight lost within 14 days. A possible explanation for the similar moisture losses is the similar amount of time the samples spent outside of the environmental chamber for testing every 2 days; the room being much warmer and with lower relative humidity.

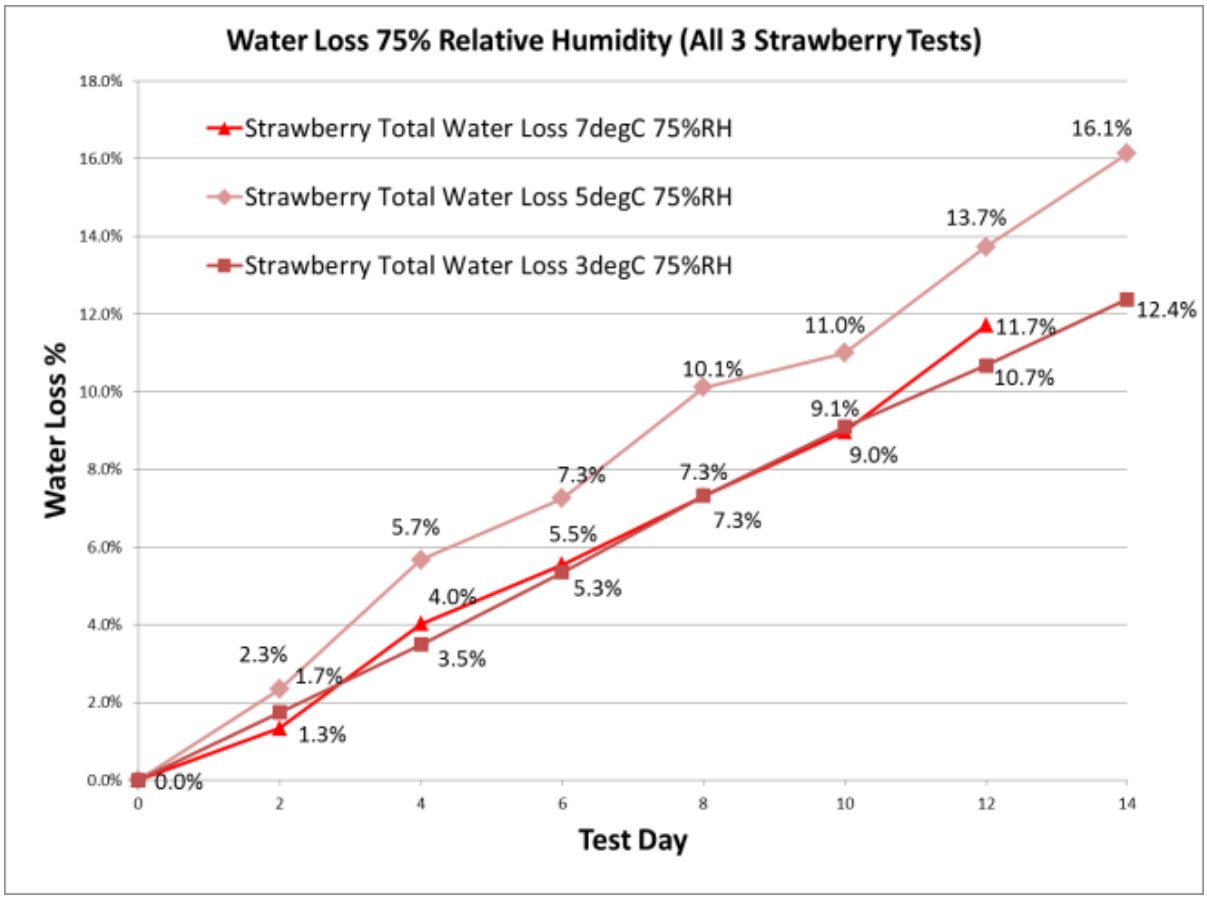


**Figure 19. Water loss data for strawberries (75% relative humidity all temperatures)**

Figure 19 depicts water loss data for lettuce at 75% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests no strawberry samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test). The samples lost moisture at very similar rates, between 11% and 14% by day 12. A possibility for the similar moisture losses is that the samples spent similar amounts of time the outside of the environmental chamber for testing; the room having been much warmer and possessing low relative humidity.

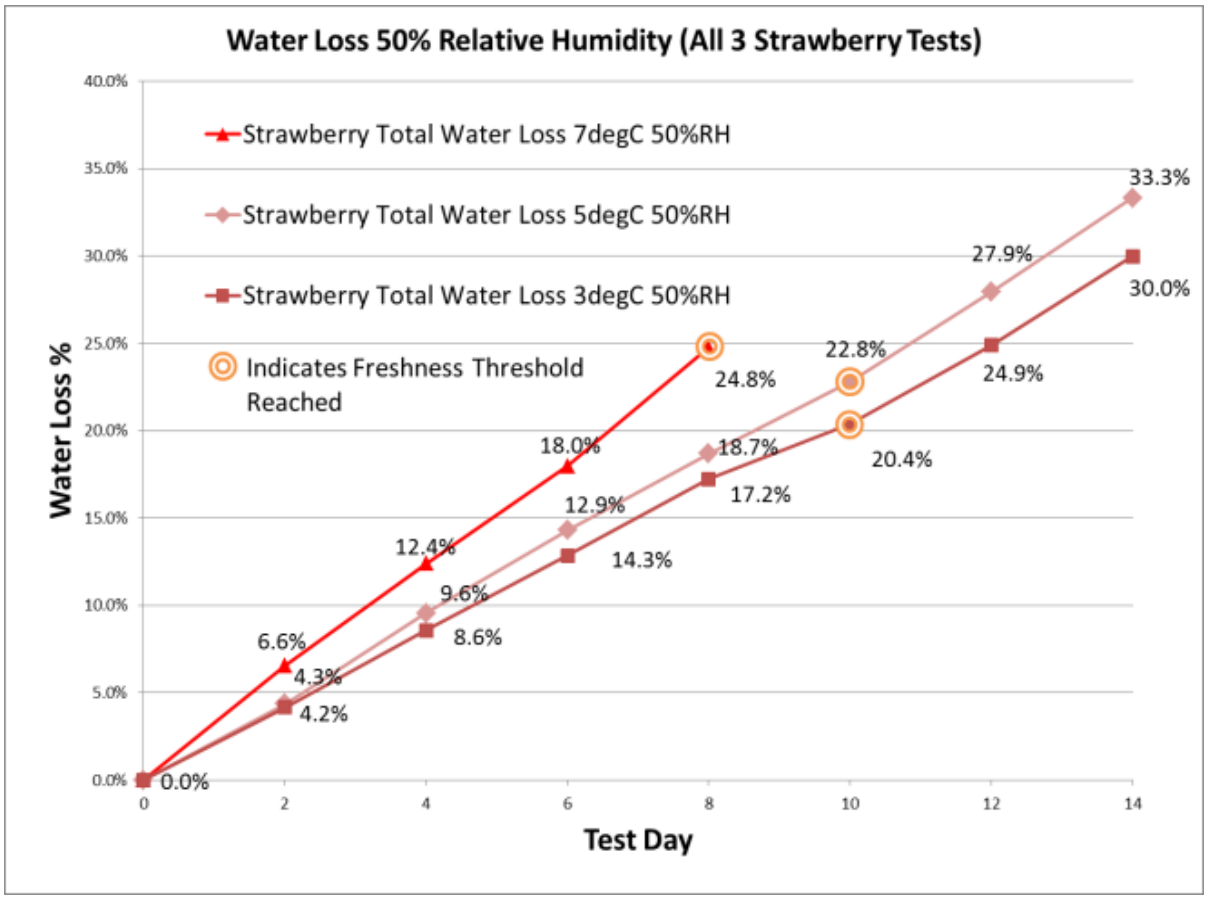


**Figure 20. Water loss data for strawberries (50% relative humidity all temperatures)**

Figure 20 depicts water loss data for strawberries at 50% relative humidity and all three temperatures (3°C, 5°C and 7°C). For these tests, all three samples reached the freshness threshold due to moisture losses (20% total weight loss according to the biological method of test), arriving at this point sometime between days 6 and 8 for the 7°C test, and between days 8 and 10 for tests at 3°C and 5°C. The three samples lost moisture at somewhat similar rates, reaching between 17% and 25% total lost by day 8.

## Moisture Loss Repeatability

The data obtained from a single two-week-test was not statistically significant enough to show repeatability in results. Exacting statistical repeatability is beyond the scope of the research, however, two extra tests were conducted for lettuce and strawberries at 5°C with 75% relative humidity. The parameters of the extra tests (see Table 1) were located in the center of the test matrix. There is value in studying the outcome of three identical spoilage tests. Results for repeatability will be shown at the end of each "RESULTS" chapter (for Water Loss, Photographs/Organoleptic Quality, and Microbial Spoilage).

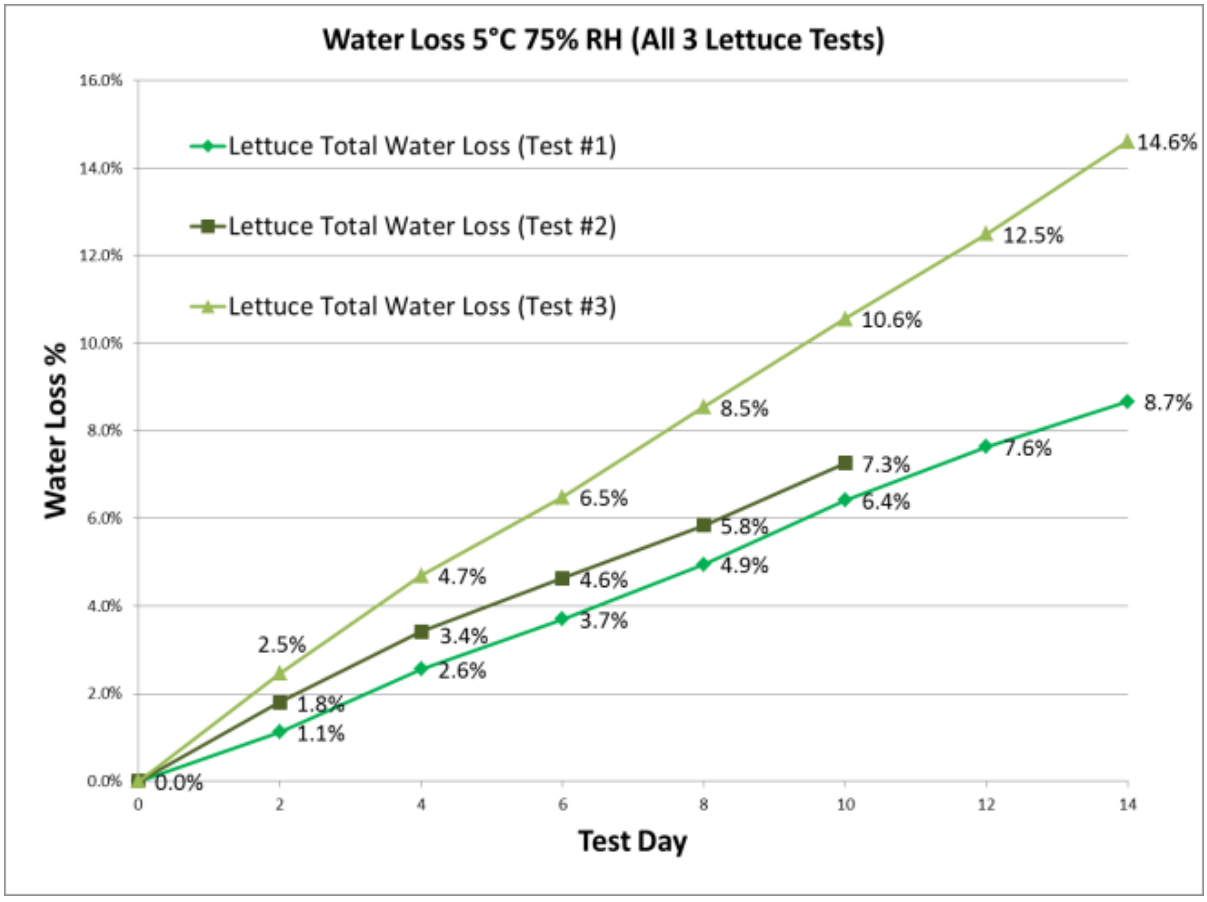


**Figure 21. Water loss data for lettuce (3 tests at 5°C with 75% relative humidity)**

Figure 21 depicts three tests of water loss data for lettuce at 5°C with 75% relative humidity. The initial test was replicated to show the degree of repeatability in determining spoilage

vectors. For these tests, the freshness threshold due to moisture loss (20% loss or greater) was not reached. Test #2 ended early due to environmental conditions in the test chamber exceeding allowable temperature tolerances, though the moisture loss trend remains clear until day 10. Analyzing these moisture loss trends, there is some degree of difference among all of them. Because the air flow rate remained the same, as well as the temperature and humidity levels, some other factor must be responsible for these discrepancies. Such factors could include uneven microbial processes, leaf arrangement or density of the samples, or perhaps uneven initial moisture levels.

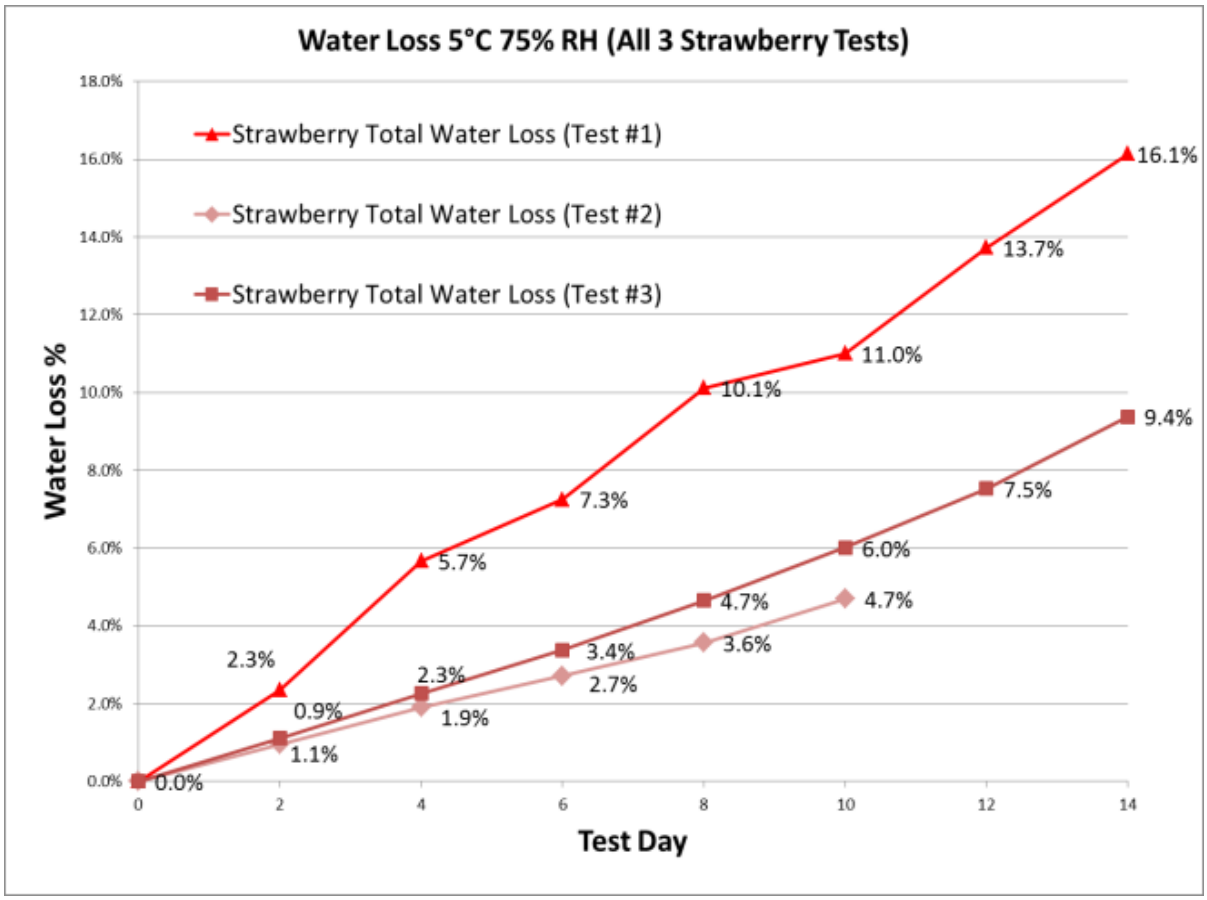


**Figure 22. Water loss data for strawberries (3 tests at 5°C with 75% relative humidity)**

Figure 22 depicts three tests of water loss data for strawberries at 5°C with 75% relative humidity. The initial test was replicated to show the degree of repeatability in determining

spoilage vectors. For these tests, the freshness threshold due to moisture loss (20% loss or greater) was not reached. Test #2 ended early due to complications in the test chamber, though the moisture loss trend remained clear until day 10. Looking at these moisture loss trends, there is some degree of difference among all of them. Because the air flow rate remained the same, as well as the temperature and humidity levels, some other factor contributed to the discrepancies. These factors could include uneven microbial processes, berry arrangement or even the size/density of the berries themselves, or perhaps uneven initial moisture levels. A possibility for this phenomenon is that microbial processes rise and fall causing variation in the degradation of the strawberries' protective skin, allowing more moisture to escape.

## Photographs/Organoleptic Quality

Every other day during the spoilage testing, samples were removed from the environmental chamber and photographed digitally. Photographic spoilage evidence was necessary to show organoleptic quality digression such as dehydration, wilting and sliming in lettuce, and dehydration, loss of firmness and molding in strawberries. When the photographs are displayed in a time-lapse sequence, these spoilage processes can be more easily recognized. Figure 23 through Figure 44 are slide-show displays of lettuce and strawberries for each test, and they correspond to the test days for moisture losses exhibited from Figure 9 to Figure 22, and microbial spoilage enumeration shown in Figure 45 through Figure 74.

Lettuce 7°C 95%RH Day 0

Lettuce 7°C 95%RH Day 2

Lettuce 7°C 95%RH Day 4

Lettuce 7°C 95%RH Day 6

The four pictures shown above as well as the four below are considered part of a single figure (Figure 23). They represent the photographic evidence of unbagged lettuce for each test day (0, 2, 4 etc.). Day '0' represents the starting state of the samples and the first test day. Substantial spoilage aspects detected on certain tests days are described, as well as corresponding relevant data from moisture loss and microbial enumeration tests.

Day 0 for the 7°C 95%RH test shows moist lettuce on a sample tray. Though the humidity level for this test was high, this excess moisture was lost by Day 2 (see Figure 23, moisture

loss for 7°C 95%RH), contributing to almost twice as much moisture loss as the rest of the test days.

Lettuce 7°C 95%RH Day 8

Lettuce 7°C 95%RH Day 10

Lettuce 7°C 95%RH Day 12

Lettuce 7°C 95%RH Day 14

**Figure 23. Collection of photographs for spoilage tests on lettuce (7°C 95% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. There were two small brown areas starting from Day 10 and growing slightly until Day 14. These brown spots were seen on the top middle leaf, and bottom left-most leaf edges.

Lettuce 7°C 75%RH Day 0

Lettuce 7°C 75%RH Day 2

Lettuce 7°C 75%RH Day 4

Lettuce 7°C 75%RH Day 6

The four pictures above as well as the four below belong to Figure 24, a collection of photographs for spoilage tests on lettuce (7°C 75% relative humidity). The Day 0 photograph shows fresh lettuce as it appeared in the test tray immediately after procurement. There is some evidence of brown spotting on the edge of the bottom-left leaf. Some wilting can be seen Day 6 on the bottom leaf, though not enough to consider the specimen beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

Lettuce 7°C 75%RH Day 8

Lettuce 7°C 75%RH Day 10

Lettuce 7°C 75%RH Day 12 Lettuce 7°C 75%RH Day 14

**Figure 24. Collection of photographs for spoilage tests on lettuce (7°C 75% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. There was one small brown area noted, growing very slightly from Day 0 until Day 14. These brown spots can be seen on the bottom left leaf edges. Some wilting did occur on the bottom leaves, however this was not substantial enough for the entire head to be considered spoiled.

Lettuce 7°C 50%RH Day 0 Lettuce 7°C 50%RH Day 2

Lettuce 7°C 50%RH Day 4

Lettuce 7°C 50%RH Day 6

The four pictures above as well as the four below belong to Figure 25, a collection of photographs for spoilage tests on lettuce (7°C 50% relative humidity). Day 0 shows fresh lettuce in a test tray immediately after procurement. There was some evidence of brown spotting on the middle top leaf, though this was not the spoilage path for this test. This test represents the fastest spoilage rate due to drying for all low humidity tests.

Lettuce 7°C 50%RH Day 8

Lettuce 7°C 50%RH Day 10

Lettuce 7°C 50%RH Day 12 (Supplemental)

Lettuce 7°C 50%RH Day 14

**Figure 25. Collection of photographs for spoilage tests on lettuce (7°C 50% relative humidity)**

Significant wilting began to occur starting on Day 6 and by Day 10 covered a significant portion of the outer leaves. This wilting did not constitute a breach of the freshness threshold because a majority of inner leaves remained firm. By Day 12, the lettuce exceeded the freshness threshold due to water loss and sufficient wilting (1/3 to 1/2 of the total surface according to the biological method of test). The Day 14 photo was recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Lettuce 5°C 95%RH Day 0

Lettuce 5°C 95%RH Day 2

Lettuce 5°C 95%RH Day 4

Lettuce 5°C 95%RH Day 6

The four pictures above as well as the four below belong to Figure 26, a collection of photographs for spoilage tests on lettuce (5°C 95% relative humidity). Day 0 shows fresh lettuce in a test tray immediately after procurement. There was some evidence of brown spotting on the top-left leaf and very faint traces of brown on the bottom-left; most easily seen on the picture for Day 6.

Lettuce 5°C 95%RH Day 8

Lettuce 5°C 95%RH Day 10

Lettuce 5°C 95%RH Day 12

Lettuce 5°C 95%RH Day 14

**Figure 26. Collection of photographs for spoilage tests on lettuce (5°C 95% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. The small brown areas mentioned previously grew slightly before the end of the test on Day 14. These brown spots did not cover a significant enough portion of the sample surface to be at the freshness threshold (5% of sample according to biological method of test).

Lettuce 5°C 75%RH Day 0

Lettuce 5°C 75%RH Day 2

Lettuce 5°C 75%RH Day 4

Lettuce 5°C 75%RH Day 6

The four pictures above as well as the three below belong to Figure 27, a collection of photographs for spoilage tests on lettuce (5°C 75% relative humidity). Day 0 showed fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting or wilting for the 6 test days shown.

Lettuce 5°C 75%RH Day 8

Lettuce 5°C 75%RH Day 10

Lettuce 5°C 75%RH Day 12

**Figure 27. Collection of photographs for spoilage tests on lettuce (5°C 75% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. By Day 12, a small amount of wilting was present and most easily seen on the bottom left leaves, though not enough to consider the samples beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

Lettuce 5°C 50%RH Day 0

Lettuce 5°C 50%RH Day 2

Lettuce 5°C 50%RH Day 4

Lettuce 5°C 50%RH Day 6

The four pictures above as well as the three below belong to Figure 28, a collection of photographs for spoilage tests on lettuce (5°C 50% relative humidity). The Day 0 photo shows fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting, but were others signs of wilting by Day 6.

Lettuce 5°C 50%RH Day 8

Lettuce 5°C 50%RH Day 10

Lettuce 5°C 50%RH Day 12

**Figure 28. Collection of photographs for spoilage tests on lettuce (5°C 50% relative humidity)**

Significant wilting began to occur starting on Day 6 and by Day 12 covered a substantial enough portion of the outer leaves to be considered beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

Lettuce 3°C 95%RH Day 0

Lettuce 3°C 95%RH Day 2

Lettuce 3°C 95%RH Day 4

Lettuce 3°C 95%RH Day 6

The four pictures above as well as the four below belong to Figure 29, a collection of photographs for spoilage tests on lettuce (3°C 95% relative humidity). Day 0 photo shows fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting and only very faint traces of wilting by Day 6.

Lettuce 3°C 95%RH Day 8

Lettuce 3°C 95%RH Day 10

Lettuce 3°C 95%RH Day 12

Lettuce 3°C 95%RH Day 14

**Figure 29. Collection of photographs for spoilage tests on lettuce (3°C 95% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. The small amount of wilting mentioned previously increased slightly before the end of the test on Day 14. Wilting mostly affected the outer leaves and was not widespread enough to consider the samples beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

Lettuce 3°C 75%RH Day 0

Lettuce 3°C 75%RH Day 2

Lettuce 3°C 75%RH Day 4

Lettuce 3°C 75%RH Day 6

The four pictures above as well as the four below belong to Figure 30, a collection of photographs for spoilage tests on lettuce (3°C 75% relative humidity). The Day 0 photo shows fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting and only very faint traces of wilting by Day 6.

Lettuce 3°C 75%RH Day 8

Lettuce 3°C 75%RH Day 10

Lettuce 3°C 75%RH Day 12

Lettuce 3°C 75%RH Day 14

**Figure 30. Collection of photographs for spoilage tests on lettuce (3°C 75% relative humidity)**

Very little evidence of spoilage occurred during the course of this test run. The small amount of wilting mentioned previously increased slightly before the end of the test on Day 14. Wilting affected mostly the outer leaves on the bottom of the sample and was not widespread enough to consider the samples beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

Lettuce 3°C 50%RH Day 0

Lettuce 3°C 50%RH Day 2

Lettuce 3°C 50%RH Day 4

Lettuce 3°C 50%RH Day 6

The four pictures above as well as the four below belong to Figure 31, a collection of photographs for spoilage tests on lettuce (3°C 50% relative humidity). The Day 0 photo shows fresh lettuce in a test tray immediately after procurement. There was some evidence of brown spotting on the bottom-left leaf, though these spots did not grow substantially during the course of this test. There was some evidence of wilting by Day 4.

Lettuce 3°C 50%RH Day 8

Lettuce 3°C 50%RH Day 10

Lettuce 3°C 50%RH Day 12

Lettuce 3°C 50%RH Day 14 (Supplemental)

**Figure 31. Collection of photographs for spoilage tests on lettuce (3°C 50% relative humidity)**

Significant wilting began to occur starting on Day 6 and by Day 12 covered a significant enough portion of the outer leaves to be considered beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test). Day 14 is recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Strawberries 7°C 95%RH Day 0

Strawberries 7°C 95%RH Day 2

Strawberries 7°C 95%RH Day 4

Strawberries 7°C 95%RH Day 6

The four pictures shown above as well as the four below are considered part of a single figure (Figure 32). They represent the photographic evidence of strawberries for each test day (0, 2, etc.). Day '0' represents the starting state of the samples. As indicated in the biological method of test, before starting the experiment, the strawberries were examined visually and the worst 5% (discolored, physically damaged, visually molded) were discarded. Substantial spoilage detected on certain tests days will be described, as well as corresponding relevant data from moisture loss and microbial enumeration tests.

Day 0 for the 7°C 95%RH test shows fresh, ripe strawberries on a sample tray. By Day 6, there was visible mold growth on the bottom-right berry.

Strawberries 7°C 95%RH Day 8

Strawberries 7°C 95%RH Day 10

Strawberries 7°C 95%RH Day 12

Strawberries 7°C 95%RH Day 14 (Supplemental)

**Figure 32. Collection of photographs for spoilage tests on strawberries (7°C 95% relative humidity)**

Mold growth continued to overtake the strawberry samples, and by Day 12, they were considered beyond the freshness threshold (25% of berries covered by mold according to the biological method of test). Day 14 is recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Strawberries 7°C 75%RH Day 0

Strawberries 7°C 75%RH Day 2

Strawberries 7°C 75%RH Day 4

Strawberries 7°C 75%RH Day 6

The four pictures above as well as the three below belong to Figure 33, a collection of photographs for spoilage tests on strawberries (7°C 75% relative humidity). The Day 0

photo shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of molding on the top-left berry that was seen as early as Day 2. By Day 6, several more berries began to grow mold.

Strawberries 7°C 75%RH Day 8

Strawberries 7°C 75%RH Day 10

Strawberries 7°C 75%RH Day 12 (Supplemental)

**Figure 33. Collection of photographs for spoilage tests on strawberries (7°C 75% relative humidity)**

Mold growth continued to overtake the strawberry samples, and by Day 10, they were considered beyond the freshness threshold (25% of berries covered by mold according to the biological method of test). Day 12 was recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Strawberries 7°C 50%RH Day 0

Strawberries 7°C 50%RH Day 2

Strawberries 7°C 50%RH Day 4

Strawberries 7°C 50%RH Day 6

The four pictures above as well as the one below belong to Figure 34, a collection of photographs for spoilage tests on strawberries (7°C 50% relative humidity). The Day 0

photo shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of molding on a berry left of middle-center that could be seen as early as Day 2. By Day 6, several more berries began to grow mold. According to Figure 9, these strawberries were considered beyond the freshness threshold due to moisture loss by Day 6, having lost more than 20% of their original water content.

Strawberries 7°C 50%RH Day 8

**Figure 34. Collection of photographs for spoilage tests on strawberries (7°C 50% relative humidity)**

Mold growth continued to overtake the strawberry samples, and by Day 8, they were again considered beyond the freshness threshold (25% of berries covered by mold according to the biological method of test).

Strawberries 5°C 95%RH Day 0

Strawberries 5°C 95%RH Day 2

Strawberries 5°C 95%RH Day 4

Strawberries 5°C 95%RH Day 6

The four pictures above as well as the four below belong to Figure 35, a collection of photographs for spoilage tests on strawberries (5°C 95% relative humidity). The Day 0 photo shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of molding on the right-most high-of-center berry that could be seen as early as Day 4.

Strawberries 5°C 95%RH Day 8

Strawberries 5°C 95%RH Day 10

Strawberries 5°C 95%RH Day 12

Strawberries 5°C 95%RH Day 14 (Supplemental)

**Figure 35. Collection of photographs for spoilage tests on strawberries (5°C 95% relative humidity)**

Mold growth continued to overtake the strawberry samples, and by Day 12, they were considered beyond the freshness threshold (25% of berries covered by mold according to the biological method of test). Day 14 was recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Strawberries 5°C 75%RH Day 0

Strawberries 5°C 75%RH Day 2

Strawberries 5°C 75%RH Day 4

Strawberries 5°C 75%RH Day 6

The four pictures above as well as the three below belong to Figure 36, a collection of photographs for spoilage tests on strawberries (5°C 75% relative humidity). The Day 0 photo shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of molding on the high-right of center berry that could be seen as early as Day 6.

Strawberries 5°C 75%RH Day 8

Strawberries 5°C 75%RH Day 10

Strawberries 5°C 75%RH Day 12

**Figure 36. Collection of photographs for spoilage tests on strawberries (5°C 75% relative humidity)**

Mold growth continues to overtake the strawberry samples, and by Day 12, they are considered beyond the freshness threshold (25% of berries covered by mold according to the biological method of test).

Strawberries 5°C 50%RH Day 0

Strawberries 5°C 50%RH Day 2

Strawberries 5°C 50%RH Day 4

Strawberries 5°C 50%RH Day 6

The four pictures above as well as the three below belong to Figure 37, a collection of photographs for spoilage tests on strawberries (5°C 50% relative humidity). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of lack of firmness due to moisture loss on several of the berries that can be seen as early as Day 6.

Strawberries 5°C 50%RH Day 8

Strawberries 5°C 50%RH Day 10

Strawberries 5°C 50%RH Day 12 (Supplemental)

**Figure 37. Collection of photographs for spoilage tests on strawberries (5°C 50% relative humidity)**

Strawberry samples continued to loose moisture and by Day 10 (according to the data in Figure 10), they were considered beyond the threshold of freshness (20% weight loss according to the biological method of test). Day 12 is recorded purely for curiosity sake and has no bearing on spoilage data for this experiment.

Strawberries 3°C 95%RH Day 0

Strawberries 3°C 95%RH Day 2

Strawberries 3°C 95%RH Day 4

Strawberries 3°C 95%RH Day 6

The four pictures above as well as the four below belong to Figure 38, a collection of photographs for spoilage tests on strawberries (3°C 95% relative humidity). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was no evidence of molding or moisture loss by Day 6.

Strawberries 3°C 95%RH Day 8 Strawberries 3°C 95%RH Day 10

Strawberries 3°C 95%RH Day 12 Strawberries 3°C 95%RH Day 14

**Figure 38. Collection of photographs for spoilage tests on strawberries (3°C 95% relative humidity)**

Mold growth can be seen on a strawberry by Day 10 (right and high of center); however there was no significant spreading of this mold by the last day of the test (Day 14). Neither mold nor moisture losses were significant enough to exceed the threshold of freshness during this test.

Strawberries 3°C 75%RH Day 0

Strawberries 3°C 75%RH Day 2

Strawberries 3°C 75%RH Day 4

Strawberries 3°C 75%RH Day 6

The four pictures above as well as the four below belong to Figure 39, a collection of photographs for spoilage tests on strawberries (3°C 75% relative humidity). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was no evidence of molding or moisture loss; however, by Day 6 there was some loss of firmness seen in a few berries (specifically the left-most center berry).

Strawberries 3°C 75%RH Day 8

Strawberries 3°C 75%RH Day 10

Strawberries 3°C 75%RH Day 12

Strawberries 3°C 75%RH Day 14

**Figure 39. Collection of photographs for spoilage tests on strawberries (3°C 75% relative humidity)**

Some loss of firmness was seen on a few strawberries by Day 8 (left of tray, and center); however this was not significant enough to be considered beyond the threshold of freshness by the last day of the test (Day 14). Neither mold nor moisture losses nor microbial numbers were significant enough to constitute threshold of freshness breach during this test.

Strawberries 3°C 50%RH Day 0

Strawberries 3°C 50%RH Day 2

Strawberries 3°C 50%RH Day 4

Strawberries 3°C 50%RH Day 6

The four pictures above as well as the four below belong to Figure 40, a collection of photographs for spoilage tests on strawberries (3°C 50% relative humidity). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was some evidence of lack of firmness due to moisture loss on several of the berries that can be seen as early as Day 4.

Strawberries 3°C 50%RH Day 8

Strawberries 3°C 50%RH Day 10

Strawberries 3°C50%RHDay12(Supplemental)

Strawberries 3°C50%RHDay14(Supplemental)

**Figure 40. Collection of photographs for spoilage tests on strawberries (3°C 50% relative humidity)**

Strawberry samples continued to loose moisture and by Day 10 (according to the data in Figure 11), they were considered beyond the threshold of freshness (20% weight loss according to the biological method of test). Days 12-14 are recorded purely for curiosity sake and have no bearing on spoilage data for this experiment.

## Photographs/Organoleptic Quality Repeatability

Two extra sets of spoilage tests were conducted for lettuce and strawberries stored at 5°C and 75% relative humidity. The following are photographs and descriptions of organoleptic quality of these tests. Noteworthy comparisons in these tests with respect to organoleptic quality are described in the "CONCLUSIONS" section.

Lettuce 5°C 75%RH TEST #2 Day 0

Lettuce 5°C 75%RH TEST #2 Day 2

Lettuce 5°C 75%RH TEST #2 Day 4

Lettuce 5°C 75%RH TEST #2 Day 6

The four pictures above as well as the two below belong to Figure 41, a collection of photographs for spoilage tests on lettuce (5°C 75% relative humidity TEST #2). Day 0 shows

fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting or wilting for the 6 test days shown.

Lettuce 5°C 75%RH TEST #2 Day 8 Lettuce 5°C 75%RH TEST #2 Day 10

**Figure 41. Collection of photographs for spoilage tests on lettuce (5°C 75% RH TEST #2)**

Very little evidence of spoilage occurred during the course of this test run. By Day 10, a small amount of wilting was present and most easily seen on the lower head on the outer leaf, though not enough to consider the samples beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test). This test ended on day 10 due to complications with the environmental chamber.

Lettuce 5°C 75%RH TEST #3 Day 0

Lettuce 5°C 75%RH TEST #3 Day 2

Lettuce 5°C 75%RH TEST #3 Day 4

Lettuce 5°C 75%RH TEST #3 Day 6

The four pictures above as well as the four below belong to Figure 42, a collection of photographs for spoilage tests on lettuce (5°C 75% relative humidity TEST #3). Day 0 shows fresh lettuce in a test tray immediately after procurement. There was no evidence of brown spotting or wilting for the 6 test days shown.

Lettuce 5°C 75%RH TEST #3 Day 8

Lettuce 5°C 75%RH TEST #3 Day 10

Lettuce 5°C 75%RH TEST #3 Day 12

Lettuce 5°C 75%RH TEST #3 Day 14

**Figure 42. Collection of photographs for spoilage tests on lettuce (5°C 75% RH TEST #3)**

Very little evidence of spoilage occurred during the course of this test run. By Day 14, a small amount of wilting was present and most easily seen on the upper head on the outer leaf, though not enough to consider the samples beyond the freshness threshold (1/3 to 1/2 of the total surface according to the biological method of test).

The two extra tests were conducted to show repeatability in this experiment. There were no significant differences in the organoleptic qualities of these samples throughout the three tests. There were some significant difference in moisture loss between the three tests; however the samples did not exhibit spoilage beyond the freshness threshold.

Strawberries 5°C 75%RH TEST #2 Day 0

Strawberries 5°C 75%RH TEST #2 Day 2

Strawberries 5°C 75%RH TEST #2 Day 4

Strawberries 5°C 75%RH TEST #2 Day 6

The four pictures above as well as the two below belong to Figure 43, a collection of photographs for spoilage tests on strawberries (5°C 75% relative humidity TEST #2). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was no evidence of molding by Day 6.

Strawberries 5°C 75%RH TEST #2 Day 8

Strawberries 5°C 75%RH TEST #2 Day 10

**Figure 43. Collection of photographs for spoilage tests on strawberries (5°C 75% RH TEST #2)**

The freshness threshold was not exceeded during the 10 days of this test. This test ended on day 10 due to complications with the environmental chamber.

