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A Proposed Method of Test for Spoilage of Fruits and Vegetables

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Abstract
This document is intended to provide all procedures and background needed for testing fruits and vegetables for deter- mination of spoilage progression during storage. This method of testing was developed for research into the effect of household refrigeration storage conditions on the shelf life of fruits and vegetables (Pate and Brehm-Stecher 2005). Instructions start with the procurement of the produce and sample preparation prior to placement within the environ- mental 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 the counting of microorgan- isms and data collection are explained step-by-step.

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A Proposed Method of Test for Spoilage of Fruits and Vegetables

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ABSTRACT

This document is intended to provide all procedures and background needed for testing fruits and vegetables for determination of spoilage progression during storage. This method of testing was developed for research into the effect of household refrigeration storage conditions on the shelf life of fruits and vegetables (Pate and Brehm-Stecher 2005). 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 the counting of microorganisms and data collection are explained step-by-step.

INTRODUCTION

Little information is available 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 setpoints, resulting in temperature fluctuations that exceed the recommended variation of ±5°C (±9°F). 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 household refrigeration systems.

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 testing, research may be conducted 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 (strawberry) were chosen for these tests. This method of test ing 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 testing starts with materials and procedures needed to procure and prepare produce samples. The sampling and plating process will be described, along with a

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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) were procured from a large Midwestern grocery chain. Arrangements were made to ensure that the sample produce were 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 2 kg/4.4 lb) were procured before being stocked/shelved. The strawberries were examined visually and the worst 5% (discolored, physically damaged, visually molded) were 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 data set for each test condition for use in subsequent correlation with microbial data. The photograph background was a simple piece of white canvas cloth. As noted elsewhere, camera settings and backlighting were 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 0: 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 (0.22 lbs) 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/lettuce slurries were then diluted and plated (the goal being to obtain both yeast/mold [strawberries] and bacterial [lettuce] counts from each sample).

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* (Downes and Ito 2001). According to the authors (Jett et al. 1997), 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 and molds (for strawberries). Growth 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 1 provides an example of track plating onto PCA for the enumeration of bacteria. Figure 2 illustrates the results of sample maceration via stomacher. Figure 3 shows microbial spoilage trends for strawberries and lettuce plated onto OGYE agar and PCA 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 and yeast count readings (OGYE agar: strawberries) were taken 48 hours after the plating process. Tests for organoleptic quality (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*.

For the purposes of these tests, *freshness threshold* is 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 may not be salable or of value to some consumers. Once it is determined the produce has exceeded its freshness threshold, as previously described, the test run is 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 sampled 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 subcultured and cellular morphology characterized via microscopy. Total genomic DNA from pure cultures of each representative organism were isolated using the PrepMan Ultra sample preparation kit (Life Technologies Corporation 2010) 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 (BLAST 2009). This enabled confirmation, on a molecular level, of the identities of the predominant microorganisms present in the initial inoculum and after spoilage had occurred.

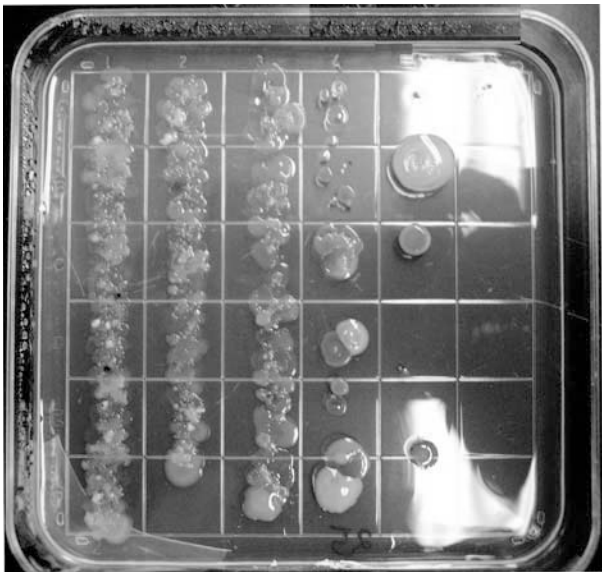

Figure 1 *Picture of PCA plate showing tenfold dilutions of microflora colonies.*

Figure 2 *Maceration (blending) of strawberry and lettuce samples.*

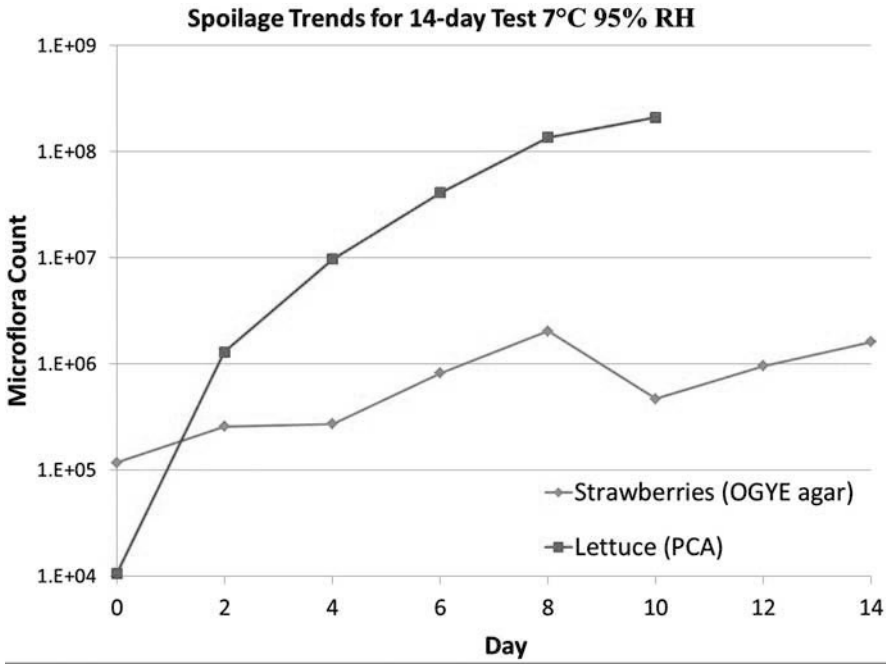

Figure 3 *Microbial spoilage trends for strawberries and lettuce plated onto OGYE (yeast and mold specific) agar and PCA (plate count agar), respectively (7°C [44.6°F] 95% RH).*

Microbial growth data were also complemented with visual characterization of produce samples (i.e., moldy, slimy, etc.).

In addition to microbial counts, other parameters providing information on the 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.

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 (+/– 0.00035 oz) 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 were 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 point in time.

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 were monitored and reported as firm, soft, or pulpous.

Data Tabulation and Summary: Data for each test were 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.

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