

POTENTIAL FOR BACTERIAL GROWTH ON THE FRESH CUT TROPICAL SQUASH, CALABAZA (*CURCUBITA MOSCHATA*), DURING STORAGE

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ABSTRACT

Calabaza (Curcubita moschata) is a tropical squash which is gaining popularity as a specialty crop for agricultural producers in the Northeast United States. It is commonly marketed by being cut in half, wrapped in plastic and may be held unrefrigerated until sold. This method of display is essential for consumer acceptance, yet unrefrigerated storage means that some potential for food safety problems exists. Experiments were conducted to determine the potential for bacterial growth during storage of cut calabaza. Freshly cut calabaza contained between 1.3 and 4.7 log₁₀CFU/g aerobic mesophiles. By 10 h, duplicate counts from some samples exceeded 4 log₁₀ CFU/g. After 24 h of room temperature storage, total aerobic plate counts ranged from 5.2 to 7.7 log₁₀ CFU/g. Rapid bacterial growth on cut calabaza stored at room temperature indicates that these products are highly perishable, and may be able to support the growth of pathogenic bacteria, should they be introduced during the slicing process.

INTRODUCTION

Over the last two decades, there has been increasing concern over produce safety as the number of foodborne disease outbreaks associated with fresh produce has steadily increased (Tauxe *et al.* 1997). Concurrent with these disease trends has been an increased attention on the microbial quality of produce. Thunberg *et al.*

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(2002) found mean aerobic populations of 8.7, 8.6, 7.5, 7.4 and 6.3 \log_{10} CFU/g on sprouts, lettuce, celery, cauliflower, and broccoli, respectively, purchased from supermarkets and farmer's markets. A survey of fresh produce from Japanese markets, including cabbage, lettuce, onions, cucumbers, radish roots and carrots, showed a range of 2.9 to 7.3 \log_{10} CFU /g in total aerobic counts (Kaneko *et al.* 1999). Extensive research has been conducted on the microbiology of melons, which have the most similar physical structure to squash and gourds. Park and Beuchat (1999) found aerobic microbial loads of 5.5 \log_{10} CFU/g on cantaloupe and 3.8 \log_{10} CFU/g on honeydew that had been stored at 5C for 10 days.

Despite the abundance of published research on bacterial levels on produce, little published research exists on the microbiology of fresh cut squash or gourds, in part because products like this are seldom cut prior to sale in the United States. Calabaza (*Curcubita moschata*), a pumpkin-like squash indigenous to Central and South America, has become increasingly popular in the United States (Cooper *et al.* 1995), in part because of recently developed hybrids (Maynard *et al.* 2002). This product is quite popular among Hispanic populations and can be purchased in many supermarkets and farmers' markets (especially in those areas with large Hispanic populations). It is added to sauces as a thickener and is used in stews and soups. The texture and flavor is similar to the Butternut squash commonly used in the U.S. It is a good source of vitamins such as beta carotene, riboflavin and thiamine (Cuniowski *et al.* 1998). Because quality is judged by flesh color, calabaza are frequently cut in half and wrapped in plastic film prior to sale. Although the recommendation is for cut calabaza to be refrigerated, storage conditions vary among retail operations. While squash and gourds are generally cooked before consumption, improper handling could still pose a significant food safety risk. The primary objective of this research was to estimate microbial stability of fresh cut calabaza stored at room temperature using total aerobic bacterial count.

MATERIALS AND METHODS

Microbiology

Two New Jersey grown calabaza cultivars ('El Dorado' and 'La Estrella') were washed with warm tap water until all dirt was removed (approximately 1 min). Calabaza were then dried using paper towels (Encore Paper Co., South Glens Falls, NY) and sliced into eighths using a sterile knife on a sterile plastic cutting board by a technician wearing sterile latex gloves, which were changed between each calabaza. Seeds were removed aseptically and slices were wrapped in plastic film and stored at room temperature (23C) for the duration of the experiment. Total mesophilic aerobic counts were determined at 24-h intervals over 4 days. Slices

from each calabaza fruit were sampled at three or four of the five sampling times (0, 1, 2, 3, and 4 days), such that 4 calabaza slices were analyzed at each time point. Bacteria were enumerated by aseptically removing a 25 g piece from the calabaza slice and placing it in a sterile stomacher bag (Fisherbrand, Pittsburgh, PA). Two hundred twenty-five mL of sterile Butterfield's buffer was added to the stomacher bag and the sample was homogenized in a stomacher (Seward, London, England) for 2 min. Serial dilutions were performed by transferring 1 mL of homogenate to 9 mL of Butterfield's buffer solution and vortexing. One-tenth mL of each dilution (up to 10^{-5}) was plated in duplicate on Plate Count Agar (Difco, Detroit, MI) and incubated at 37C for 24 h (FDA 1998).