Strawberries 5°C 75%RH TEST #3 Day 0

Strawberries 5°C 75%RH TEST #3 Day 2

Strawberries 5°C 75%RH TEST #3 Day 4

Strawberries 5°C 75%RH TEST #3 Day 6

The four pictures above as well as the four below belong to Figure 44, a collection of photographs for spoilage tests on strawberries (5°C 75% relative humidity TEST #3). Day 0 shows fresh strawberries in a test tray immediately after procurement and discarding the worst 5%. There was no evidence of molding by Day 6.

Strawberries 5°C 75%RH TEST #3 Day 8

Strawberries 5°C 75%RH TEST #3 Day 10

Strawberries 5°C 75%RH TEST #3 Day 12

Strawberries 5°C 75%RH TEST #3 Day 14

**Figure 44. Collection of photographs for spoilage tests on strawberries (5°C 75% RH TEST #3)**

Some evidence of mold growth was seen by Day 10, worsening until the last day (Day 14). These samples were not considered beyond the threshold of freshness (25% of berries covered by mold according to the biological method of test) during the course of this 14-day test.

The two extra tests at this temperature and humidity (5°C 75%) were conducted to show repeatability in this experiment. The results show that only the first test exceeded the freshness threshold during the 14 days. The results for the second test were inconclusive. As for the third test, significant mold coverage occurred on the last day, showing that the samples would have gone beyond the threshold of freshness about 1-2 days afterward. One factor most likely affecting this discrepancy could be initial microflora count, which will be compared later in this chapter.

## Microbial Spoilage

Microbial spoilage is one of the most important aspects of this research project. Microbial enumeration testing can show the progression of organisms' development in and on produce. However, as mentioned earlier, microbial counts alone are not the only descriptors of spoilage, as abiotic processes such as moisture/turgor loss, oxidation, etc. can also detract from the organoleptic quality of the produce. Additionally, even with relatively high numbers of microbial counts, some produce may not appear visually spoiled. However, it is generally undesirable to have microbial counts over the threshold level defined earlier, and microbial counts are an important component describing overall spoilage (biotic abiotic processes, combined). The streak plating method used to determine the CFU/g (colony forming units/gram) is explained in the biological method of test section of this report.

The microbial spoilage counts for each sample (lettuce then strawberries) and each test day (every 48 hours) are shown below. For each test, an explanation of the results and in some cases corresponding substantial moisture loss and visual inspection results will be given. As mentioned previously, lettuce microbial spoilage tests were performed on samples contained within resealable (Whirl-Pak) bags, not on open trays as was the case with strawberries. Therefore, ambient humidity levels are assumed to have had less of an

impact on the lettuce samples than on the strawberries. Two full tests were completed for lettuce at each temperature (four tests @ 5°C) to develop more meaningful and comparable results. Figure 45 to Figure 53 show microbial spoilage results for romaine lettuce, and Figure 54 to Figure 70 results for strawberries.

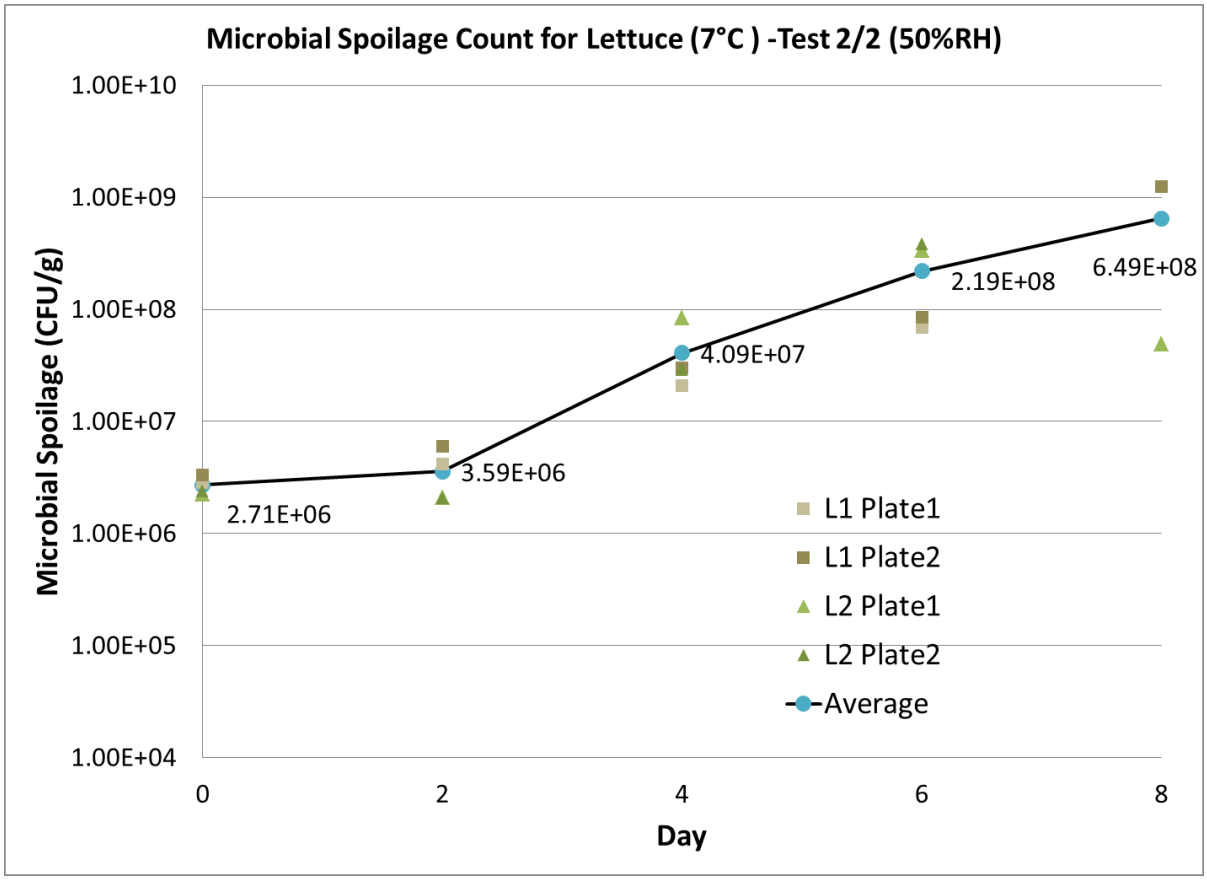


**Figure 45. Microbial spoilage data for lettuce (7°C – Test 1/2)**

Figure 45 shows the first microbial spoilage test for lettuce at 7°C. The "(95%)" shown in the title is only a reference to the test that was used for the temperature listed (i.e. this test was conducted during the 7°C-95% relative humidity run). Each test day (0, 2, 4 etc…) shows up to four CFU/g count numbers and one average (blue) number. The 'average' number is an overall average over all countable plates for that test day. The actual average spoilage count is shown near its data point. The meaning of the legend is as follows:

sample numbers (L1 or L2) represent the specific sample (25-60 grams) used for enumeration. The plate numbers (Plate1 or Plate2) represent the duplicate plates used for each sample. In some cases, there are less than four data points for each average. This could mean that either a plate count was not taken, or was unsuccessful (i.e. too numerous to count, overgrown plate, etc.). Plate count numbers are tabulated in Appendix C.

The starting CFU/g for this test (Figure 45) was low compared to other tests. Bacterial growth progressed steadily throughout the test period, peaking at an average $2.1x10^8$ CFU/g. According to the biological method of test shown earlier in this report, lettuce is considered to be beyond the threshold of freshness at $1.0x10^7$ CFU/g. Therefore, these samples were considered beyond the threshold of freshness between day 4 and day 6 of this test run. As noted previously, the freshness threshold specified is a general guideline based on the average expected count in fresh bagged lettuce, which is typically ~ $1 \times 10^5$ CFU/g lettuce, but the total microbial count is less important than the actual type of bacteria present, as some organisms are more active spoilers (e.g. pectinolytic bacteria) and the presence of pathogens of any type would be intolerable at any detectable concentration. Suggestions for a more uniform spoilage method of test with only one or two types of microflora present can be seen in the Future Recommended Work section of this report. As for the organoleptic quality of the samples, there were no substantial changes throughout the test; no wilting was observed and only minimal sliming was seen.

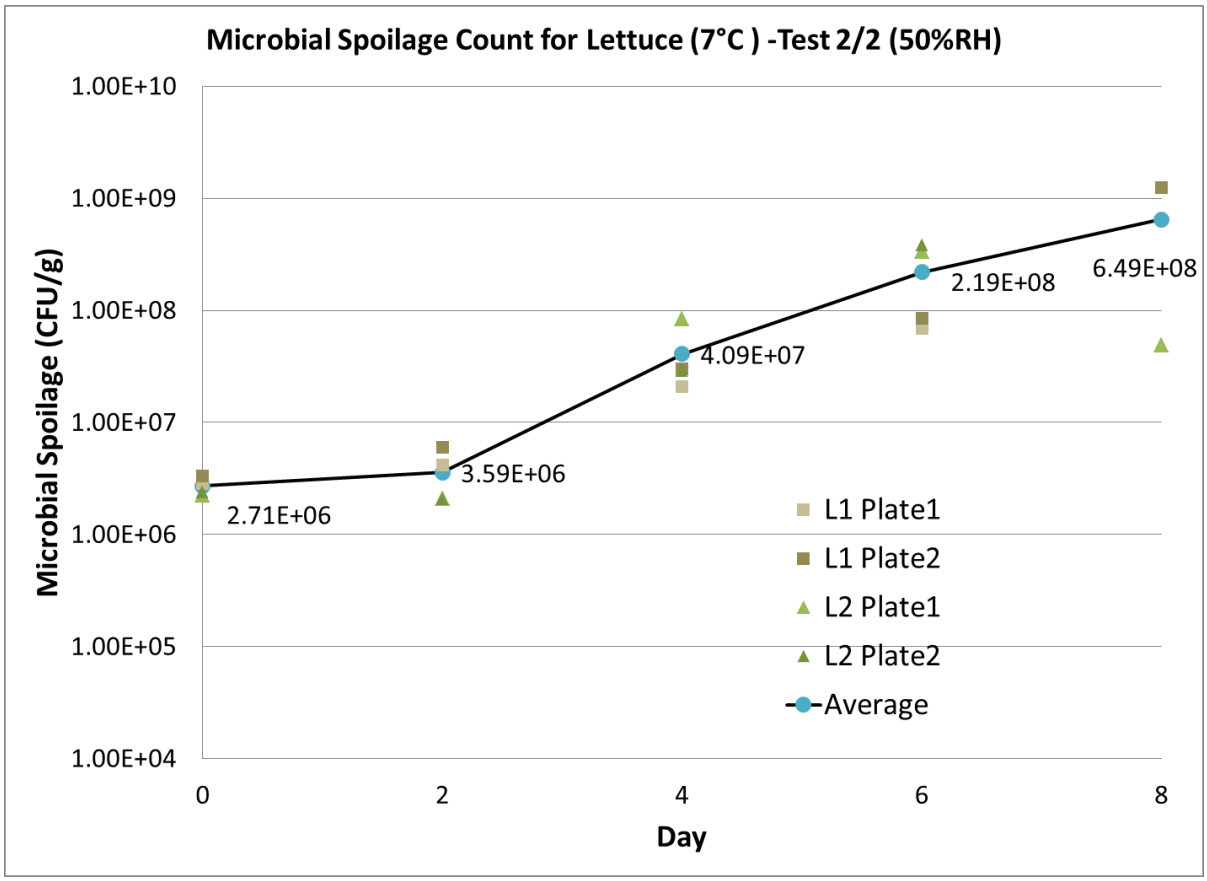


**Figure 46. Microbial spoilage data for lettuce (7°C – Test 2/2)**

Figure 46 shows the second microbial spoilage test for lettuce at 7°C. The starting spoilage number ($2.7x10^6$ CFU/g lettuce) for this test was higher than most others for lettuce, due to the inherent variability in microbial load between lettuce samples sourced from the distributor. Such variability is a hallmark of biological systems, and was addressed as fully as possible through the experimental design. Bacterial numbers rose steadily throughout the test period, peaking at an average $6.5x10^8$ CFU/g lettuce. According to the biological method of test, lettuce was considered beyond the threshold of freshness at $1.0x10^7$ CFU/g lettuce, and these samples reached this threshold between day 2 and day 4 of this test run. As for the organoleptic quality of the samples, there were no substantial changes throughout the test; no wilting was observed and only minimal sliming was seen.

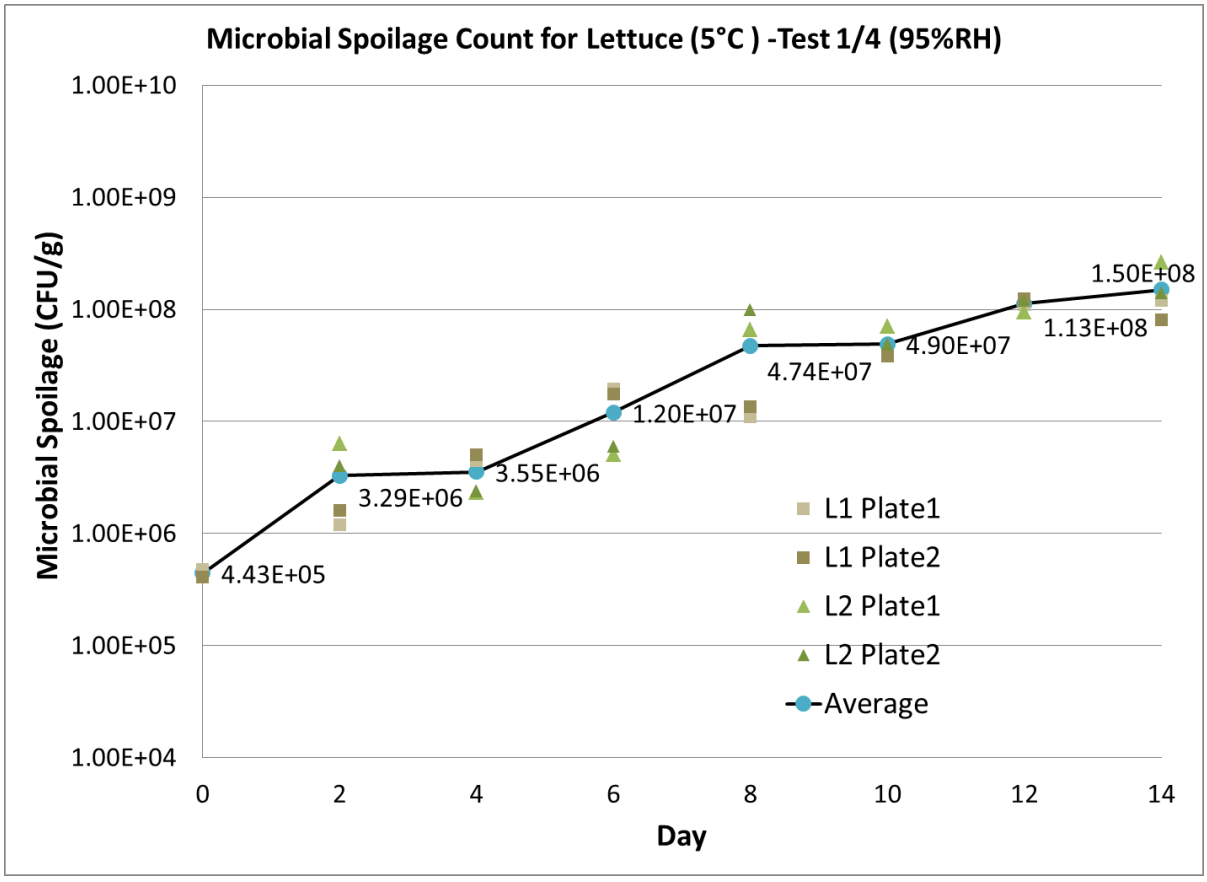


**Figure 47. Microbial spoilage data for lettuce (5°C – Test 1/4)**

Figure 47 shows the first of four microbial spoilage tests for lettuce at 5°C. The starting spoilage number ($4.4 \times 10^5$ CFU/g) for this test is about average for lettuce. Bacterial growth peaked at an average of $1.1 \times 10^8$ CFU/g lettuce. According to the biological method of test, these samples were considered beyond the threshold of freshness between day 4 and day 6 of this test run. As for the organoleptic quality of the samples, there were no substantial changes throughout the test; no wilting was observed and no sliming was noted.

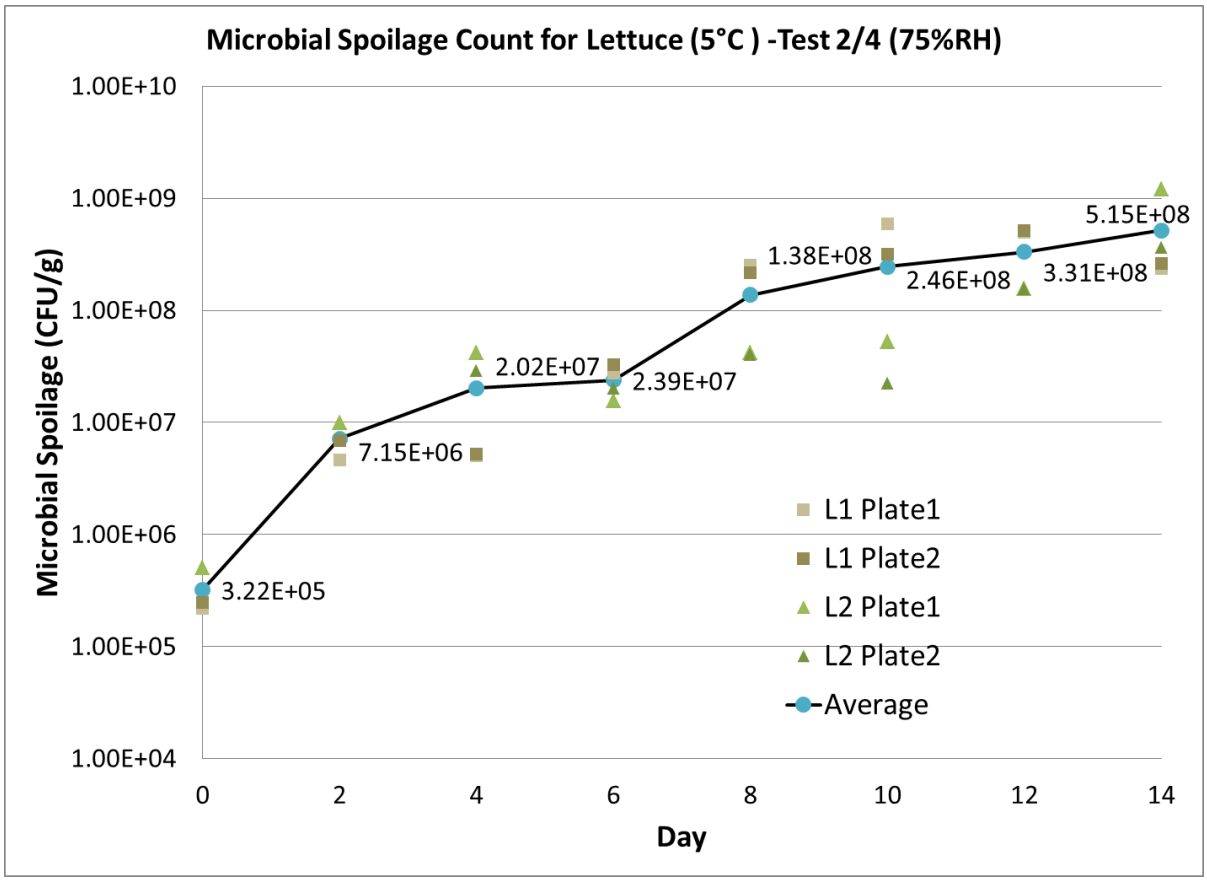


**Figure 48. Microbial spoilage data for lettuce (5°C – Test 2/4)**

Figure 48 shows the second microbial spoilage test for lettuce at 5°C. The starting spoilage number ($3.2x10^5$ CFU/g) for this test is about average for lettuce. Bacterial growth peaked at an average of $5.2x10^8$ CFU/g lettuce. According to the biological method of test, these samples were considered beyond the threshold of freshness between day 2 and day 4 of this test run. As for the organoleptic quality of the samples, there were no significant changes throughout the test; no wilting was observed and no sliming was present.

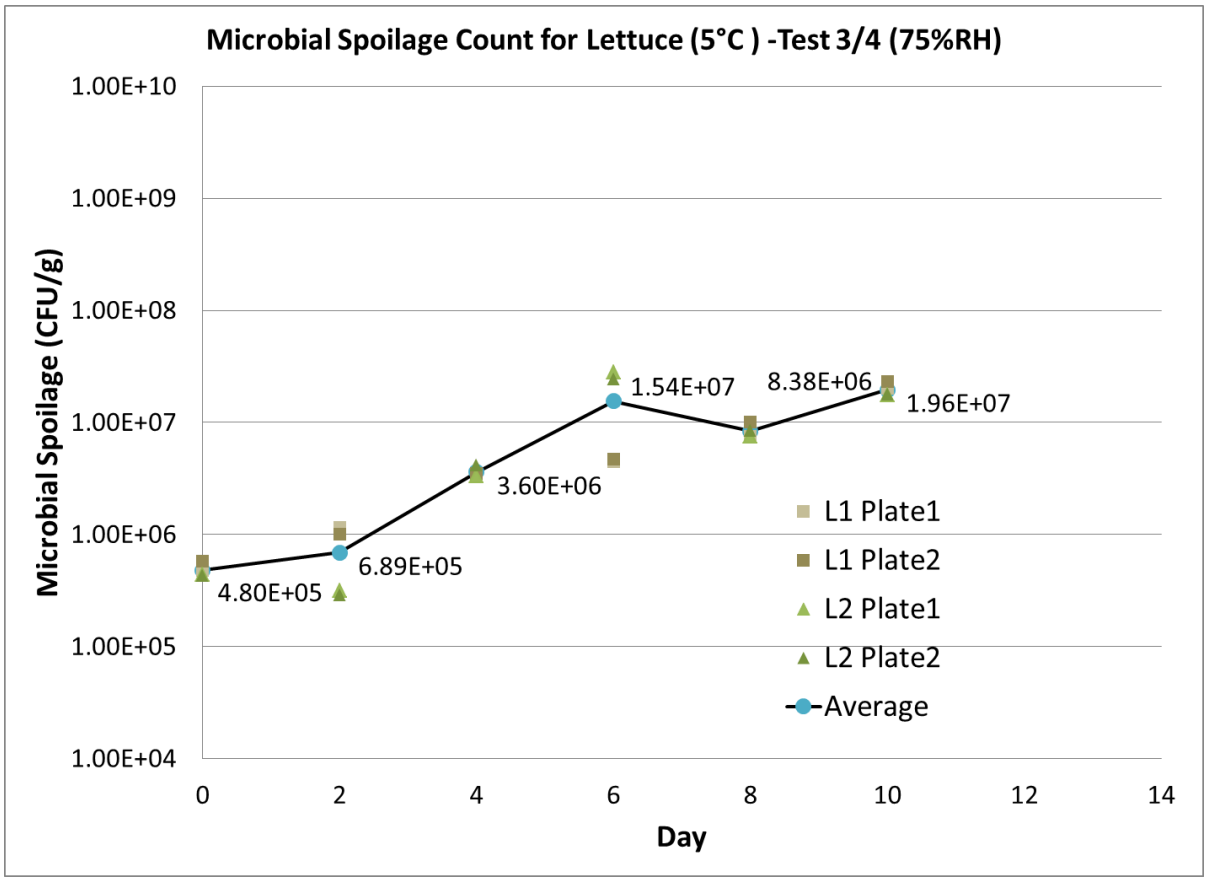


**Figure 49. Microbial spoilage data for lettuce (5°C – Test 3/4)**

Figure 49 shows the third microbial spoilage test for lettuce at 5°C. The starting spoilage number ($4.8x10^5$ CFU/g lettuce) for this test was about average for lettuce. As expected, growth rate for the bacteria was exponential and rose throughout the test period, peaking at an average $1.96x10^7$ CFU/g. According to the biological method of test, these samples were considered beyond the threshold of freshness between day 4 and day 6 of this test run. As for the organoleptic quality of the samples, there were no substantial changes throughout the test; no wilting was observed and no sliming was present. The test was cut short 4 days early because of complications with the test chamber. The basic microbial growth trend remains.

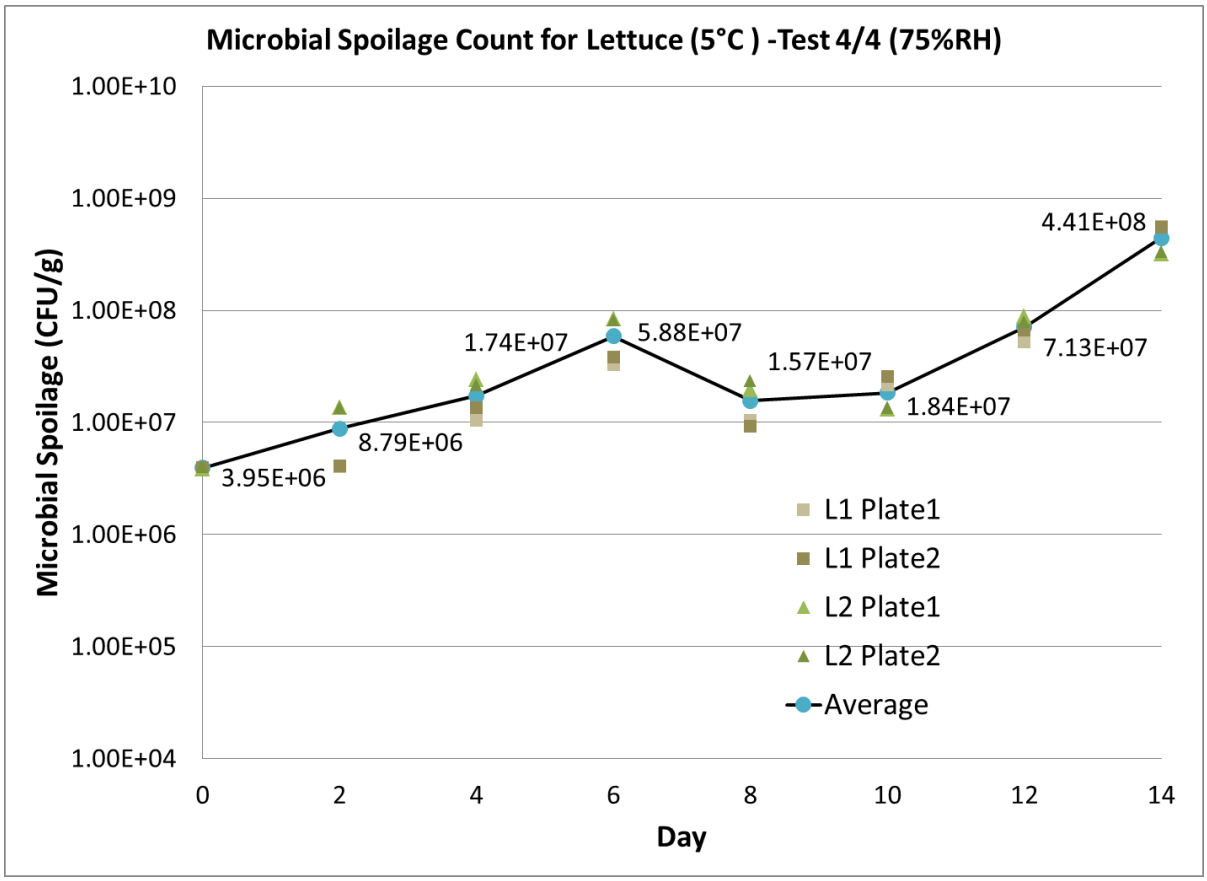


**Figure 50. Microbial spoilage data for lettuce (5°C – Test 4/4)**

Figure 50 shows the fourth microbial spoilage test for lettuce at 5°C. The starting spoilage number ($3.95x10^6$ CFU/g lettuce) for this test is an entire order of magnitude higher than the average for lettuce. As expected, growth rate for the bacteria was exponential and rose throughout the test period, peaking at an average $4.41x10^8$ CFU/g on the last test day. According to the biological method of test, these samples were considered beyond the threshold of freshness between day 2 and day 4 of this test run. As for the organoleptic quality of the samples, there were no significant changes throughout the test; no wilting was observed and no sliming was present.

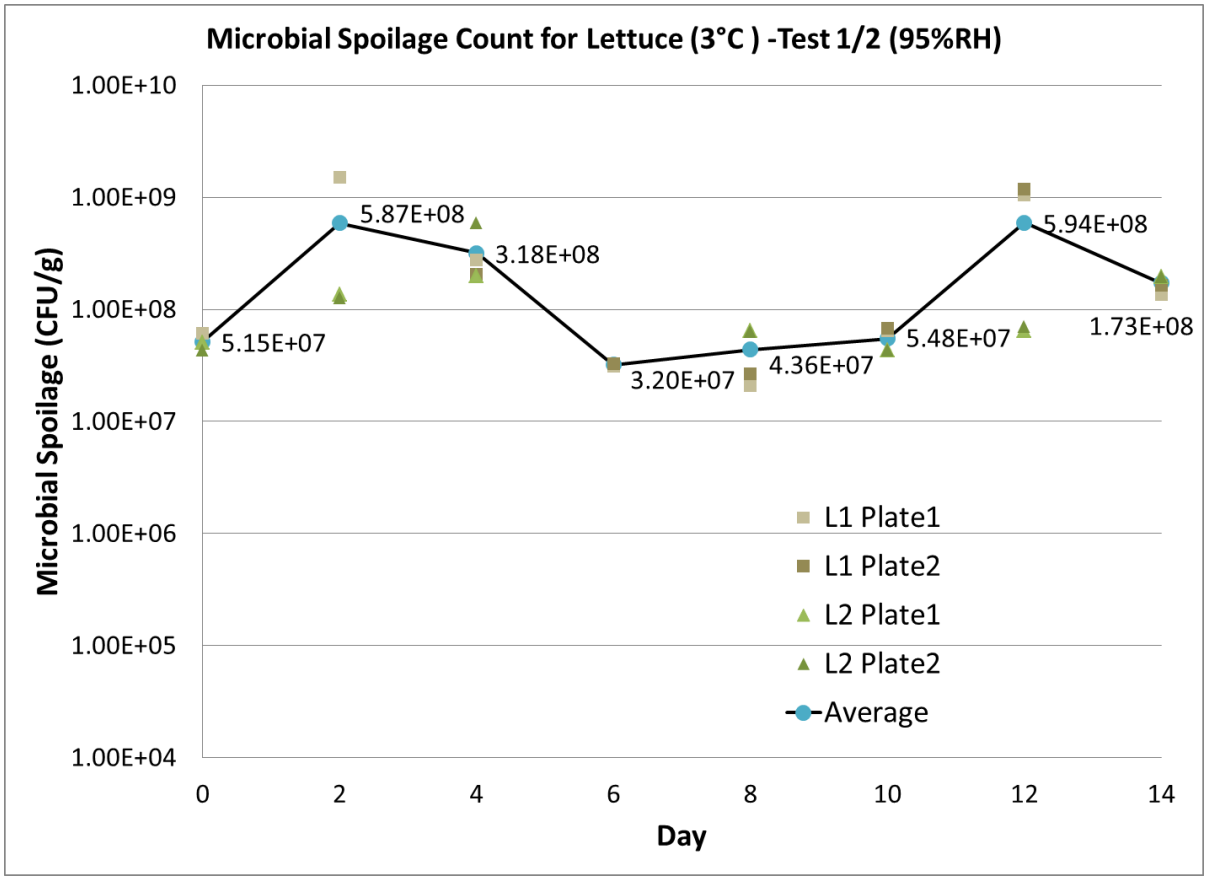


**Figure 51. Microbial spoilage data for lettuce (3°C – Test 1/2)**

Figure 51 shows the first microbial spoilage test for lettuce at 3°C. The starting spoilage number ($5.2x10^7$ CFU/g lettuce) for this test was the highest of all tests. Dissimilar from other tests, growth rate for the bacteria was exponential and rising for day 2, peaking at an average $5.9x10^8$ CFU/g then the colony count began to die off. After reaching a low of $3.2x10^7$ CFU/g lettuce on day 6, possibly due to toxin levels or competing microbial processes or temperature/environment shock factors, the spoilage level increased again to about the same level as day 0 ($5.2x10^7$ CFU/g lettuce). According to the biological method of test, these samples were considered beyond the freshness threshold from day 0 and

beyond in this test run. As for the organoleptic quality of the samples, there were no significant changes throughout the test; no wilting was observed and no sliming was noted.

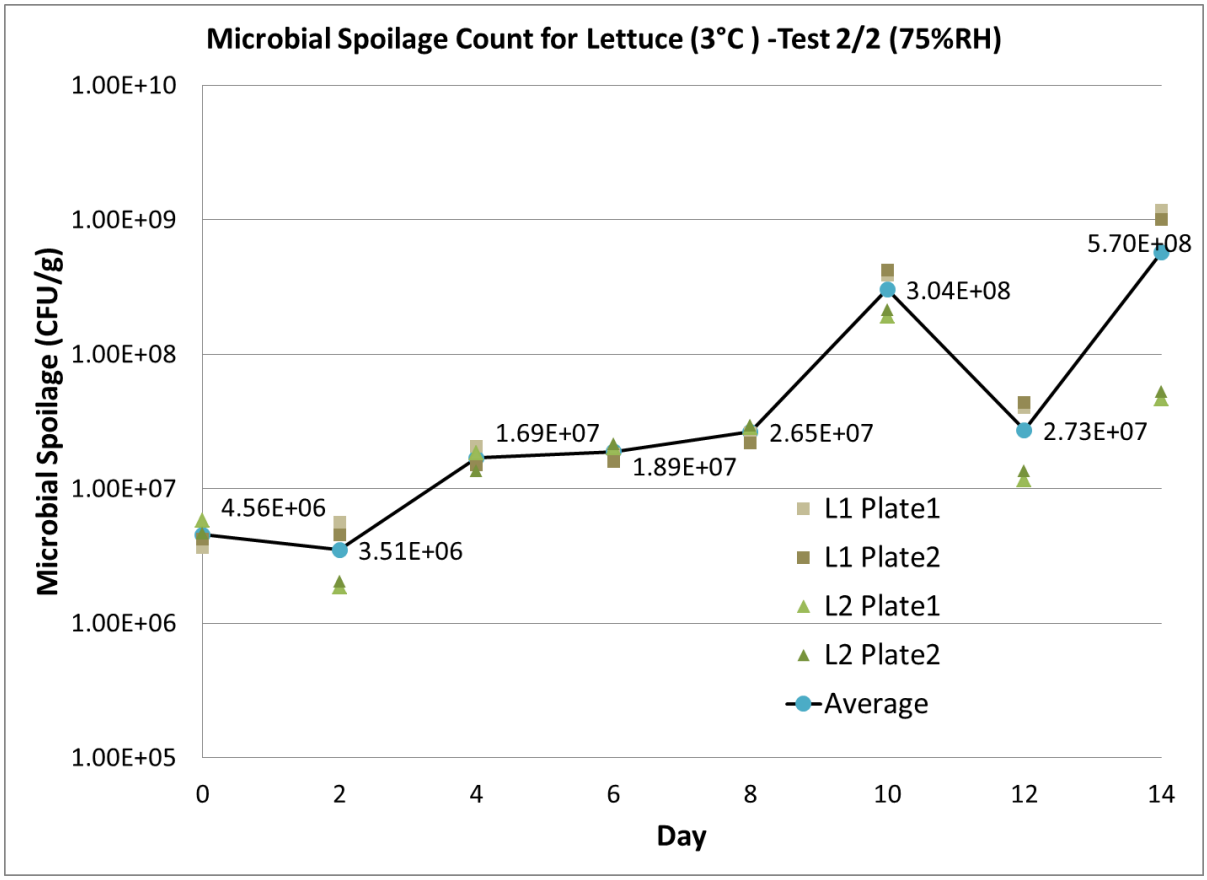


**Figure 52. Microbial spoilage data for lettuce (3°C – Test 2/2)**

Figure 52 shows the second microbial spoilage test for lettuce at 3°C. The starting spoilage number ($4.6x10^6$ CFU/g) for this test is high compared to other tests. Dissimilar from other tests, the bacteria numbers shrank for day 2 to $3.5x10^6$, then begin rising exponentially to an average $3.0x10^8$ CFU/g. The colony count began to die off until day 12, where a dip occurred, possibly due to bacterial competition and succession in microbial communities; counts then rose again for a final count of $5.7x10^8$ CFU/g. According to the biological method of test, these samples were considered beyond the freshness threshold between

day 2 and 4 during this test run. As for the organoleptic quality of the samples, there were no substantial changes throughout the test; no wilting was observed and no sliming was present.

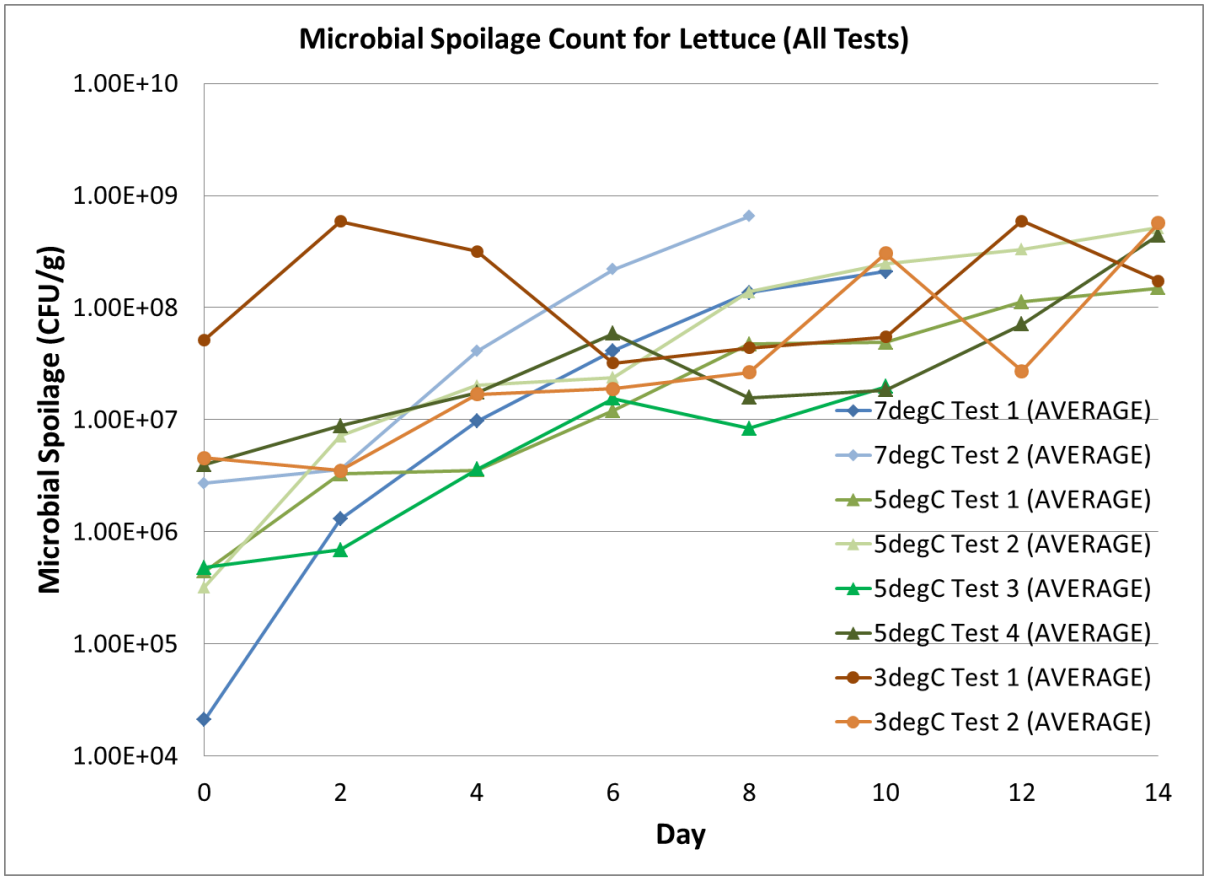


**Figure 53. Microbial spoilage data for lettuce (average count - all tests)**

Figure 53 depicts the averages of all microbial spoilage test run for lettuce at 7°C, 5°C, and 3°C. The starting spoilage numbers differed substantially, ranging from $2.1x10^4$ CFU/g to $5.2x10^7$ CFU/g lettuce. Growth rates for 7°C and 5°C tests are similar in that they rose continuously throughout the test period, peaking at the last test day. The 7°C samples were considered beyond the freshness threshold between day 2 and day 6. The 5°C samples were considered beyond the freshness threshold between day 2 and day 6. The 3°C

samples are considered beyond the freshness threshold between day 0 and day 4. It was expected that occurrence of spoilage should be relative to temperature, with lettuce at higher temperatures spoiling faster. However, this was not observed. Ignoring overall microbial counts and focusing on organoleptic quality, the observation was made that lower temperatures yielded longer lasting, crisper lettuce. This suggests that the method of storing the romaine (aseptically tearing apart leaves and placing into a sterile sealed bag) may be a way to improve shelf life, although this is not likely a practical approach.

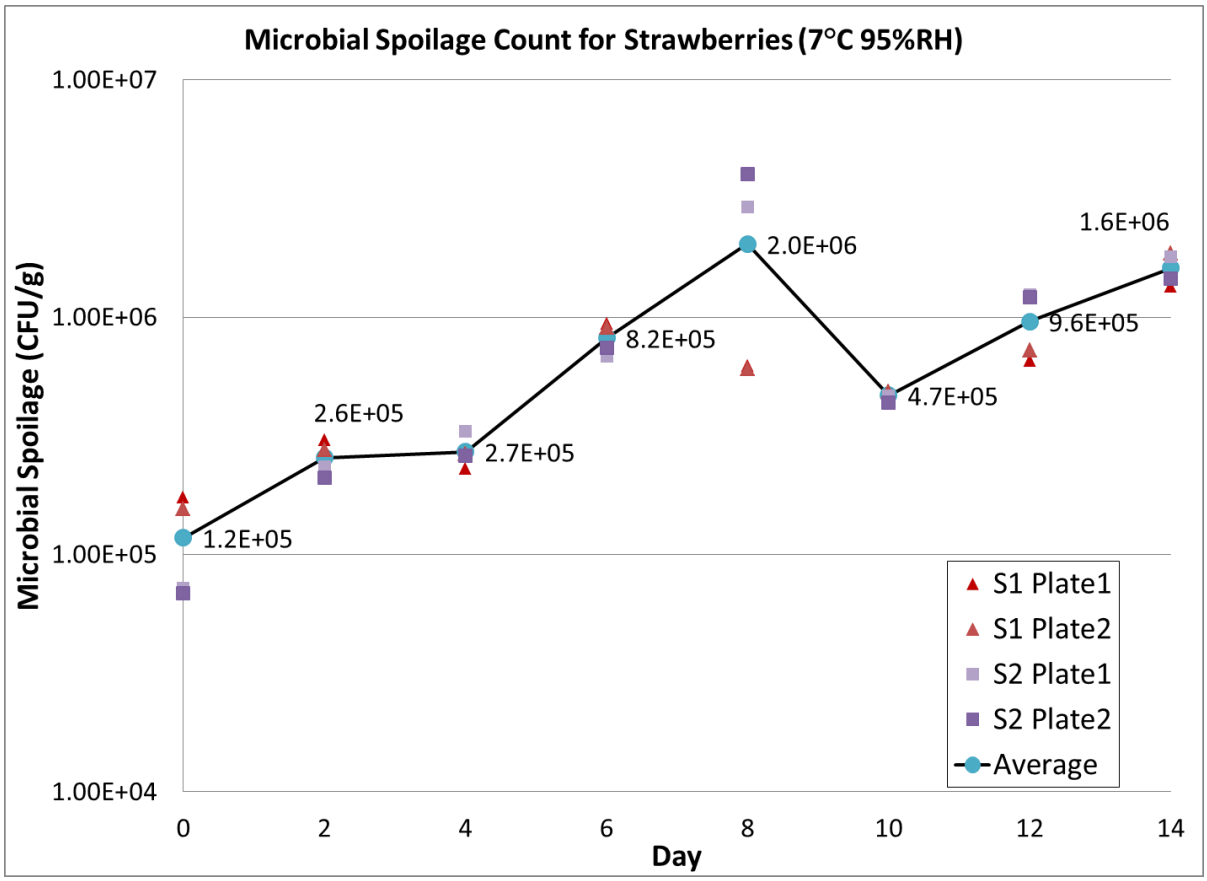


**Figure 54. Microbial spoilage data for strawberries (7°C 95% relative humidity)**

Figure 54 shows the microbial spoilage test data for strawberries at 7°C and 95% relative humidity. Strawberry samples were taken randomly from an aluminum tray identical to the one used for photographing and weighing. Each test day (0, 2, 4 etc…) shows up to four

CFU/g strawberries count numbers and one average (blue) number. The 'average' number is a general average over all countable plates for that test day. The average spoilage count number is labeled near its data point. The meaning of the legend is as follows: sample number (S1 or S2) represent the specific sample (25-75 grams) used for enumeration. The plate number (Plate1 or Plate2) represents the duplicate plates used for each sample. In some cases, there are less than four data points for each average. This could mean that either a plate count was not taken, or was unsuccessful (i.e. too numerous to count, contamination suspected etc.). Specific plate count numbers can be seen in Appendix D.

The starting spoilage number ($1.2x10^5$ CFU/g strawberries) for this test was similar to results obtained in other tests on strawberries. The spoilage count rose continuously, peaking on day 8 at $2.0x10^6$ CFU/g strawberries and then began to fall until around day 10. Colony counts dropped at day 8, possibly due to exhaustion of nutrients or overcompetition and buildup of toxic waste products. However, at day 10, the spoilage count rose again for a final high of $1.6x10^6$ CFU/g strawberries. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial count ($1.0 \times 10^6$ CFU/g strawberries between day 6 and 8 of this test run. As for the organoleptic quality of the samples, berries were firm and ripe until visual molding occurred around day 8, reaching the freshness threshold level (25% mold coverage, according to the biological method of test) around day 12. It should be noted that although there was a correlation between the peak microbial count and occurrence of mold growth, (both on test day 8) and that colony forming units (CFU) resulted mostly from yeast growth and not from mold colonies. In most tests, on average, the number of mold CFU counts was less than 10% of the total recorded CFU/g strawberries.

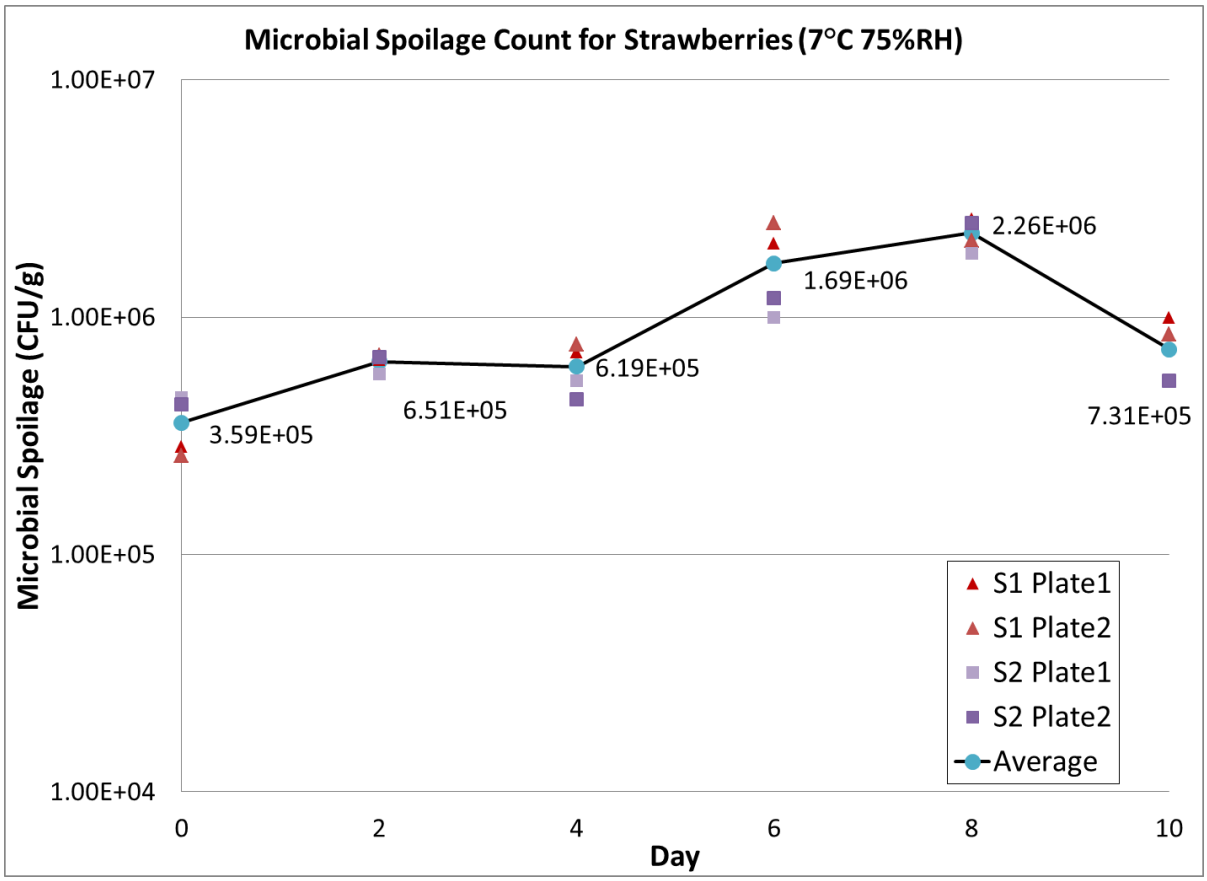


**Figure 55. Microbial spoilage data for strawberries (7°C 75% relative humidity)**

Figure 55 shows the microbial spoilage test data for strawberries at 7°C and 75% relative humidity. The starting spoilage number ($3.6x10^5$ CFU/g strawberries) for this test was similar to results obtained from other tests on strawberries. The spoilage count rose continuously, peaking on day 8 at a count of $2.26x10^6$ CFU/g strawberries and then began to fall until around day 10. The colony count began to die off at day 8, possibly due to overcompetition and build up of waste products. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial count ($1.0x10^6$ CFU/g strawberries) between day 4 and 6 during this test run. As for the organoleptic quality of the samples, berries were firm and ripe until visual molding occurred around day 6, reaching the freshness threshold level (25% mold coverage, according to the biological method of test) around day 10.

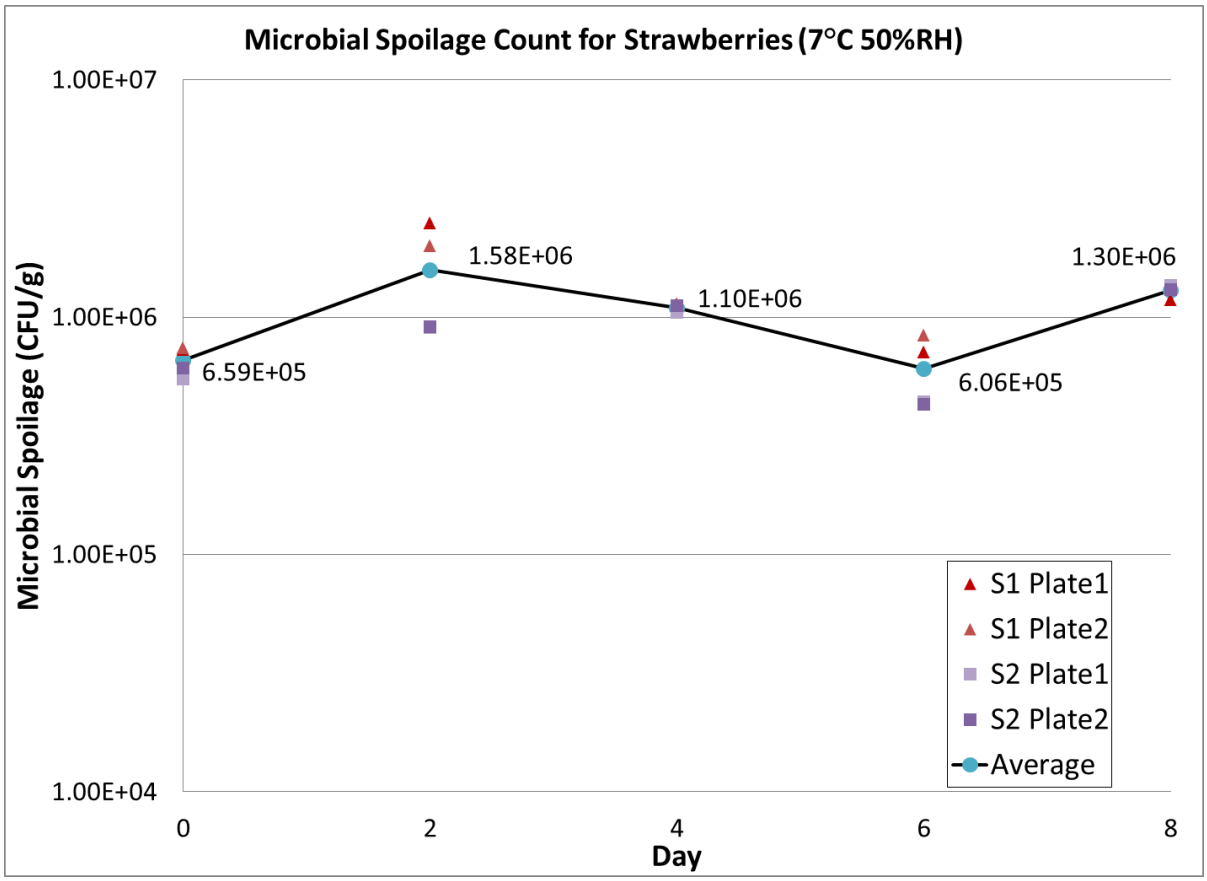


**Figure 56. Microbial spoilage data for strawberries (7°C 50% relative humidity)**

Figure 56 shows the microbial spoilage test data for strawberries at 7°C and 50% relative humidity. The starting spoilage number ($6.6x10^5$ CFU/g strawberries) for this test was very high compared to other tests on strawberries. The colony count rose to the established freshness threshold level by day 2 peaking to an all-time high on of $1.6x10^6$ CFU/g and then began to fall until around day 6. The colony count began to die off, again potentially due to nutrient exhaustion or competition. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial count ($1.0x10^6$ CFU/g strawberries) between day 0 and 2 during this test run. As for the organoleptic quality of the samples, berries were firm and ripe until visual molding and loss of firmness occurred around day 2. The sample lost enough moisture by day 6 to be considered beyond the freshness threshold according to the established cutoff for physical processes (20%

moisture loss, according to biological method of test). The samples went beyond the freshness threshold due to mold (25% mold coverage, according to the biological method of test) around day 8.

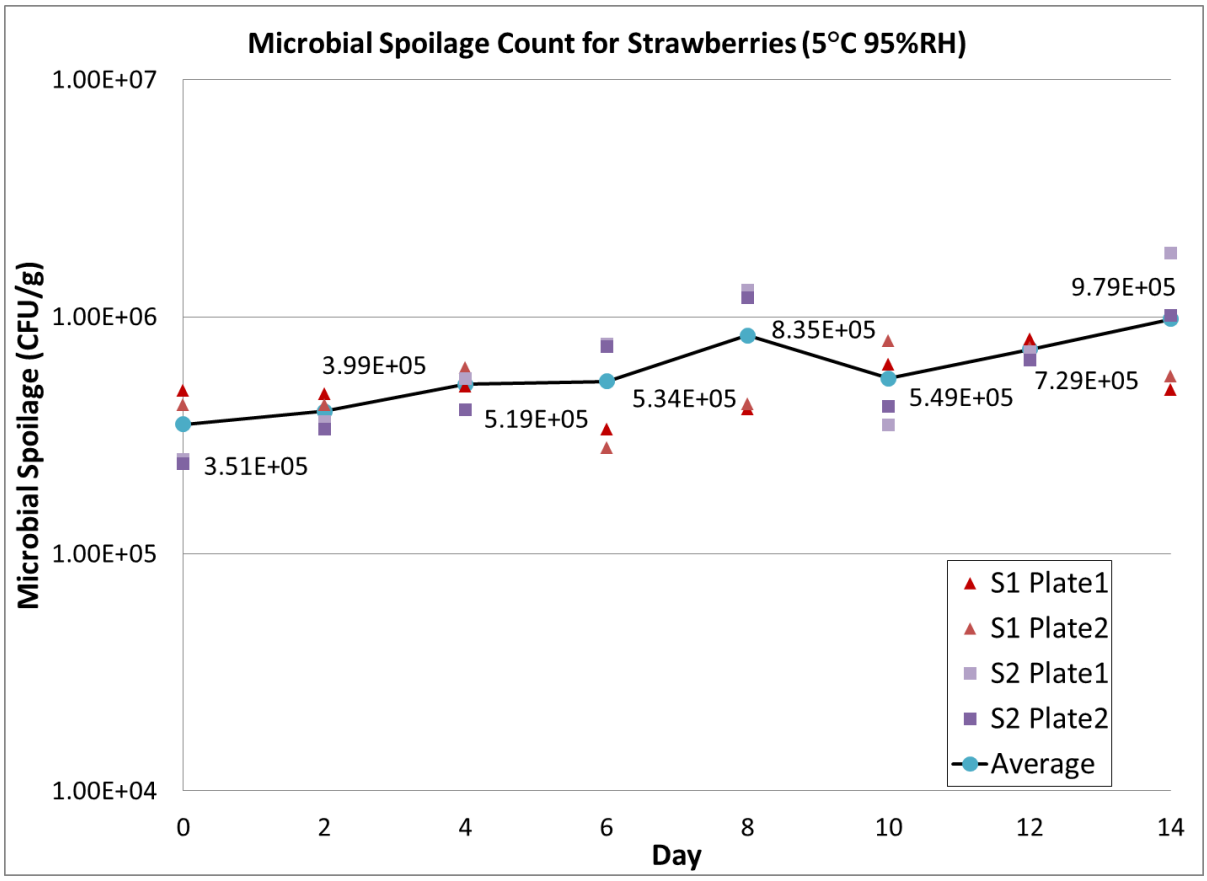


**Figure 57. Microbial spoilage data for strawberries (5°C 95% relative humidity)**

Figure 57 depicts the microbial spoilage test data for strawberries at 5°C and 95% relative humidity. The starting spoilage number ($3.5x10^5$ CFU/g) for this test was about average compared to other tests on strawberries. The spoilage count rose continuously, peaking to an all-time high on the last test day (day 14) at $9.8x10^5$ CFU/g strawberries. The colony count began to die off at day 8 until day 10. According to the biological method of test, these samples exceeded the established freshness threshold due to microbial counts ($1.0x10^6$ CFU/g strawberries) during this test run. As for the organoleptic quality of the

samples, berries were firm and ripe until visual molding occurred around day 4, reaching the freshness threshold level (25% mold coverage, according to the biological method of test) around day 12.

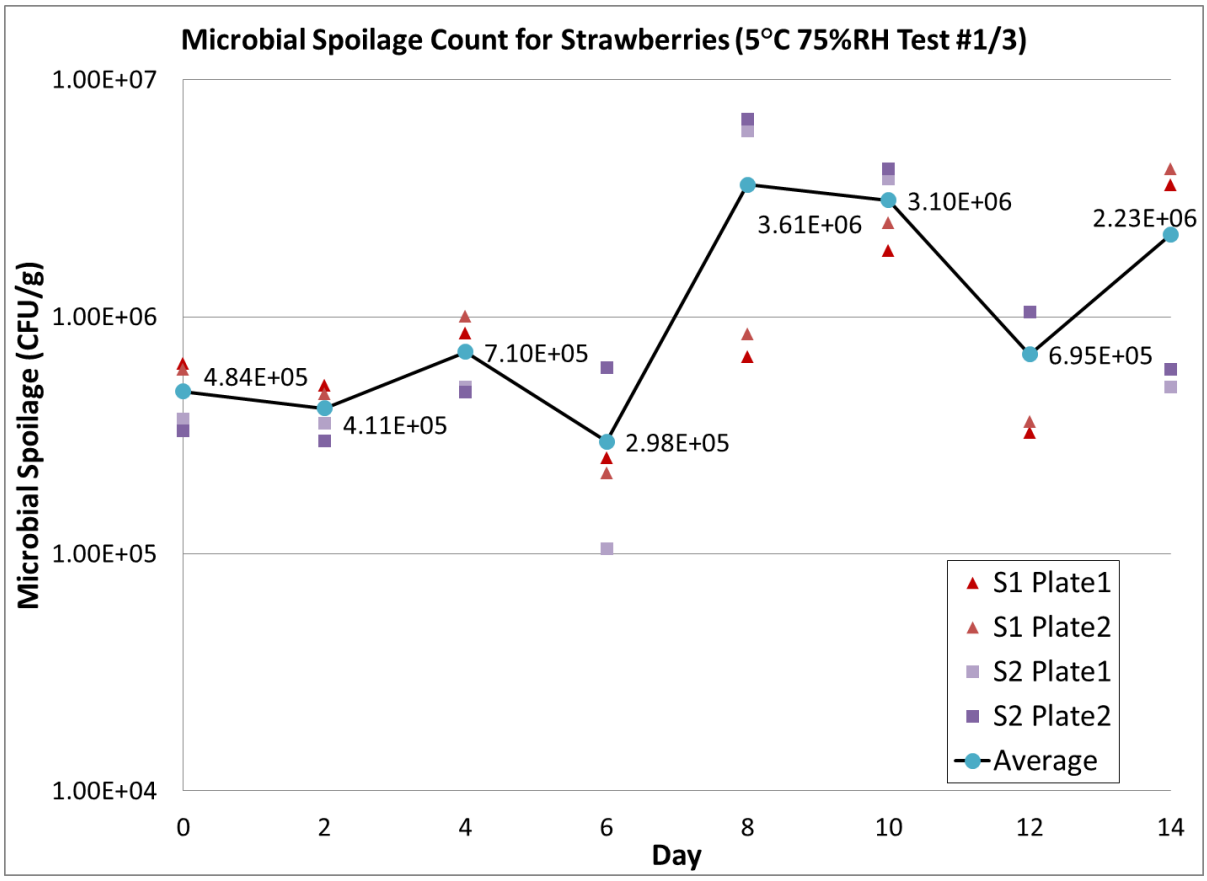


**Figure 58. Microbial spoilage data for strawberries (5°C 75% relative humidity Test #1/3)**

Figure 58 shows data for the first of three microbial spoilage tests for strawberries at 5°C and 75% relative humidity. The starting spoilage number ($4.8 \times 10^5$ CFU/g) for this test was slightly higher than average compared to other tests on strawberries. The spoilage count rose continuously, peaking on day 8 at $3.6 \times 10^6$ CFU/g, and then began to fall until around day 12. Colony counts began to fall at day 8, possibly due to nutrient exhaustion or buildup of metabolic waste products. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial count ($1.0 \times 10^6$ CFU/g

strawberries) between day 6 and 8 during this test run. As for the organoleptic quality of the samples, berries were firm and ripe until visual molding occurred around day 6, reaching the freshness threshold level (25% mold coverage, according to the biological method of test) around day 12.

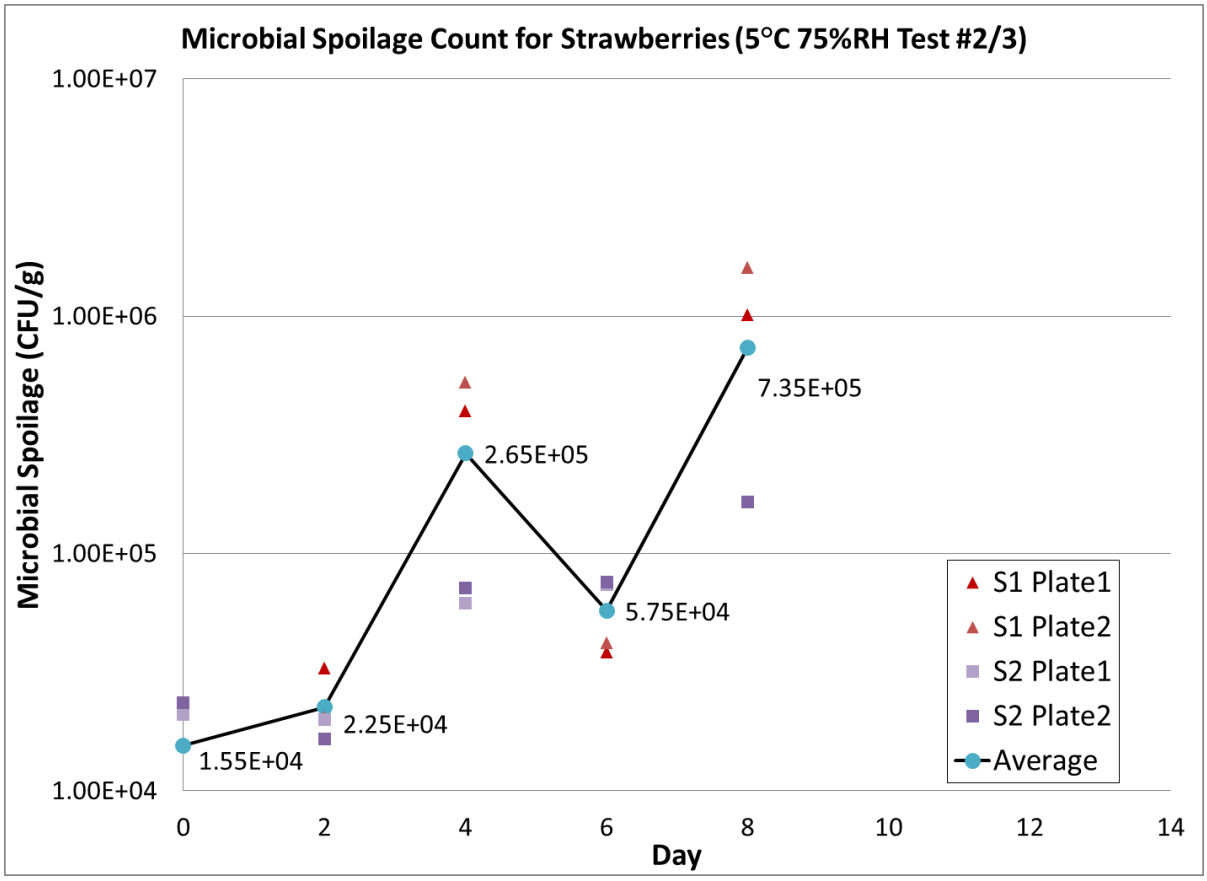


**Figure 59. Microbial spoilage data for strawberries (5°C 75% relative humidity Test #2/3)**

Figure 59 shows data for the second of three microbial spoilage tests for strawberries at 5°C and 75% relative humidity. The starting spoilage number ($1.6x10^4$ CFU/g) for this test was similar to numbers seen in other tests on strawberries. The spoilage count rises continuously, peaking on day 4 at $2.7x10^5$ CFU/g strawberries and then began to fall until around day 6. The colony count rose until day 8 when the test was ended due to complications with the test chamber. According to the biological method of test, these samples did not reach the freshness threshold due to microbial count ($1.0x10^6$ CFU/g)

during this test run. As for the organoleptic quality of the samples, berries were firm and ripe until the end of the test.

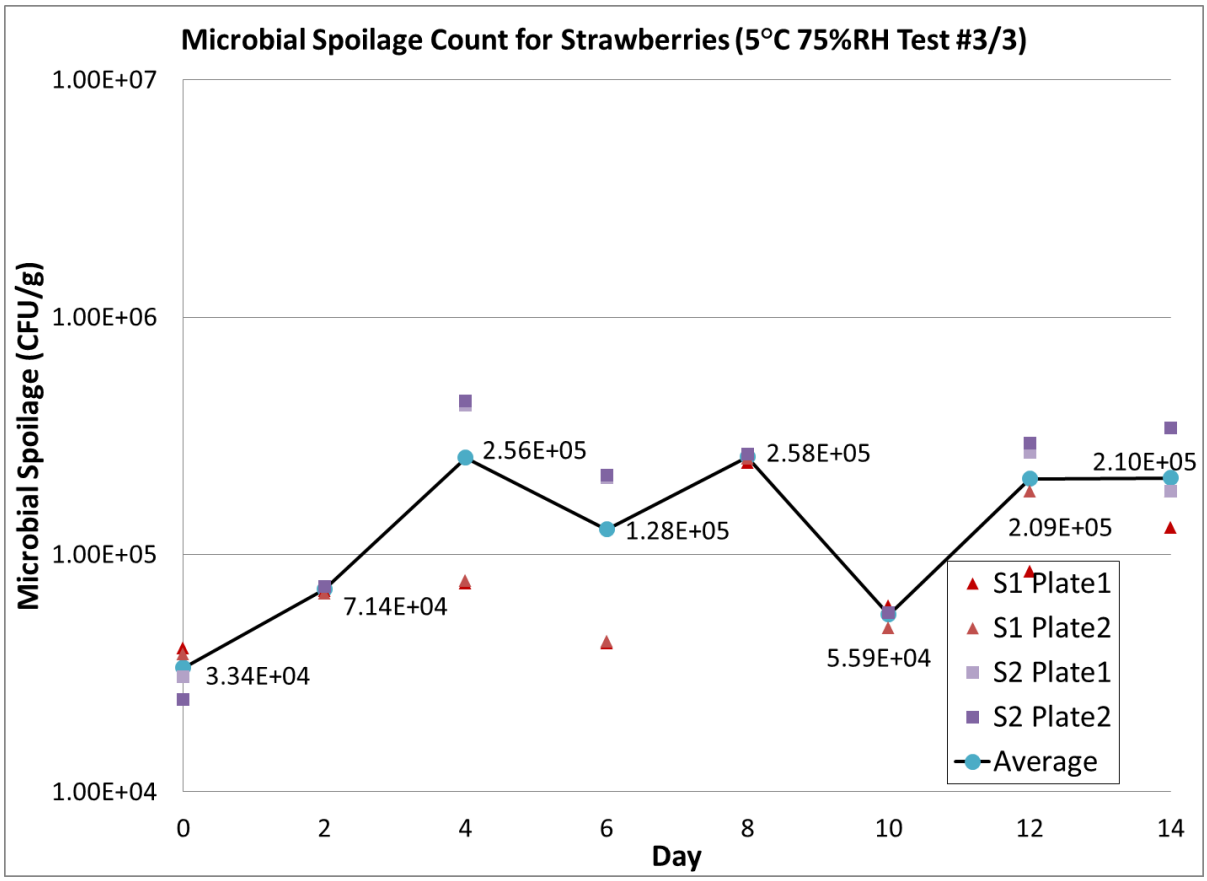


**Figure 60. Microbial spoilage data for strawberries (5°C 75% relative humidity Test #3/3)**

Figure 60 shows data for the third of three microbial spoilage tests for strawberries at 5°C and 75% relative humidity. The starting spoilage number ($3.3x10^4$ CFU/g) for this test was similar to that seen in other tests on strawberries. The spoilage count rose continuously, peaking on day 4 at $2.6x10^5$ CFU/g strawberries and then began to fall until around day 12. The colony count began to fall at day 8, again possibly due to nutrient exhaustion or buildup of metabolic waste products. According to the biological method of test, these samples did not exceed the established freshness threshold due to microbial count ($1.0x10^6$ CFU/g strawberries) during this test run. As for the organoleptic quality of the samples, berries

were firm and ripe until visual molding occurred around day 12, though the freshness threshold was not reached within 14 days.

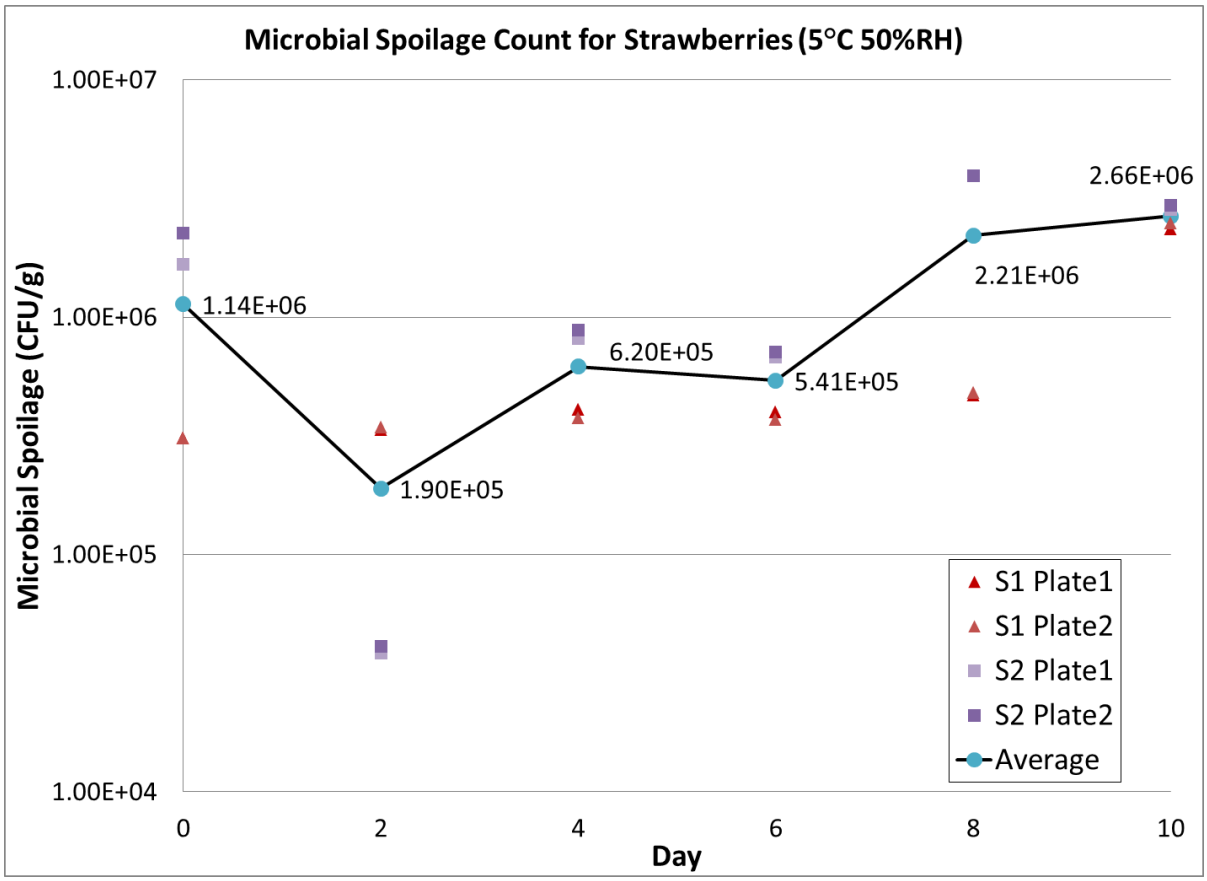


**Figure 61. Microbial spoilage data for strawberries (5°C 50% relative humidity)**

Figure 61 shows the microbial spoilage test data for strawberries at 5°C and 50% relative humidity. The starting spoilage number ($1.1x10^6$ CFU/g strawberries) for this test was higher than any other test on strawberries. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial count ($1.0x10^6$ CFU/g) day 0 of this test run. The CFU count dropped sharply on day 2, and began to rise again, peaking at $2.7x10^6$ CFU/g by day 10. As for the organoleptic quality of the samples, berries were firm and ripe until loss of firmness due to dehydration. The samples

lost enough moisture by day 10 to be considered beyond the freshness threshold (20% according to biological method of test).