Results from these experiments indicated that the majority of microbial growth occurred in the first 24 h after slicing, so the objective of the next series of experiments was characterize the growth of microorganisms during the first 24 h after cutting. Methods were as described above, except 25 g pieces from four different calabaza fruit were placed directly into stomacher bags and held at room temperature until tested. Two 25 g portions from each calabaza fruit were analyzed at every time point. One pair of calabaza fruit was analyzed in duplicate at times 0 and 2 h, then at two-hour intervals from 14 to 22 h. The second pair of calabaza fruit were analyzed in duplicate from times 0 to time 10 h, again at 2 h intervals. The fruit were analyzed in pairs to account for some of the natural variability in bacterial levels.

Modeling

Data were compiled in Excel spreadsheets (Microsoft, Redmond, WA). The mean aerobic plate count was obtained for each time point by averaging the \log_{10} CFU/g values for all replicates. Averaging logarithmically transformed data has been shown to be appropriate where data are logarithmically distributed (Jarvis 1989). The mean values were then fit to a four-parameter Gompertz curve (Zwietering *et al.* 1990) using Sigmaplot (Jandel Scientific, San Rafael, CA).

Risk Assessment

Information on growth rates (\log_{10} CFU/h) of *Salmonella* and *Escherichia coli* were obtained from the Common Database of Predictive Microbiology Information (ComBase) <http://wyndmoor.arserrc.gov/combase/> for growth conditions appropriate for calabaza in this study: temperature between 20 and 25C, pH 4.8 and 5.5, and water activity 0.97 to 1.00. Growth data obtained from experiments in broth or food were selected. Since melon and squash have similar physical characteristics, and similar pH (greater than 5 for melons and between 4.8 and 5.5 for squash) (Beuchat and Brackett 1987), growth rates for pathogens in melons and related fruits were also obtained from relevant publications (Escartin *et al.* 1989; Golden *et al.* 1993; Del Rosario and Beuchat 1995). Growth rates for change in

total bacterial populations during exponential growth on calabaza from the data described above were obtained using the consecutive pairs of points method (Duffy and Schaffner 2001). Histograms for distribution of ComBase, literature and calabaza growth rates were constructed using Microsoft Excel (Redmond, WA).

RESULTS AND DISCUSSION

Results from the first phase of experiments indicated a range of 1.3 - 4.7 \log_{10} CFU/g on calabaza flesh shortly after slicing. No difference in microbial quality between calabaza varieties was detected ($P=0.67$). Total aerobic plate counts ranged from 5.2 to 7.7 \log_{10} CFU/g after 24 h, showing a greater than 6 \log_{10} CFU/g of growth in one replicate. Bacterial populations exhibited less than 1 \log_{10} CFU/g of additional growth after 1 day. Visual quality had decreased noticeably by 3 days and some fruit were visibly slimy or smelled spoiled. Total bacterial counts ranged from 6.7 to 8.4 \log_{10} CFU/g after 4 days, and some fruit were visibly moldy.

Data from the second phase of experiments confirmed that a large increase in total aerobic count occurs in the first 24 h after slicing. Immediately after slicing (0 h), total aerobic counts on calabaza flesh ranged from 2.2 to 4.4 \log_{10} CFU/g (similar to the range seen in experiments described above). By 10 h, duplicate counts on two different calabaza fruit exceeded 4 \log_{10} CFU/g and after 22 h, counts ranged from 6.1 to 7.5 \log_{10} CFU/g. Population increase was approximately log-linear after a lag time of 4 to 6 h.

The Gompertz equation (see Fig. 1) fit to the data was as follows:

$$\text{Log}(N_t) = A + Ce^{-e^{-B(t-M)}}$$

Where $a= 3.14$, $c= 3.88$, $b=0.1217$, $m = 14.04$. The goodness of fit (r^2) was 0.86. The calculated lag from these parameters was 5.8 h and the calculated exponential growth rate was 0.174 Log_{10} CFU/gm/h.

Fourteen *E. coli* growth rates and eight *Salmonella* growth rates met the selection criteria input into ComBase, and frequency distributions for those data are shown in Fig. 2, panels A (*E. coli*) and B (*Salmonella*). Both datasets show peaks at 0.3 \log_{10} CFU/h, and tails with higher growth rates, some as high as 0.9 \log_{10} CFU/h (*E. coli*) or 0.7-0.9 \log_{10} CFU/h (*Salmonella*).