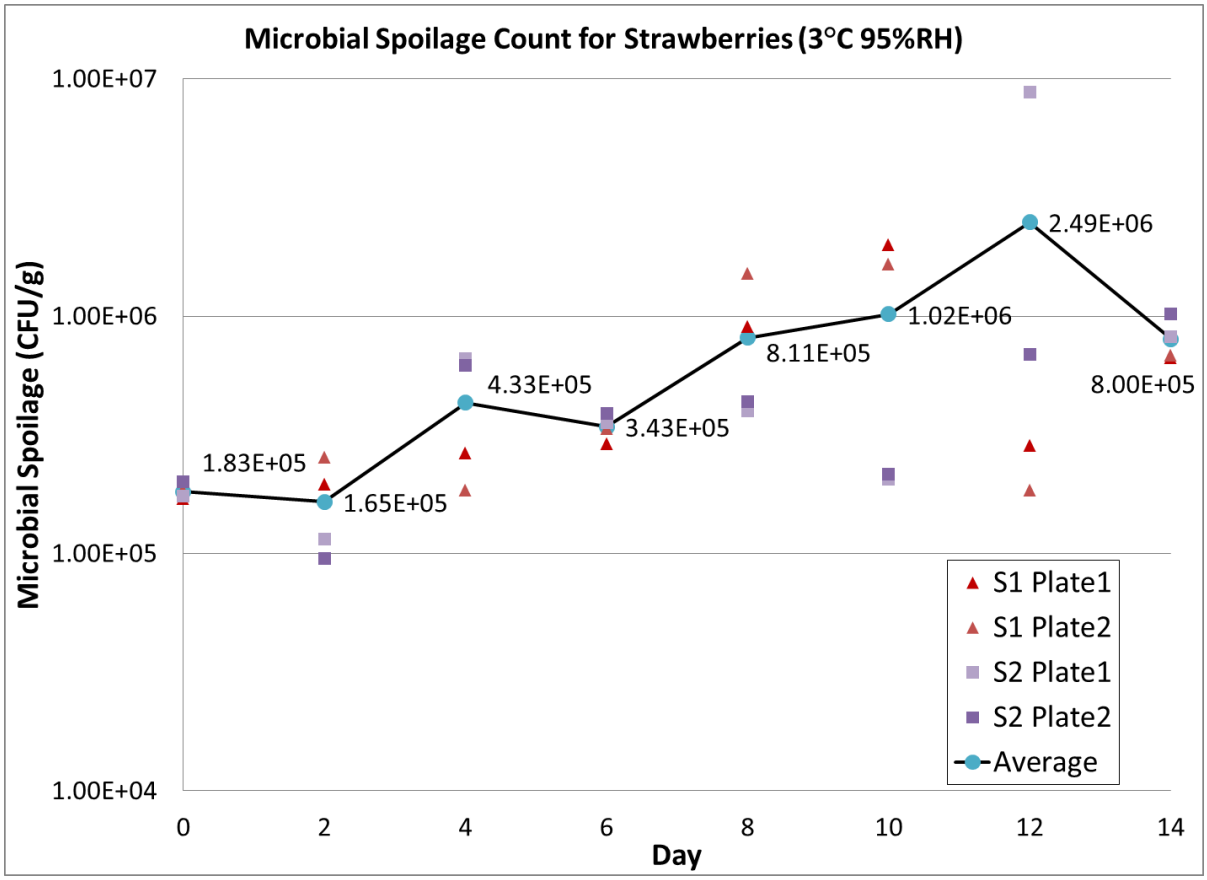


**Figure 62. Microbial spoilage data for strawberries (3°C 95% relative humidity)**

Figure 62 shows the microbial spoilage test data for strawberries at 3°C and 95% relative humidity. The starting spoilage number ($1.8x10^5$ CFU/g strawberries) for this test was relatively low compared to other tests on strawberries. Counts rose continuously, peaking to on day 12 at $2.5x10^6$ CFU/g and then began to fall until the end of the test (day 14). Counts began to fall at day 12. According to the biological method of test, these samples were considered beyond the freshness threshold due to microbial counts ($1.0x10^6$ CFU/g) between day 8 and 10 during this test run, although there was some indication that spoilage

in some areas of the sample tray never reached this spoilage level. As for the organoleptic quality of the samples, berries were firm and ripe until visual molding occurred around day 10, but never reached the freshness threshold level (25% mold coverage, according to the biological method of test).

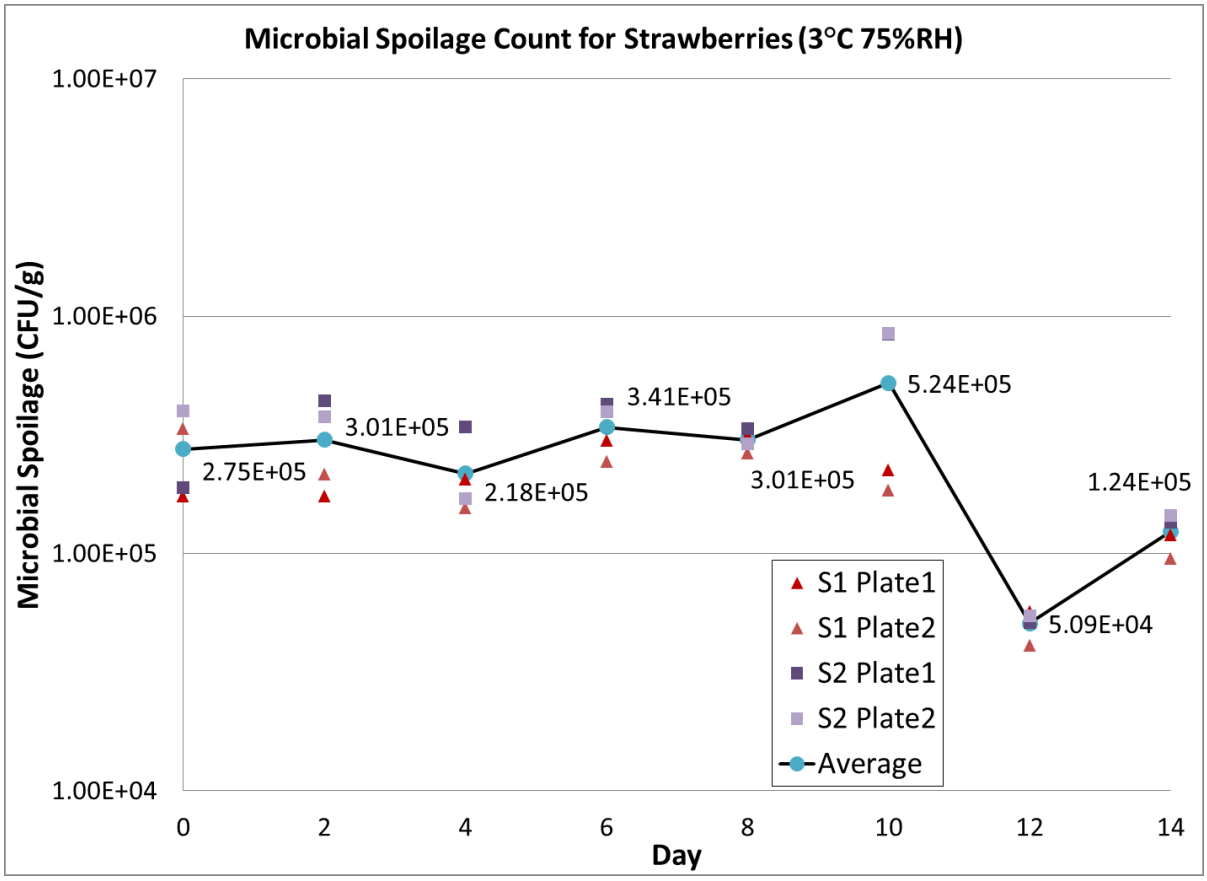


**Figure 63. Microbial spoilage data for strawberries (3°C 75% relative humidity)**

Figure 63 shows the microbial spoilage test data for strawberries at 3°C and 75% relative humidity. The starting spoilage number ($2.8x10^5$ CFU/g strawberries) for this test was similar to that seen in other tests on strawberries. The spoilage count rose very slowly, peaking on day 10 at $5.2x10^5$ CFU/g, and then began to fall until day 12, rising again until the end of the test (day 14). Counts began to fall again on day 10, possibly due to nutrient exhaustion or buildup of metabolic waste products. According to the biological method of

test, these samples were never considered beyond the freshness threshold due to microbial count or organoleptic quality. These data suggest that this combination of temperature and humidity level may be useful for optimal preservation of freshness in strawberries.

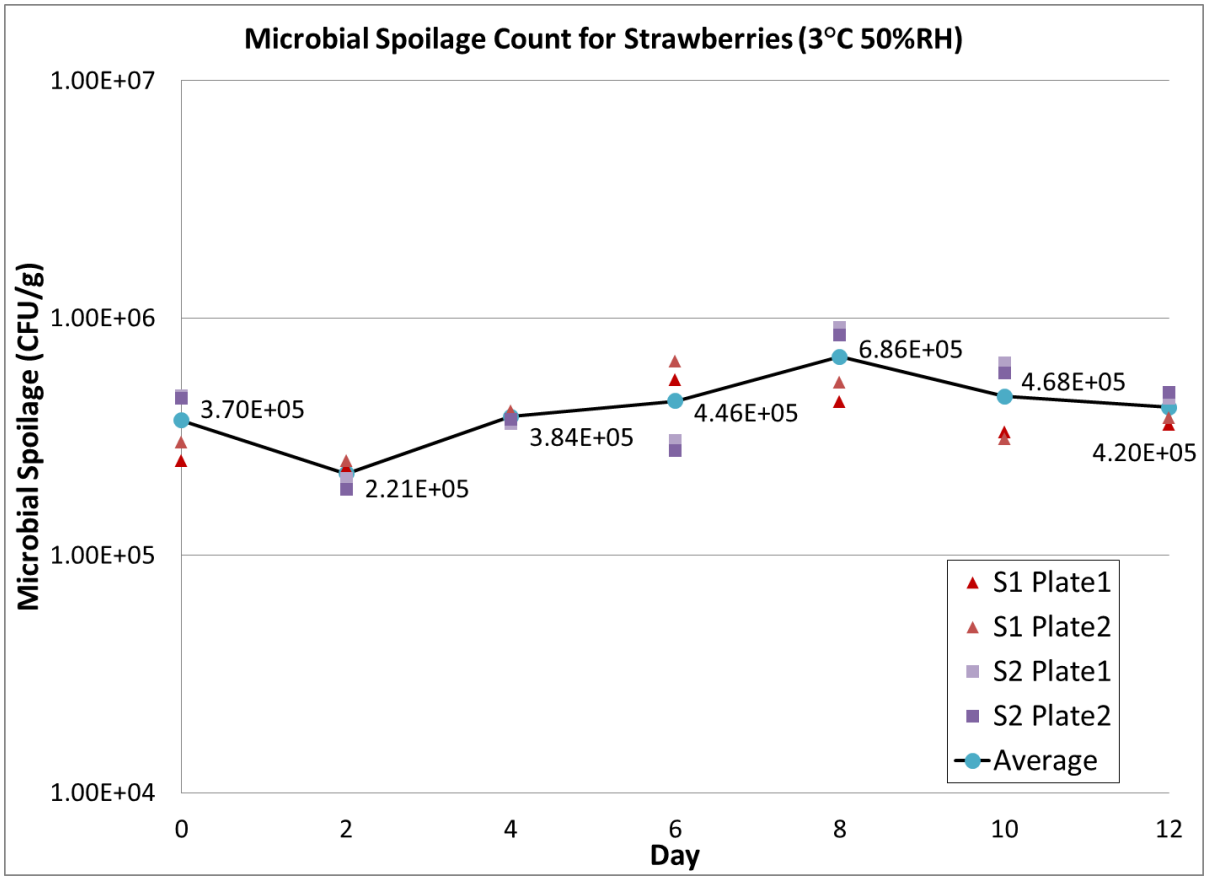


**Figure 64. Microbial spoilage data for strawberries (3°C 50% relative humidity)**

Figure 64 shows the microbial spoilage test data for strawberries at 3°C and 50% relative humidity. The starting spoilage number ($3.7x10^6$ CFU/g strawberries) for this test was higher than any other test on strawberries. The spoilage count dropped somewhat until day 2, and began to rise again afterward, peaking by day 8 at $6.7x10^5$ CFU/g, followed by another drop. The microbial count for this test did not reach or exceed the established freshness threshold at any point during the test. As for the organoleptic quality of the

samples, berries were firm and ripe until loss of firmness due to dehydration. The sample lost enough moisture by day 10 to be considered beyond the freshness threshold (20% loss according to biological method of test). This loss of moisture likely played an important role in preservation against microbial spoilage, although it led to an organoleptically unacceptable outcome in produce quality.

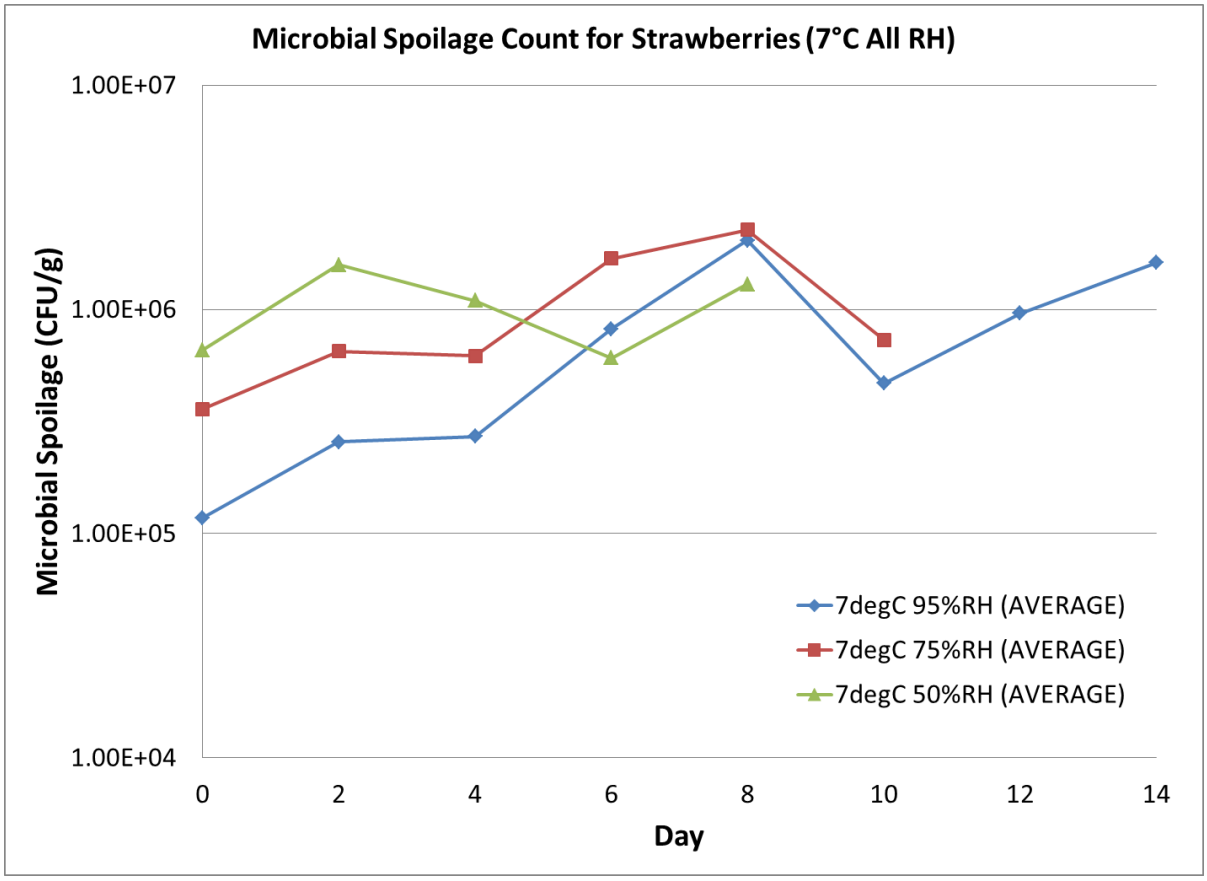


**Figure 65. Microbial spoilage data for strawberries (7°C - all relative humidities)**

Figure 65 shows all three microbial spoilage test averages for strawberries at 7°C and all relative humidities. The starting microbial load for each test varied greatly, though remained within one $\log_{10}$ between tests. Because of these differences, it is difficult to compare the tests directly, although there are some similarities. Firstly, the averages for

each test reached a peak and then fell by more than 50% (half of one $\log_{10}$), though this happened at different times during the course of the tests. Another interesting similarity is that after the initial peak CFU/g, and within 2 days of reaching the next low point, all three samples exceeded the freshness threshold due to mold growth (25% mold coverage, according to the biological method of test). This could be an indication of biological competition between molds and yeasts. Another interesting observation was that the microbial count was largely unaffected by humidity levels. This is not greatly surprising, as yeasts and molds are generally more xerotolerant (require less moisture) than are bacteria; however, the extent of mold growth did appear to be dependent on inoculum size (day 12, 10 and 6 for 95%, 75% and 50% respectively).

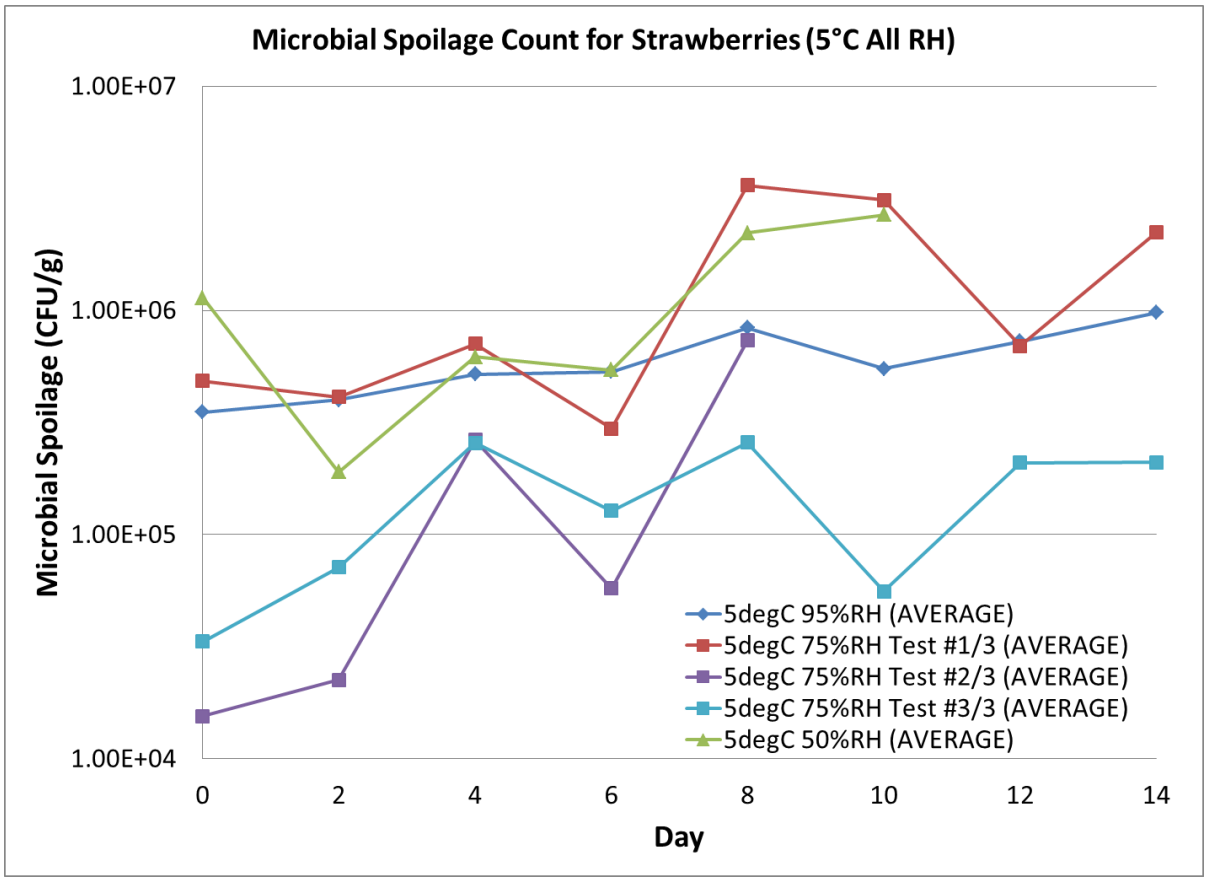


**Figure 66. Microbial spoilage data for strawberries (5°C - all relative humidities)**

Figure 66 shows all five microbial spoilage test averages for strawberries at 5°C and all relative humidities. The starting CFU/g strawberries for each test varied greatly, especially with the 75% RH tests. Because of these differences, it is difficult to compare the tests directly, although there are some similarities. Firstly, all CFU counts peaked in 2-4 days, then fell around day 6, rising again and then falling around days 10-12. This may indicate that similar microflora were present and that similar microbial processes were occurring. One more interesting observation and counterintuitive to the expectation that moisture would promote microbial growth, is that the microbial counts were lower at the highest humidity level (95%RH). One possible explanation for this result is that mold growth predominated, and yeast growth was suppressed. Yeasts occur as individual cells – one cell will result in one colony on a plate. However, molds are more difficult to enumerate, as they occur as hyphae (branched, tube-like structures), rather than as individual cells. A small fragment of a longer hypha, a spore or a large mass of hyphae will all produce a single colony, and eventually, confluent growth on the plate, making interpretation of these tests more difficult. However, from observations, the majority of microorganisms contributing to the spoilage count on strawberries were yeast, suggesting that this theory is not correct.

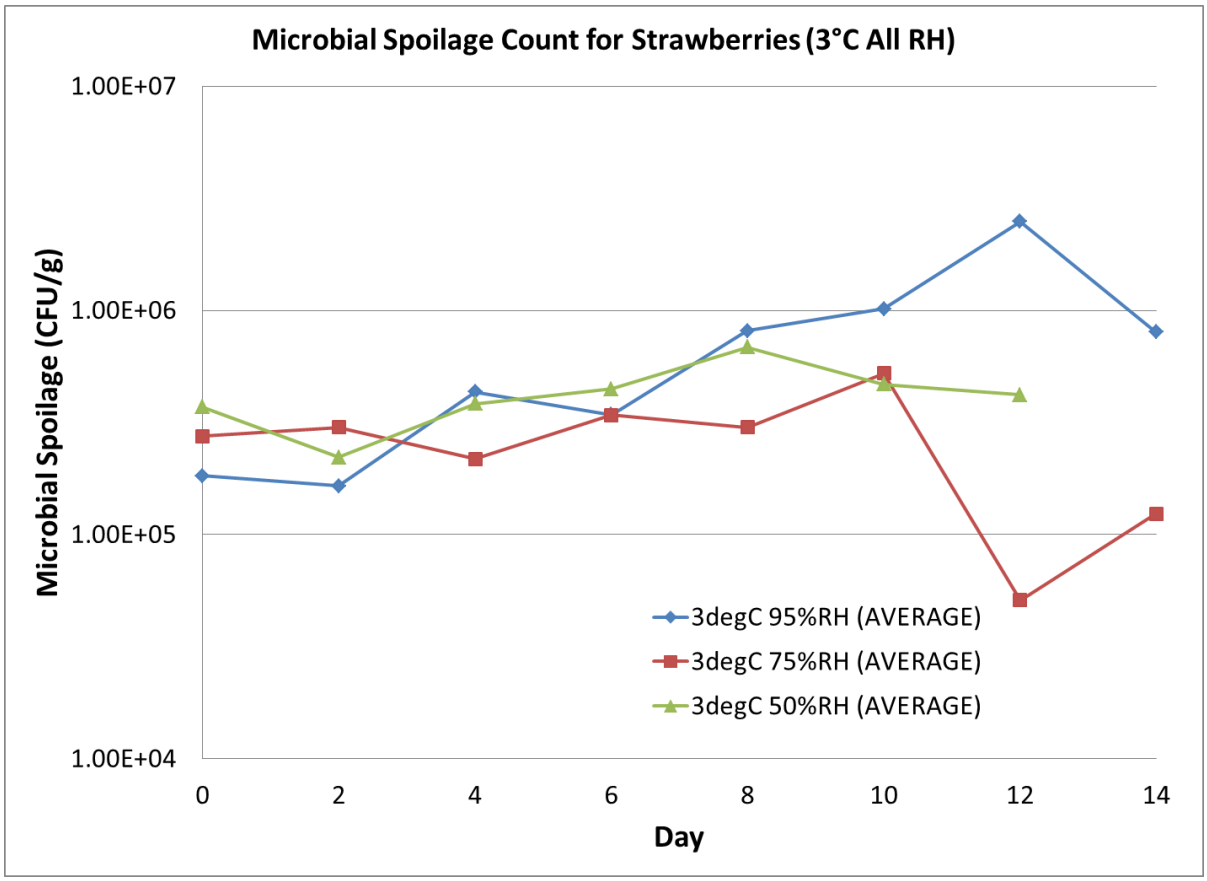


**Figure 67. Microbial spoilage data for strawberries (3°C - all relative humidities)**

Figure 67 shows all three microbial spoilage test averages for strawberries at 3°C and all relative humidities. The starting CFU/g counts for each test were similar (within 50% - 1/2 $\log_{10}$) to each other. These averages remained within 50% of each other until day 10, when they diverged greatly. Throughout the course of these three tests, none exceeded the established freshness threshold due to mold growth, and only the 50%-relative-humidity-test exceeded the freshness threshold due to organoleptic factors (moisture loss by day 10). In previous tests at higher temperatures, peaking of microbial counts, followed by a drastic drop was observed as a precursor to the occurrence of substantial mold spoilage (in about 2 days for 7°C and 4 days for 5°C tests). This may well be the case for these lower temperature tests, as some evidence of mold was seen before day 14. The lack of spoilage due to mold growth suggests that lower temperatures had an inhibiting effect.

Previously, strawberry spoilage data were shown individually or as sets of temperatures (i.e. 7°C all relative humidities etc.). It is useful to compare results in the other dimension of the test matrix (humidity). Figure 68 through Figure 70 show combined spoilage data for all temperatures at one humidity to more easily compare results in a meaningful way.

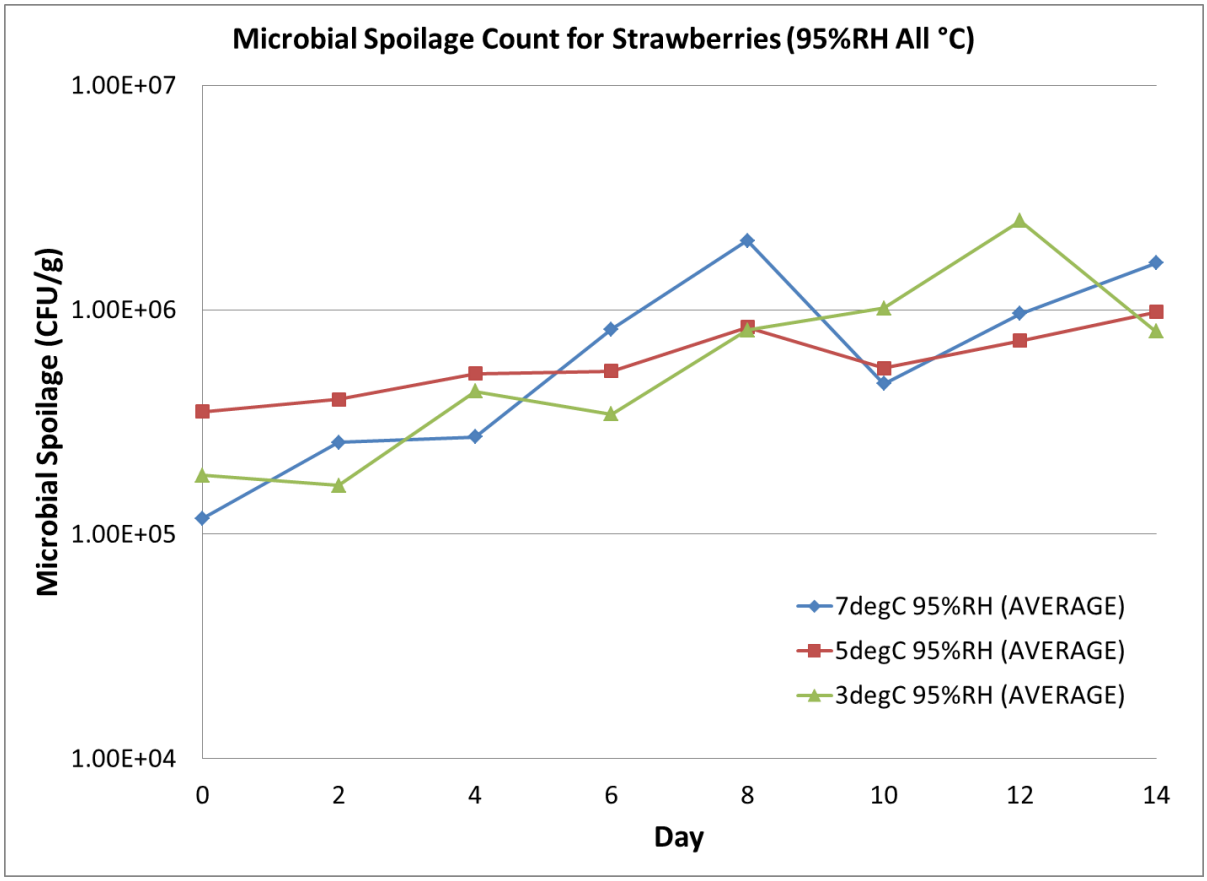


**Figure 68. Microbial spoilage data for strawberries (95% relative humidity - all temperatures)**

Figure 68 shows all three microbial spoilage test averages for strawberries at 95% relative humidity and all temperatures. The starting CFU/g counts for each test were similar (within 50% - 1/2 $\log_{10}$) to each other. These averages remained within 50% until the end of the test (day 14). In previous tests, and for the 7°C and 5°C tests in this comparison, a peak in microbial count peaking followed by a rapid drop, suggested that substantial mold spoilage

was incipient (in about 2-4 days for 7°C and 5°C tests). The lack of spoilage due to mold growth suggests that the lower temperature (3°C test) had an inhibiting effect.

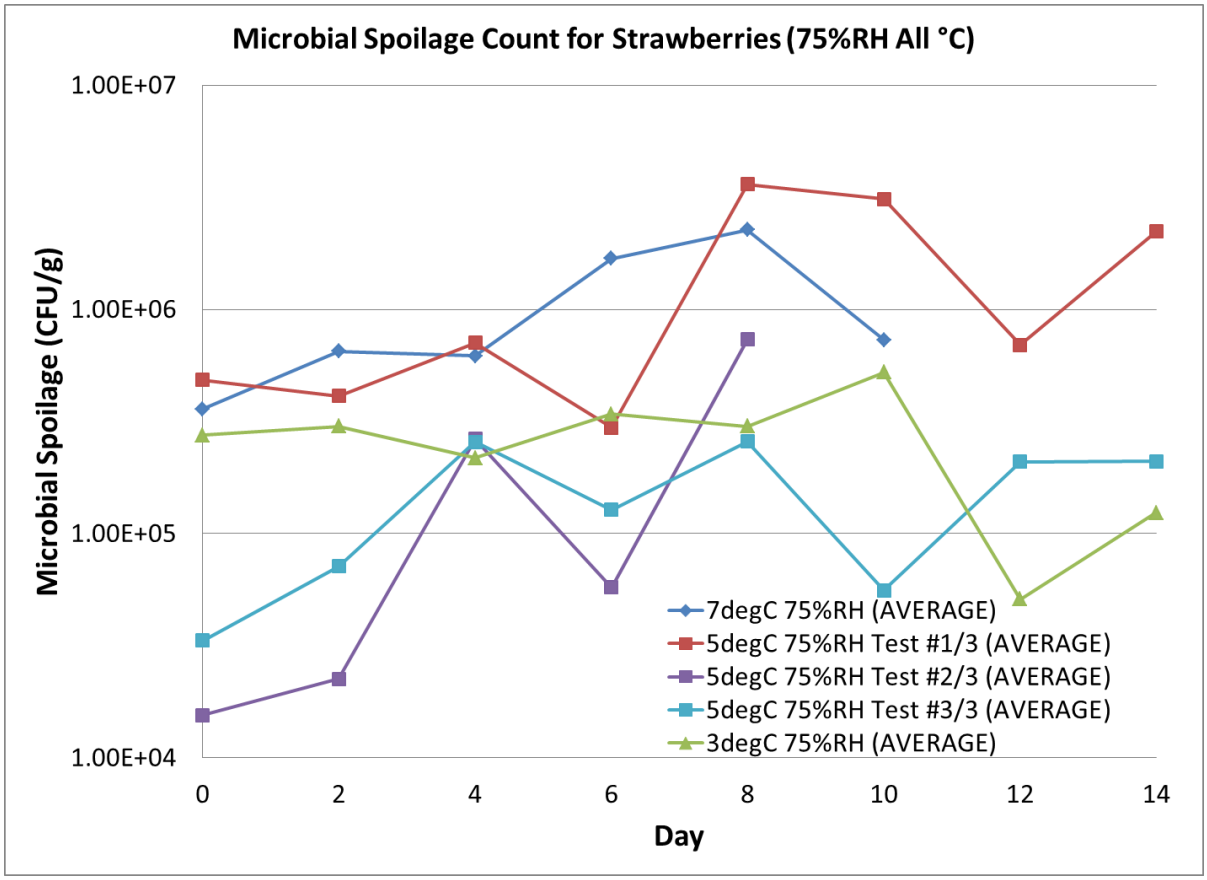


**Figure 69. Microbial spoilage data for strawberries (75% relative humidity - all temperatures)**

Figure 69 shows all five microbial spoilage test averages for strawberries at 75% relative humidity and all temperatures. The starting CFU/g strawberries counts for each test were similar (within 50% - 1/2 $\log_{10}$) except for 5°C 75%RH tests #2 and #3. These averages began to vary substantially around day 6, sometimes by as much as an entire $\log_{10}$. Though the differences in microbial count were large, similar trends were apparent, including a rapid drop in microbial counts following peak levels (days 8 for 7°C, day 8 for 5°C, day 10 for 3°C).

The lack of spoilage due to mold growth suggests that the lower temperature (3°C test) had an inhibiting effect. Spoilage due to mold growth occurred on day 10 for the 7°C test, day 12 for the 5°C test #1 and did not occur within 14 days for the 3°C test or remaining 5°C tests. In fact, no measureable factors for spoilage were achieved during the course of the 3°C 75% or 5°C 75% relative humidity (tests #2 and #3), suggesting that around these two set points may be a suitable combination of environmental factors useful in preventing spoilage.

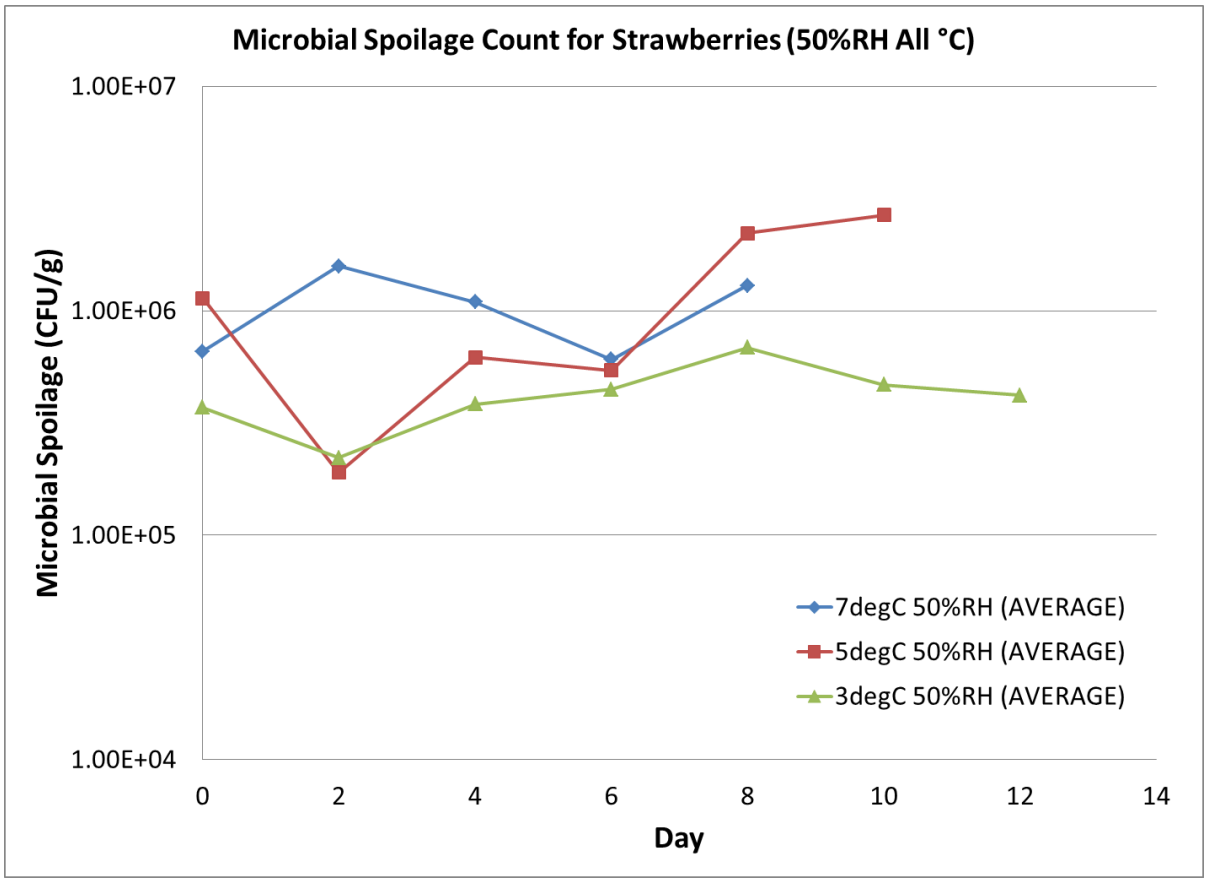


**Figure 70. Microbial spoilage data for strawberries (50% relative humidity - all temperatures)**

Figure 70 shows all three microbial spoilage test averages for strawberries at 50% relative humidity and all temperatures. The starting CFU/g strawberries counts for each test were similar (little more than 50% - 1/2 $\log_{10}$) to each other. The average for the 7°C test varied

substantially from the 5°C and 3°C tests by day 2; the 5°C and 3°C tests began to diverge around day 8. All three of these tests exceeded the freshness threshold due to moisture losses: the 7°C sample after 8 days, and the 5°C and 3°C samples after 10 days. Spoilage beyond the freshness threshold due to mold growth occurred only for the 7°C test on day 6 and did not occur within 10 days for the 5°C test or within 12 days for the 3°C test. It is interesting to note that the 3°C 50% relative humidity test was the only test not showing visual of mold growth by the end. In fact, the only measureable factor for spoilage achieved during the course of the 3°C 50% relative humidity test was moisture loss. These results suggest that if some dehydration is not a concern for the ultimate use of these strawberries (e.g. strawberries to be used in smoothies, pies, etc.), then this could be an appropriate environmental condition for preservative storage of these berries prior to use.

The relative humidity generator/environmental chamber used in this experiment has the capability to record selected data points throughout the test runs. Chamber temperatures were logged throughout the tests to confirm that accurate temperatures were achieved. These data were converted to charts for each test and can be seen in Appendix A. Chamber humidities were also logged to confirm that environmental conditions remained within tolerance. These data were converted to charts for each test and can be seen in Appendix B.

## Microbial Spoilage Testing: Repeatability

Because lettuce samples undergoing microbial testing were sealed in a plastic bag, the moisture level is expected to be saturated and constant. For this reason, only one set of lettuce tests were used for microbial spoilage testing. For the purposes demonstrating test repeatability, two microbial spoilage counts were taken for each temperature (four at 5°C). Three standard tests for microbial spoilage in strawberries stored at 5°C 75% relative humidity are shown after the results for the lettuce in Figure 74.

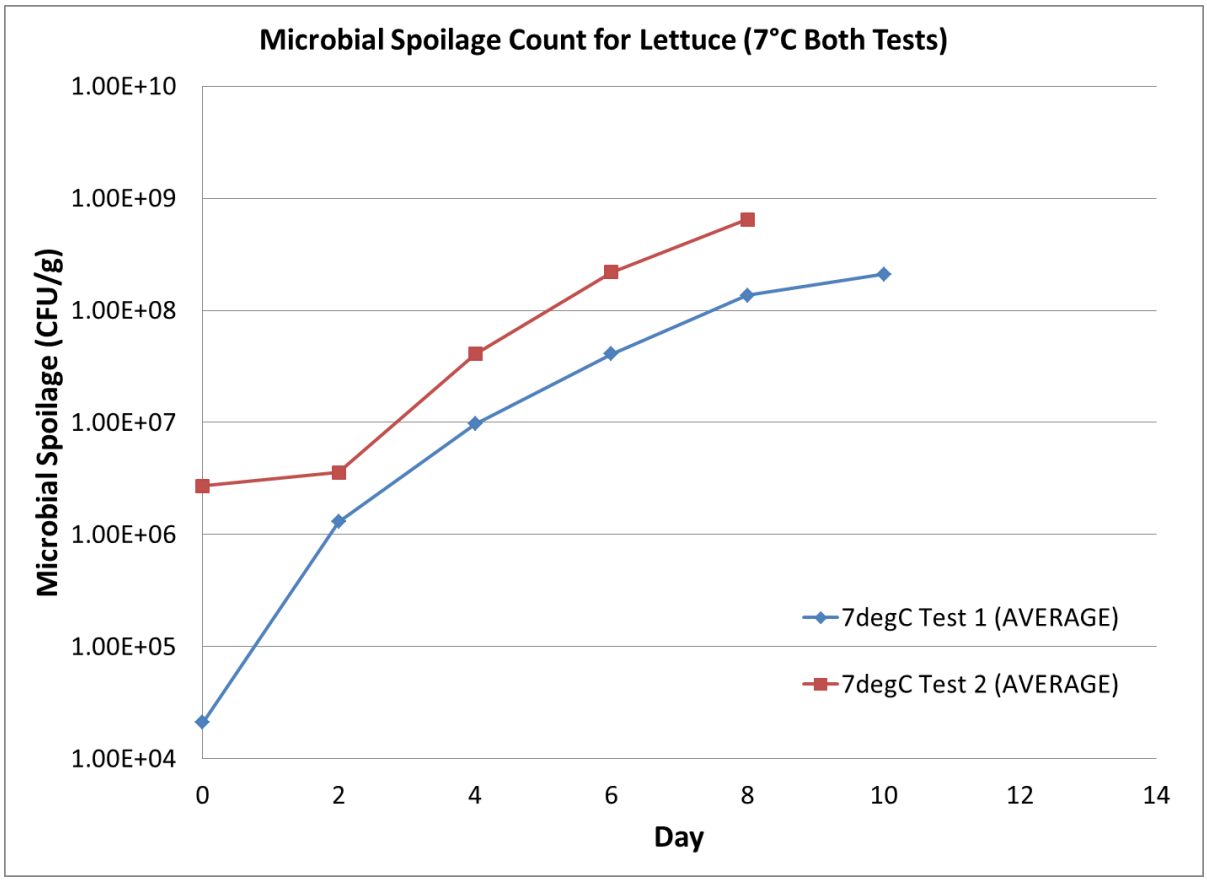


**Figure 71. Microbial spoilage data for lettuce (7°C – average count - both tests)**

Figure 71 shows the first and second microbial spoilage test run averages for lettuce at 7°C. The starting spoilage numbers were substantially different ($2.7x10^6$ CFU/g lettuce vs. $2.1x10^4$ CFU/g), differing by two orders of magnitude. Growth progression showed a steady rise throughout the test period, so that both tests were within one order of magnitude by the second test day ($1.3x10^6$ CFU/g lettuce vs. $3.4x10^6$ CFU/g). These separate samples remained within one log of each other until the threshold of freshness was reached ($1.0x10^7$ CFU/g lettuce). These samples were considered beyond the threshold of freshness between day 2 and day 6 of these test runs.

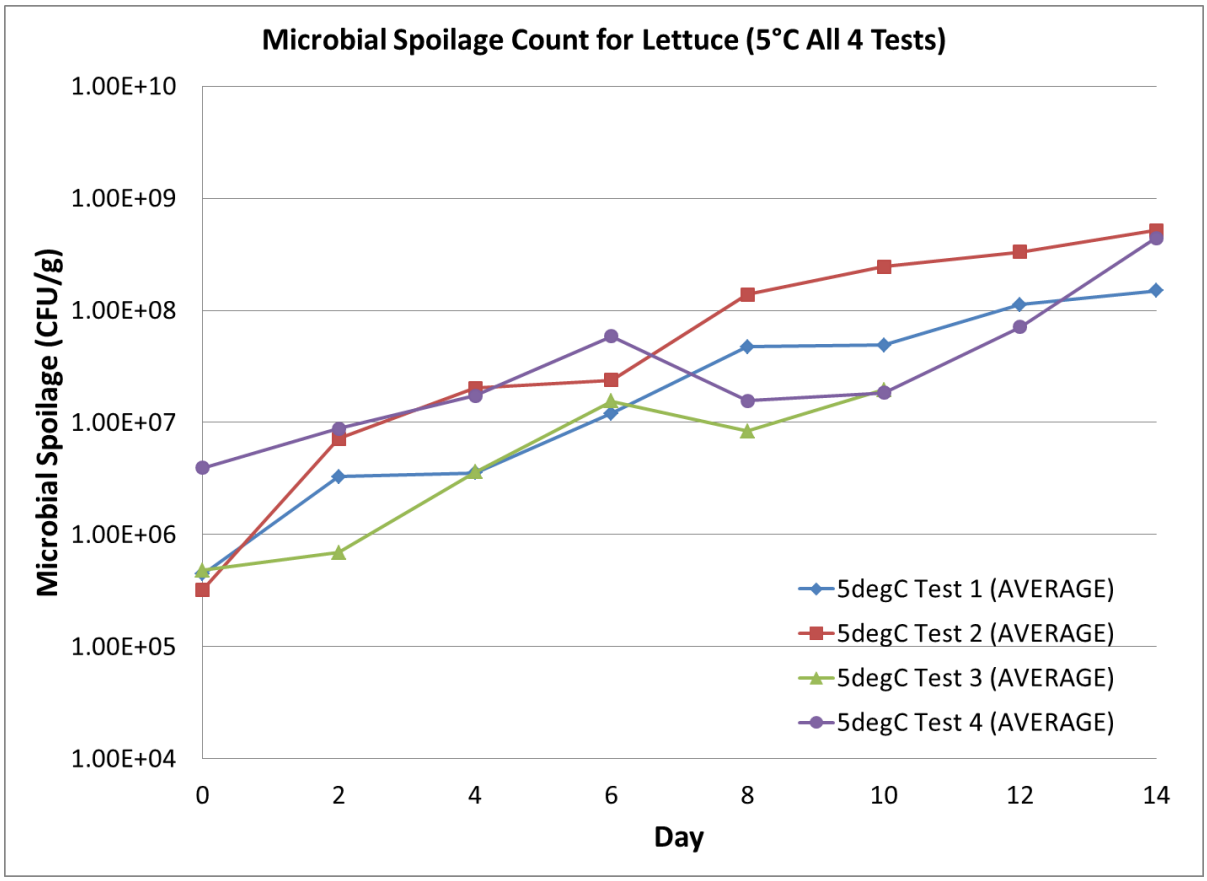


**Figure 72. Microbial spoilage data for lettuce (5°C – average count - all 4 tests)**

Figure 72 shows the four microbial spoilage test run averages for lettuce at 5°C. The initial microbial loads were strikingly similar except for #4 ($3.2x10^5$ CFU/g lettuce, $4.4x10^5$ CFU/g, $4.8x10^5$ CFU/g vs. $4.0x10^6$ CFU/g). Growth rates for all four tests rose throughout the test period, remaining within about one order of magnitude until the end of the test period. The similarity between these growth curves suggests that similar microflora were present on all samples. All test samples were considered beyond the freshness threshold between day 2 and day 6 of these test runs.

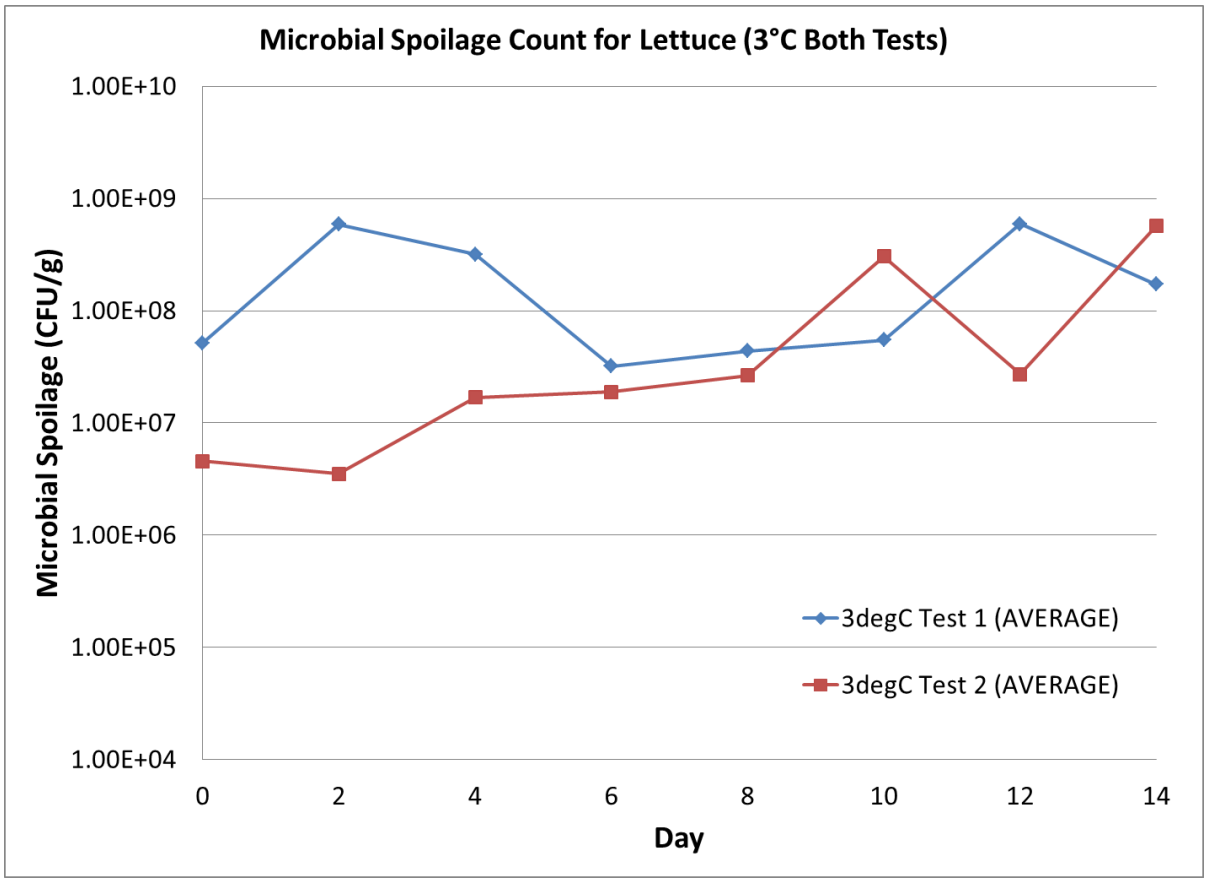


**Figure 73. Microbial spoilage data for lettuce (3°C – average count - both tests)**

Figure 73 shows the first and second microbial spoilage test run averages for lettuce at 3°C. The initial spoilage levels differed by an order of magnitude ($4.6x10^6$ CFU/g lettuce vs. $5.2x10^7$ CFU/g). Differences observed between these two samples may reflect differences in the microbial spoilage flora, or the levels of this flora between the strawberries used in these tests. These samples were considered beyond the freshness threshold between day 0 and day 4 of these test runs.

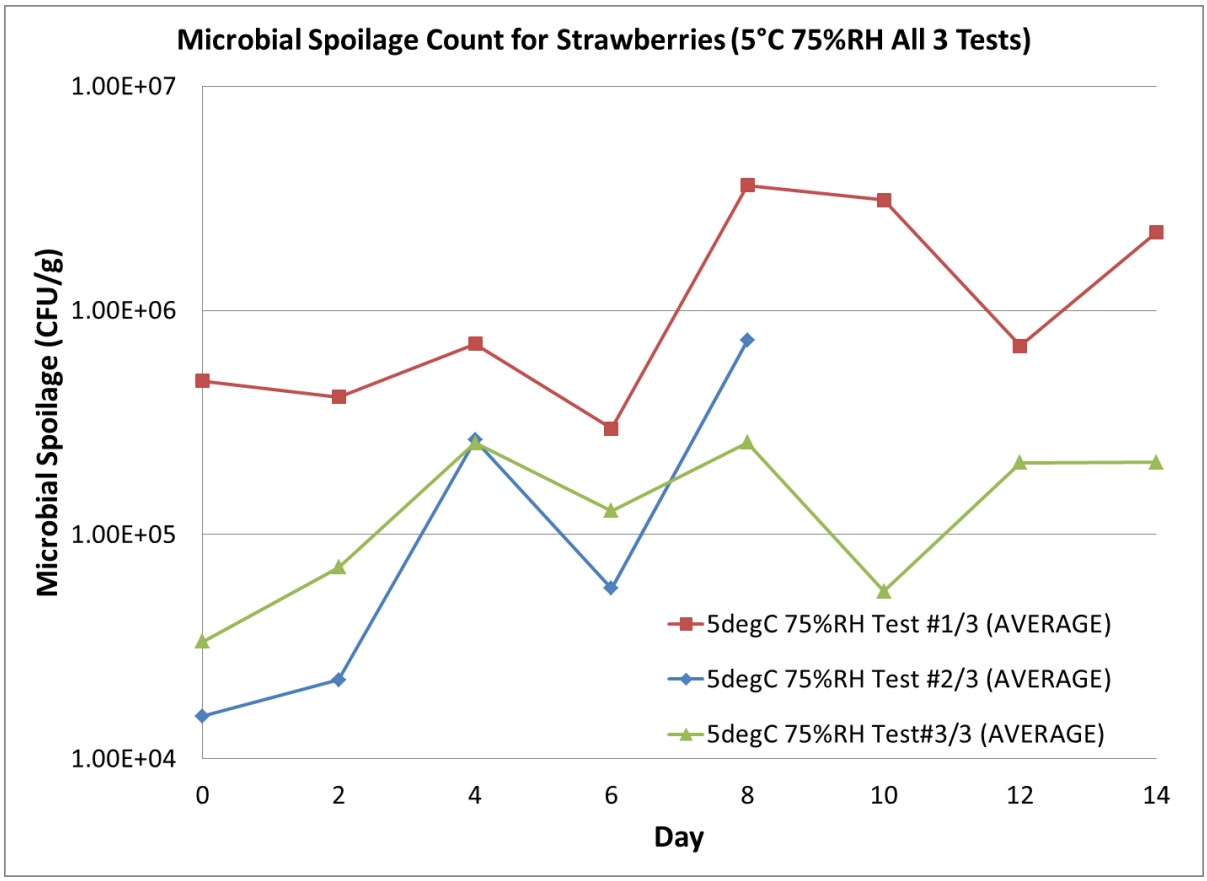


**Figure 74. Microbial spoilage data for strawberries (5°C 75% all 3 Tests)**

Figure 74 shows all three microbial spoilage test averages for strawberries at 5°C 75% relative humidity. The starting CFU/g count for each test varied greatly, though they came to within about 50% (half of one $\log_{10}$) of each other by day 4. Despite likely dissimilarities in the initial inoculum levels present on these different batches of strawberries, similar trends were seen across these samples, suggesting that similar processes of growth and die-off occurred within these different tests.

# CHAPTER 4. SUMMARY AND DISCUSSION

## Repeatability

Two extra tests for lettuce and strawberries at 5°C and 75% relative humidity were used to show the repeatability (or lack thereof) within this experiment. This test condition was chosen for repetition because it resided within the center of the test matrix (Table 1). The following are conclusions drawn regarding the repeatability of this experiment with respect to the three repeated tests:

Moisture Losses: Lettuce (Figure 21): For these tests, the freshness threshold due to moisture loss (20% loss or greater according to the biological method of test) was not reached. Because the air flow rate remained the same, as well as the temperature and humidity levels, some other factor contributed to these discrepancies. Potentially confounding factors could include uneven microbial inocula or growth, leaf arrangement or density of the samples, or perhaps uneven initial moisture levels. The largest difference in moisture loss was about 6% between Test #1 and Test #3 by Day 14.

Strawberries (Figure 22): These samples exhibited uneven moisture losses. Potential complicating factors could include uneven microbial processes (inoculum type or level), berry arrangement, the size/density of the berries themselves, or perhaps uneven initial moisture levels. The largest difference in moisture loss was about 6% between Test #1 and Test #2 by Day 14.

Photographs/Organoleptic Quality: Lettuce (Figure 27, Figure 41 and Figure 42): There were no substantial differences in the organoleptic qualities of these samples throughout the three tests. Differences in moisture loss were observed between the three tests; however the samples did not exhibit spoilage beyond the freshness threshold within 14 days.

Strawberries (Figure 36, Figure 43 and Figure 44): The results show that only the first test exceeded the freshness threshold during the 14 days. The results for the second test were inconclusive due to the failure in the test chamber at day 10. As for the third test, substantial mold coverage occurred on the last day, demonstrating that the samples would have surpassed the threshold of freshness about 1-2 days afterward. One factor most likely affecting this discrepancy could be initial inoculum (type or level).

Microbial Growth: Lettuce (Figure 72): Four microbial spoilage test runs were taken for lettuce at 5°C. The initial inocula were strikingly similar except for test #4 ($3.2x10^5$ CFU/g lettuce, $4.4x10^5$ CFU/g, $4.8x10^5$ CFU/g vs. $4.0x10^6$ CFU/g). Growth rose steadily throughout the test period, remaining within about one order of magnitude until the end. The similar growth curves of these samples may indicate similar microflora were present. All test samples were considered beyond the freshness threshold between day 2 and day 6 of these test runs.

Strawberries (Figure 74. Microbial spoilage data for strawberries (5°C 75% all 3 Tests): Three microbial spoilage tests were performed for strawberries at 5°C 75% relative humidity. The starting CFU/g for each test varied greatly, but came to within about 50% (half of one $\log_{10}$) of each other by day 4. Because of these differences, especially in the first test, it is difficult to compare the tests directly, although there are some similarities. Firstly, the averages for each test reached similar CFU/g counts by day 4, and remained similar until day 8. The colony count dropped for each test by day 6, then rose until day 8, and fell again. This suggests that similar microbial processes were taking place and that there was some degree of repeatability with these tests.

Conclusions regarding microbial test repeatability: Tests were conducted using different lots of produce having varying degrees of initial microbial inocula (and possibly different distributions in the types of microbes present). Despite this substantial biological variation, the tests still yielded results that were similar within about a factor of 10, indicating an

overall good level of repeatability across different lots of produce. This outcome suggests that the data collected here provided a good indication of the inherent similarities across different lots of produce and that similar results may be expected in future experiments with strawberries and romaine lettuce.

## Experiment Results

The results of the entire experiment with regards to freshness threshold are shown in Table 2. The table compares the spoilage paths of moisture loss, organoleptic quality and microbial count for each test run.

| Strawberries | 1st Day Freshness Threshold Exceeded | Reason | 2nd Day Freshness Threshold Exceeded | Reason | 3rd Day Freshness Threshold Exceeded | Reason |
|---|---|---|---|---|---|---|
| 7degC 95% | 8,14 | Micro | 12 | Mold | | |
| 7degC 75% | 8 | Micro | 10 | Mold | | |
| 7degC 50% | 2,4,8 | Micro | 6 | Mold | 8 | Loss W |
| 5degC 95% | 12 | Mold | | | | |
| 5degC 75% | 8,10,14 | Micro | 12 | Mold | | |
| 5degC 75% #2 | none* | | | | | |
| 5degC 75% #3 | none | | | | | |
| 5degC 50% | 0,8,10 | Micro | 10 | Loss W | | |
| 3degC 95% | 10,12 | Micro | | | | |
| 3degC 75% | none | | | | | |
| 3degC 50% | 10 | Loss W | | | | |
| | | | | | | |
| Bagged Lettuce | | | | | | |
| 7degC Test 1 | 6 to 10 | Micro | | | | |
| 7degC Test 2 | 4 to 8 | Micro | | | | |
| 5degC Test 1 | 6 to 14 | Micro | | | | |
| 5degC Test 2 | 4 to 14 | Micro | | | | |
| 5degC Test #3 | 6,10 | Micro | | | | |
| 5degC Test #4 | 4 to 14 | Micro | | | | |
| 3degC Test 1 | 0 to 14 | Micro | | | | |
| 3degC Test 2 | 4 to 14 | Micro | | | | |
| | | | | | | |
| Unbagged Lettuce | | | | | | |
| 7degC 95% | none | | | | | |
| 7degC 75% | none | | | | | |
| 7degC 50% | 12 | Loss W | | | | |
| 5degC 95% | none | | | | | |
| 5degC 75% | none | | | | | |
| 5degC 75% #2 | none | | | | | |
| 5degC 75% #3 | none | | | | | |
| 5degC 50% | 12 | Wilt | | | | |
| 3degC 95% | none | | | | | |
| 3degC 75% | none | | | | | |
| 3degC 50% | 12 | Wilt | | | | |

*5degC 75% #2 ended on day 10 due to complications in test chamber

**Table 2. Spoilage results for strawberries and bagged/un-bagged lettuce**

Table 2 shows the spoilage results for strawberries and bagged/non-bagged lettuce tests. The "Day" indicates on what day or days the samples were considered beyond the freshness threshold according to the biological method of test. These event days are listed in order of appearance, therefore two, and in one case, three separate days are shown for different spoilage pathways. The reasons for exceeding the freshness threshold are as follows: 'Micro' indicates the average quantity of microbes (CFU/g produce) met or exceeded the freshness threshold level defined by the biological method of test ($1x10^6$ for strawberries and $1x10^7$ for lettuce). 'Loss W' indicates the percentage of moisture lost from the sample, with a freshness threshold level of 20% loss for lettuce and strawberries. 'Mold' indicates that mold was observed on 25% or more of the sample (only relevant to strawberries). 'Wilt' indicates that wilting occurred on over 1/3 of the total surface area (only relevant to lettuce).

Romaine lettuce heads were found to last the longest, and stay the freshest at high humidity (~95%) with little impact from temperature. If humidity control is not possible, aseptically separating leaves and storing them in a sterile, sealable bag was found to preserve organoleptic quality at low temperatures (~3°C), although this is not a very practical recommendation for routine storage of lettuce.

Strawberries stored at low temperature and humidity (~3°C and ~50%) were found to resist molding longest; however, firmness was lost after 1-2 weeks. To preserve desirable organoleptic qualities, it was found that storage at low temperature and high humidity (~3°C and ~95%) yielded the best results. Initial microbial count impacted final mold growth.

## Future Work Recommended

The following suggestions may be used in future experiments to help maximize the repeatability of these types of experiments.

Natural variation in microbial inocula across produce lots was a potentially confounding factor, although it is believed that the type of flora colonizing/naturally present on each produce type were relatively similar across lots and even across growing regions (although seasonal difference would still be expected), based on this work and on survey of the scientific literature. Still, by sterilizing lettuce and strawberries (via irradiation, or gas, for example), then inoculating with controlled, lab-grown microflora, it is expected that more uniform growth, under specified environmental conditions, could be achieved than were found using naturally occurring microflora. The biggest drawback to this design would be that it is not representative of real-world scenario, in which varied levels and, to a lesser extent, types of inocula are expected. Although the data were inherently variable, it is apparent that these results are those that would be expected from conditions occurring in real produce samples, and are therefore more valuable than an idealized albeit unrealistic scenario relevant only to a scientific laboratory.

Another recommendation is to limit the variability between produce of the same type throughout a several-month test-span. By using 9 separate environmental chambers at once, the entire experiment will utilize produce from exactly the same season and region. The biggest drawback would be the impracticality of obtaining 9 identical environmental chambers with the same exacting specifications as was utilized in this experiment.

# APPENDIX A: TEMPERATURE LOGS FOR ENVIRONMENTAL CHAMBER TEST RUNS

Spikes in temperature appeared when the chamber door was opened to access the samples.

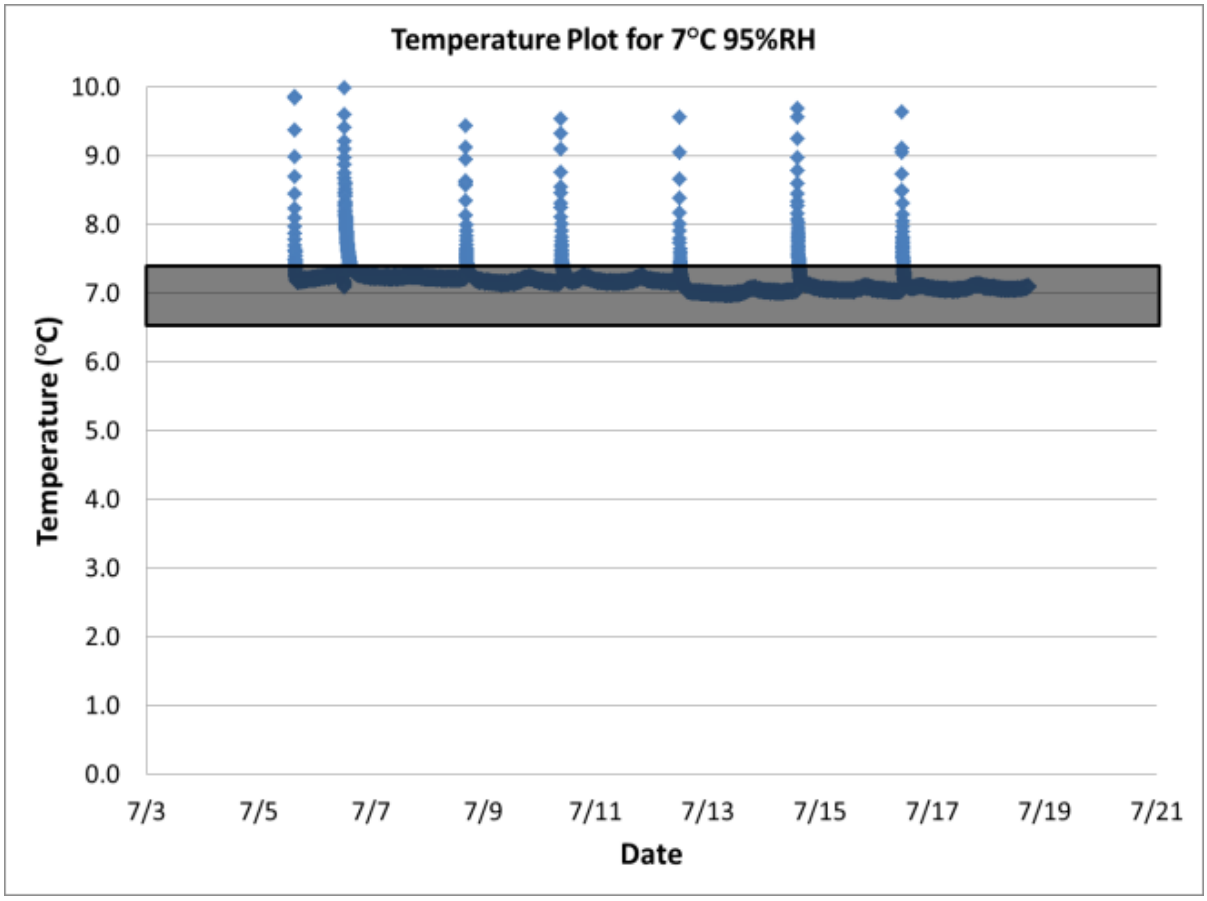


**Figure 75. Temperature plot for 7°C 95% relative humidity test run**

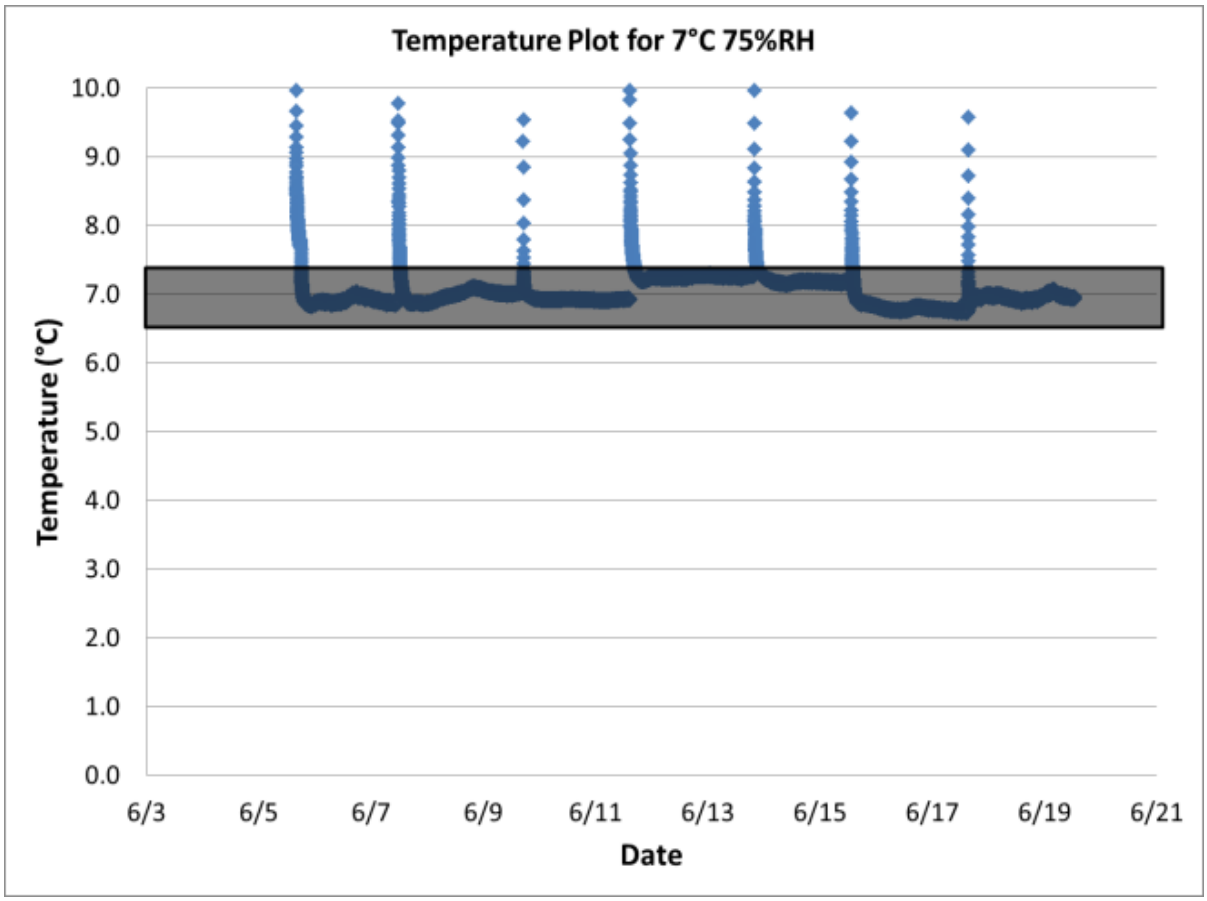


**Figure 76. Temperature plot for 7°C 75% relative humidity test run**

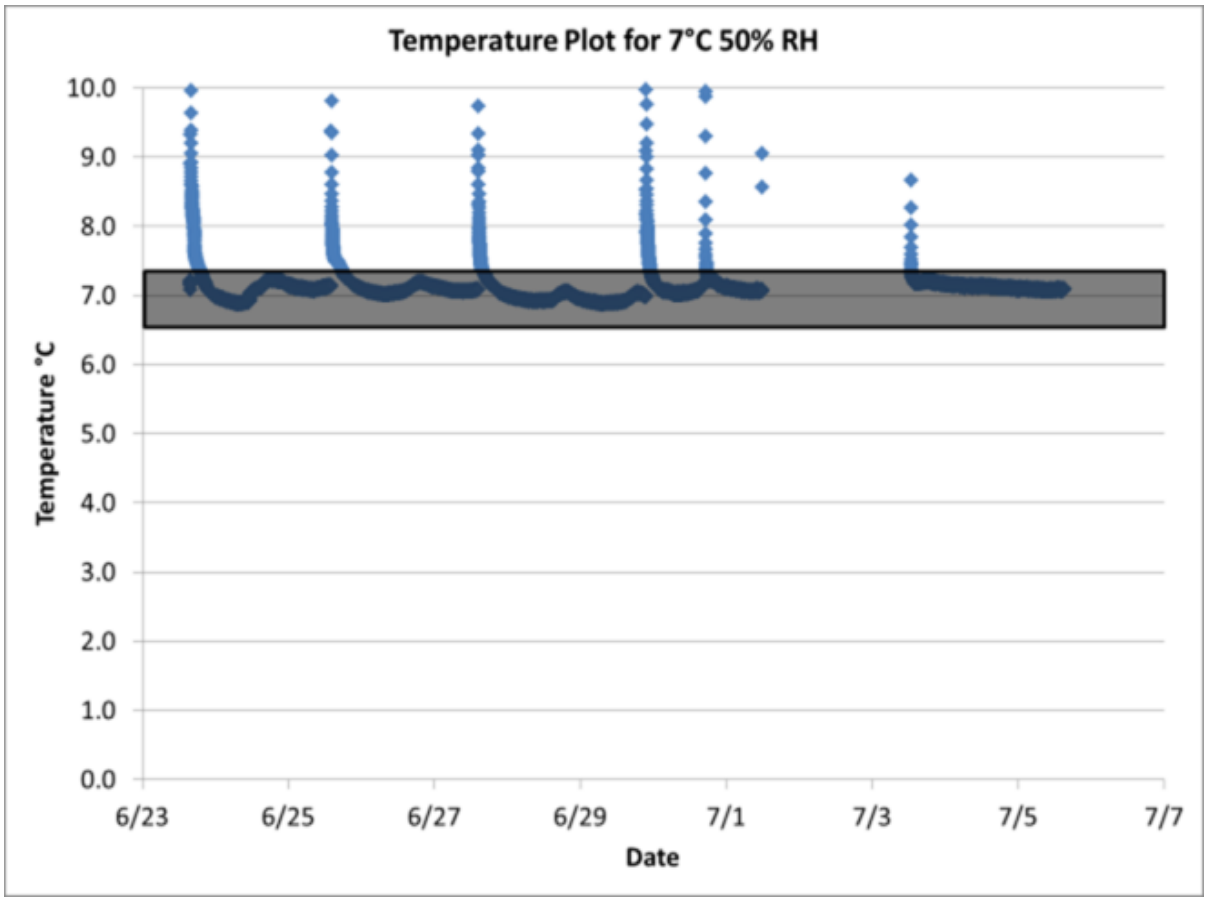


**Figure 77. Temperature plot for 7°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors which resulted in a failure in data collection. The physical integrity of the environmental chamber was not affected by the computer error, and power logs (Outage 2011) were checked to verify that no outages or other anomalies were present during this time.

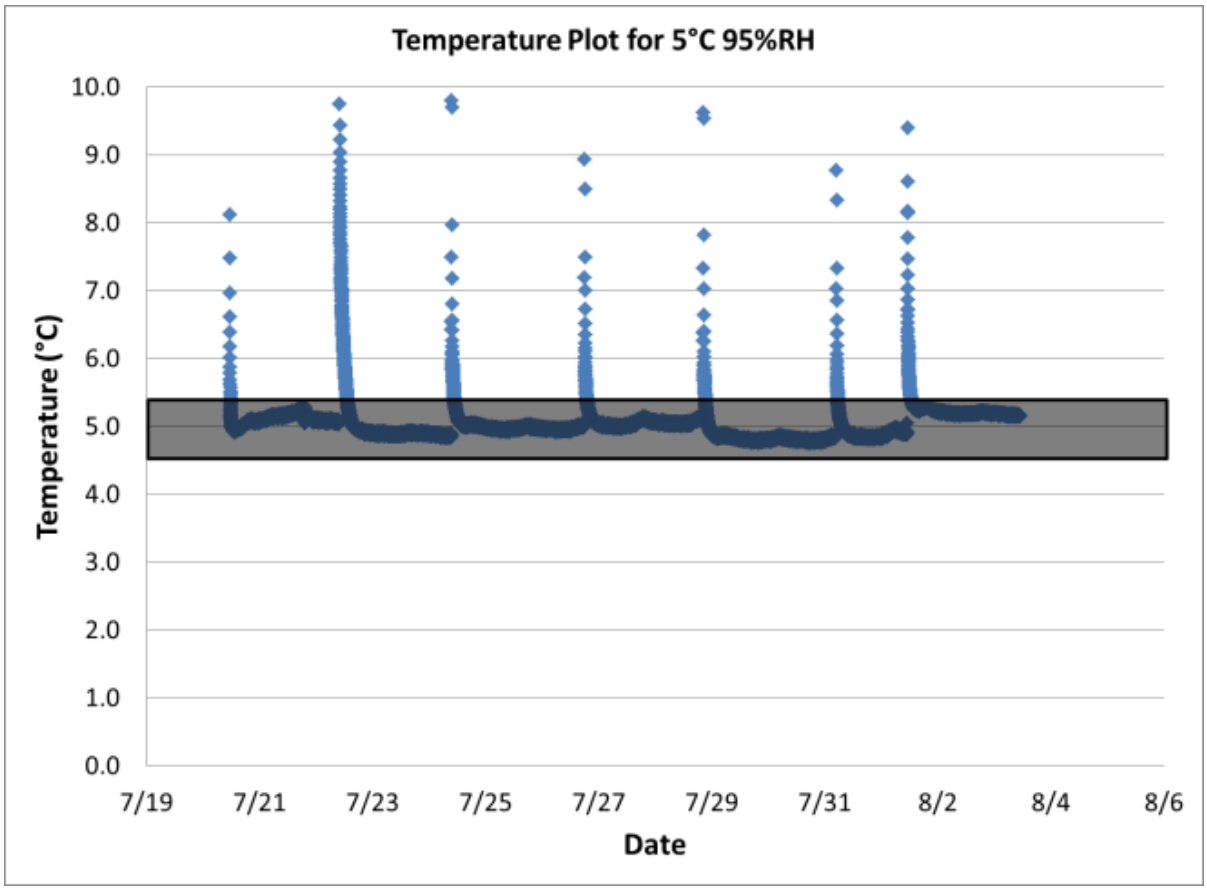


**Figure 78. Temperature plot for 5°C 95% relative humidity test run**

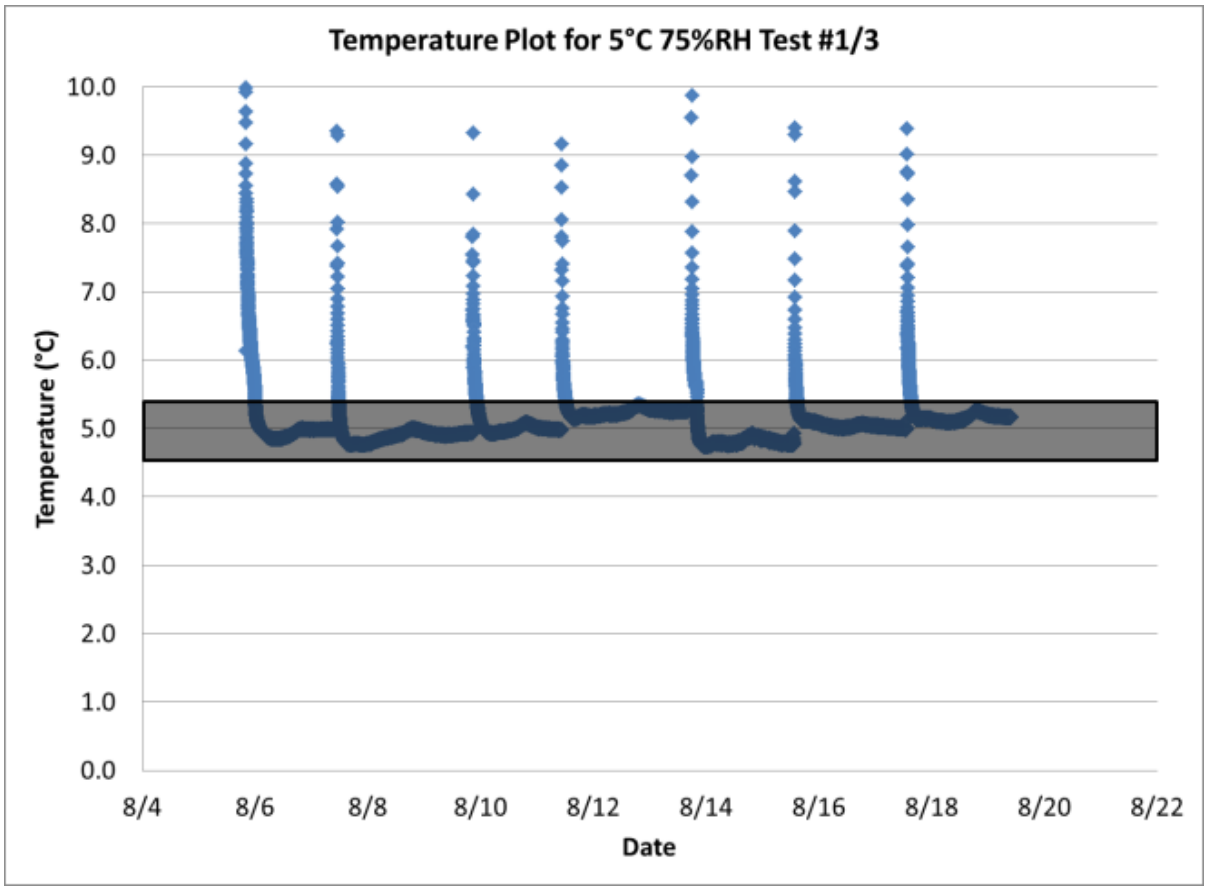


**Figure 79. Temperature plot for 5°C 75% relative humidity test run #1/3**

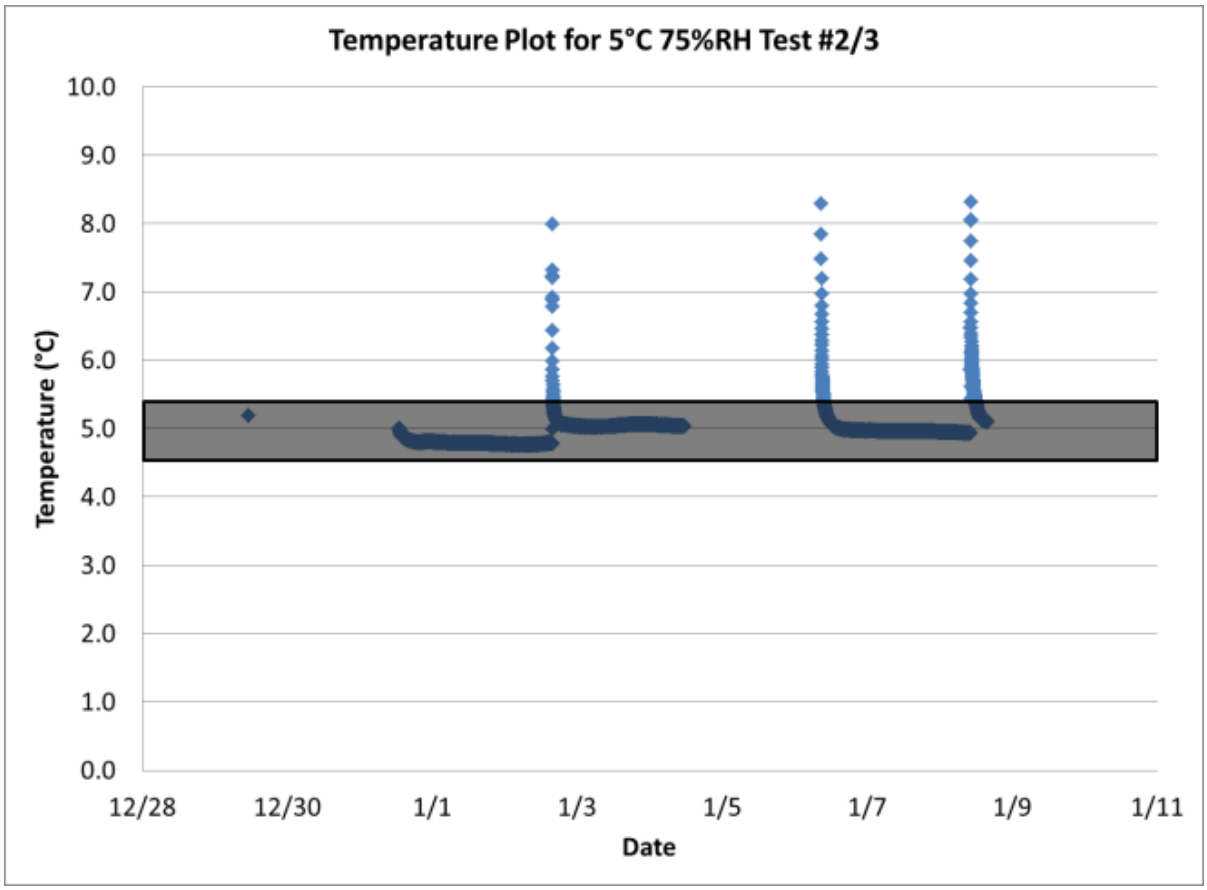


**Figure 80. Temperature plot for 5°C 75% relative humidity test run #2/3**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The physical integrity of the environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or other anomalies were present during this time.

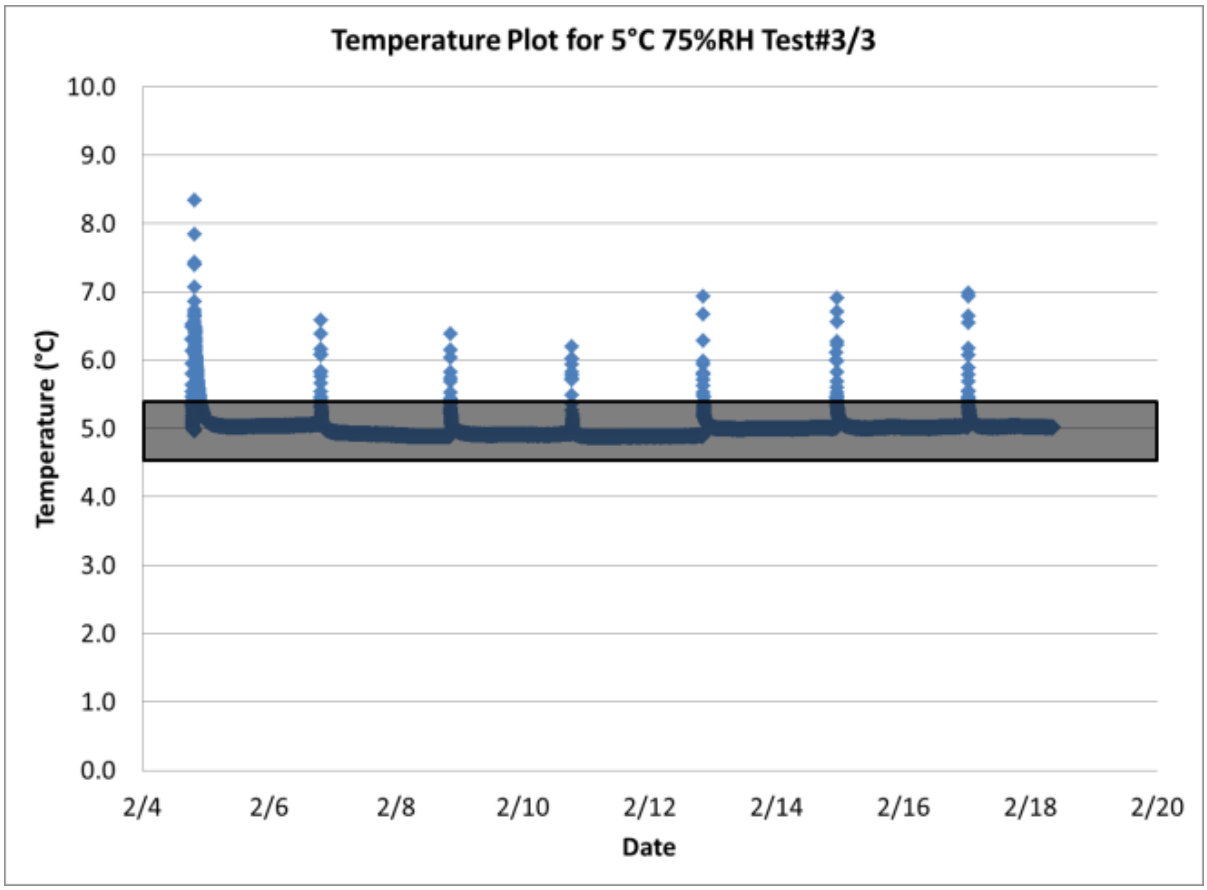


**Figure 81. Temperature plot for 5°C 75% relative humidity test run #3/3**

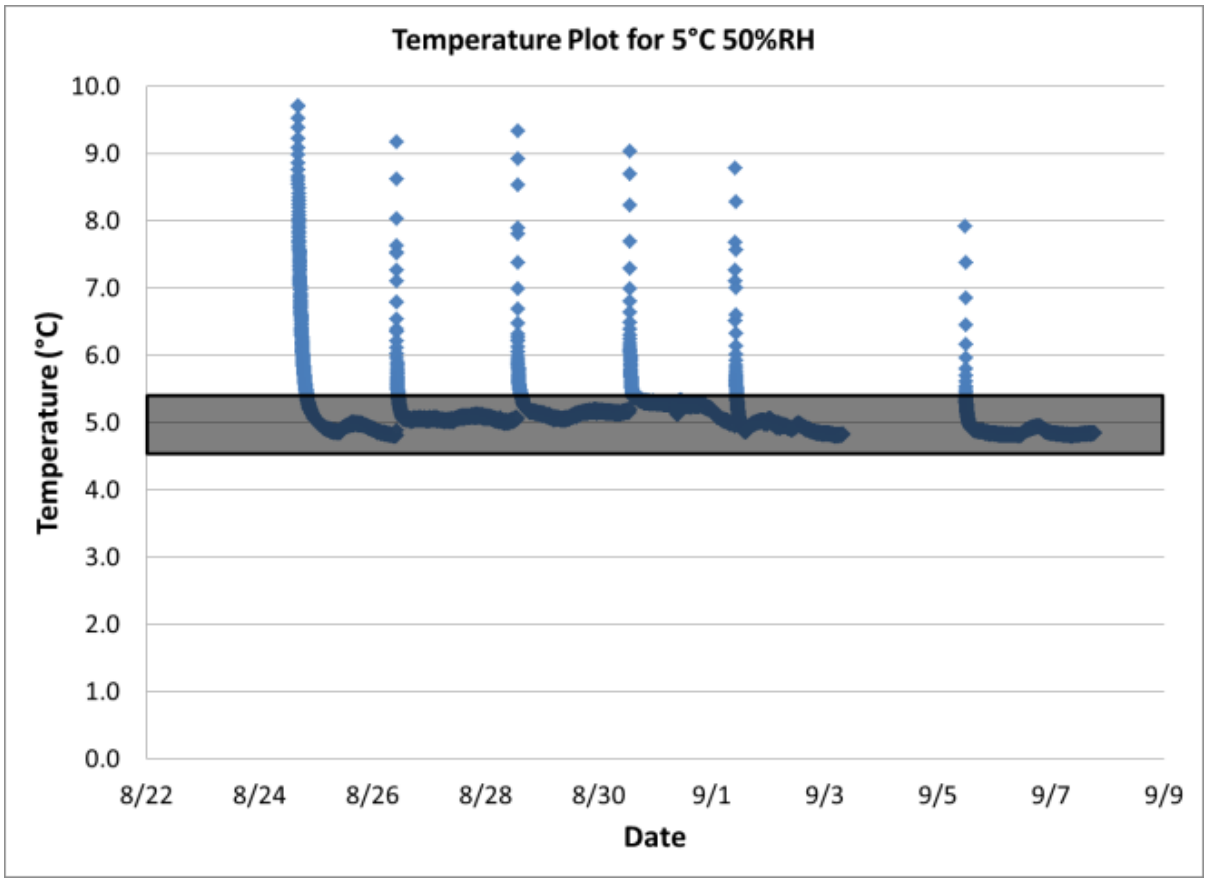


**Figure 82. Temperature plot for 5°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

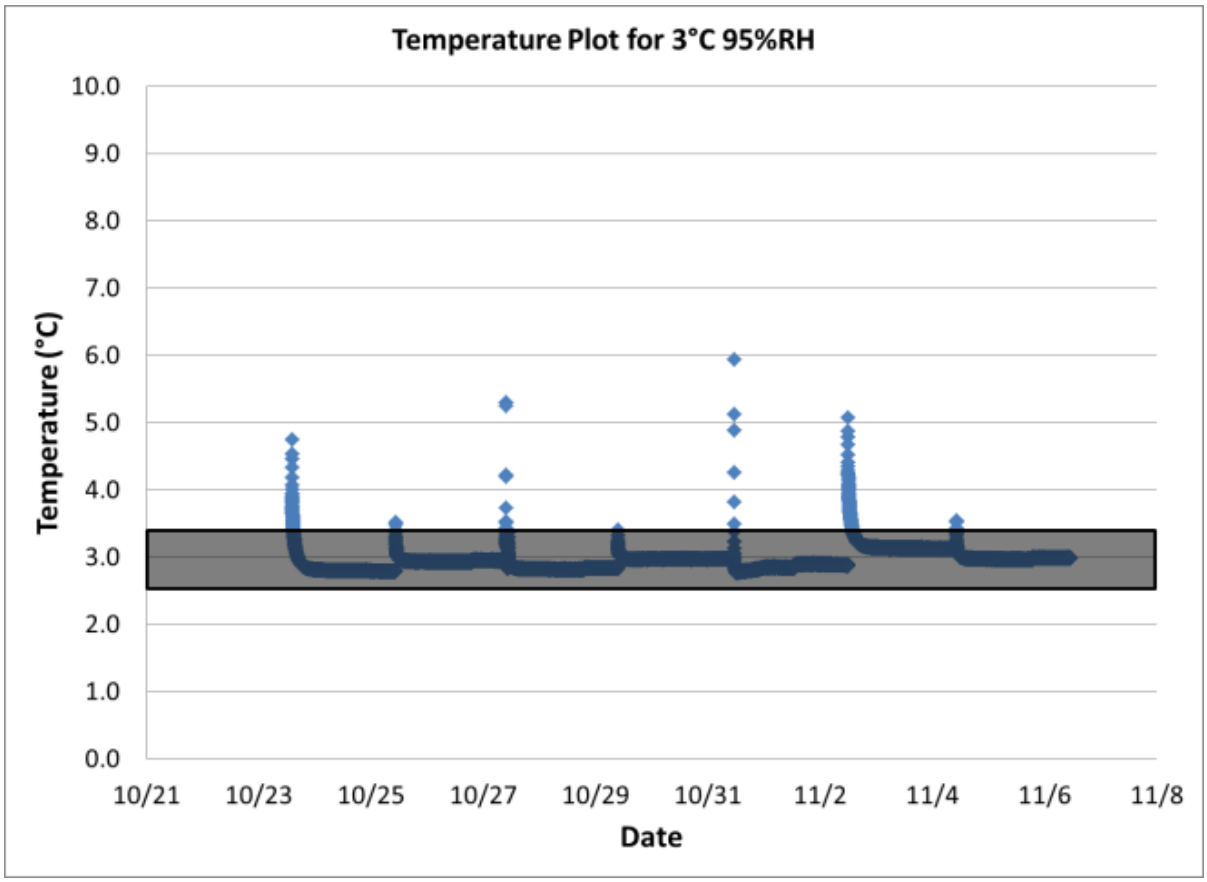


**Figure 83. Temperature plot for 3°C 95% relative humidity test run**

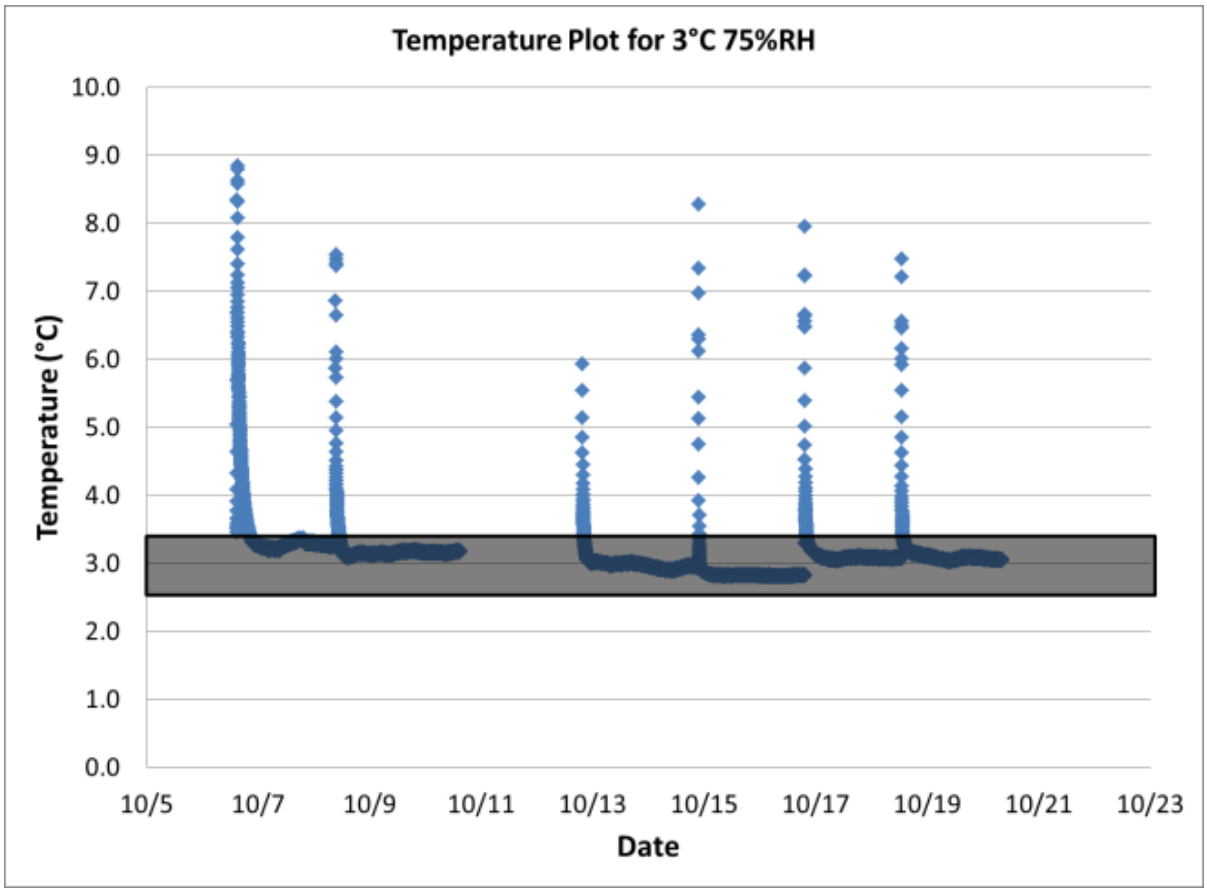


**Figure 84. Temperature plot for 3°C 75% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

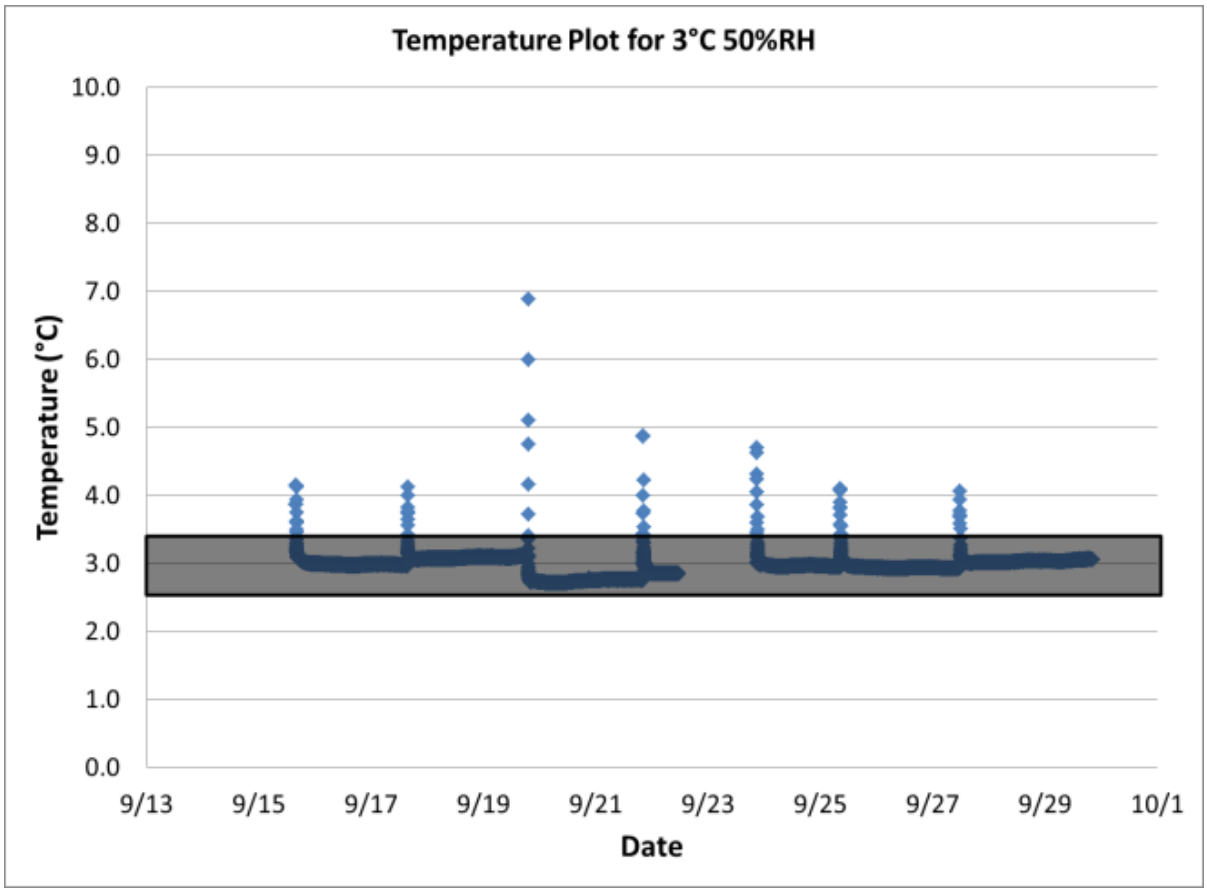


**Figure 85. Temperature plot for 3°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or other anomalies were present during this time.

# APPENDIX B: HUMIDITY LOGS FOR ENVIRONMENTAL CHAMBER TEST RUNS

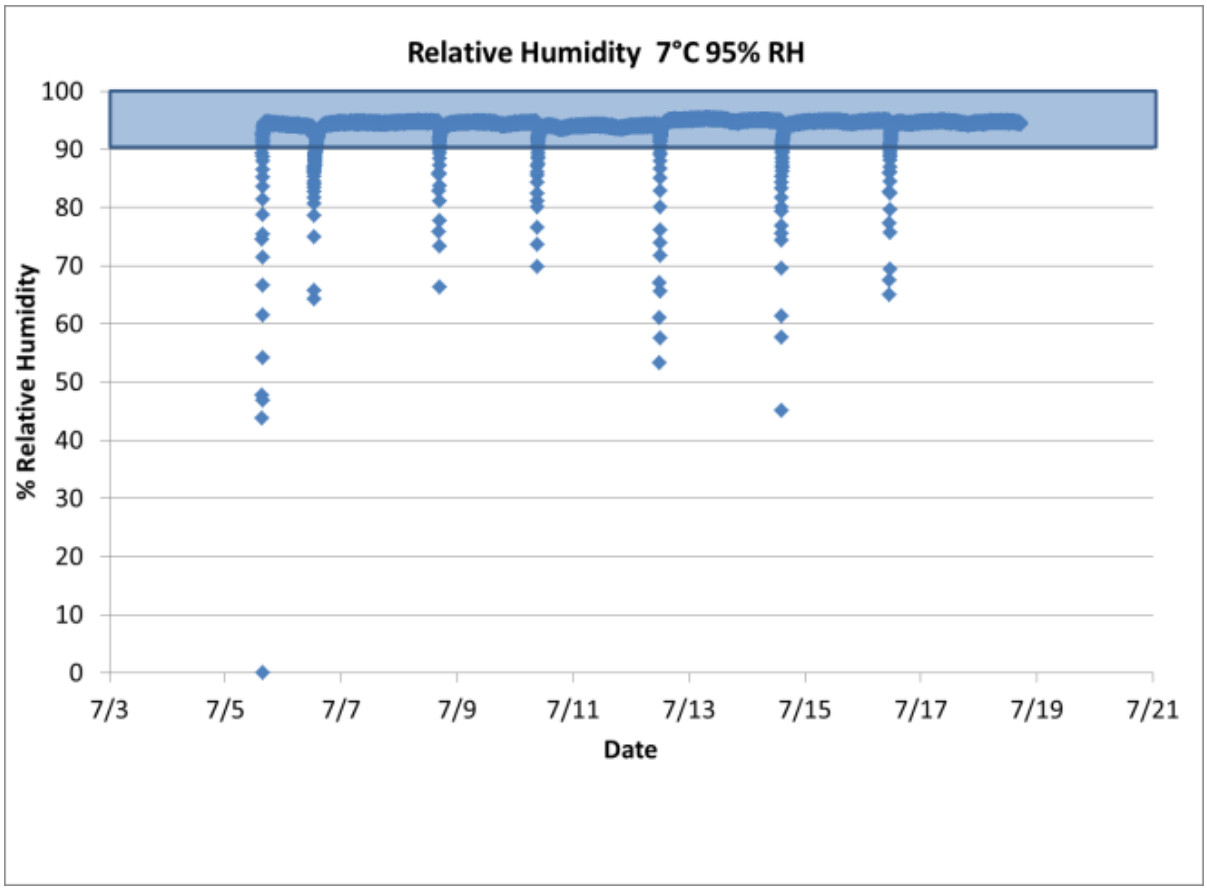


**Figure 86. Humidity plot for 7°C 95% relative humidity test run**

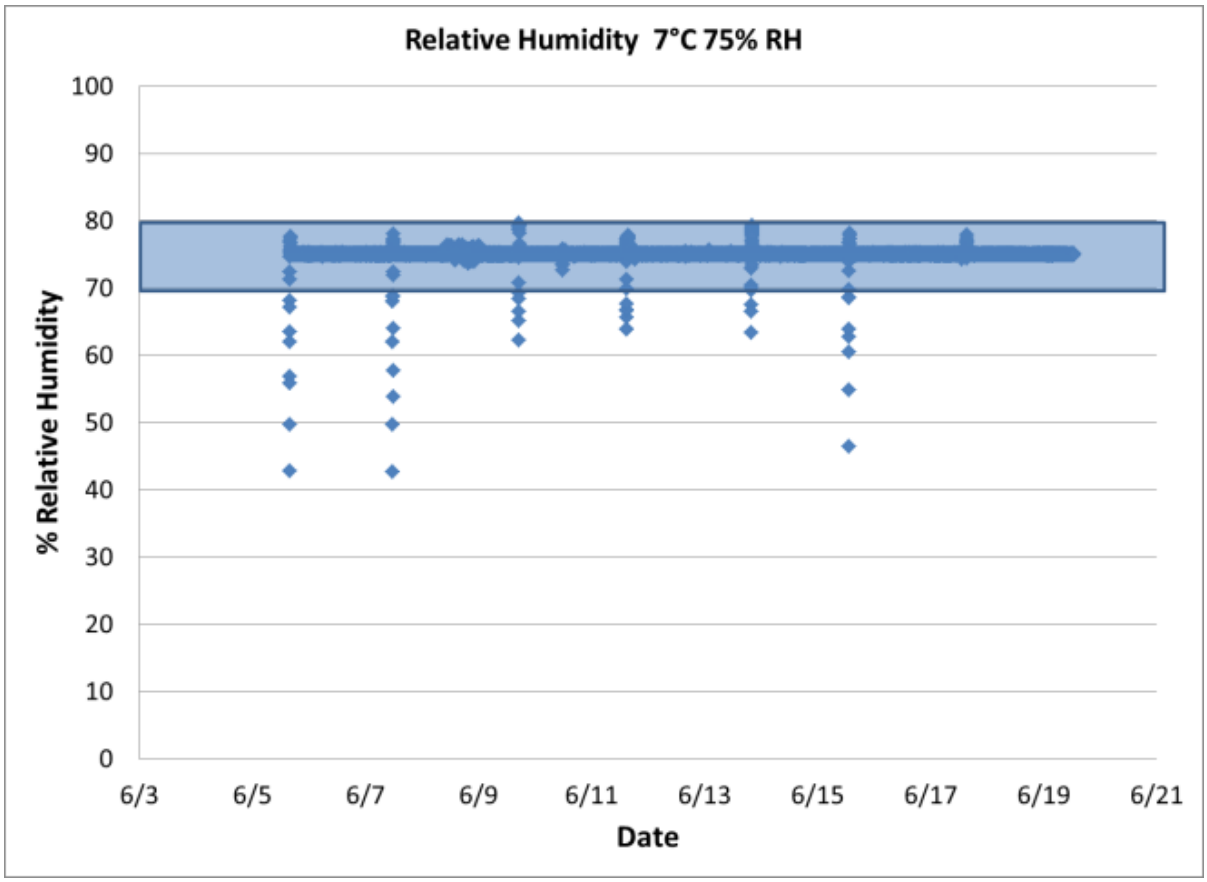


**Figure 87. Humidity plot for 7°C 75% relative humidity test run**

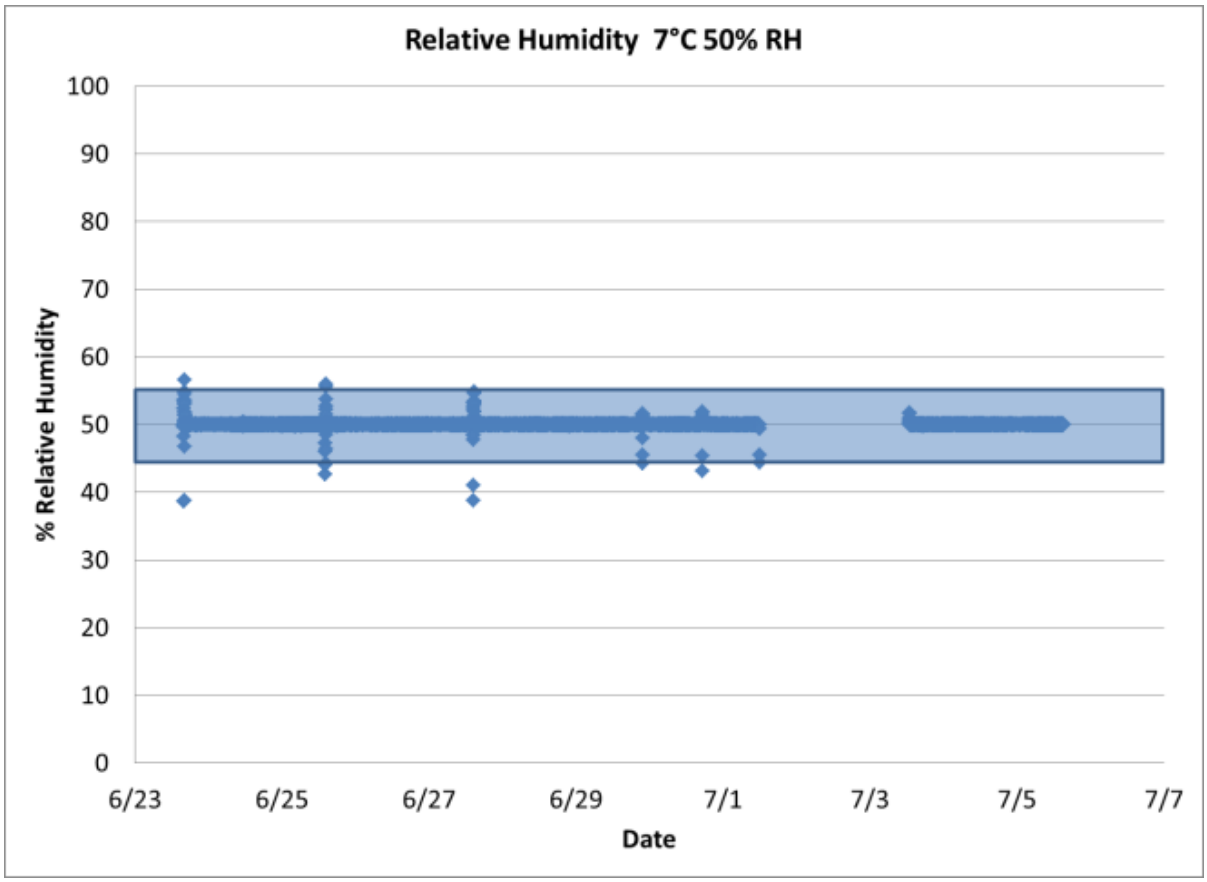


**Figure 88. Humidity plot for 7°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The physical integrity of the environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

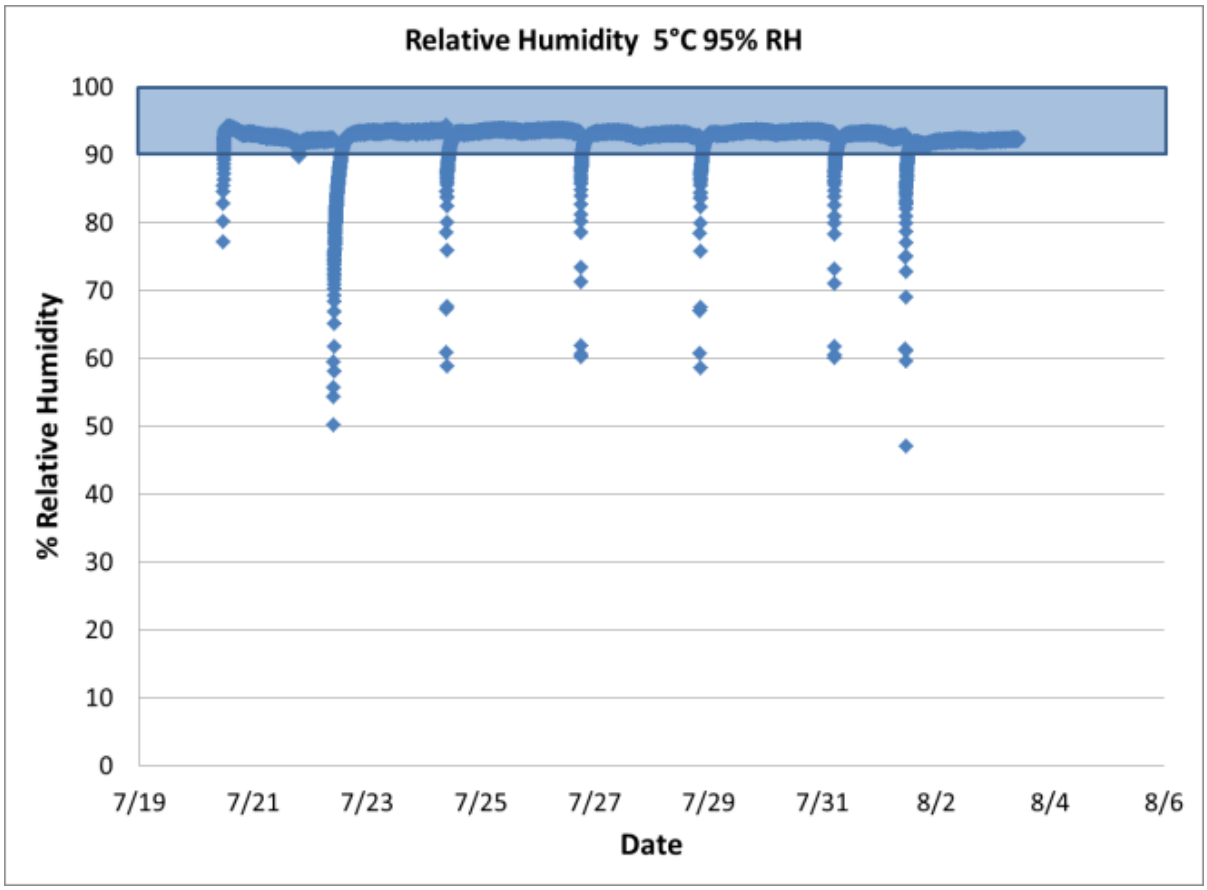


**Figure 89. Humidity plot for 5°C 95% relative humidity test run**

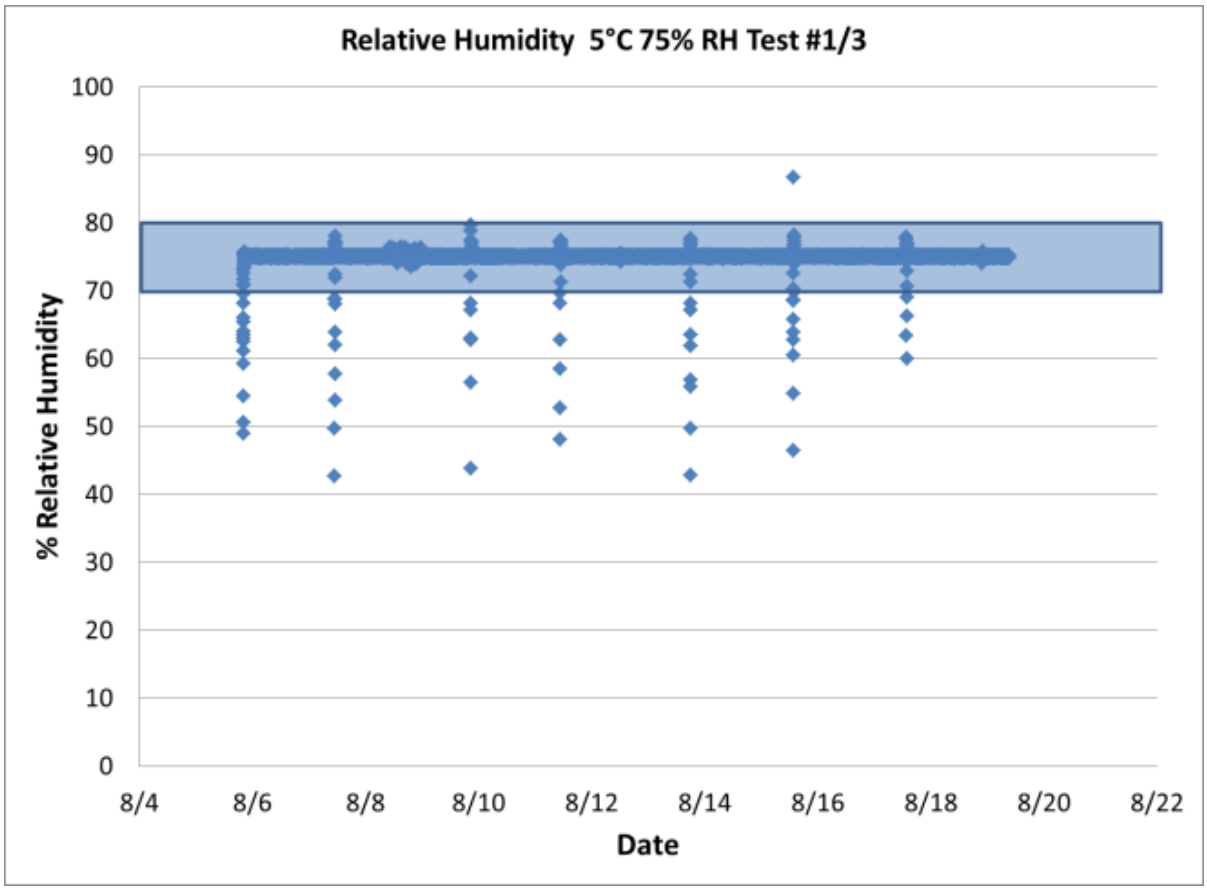


**Figure 90. Humidity plot for 5°C 75% relative humidity test run #1/3**

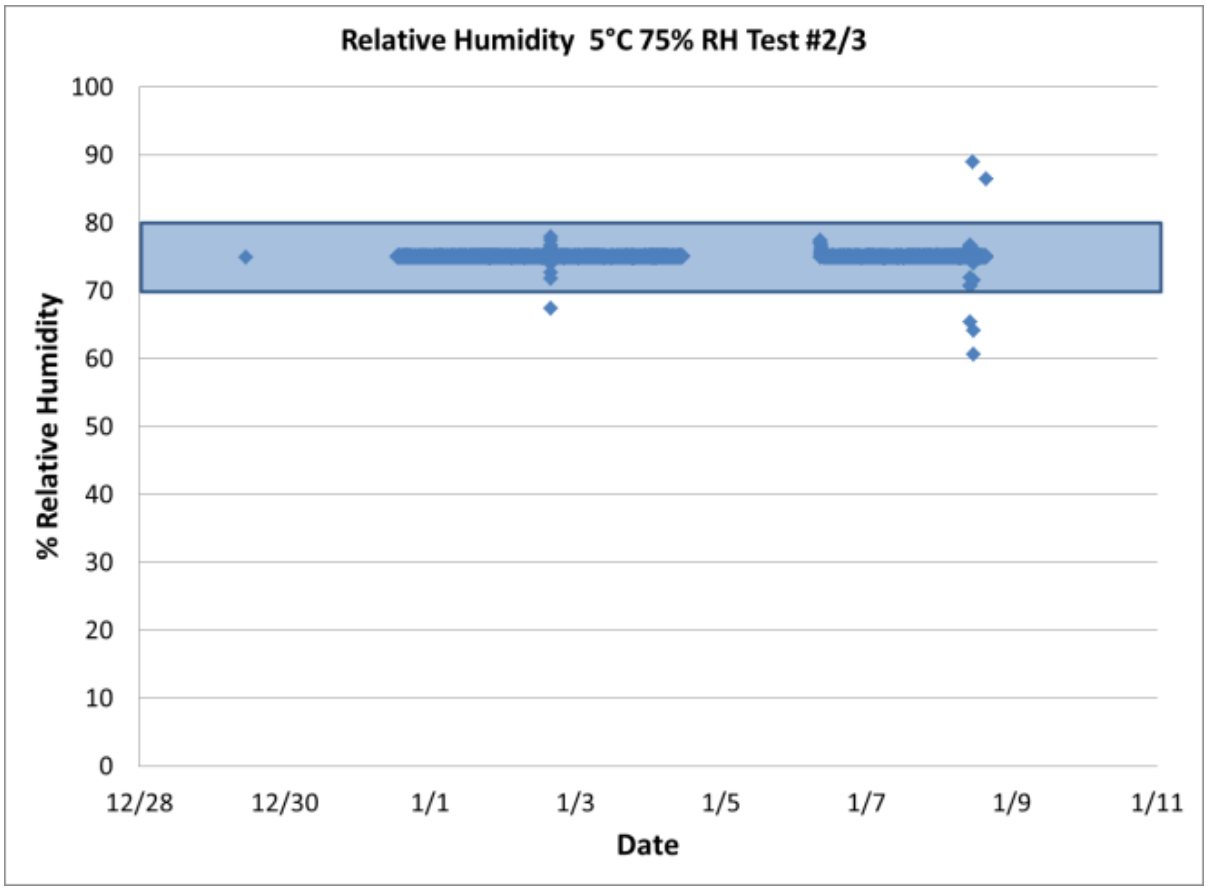


**Figure 91. Humidity plot for 5°C 75% relative humidity test run #2/3**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

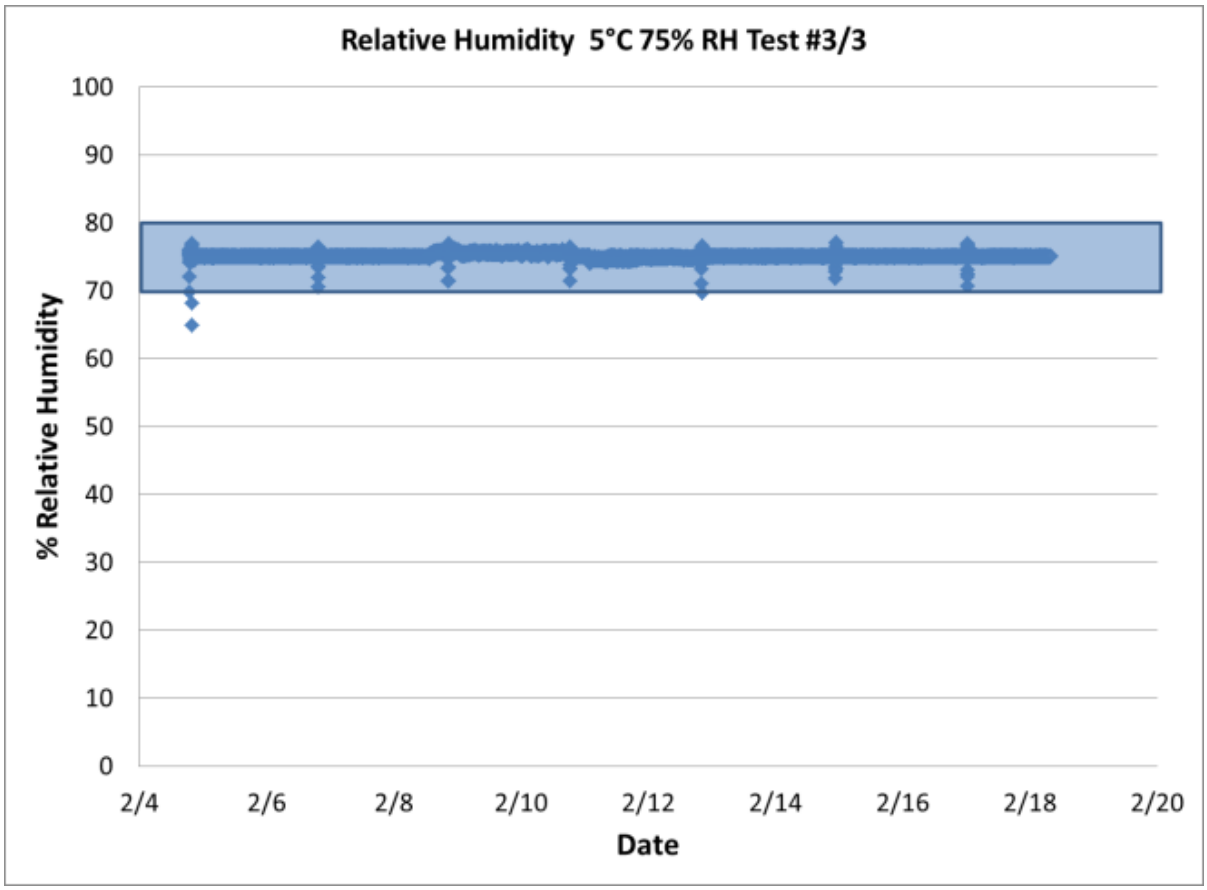


**Figure 92. Humidity plot for 5°C 75% relative humidity test run #3/3**

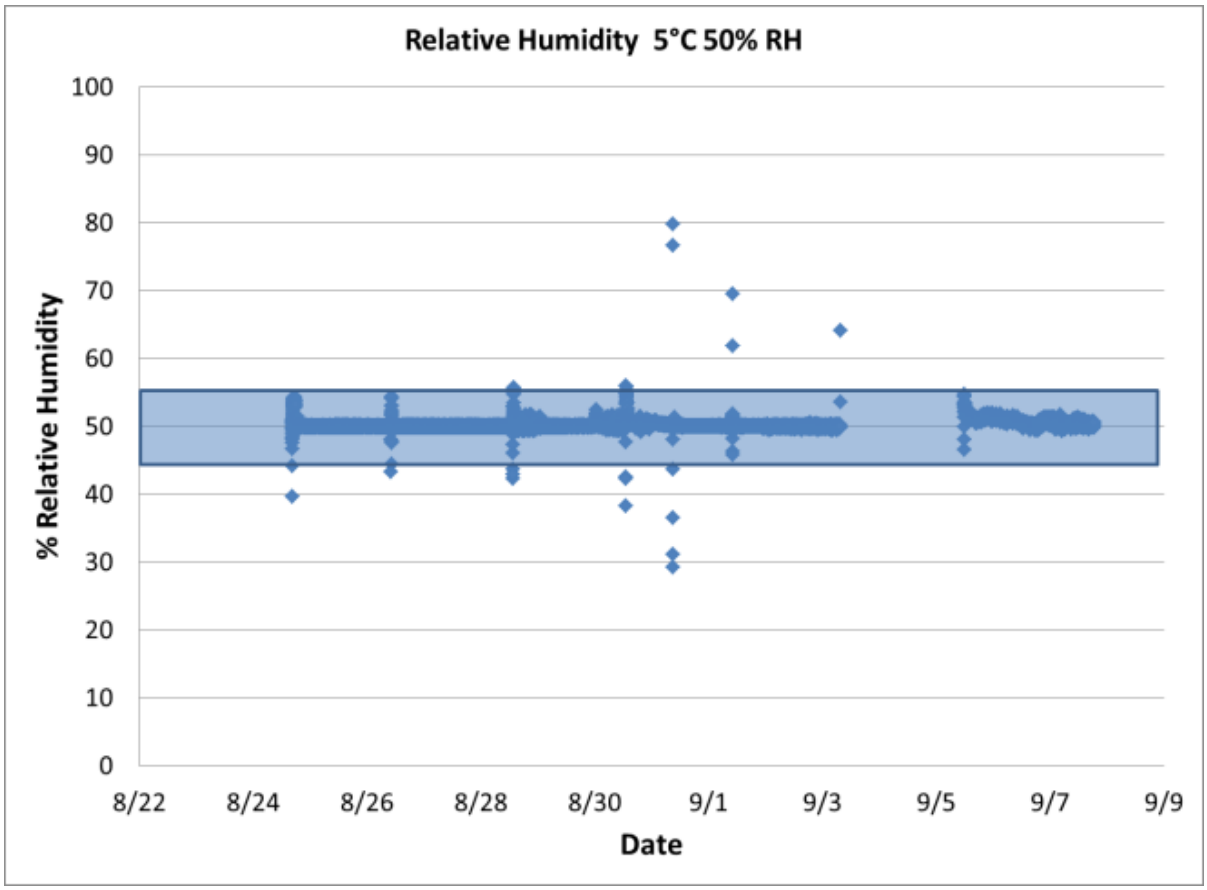


**Figure 93. Humidity plot for 5°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

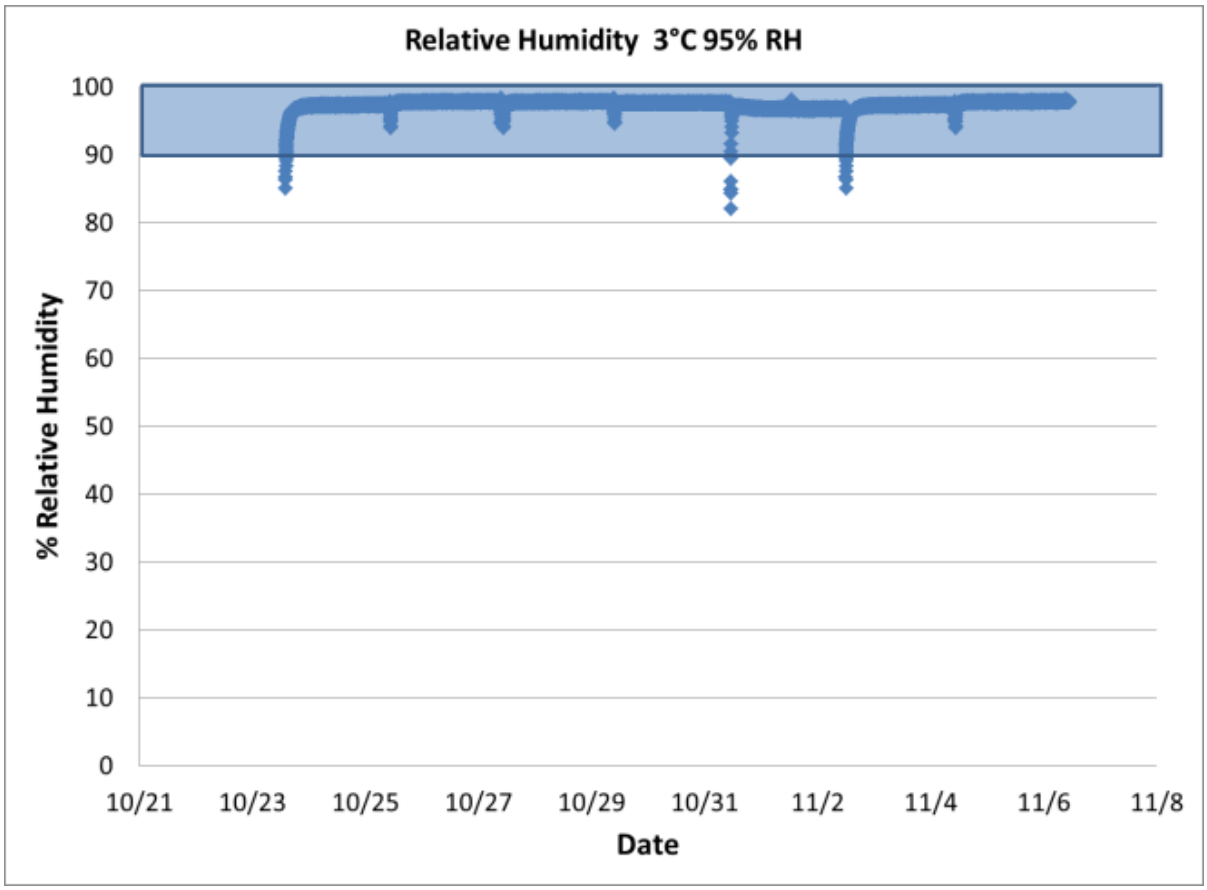


**Figure 94. Humidity plot for 3°C 95% relative humidity test run**

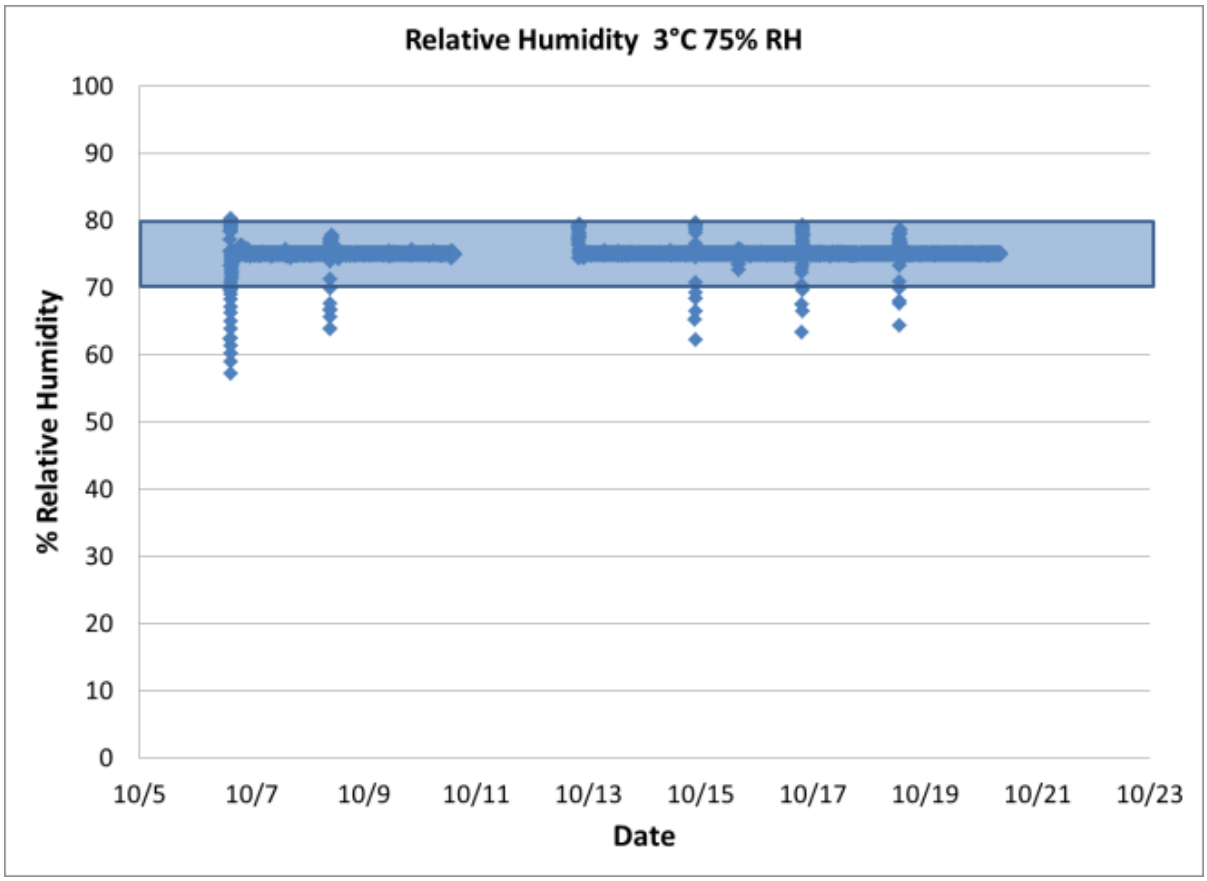


**Figure 95. Humidity plot for 3°C 75% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

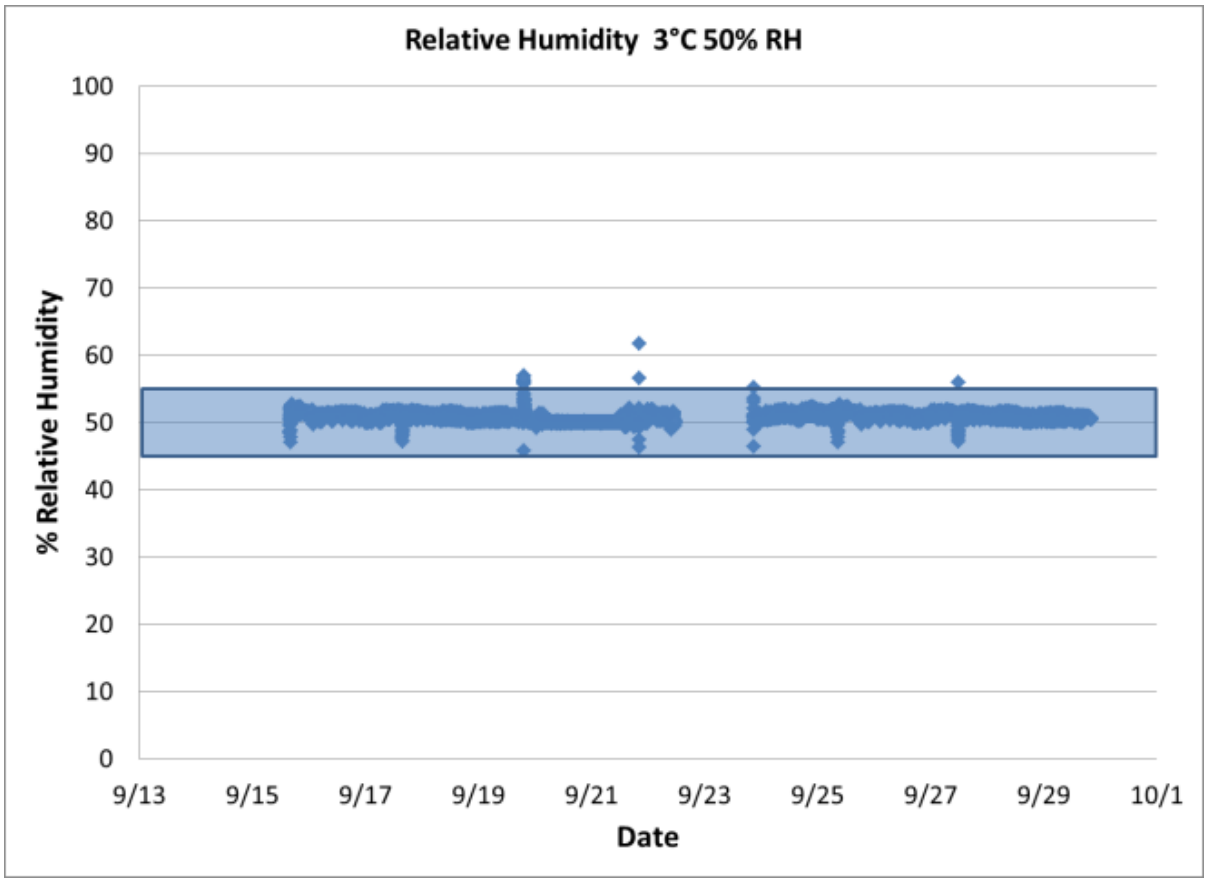


**Figure 96. Humidity plot for 3°C 50% relative humidity test run**

This plot is missing data during one testing period because of computer hardware or software errors causing a failure in data collection. The environmental chamber was not affected by the computer error, and power logs were checked to verify that no outages or anomalies were present during this time.

# APPENDIX C: TABLE OF RAW MICROBIAL SPOILAGE RESULTS FOR LETTUCE

(Yellow figures represent uncountable plates, unused measurements or substantial outliers-note: outliers were still used in final calculation of averages)

| | 7degC 95% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 10/2/2008 | 4.10E+02 | 4.30E+02 | not used | not used | 5.00E+01 | 2.05E+04 | 2.15E+04 | not used | not used | 2.10E+04 |
| 2 | 10/4/2008 | 6.60E+03 | 6.00E+03 | 4.00E+04 | 5.10E+04 | 5.00E+01 | 3.30E+05 | 3.00E+05 | 2.00E+06 | 2.55E+06 | 1.30E+06 |
| 4 | 10/6/2008 | 2.80E+05 | 1.80E+05 | 1.60E+05 | 1.55E+05 | 5.00E+01 | 1.40E+07 | 9.00E+06 | 8.00E+06 | 7.75E+06 | 9.69E+06 |
| 6 | 10/8/2008 | 7.80E+05 | 7.50E+05 | 8.90E+05 | 8.60E+05 | 5.00E+01 | 3.90E+07 | 3.75E+07 | 4.45E+07 | 4.30E+07 | 4.10E+07 |
| 8 | 10/10/2008 | 3.60E+06 | 3.40E+06 | 1.90E+06 | 2.00E+06 | 5.00E+01 | 1.80E+08 | 1.70E+08 | 9.50E+07 | 1.00E+08 | 1.36E+08 |
| 10 | 10/12/2008 | 7.40E+06 | 7.50E+06 | 1.05E+06 | 8.60E+05 | 5.00E+01 | 3.70E+08 | 3.75E+08 | 5.25E+07 | 4.30E+07 | 2.10E+08 |
| | | | | | | | | | | | |
| | 7degC 50% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 6/21/2010 | 5.70E+04 | 6.70E+04 | 4.50E+04 | 4.80E+04 | 5.00E+01 | 2.85E+06 | 3.35E+06 | 2.25E+06 | 2.40E+06 | 2.71E+06 |
| 2 | 6/23/2010 | 8.30E+04 | 1.20E+05 | 4.20E+04 | 4.20E+04 | 5.00E+01 | 4.15E+06 | 6.00E+06 | 2.10E+06 | 2.10E+06 | 3.59E+06 |
| 4 | 6/25/2010 | 4.20E+05 | 6.00E+05 | 1.67E+06 | 5.80E+05 | 5.00E+01 | 2.10E+07 | 3.00E+07 | 8.35E+07 | 2.90E+07 | 4.09E+07 |
| 6 | 6/27/2010 | 1.38E+06 | 1.71E+06 | 6.70E+06 | 7.70E+06 | 5.00E+01 | 6.90E+07 | 8.55E+07 | 3.35E+08 | 3.85E+08 | 2.19E+08 |
| 8 | 6/29/2010 | uncountab | 2.50E+07 | 9.70E+05 | uncountab | 5.00E+01 | uncountab | 1.25E+09 | 4.85E+07 | uncountab | 6.49E+08 |
| | | | | | | | | | | | |
| | 5degC 95% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 11/28/2008 | 9.50E+03 | 8.20E+03 | not used | not used | 5.00E+01 | 4.75E+05 | 4.10E+05 | not used | not used | 4.43E+05 |
| 2 | 11/30/2008 | 2.40E+04 | 3.20E+04 | 1.26E+05 | 8.10E+04 | 5.00E+01 | 1.20E+06 | 1.60E+06 | 6.30E+06 | 4.05E+06 | 3.29E+06 |
| 4 | 12/2/2008 | 9.00E+04 | 1.00E+05 | 4.60E+04 | 4.80E+04 | 5.00E+01 | 4.50E+06 | 5.00E+06 | 2.30E+06 | 2.40E+06 | 3.55E+06 |
| 6 | 12/4/2008 | 3.90E+05 | 3.50E+05 | 1.00E+05 | 1.20E+05 | 5.00E+01 | 1.95E+07 | 1.75E+07 | 5.00E+06 | 6.00E+06 | 1.20E+07 |
| 8 | 12/6/2008 | 2.20E+05 | 2.70E+05 | 1.30E+06 | 2.00E+06 | 5.00E+01 | 1.10E+07 | 1.35E+07 | 6.50E+07 | 1.00E+08 | 4.74E+07 |
| 10 | 12/8/2008 | 7.70E+05 | 7.60E+05 | 1.41E+06 | 9.80E+05 | 5.00E+01 | 3.85E+07 | 3.80E+07 | 7.05E+07 | 4.90E+07 | 4.90E+07 |
| 12 | 12/10/2008 | 2.20E+06 | 2.50E+06 | 1.90E+06 | 2.40E+06 | 5.00E+01 | 1.10E+08 | 1.25E+08 | 9.50E+07 | 1.20E+08 | 1.13E+08 |
| 14 | 12/12/2008 | 2.40E+06 | 1.60E+06 | 5.20E+06 | 2.80E+06 | 5.00E+01 | 1.20E+08 | 8.00E+07 | 2.60E+08 | 1.40E+08 | 1.50E+08 |
| | | | | | | | | | | | |
| | 5degC 75% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 8/5/2010 | 4.40E+03 | 4.90E+03 | 1.00E+04 | uncountab | 5.00E+01 | 2.20E+05 | 2.45E+05 | 5.00E+05 | uncountab | 3.22E+05 |
| 2 | 8/7/2010 | 9.20E+04 | 1.38E+05 | 1.99E+05 | uncountab | 5.00E+01 | 4.60E+06 | 6.90E+06 | 9.95E+06 | uncountab | 7.15E+06 |
| 4 | 8/9/2010 | 1.00E+05 | 1.04E+05 | 8.30E+05 | 5.80E+05 | 5.00E+01 | 5.00E+06 | 5.20E+06 | 4.15E+07 | 2.90E+07 | 2.02E+07 |
| 6 | 8/11/2010 | 5.50E+05 | 6.50E+05 | 3.10E+05 | 4.00E+05 | 5.00E+01 | 2.75E+07 | 3.25E+07 | 1.55E+07 | 2.00E+07 | 2.39E+07 |
| 8 | 8/13/2010 | 5.10E+06 | 4.30E+06 | 8.30E+05 | 8.00E+05 | 5.00E+01 | 2.55E+08 | 2.15E+08 | 4.15E+07 | 4.00E+07 | 1.38E+08 |
| 10 | 8/15/2010 | 1.19E+07 | 6.30E+06 | 1.04E+06 | 4.50E+05 | 5.00E+01 | 5.95E+08 | 3.15E+08 | 5.20E+07 | 2.25E+07 | 2.46E+08 |
| 12 | 8/17/2010 | 1.00E+07 | 1.03E+07 | 3.10E+06 | 3.10E+06 | 5.00E+01 | 5.00E+08 | 5.15E+08 | 1.55E+08 | 1.55E+08 | 3.31E+08 |
| 14 | 8/19/2010 | 4.70E+06 | 5.20E+06 | 2.40E+07 | 7.30E+06 | 5.00E+01 | 2.35E+08 | 2.60E+08 | 1.20E+09 | 3.65E+08 | 5.15E+08 |

| TEST #2 | 5degC 75% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 12/29/2011 | 9.50E+03 | 1.15E+04 | 8.80E+03 | 8.60E+03 | 5.00E+01 | 4.75E+05 | 5.75E+05 | 4.40E+05 | 4.30E+05 | 4.80E+05 |
| 2 | 12/31/2011 | 2.30E+04 | 2.00E+04 | 6.30E+03 | 5.80E+03 | 5.00E+01 | 1.15E+06 | 1.00E+06 | 3.15E+05 | 2.90E+05 | 6.89E+05 |
| 4 | 1/2/2012 | 6.80E+04 | 7.00E+04 | 6.70E+04 | 8.30E+04 | 5.00E+01 | 3.40E+06 | 3.50E+06 | 3.35E+06 | 4.15E+06 | 3.60E+06 |
| 6 | 1/4/2012 | 9.00E+04 | 9.40E+04 | 5.60E+05 | 4.90E+05 | 5.00E+01 | 4.50E+06 | 4.70E+06 | 2.80E+07 | 2.45E+07 | 1.54E+07 |
| 8 | 1/6/2012 | 1.50E+05 | 2.00E+05 | 1.50E+05 | 1.70E+05 | 5.00E+01 | 7.50E+06 | 1.00E+07 | 7.50E+06 | 8.50E+06 | 8.38E+06 |
| 10 | 1/8/2012 | 4.00E+05 | 4.60E+05 | 3.50E+05 | 3.60E+05 | 5.00E+01 | 2.00E+07 | 2.30E+07 | 1.75E+07 | 1.80E+07 | 1.96E+07 |
| | | | | | | | | | | | |
| TEST #3 | 5degC 75% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 2/4/2011 | 7.90E+04 | 7.90E+04 | 7.70E+04 | 8.10E+04 | 5.00E+01 | 3.95E+06 | 3.95E+06 | 3.85E+06 | 4.05E+06 | 3.95E+06 |
| 2 | 2/6/2011 | 8.10E+04 | 8.20E+04 | 2.70E+05 | 2.70E+05 | 5.00E+01 | 4.05E+06 | 4.10E+06 | 1.35E+07 | 1.35E+07 | 8.79E+06 |
| 4 | 2/8/2011 | 2.10E+05 | 2.70E+05 | 4.80E+05 | 4.30E+05 | 5.00E+01 | 1.05E+07 | 1.35E+07 | 2.40E+07 | 2.15E+07 | 1.74E+07 |
| 6 | 2/10/2011 | 6.50E+05 | 7.60E+05 | 1.66E+06 | 1.63E+06 | 5.00E+01 | 3.25E+07 | 3.80E+07 | 8.30E+07 | 8.15E+07 | 5.88E+07 |
| 8 | 2/12/2011 | 2.08E+05 | 1.85E+05 | 3.90E+05 | 4.70E+05 | 5.00E+01 | 1.04E+07 | 9.25E+06 | 1.95E+07 | 2.35E+07 | 1.57E+07 |
| 10 | 2/14/2011 | 4.30E+05 | 5.10E+05 | 2.60E+05 | 2.70E+05 | 5.00E+01 | 2.15E+07 | 2.55E+07 | 1.30E+07 | 1.35E+07 | 1.84E+07 |
| 12 | 2/16/2011 | 1.04E+06 | 1.33E+06 | 1.75E+06 | 1.58E+06 | 5.00E+01 | 5.20E+07 | 6.65E+07 | 8.75E+07 | 7.90E+07 | 7.13E+07 |
| 14 | 2/18/2011 | 1.13E+07 | 1.10E+07 | 6.30E+06 | 6.70E+06 | 5.00E+01 | 5.65E+08 | 5.50E+08 | 3.15E+08 | 3.35E+08 | 4.41E+08 |
| | | | | | | | | | | | |
| | 5degC 50% | | | | | | | | | | |
| | no data taken | | | | | | | | | | |
| | | | | | | | | | | | |
| | 3degC 50% | | | | | | | | | | |
| | no data taken | | | | | | | | | | |
| | | | | | | | | | | | |
| | 3degC 95% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 10/23/2010 | 1.22E+06 | uncountab | 1.01E+06 | 8.60E+05 | 5.00E+01 | 6.10E+07 | uncountat | 5.05E+07 | 4.30E+07 | 5.15E+07 |
| 2 | 10/25/2010 | 3.00E+07 | uncountab | 2.69E+06 | 2.54E+06 | 5.00E+01 | 1.50E+09 | uncountat | 1.35E+08 | 1.27E+08 | 5.87E+08 |
| 4 | 10/27/2010 | 5.50E+06 | 4.10E+06 | 4.00E+06 | 1.18E+07 | 5.00E+01 | 2.75E+08 | 2.05E+08 | 2.00E+08 | 5.90E+08 | 3.18E+08 |
| 6 | 10/29/2010 | 6.20E+05 | 6.60E+05 | uncountab | uncountab | 5.00E+01 | 3.10E+07 | 3.30E+07 | uncountat | uncountat | 3.20E+07 |
| 8 | 10/31/2010 | 4.20E+05 | 5.30E+05 | 1.28E+06 | 1.26E+06 | 5.00E+01 | 2.10E+07 | 2.65E+07 | 6.40E+07 | 6.30E+07 | 4.36E+07 |
| 10 | 11/2/2010 | 1.29E+06 | 1.36E+06 | 8.70E+05 | 8.60E+05 | 5.00E+01 | 6.45E+07 | 6.80E+07 | 4.35E+07 | 4.30E+07 | 5.48E+07 |
| 12 | 11/4/2010 | 2.10E+07 | 2.38E+07 | 1.29E+06 | 1.40E+06 | 5.00E+01 | 1.05E+09 | 1.19E+09 | 6.45E+07 | 7.00E+07 | 5.94E+08 |
| 14 | 11/6/2010 | 2.70E+06 | 3.30E+06 | 3.90E+06 | 3.90E+06 | 5.00E+01 | 1.35E+08 | 1.65E+08 | 1.95E+08 | 1.95E+08 | 1.73E+08 |
| | | | | | | | | | | | |
| | 3degC 75% | Reading | Reading | Reading | Reading | | Actual | Actual | Actual | Actual | |
| Day | Date | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Dilution Corr. | L1 Plate1 | L1 Plate2 | L2 Plate1 | L2 Plate2 | Average |
| 0 | 10/6/2010 | 7.30E+04 | 8.40E+04 | 1.15E+05 | 9.30E+04 | 5.00E+01 | 3.65E+06 | 4.20E+06 | 5.75E+06 | 4.65E+06 | 4.56E+06 |
| 2 | 10/8/2010 | 1.13E+05 | 9.00E+04 | 3.70E+04 | 4.10E+04 | 5.00E+01 | 5.65E+06 | 4.50E+06 | 1.85E+06 | 2.05E+06 | 3.51E+06 |
| 4 | 10/10/2010 | 4.10E+05 | 3.00E+05 | 3.70E+05 | 2.70E+05 | 5.00E+01 | 2.05E+07 | 1.50E+07 | 1.85E+07 | 1.35E+07 | 1.69E+07 |
| 6 | 10/12/2010 | 3.60E+05 | 3.20E+05 | 4.00E+05 | 4.30E+05 | 5.00E+01 | 1.80E+07 | 1.60E+07 | 2.00E+07 | 2.15E+07 | 1.89E+07 |
| 8 | 10/14/2010 | 5.20E+05 | 4.40E+05 | 5.70E+05 | 5.90E+05 | 5.00E+01 | 2.60E+07 | 2.20E+07 | 2.85E+07 | 2.95E+07 | 2.65E+07 |
| 10 | 10/16/2010 | 7.70E+06 | 8.50E+06 | 3.80E+06 | 4.30E+06 | 5.00E+01 | 3.85E+08 | 4.25E+08 | 1.90E+08 | 2.15E+08 | 3.04E+08 |
| 12 | 10/18/2010 | 8.00E+05 | 8.80E+05 | 2.30E+05 | 2.70E+05 | 5.00E+01 | 4.00E+07 | 4.40E+07 | 1.15E+07 | 1.35E+07 | 2.73E+07 |
| 14 | 10/20/2010 | 2.36E+07 | 2.00E+07 | 9.30E+05 | 1.05E+06 | 5.00E+01 | 1.18E+09 | 1.00E+09 | 4.65E+07 | 5.25E+07 | 5.70E+08 |

**Table 3. Microbial spoilage results for lettuce**

# APPENDIX D: TABLE OF RAW MICROBIAL SPOILAGE RESULTS FOR STRAWBERRIES

| | 7degC 95% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 7/6/2010 | 3.50E+03 | 3.10E+03 | 1.44E+03 | 1.37E+03 | 5.00E+01 | | 1.75E+05 | 1.55E+05 | 7.20E+04 | 6.85E+04 | 1.18E+05 |
| 2 | 7/8/2010 | 6.10E+03 | 5.50E+03 | 4.70E+03 | 4.20E+03 | 5.00E+01 | | 3.05E+05 | 2.75E+05 | 2.35E+05 | 2.10E+05 | 2.56E+05 |
| 4 | 7/10/2010 | 4.60E+03 | 5.30E+03 | 6.60E+03 | 5.20E+03 | 5.00E+01 | | 2.30E+05 | 2.65E+05 | 3.30E+05 | 2.60E+05 | 2.71E+05 |
| 6 | 7/12/2010 | 1.89E+04 | 1.80E+04 | 1.37E+04 | 1.48E+04 | 5.00E+01 | | 9.45E+05 | 9.00E+05 | 6.85E+05 | 7.40E+05 | 8.18E+05 |
| 8 | 7/14/2010 | 1.25E+04 | 1.21E+04 | 5.80E+04 | 8.00E+04 | 5.00E+01 | | 6.25E+05 | 6.05E+05 | 2.90E+06 | 4.00E+06 | 2.03E+06 |
| 10 | 7/16/2010 | 9.80E+03 | 9.70E+03 | 9.30E+03 | 8.70E+03 | 5.00E+01 | | 4.90E+05 | 4.85E+05 | 4.65E+05 | 4.35E+05 | 4.69E+05 |
| 12 | 7/18/2010 | 1.31E+04 | 1.45E+04 | 2.48E+04 | 2.43E+04 | 5.00E+01 | | 6.55E+05 | 7.25E+05 | 1.24E+06 | 1.22E+06 | 9.59E+05 |
| 14 | 7/20/2010 | 2.70E+04 | 3.70E+04 | 3.60E+04 | 2.90E+04 | 5.00E+01 | | 1.35E+06 | 1.85E+06 | 1.80E+06 | 1.45E+06 | 1.61E+06 |
| | | | | | | | | | | | | |
| | 7degC 75% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 6/5/2010 | 5.70E+03 | 5.20E+03 | 9.20E+03 | 8.60E+03 | 5.00E+01 | | 2.85E+05 | 2.60E+05 | 4.60E+05 | 4.30E+05 | 3.59E+05 |
| 2 | 6/7/2010 | 1.32E+04 | 1.38E+04 | 1.15E+04 | 1.36E+04 | 5.00E+01 | | 6.60E+05 | 6.90E+05 | 5.75E+05 | 6.80E+05 | 6.51E+05 |
| 4 | 6/9/2010 | 1.43E+04 | 1.54E+04 | 1.08E+04 | 9.00E+03 | 5.00E+01 | | 7.15E+05 | 7.70E+05 | 5.40E+05 | 4.50E+05 | 6.19E+05 |
| 6 | 6/11/2010 | 4.10E+04 | 5.00E+04 | 2.00E+04 | 2.40E+04 | 5.00E+01 | | 2.05E+06 | 2.50E+06 | 1.00E+06 | 1.20E+06 | 1.69E+06 |
| 8 | 6/13/2010 | 5.20E+04 | 4.20E+04 | 3.70E+04 | 5.00E+04 | 5.00E+01 | | 2.60E+06 | 2.10E+06 | 1.85E+06 | 2.50E+06 | 2.26E+06 |
| 10 | 6/15/2010 | 1.99E+04 | 1.69E+04 | 1.09E+04 | 1.08E+04 | 5.00E+01 | | 9.95E+05 | 8.45E+05 | 5.45E+05 | 5.40E+05 | 7.31E+05 |
| | | | | | | | | | | | | |
| | 7degC 50% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 6/21/2010 | 1.46E+04 | 1.49E+04 | 1.10E+04 | 1.22E+04 | 5.00E+01 | | 7.30E+05 | 7.45E+05 | 5.50E+05 | 6.10E+05 | 6.59E+05 |
| 2 | 6/23/2010 | 5.00E+04 | 4.00E+04 | 1.81E+04 | 1.82E+04 | 5.00E+01 | | 2.50E+06 | 2.00E+06 | 9.05E+05 | 9.10E+05 | 1.58E+06 |
| 4 | 6/25/2010 | 2.28E+04 | 2.15E+04 | 2.09E+04 | 2.24E+04 | 5.00E+01 | | 1.14E+06 | 1.08E+06 | 1.05E+06 | 1.12E+06 | 1.10E+06 |
| 6 | 6/27/2010 | 1.43E+04 | 1.68E+04 | 8.80E+03 | 8.60E+03 | 5.00E+01 | | 7.15E+05 | 8.40E+05 | 4.40E+05 | 4.30E+05 | 6.06E+05 |
| 8 | 6/29/2010 | 2.36E+04 | 2.68E+04 | 2.72E+04 | 2.61E+04 | 5.00E+01 | | 1.18E+06 | 1.34E+06 | 1.36E+06 | 1.31E+06 | 1.30E+06 |
| | | | | | | | | | | | | |
| | 5degC 95% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 7/22/2010 | 9.80E+03 | 8.50E+03 | 5.00E+03 | 4.80E+03 | 5.00E+01 | | 4.90E+05 | 4.25E+05 | 2.50E+05 | 2.40E+05 | 3.51E+05 |
| 2 | 7/24/2010 | 9.50E+03 | 8.50E+03 | 7.20E+03 | 6.70E+03 | 5.00E+01 | | 4.75E+05 | 4.25E+05 | 3.60E+05 | 3.35E+05 | 3.99E+05 |
| 4 | 7/26/2010 | 1.02E+04 | 1.22E+04 | 1.10E+04 | 8.10E+03 | 5.00E+01 | | 5.10E+05 | 6.10E+05 | 5.50E+05 | 4.05E+05 | 5.19E+05 |
| 6 | 7/28/2010 | 6.70E+03 | 5.60E+03 | 1.54E+04 | 1.50E+04 | 5.00E+01 | | 3.35E+05 | 2.80E+05 | 7.70E+05 | 7.50E+05 | 5.34E+05 |
| 8 | 7/30/2010 | 8.20E+03 | 8.60E+03 | 2.60E+04 | 2.40E+04 | 5.00E+01 | | 4.10E+05 | 4.30E+05 | 1.30E+06 | 1.20E+06 | 8.35E+05 |
| 10 | 8/1/2010 | 1.26E+04 | 1.59E+04 | 7.00E+03 | 8.40E+03 | 5.00E+01 | | 6.30E+05 | 7.95E+05 | 3.50E+05 | 4.20E+05 | 5.49E+05 |
| 12 | 8/3/2010 | 1.61E+04 | 1.48E+04 | 1.43E+04 | 1.31E+04 | 5.00E+01 | | 8.05E+05 | 7.40E+05 | 7.15E+05 | 6.55E+05 | 7.29E+05 |
| 14 | 8/5/2010 | 9.90E+03 | 1.12E+04 | 3.70E+04 | 2.02E+04 | 5.00E+01 | | 4.95E+05 | 5.60E+05 | 1.85E+06 | 1.01E+06 | 9.79E+05 |
| | | | | | | | | | | | | |
| | 5degC 75% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 8/5/2010 | 1.27E+04 | 1.20E+04 | 7.40E+03 | 6.60E+03 | 5.00E+01 | | 6.35E+05 | 6.00E+05 | 3.70E+05 | 3.30E+05 | 4.84E+05 |
| 2 | 8/7/2010 | 1.03E+04 | 9.50E+03 | 7.10E+03 | 6.00E+03 | 5.00E+01 | | 5.15E+05 | 4.75E+05 | 3.55E+05 | 3.00E+05 | 4.11E+05 |
| 4 | 8/9/2010 | 1.70E+04 | 2.01E+04 | 1.01E+04 | 9.60E+03 | 5.00E+01 | | 8.50E+05 | 1.01E+06 | 5.05E+05 | 4.80E+05 | 7.10E+05 |
| 6 | 8/11/2010 | 5.10E+03 | 4.40E+03 | 2.10E+03 | 1.22E+04 | 5.00E+01 | | 2.55E+05 | 2.20E+05 | 1.05E+05 | 6.10E+05 | 2.98E+05 |
| 8 | 8/13/2010 | 1.36E+04 | 1.69E+04 | 1.22E+05 | 1.36E+05 | 5.00E+01 | | 6.80E+05 | 8.45E+05 | 6.10E+06 | 6.80E+06 | 3.61E+06 |
| 10 | 8/15/2010 | 3.80E+04 | 5.00E+04 | 7.60E+04 | 8.40E+04 | 5.00E+01 | | 1.90E+06 | 2.50E+06 | 3.80E+06 | 4.20E+06 | 3.10E+06 |
| 12 | 8/17/2010 | 6.50E+03 | 7.20E+03 | 2.10E+04 | 2.09E+04 | 5.00E+01 | | 3.25E+05 | 3.60E+05 | 1.05E+06 | 1.05E+06 | 6.95E+05 |
| 14 | 8/19/2010 | 7.20E+04 | 8.40E+04 | 1.01E+04 | 1.20E+04 | 5.00E+01 | | 3.60E+06 | 4.20E+06 | 5.05E+05 | 6.00E+05 | 2.23E+06 |

| TEST #2 | 5degC 75% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 12/29/2011 | 1.70E+02 | 1.80E+02 | 4.20E+02 | 4.70E+02 | 5.00E+01 | | 8.50E+03 | 9.00E+03 | 2.10E+04 | 2.35E+04 | 1.55E+04 |
| 2 | 12/31/2011 | 6.60E+02 | 4.10E+02 | 4.00E+02 | 3.30E+02 | 5.00E+01 | | 3.30E+04 | 2.05E+04 | 2.00E+04 | 1.65E+04 | 2.25E+04 |
| 4 | 1/2/2012 | 8.00E+03 | 1.05E+04 | 1.23E+03 | 1.43E+03 | 5.00E+01 | | 4.00E+05 | 5.25E+05 | 6.15E+04 | 7.15E+04 | 2.65E+05 |
| 6 | 1/4/2012 | 7.70E+02 | 8.40E+02 | 1.48E+03 | 1.51E+03 | 5.00E+01 | | 3.85E+04 | 4.20E+04 | 7.40E+04 | 7.55E+04 | 5.75E+04 |
| 8 | 1/6/2012 | 2.02E+04 | 3.20E+04 | 3.30E+03 | 3.30E+03 | 5.00E+01 | | 1.01E+06 | 1.60E+06 | 1.65E+05 | 1.65E+05 | 7.35E+05 |
| | | | | | | | | | | | | |
| TEST #3 | 5degC 75% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 2/4/2011 | 8.10E+02 | 7.60E+02 | 6.10E+02 | 4.90E+02 | 5.00E+01 | | 4.05E+04 | 3.80E+04 | 3.05E+04 | 2.45E+04 | 3.34E+04 |
| 2 | 2/6/2011 | 1.41E+03 | 1.37E+03 | 1.47E+03 | 1.46E+03 | 5.00E+01 | | 7.05E+04 | 6.85E+04 | 7.35E+04 | 7.30E+04 | 7.14E+04 |
| 4 | 2/8/2011 | 1.51E+03 | 1.55E+03 | 8.50E+03 | 8.90E+03 | 5.00E+01 | | 7.55E+04 | 7.75E+04 | 4.25E+05 | 4.45E+05 | 2.56E+05 |
| 6 | 2/10/2011 | 8.50E+02 | 8.60E+02 | 4.20E+03 | 4.30E+03 | 5.00E+01 | | 4.25E+04 | 4.30E+04 | 2.10E+05 | 2.15E+05 | 1.28E+05 |
| 8 | 2/12/2011 | 4.90E+03 | 5.10E+03 | 5.30E+03 | 5.30E+03 | 5.00E+01 | | 2.45E+05 | 2.55E+05 | 2.65E+05 | 2.65E+05 | 2.58E+05 |
| 10 | 2/14/2011 | 1.21E+03 | 9.80E+02 | 1.14E+03 | 1.14E+03 | 5.00E+01 | | 6.05E+04 | 4.90E+04 | 5.70E+04 | 5.70E+04 | 5.59E+04 |
| 12 | 2/16/2011 | 1.70E+03 | 3.70E+03 | 5.40E+03 | 5.90E+03 | 5.00E+01 | | 8.50E+04 | 1.85E+05 | 2.70E+05 | 2.95E+05 | 2.09E+05 |
| 14 | 2/18/2011 | 2.60E+03 | 3.70E+03 | 3.70E+03 | 6.80E+03 | 5.00E+01 | | 1.30E+05 | 1.85E+05 | 1.85E+05 | 3.40E+05 | 2.10E+05 |
| | | | | | | | | | | | | |
| | 5degC 50% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 8/24/2010 | 6.20E+03 | 6.20E+03 | 3.34E+04 | 4.50E+04 | 5.00E+01 | | 3.10E+05 | 3.10E+05 | 1.67E+06 | 2.25E+06 | 1.14E+06 |
| 2 | 8/26/2010 | 6.70E+03 | 6.90E+03 | 7.70E+02 | 8.20E+02 | 5.00E+01 | | 3.35E+05 | 3.45E+05 | 3.85E+04 | 4.10E+04 | 1.90E+05 |
| 4 | 8/28/2010 | 8.20E+03 | 7.50E+03 | 1.62E+04 | 1.77E+04 | 5.00E+01 | | 4.10E+05 | 3.75E+05 | 8.10E+05 | 8.85E+05 | 6.20E+05 |
| 6 | 8/30/2010 | 8.00E+03 | 7.40E+03 | 1.36E+04 | 1.43E+04 | 5.00E+01 | | 4.00E+05 | 3.70E+05 | 6.80E+05 | 7.15E+05 | 5.41E+05 |
| 8 | 9/1/2010 | 9.40E+03 | 9.60E+03 | 7.90E+04 | 7.90E+04 | 5.00E+01 | | 4.70E+05 | 4.80E+05 | 3.95E+06 | 3.95E+06 | 2.21E+06 |
| 10 | 9/3/2010 | 4.70E+04 | 5.00E+04 | 5.70E+04 | 5.90E+04 | 5.00E+01 | | 2.35E+06 | 2.50E+06 | 2.85E+06 | 2.95E+06 | 2.66E+06 |
| | | | | | | | | | | | | |
| | 3degC 95% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 10/23/2010 | 3.40E+03 | 3.70E+03 | 3.50E+03 | 4.00E+03 | 5.00E+01 | | 1.70E+05 | 1.85E+05 | 1.75E+05 | 2.00E+05 | 1.83E+05 |
| 2 | 10/25/2010 | 3.90E+03 | 5.10E+03 | 2.30E+03 | 1.90E+03 | 5.00E+01 | | 1.95E+05 | 2.55E+05 | 1.15E+05 | 9.50E+04 | 1.65E+05 |
| 4 | 10/27/2010 | 5.30E+03 | 3.70E+03 | 1.32E+04 | 1.24E+04 | 5.00E+01 | | 2.65E+05 | 1.85E+05 | 6.60E+05 | 6.20E+05 | 4.33E+05 |
| 6 | 10/29/2010 | 5.80E+03 | 6.70E+03 | 7.10E+03 | 7.80E+03 | 5.00E+01 | | 2.90E+05 | 3.35E+05 | 3.55E+05 | 3.90E+05 | 3.43E+05 |
| 8 | 10/31/2010 | 1.80E+04 | 3.02E+04 | 8.00E+03 | 8.70E+03 | 5.00E+01 | | 9.00E+05 | 1.51E+06 | 4.00E+05 | 4.35E+05 | 8.11E+05 |
| 10 | 11/2/2010 | 4.00E+04 | 3.30E+04 | 4.10E+03 | 4.30E+03 | 5.00E+01 | | 2.00E+06 | 1.65E+06 | 2.05E+05 | 2.15E+05 | 1.02E+06 |
| 12 | 11/4/2010 | 5.70E+03 | 3.70E+03 | 1.76E+05 | 1.38E+04 | 5.00E+01 | | 2.85E+05 | 1.85E+05 | 8.80E+06 | 6.90E+05 | 2.49E+06 |
| 14 | 11/6/2010 | 1.34E+04 | 1.37E+04 | 1.64E+04 | 2.05E+04 | 5.00E+01 | | 6.70E+05 | 6.85E+05 | 8.20E+05 | 1.03E+06 | 8.00E+05 |

| | 3degC 75% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 10/6/2010 | 3.50E+03 | 6.70E+03 | 3.80E+03 | 8.00E+03 | 5.00E+01 | | 1.75E+05 | 3.35E+05 | 1.90E+05 | 4.00E+05 | 2.75E+05 |
| 2 | 10/8/2010 | 3.50E+03 | 4.30E+03 | 8.80E+03 | 7.50E+03 | 5.00E+01 | | 1.75E+05 | 2.15E+05 | 4.40E+05 | 3.75E+05 | 3.01E+05 |
| 4 | 10/10/2010 | 4.10E+03 | 3.10E+03 | 6.80E+03 | 3.40E+03 | 5.00E+01 | | 2.05E+05 | 1.55E+05 | 3.40E+05 | 1.70E+05 | 2.18E+05 |
| 6 | 10/12/2010 | 6.00E+03 | 4.90E+03 | 8.50E+03 | 7.90E+03 | 5.00E+01 | | 3.00E+05 | 2.45E+05 | 4.25E+05 | 3.95E+05 | 3.41E+05 |
| 8 | 10/14/2010 | 6.30E+03 | 5.30E+03 | 6.70E+03 | 5.80E+03 | 5.00E+01 | | 3.15E+05 | 2.65E+05 | 3.35E+05 | 2.90E+05 | 3.01E+05 |
| 10 | 10/16/2010 | 4.50E+03 | 3.70E+03 | 1.68E+04 | 1.69E+04 | 5.00E+01 | | 2.25E+05 | 1.85E+05 | 8.40E+05 | 8.45E+05 | 5.24E+05 |
| 12 | 10/18/2010 | 1.14E+03 | 8.20E+02 | 1.02E+03 | 1.09E+03 | 5.00E+01 | | 5.70E+04 | 4.10E+04 | 5.10E+04 | 5.45E+04 | 5.09E+04 |
| 14 | 10/20/2010 | 2.40E+03 | 1.90E+03 | 2.70E+03 | 2.90E+03 | 5.00E+01 | | 1.20E+05 | 9.50E+04 | 1.35E+05 | 1.45E+05 | 1.24E+05 |
| | | | | | | | | | | | | |
| | 3degC 50% | Reading | Reading | Reading | Reading | | | Actual | Actual | Actual | Actual | |
| Day | Date | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Dilution Corr. | | S1 Plate1 | S1 Plate2 | S2 Plate1 | S2 Plate2 | Average |
| 0 | 9/15/2010 | 5.00E+03 | 6.00E+03 | 9.40E+03 | 9.20E+03 | 5.00E+01 | | 2.50E+05 | 3.00E+05 | 4.70E+05 | 4.60E+05 | 3.70E+05 |
| 2 | 9/17/2010 | 4.70E+03 | 5.00E+03 | 4.20E+03 | 3.80E+03 | 5.00E+01 | | 2.35E+05 | 2.50E+05 | 2.10E+05 | 1.90E+05 | 2.21E+05 |
| 4 | 9/19/2010 | 7.90E+03 | 8.10E+03 | 7.20E+03 | 7.50E+03 | 5.00E+01 | | 3.95E+05 | 4.05E+05 | 3.60E+05 | 3.75E+05 | 3.84E+05 |
| 6 | 9/21/2010 | 1.10E+04 | 1.31E+04 | 6.10E+03 | 5.50E+03 | 5.00E+01 | | 5.50E+05 | 6.55E+05 | 3.05E+05 | 2.75E+05 | 4.46E+05 |
| 8 | 9/23/2010 | 8.90E+03 | 1.07E+04 | 1.83E+04 | 1.70E+04 | 5.00E+01 | | 4.45E+05 | 5.35E+05 | 9.15E+05 | 8.50E+05 | 6.86E+05 |
| 10 | 9/25/2010 | 6.60E+03 | 6.20E+03 | 1.29E+04 | 1.17E+04 | 5.00E+01 | | 3.30E+05 | 3.10E+05 | 6.45E+05 | 5.85E+05 | 4.68E+05 |
| 12 | 9/27/2010 | 7.10E+03 | 7.60E+03 | 9.20E+03 | 9.70E+03 | 5.00E+01 | | 3.55E+05 | 3.80E+05 | 4.60E+05 | 4.85E+05 | 4.20E+05 |

**Table 4. Microbial spoilage results for strawberries**

# APPENDIX E: THE ORIGINAL TEST CHAMBER

Introduction:

Though a small humidity-testing chamber was used for the spoilage testing, initially the environmental chamber was to be designed and constructed from scratch. Except for humidity generation, the constructed chamber system was operational within the parameters specified by the ASHRAE technical committee. Over the course of this project, much iteration from professors and the technical committee caused the test requirements and scope to change dramatically.

The following sections will briefly describe the design and construction of the original environmental chamber. The chambers, chiller, thermal mass storage tank, heat exchangers and data acquisition system will be shown. The operation of the environmental chamber will be described, as well as the reasons for eventually using the smaller humidity testing chamber.

Design and Construction:

The original design consisted of a two-chamber system. The inner chamber held the produce samples, and had an airflow limitation of 1.0 ft/min. This limitation required the use of an outer chamber with higher airflow to serve as an insulating buffer. The original diagram of the proposed system can be seen in Figure 97. Sizing the chamber walls required many calculations for heat transfer including convection from the air passing over the samples, conduction through the walls, and radiation effects. The CAD model of the designed two-chamber system can be seen in Figure 98 and the actual constructed chambers can be seen in Figure 99.

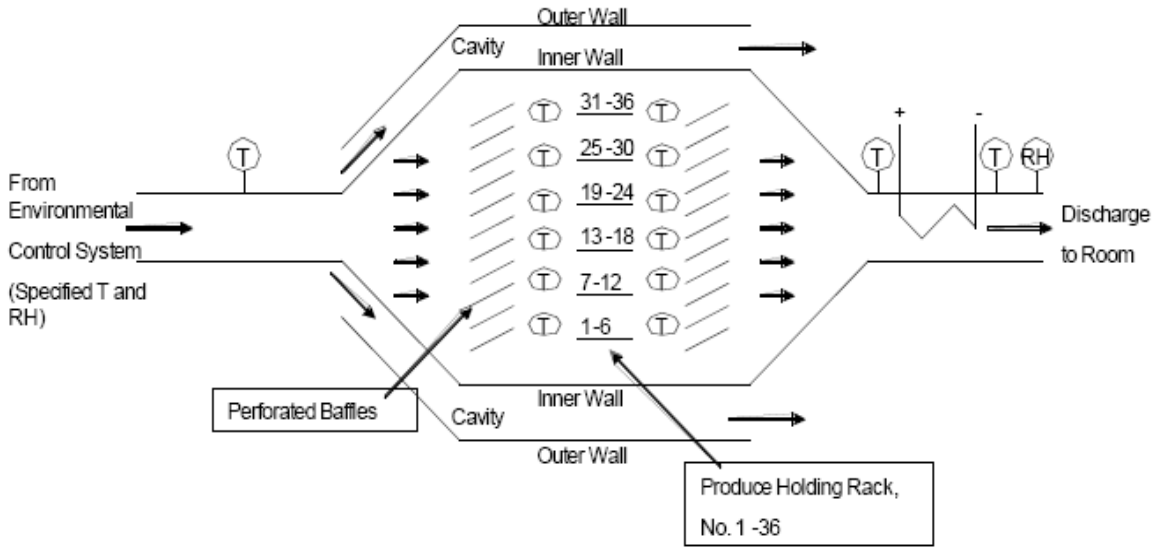


**Figure 97. Diagram of original environmental chamber from proposal**

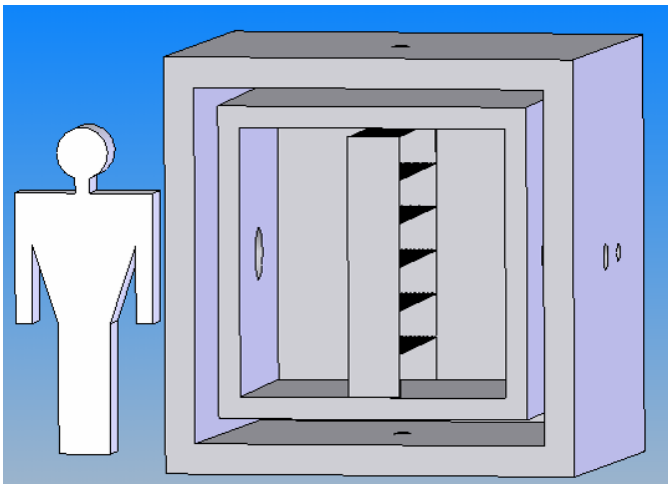

**Figure 98. CAD drawing of chamber and shelves (reference model is 6' tall)**

**Figure 99. Original environmental chamber.**

The design and sizing of the chiller, heat exchangers and thermal storage tank started with ideal measurements for each. After some researching, the availability and affordability of the equipment became the biggest factors for this design. A standard 55 gallon plastic drum was selected for the water-glycol storage Figure 100. A 2.5 ton chiller/pump assembly was built from a kit with customized fine-tuning temperature control system Figure 101. The required heat exchangers were custom-built to the exact specifications calculated and then assembled into a duct system Figure 102.

**Figure 100. 55 gallon drum for glycol/water thermal storage**


**Figure 101. 2.5 ton chiller built from kit**

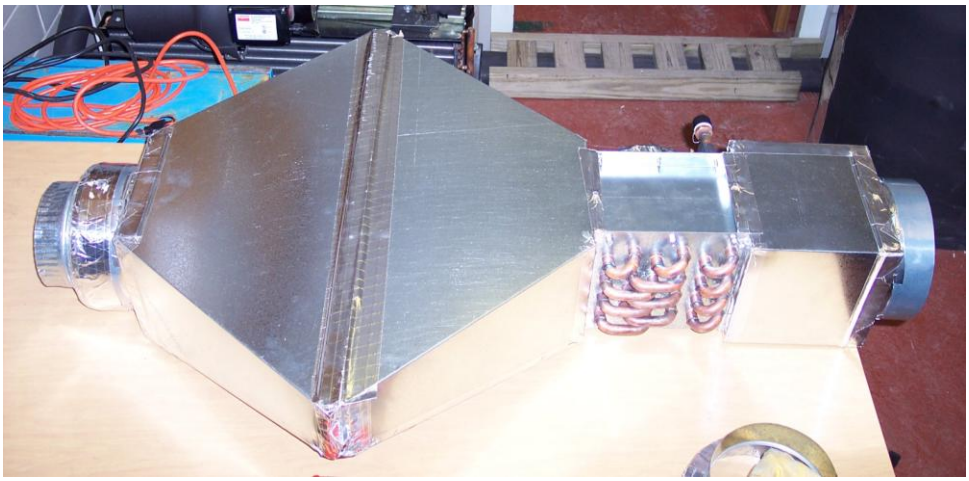

**Figure 102. Inner chamber heat exchanger in duct**

Duct fans, fin heaters and insulation were also sized and fitted into the system, along with condensation management and a data acquisition system. These systems will be described in more detail in the 'Operation:' section of this appendix.

In order to control the humidity in the inner chamber, a two-pressure relative humidity generation system was designed and several parts sized and procured. This humidity generation system was not fully constructed for several reasons. First, after performing many simulation calculations, it was found that the pressure control tolerance required to dial in an exact relative humidity (+/- 5%) was not reasonable for the off-the shelf equipment. Also, a major issue with reheating the inner chamber air stream was discovered. This issue will be described in detail along with the environmental chamber operation in the next section.

Operation:

Because exacting temperature and humidity control is paramount to the produce spoilage test, a highly calibrated data collection system was utilized (Figure 103). The temperature and humidity logging system took measurements every 60 seconds using 17 platinum RTDs with and accuracy of +/- 0.06°C, and two reference-quality humidity sensors calibrated at

low temperatures. A hot-wire anemometer was originally to be used as well, however issues with low temperatures and laminar flow created inconsistent accuracies.

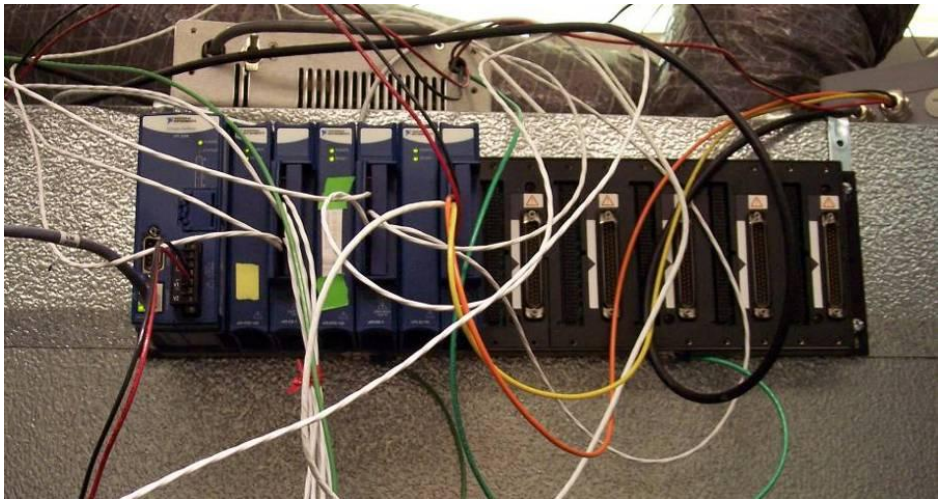

**Figure 103. Data collection system**

In order to visualize the operation of the two-chamber test system, it is useful to follow the path of air movement. The outer chamber, acting as a thermal barrier to the inner chamber and produce samples, exhibits the majority of heat exchange. Air circulates through a large squirrel-cage fan, through a heat exchanger, past a fin heater, and into one of four flex-duct paths. From there, chilled air moves over the inner chamber, on all 6 sides, and circulates back to the fan.

The inner chamber, requiring a maximum air flow velocity of 1ft/sec, could only be operated at 25CFM or lower. Air passes through a residential furnace-mounted humidifier (Figure 104), then into a small duct fan, then through the heat exchanger. The air, having been chilled to a lower temperature than required, is reheated using a fine-tuned fin heater system with a PID control. The air then passes through to the inner chamber and is diffused by several layers of screens to create homogenous flow over the produce. The air then passes through several more diffusers and exits the inner chamber.

**Figure 104. Humidifier typical for residential furnace**

Humidity generation was a very important part of this environmental chamber. Two methods of generation were looked into as possibilities for this system. The first method resembled exactly the two-pressure humidity generation system employed by this project. The second was a desiccant–based system. The concept was simple; the precisely humidified air was pumped through a heat exchanger to about the correct temperature, then a re-heater was used to bring the temperature to within 0.3°C of specified. There was a problem with this reheat approach. Looking at a standard psychometric chart, when very cold saturated air is brought from 1.5°C to 3°C there is a significant drop in relative humidity. This issue was the main reason for abandoning the customized environmental chamber, and why using an existing chamber was so crucial.

Conclusion:

Throughout most of the design and construction of the above-explained environmental chamber, the biological method of test (how to test “freshness”) was still being written. This included all of the procedures for testing freshness and exactly what measurements were needed. After several trial runs and countless iteration from professors and

colleagues in the food science department, a suitable method of test was written and approved. This method of test did not require a large-scale wide shelf system and a huge environmental chamber. Once the method of test was complete, more options opened for substitutes for an environmental chamber, and thus the humidity sensor calibration machine was used (Figure 105).

**Figure 105. Small environmental chamber actually used for the experiment**

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