Published growth rates for pathogens in melons are shown in Fig. 2, panel C. Data for 3 different *Shigella* species, in papaya and jicama at room temperature published by Escartin *et al.* (1989) are shown by the dark bars and show growth rates from 0.2 to 0.4 \log_{10} CFU/h. Del Rosario and Beuchat (1995) published

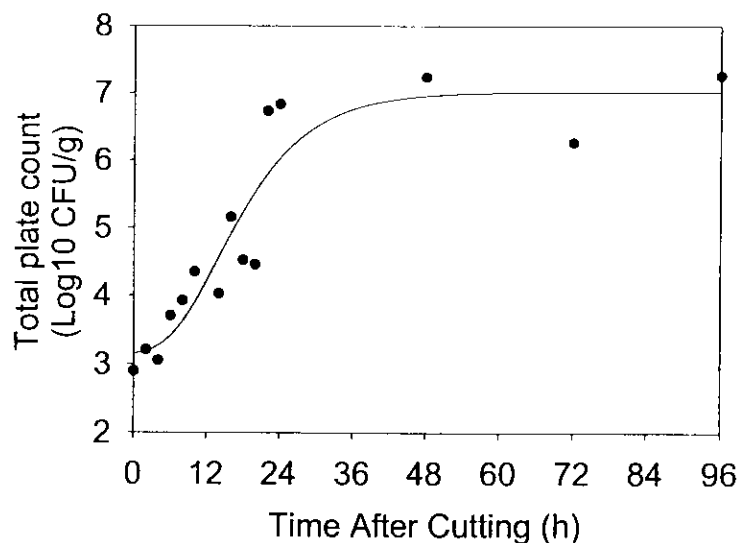


FIG. 1. GROWTH OF NATIVE MICROFLORA ON CUT CALABAZA SURFACE OVER THE PERIOD OF 4 DAYS

Each point represents an average of a minimum of 4 and a maximum of 8 replicates. The fitted line is a Gompertz equation with parameters $a=3.14$, $c=3.88$, $b=0.1217$, $m=14.04$.

growth rates for *E. coli* O157:H7 in cantaloupe and watermelon at room temperature, (light bars) which overlaps the lower end of the *Shigella* data. *Salmonella* growth rates (shown in white bars) from Golden *et al.* (1993) for *Salmonella* growth in cantaloupe, watermelon and honeydew melons at room temperature fall in the middle of the *Shigella* dataset, with growth rates of about 0.3 Log₁₀ CFU/h.

Data for total plate count changes in calabaza are shown in Fig. 2, panel D. These growth rates span a wide range, and show one observed growth rate as fast as 0.6 Log₁₀ CFU/h. Tests were not performed to determine whether or not calabaza were contaminated with pathogenic bacteria, but occurrence of any pathogens in such a small number of samples is unlikely, as pathogen prevalence on produce is generally low (Thunberg *et al.* 2002). Although this work only determined total bacterial counts for aerobic mesophiles, Fig. 2 supports the assumption that similar growth patterns are observed with pathogens in related environments.

Until such data is available cut calabaza or properly refrigerated to reduce the risk of substantial microbial growth. If adequate refrigeration is not available, cut calabaza should be held at room temperature for a limited amount of time. While an exact estimate for that amount of time is not yet available, the data presented in

Fig. 2 offers some possibilities. If the criteria suggested by Institute of Food Technologist Expert Panel on Evaluation and Definition of Potentially Hazardous Foods (2001) were used to assess safety, this product could be held at

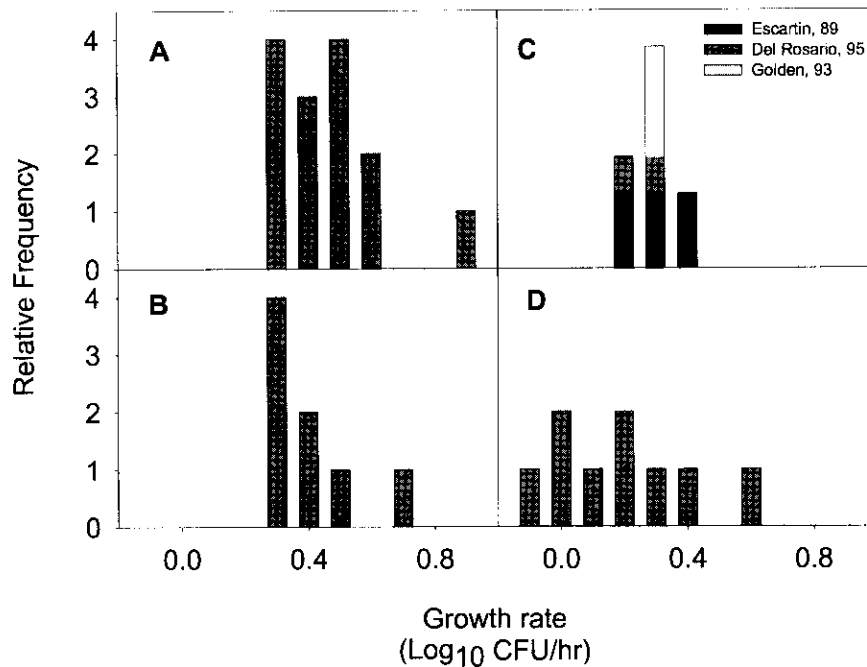


FIG. 2. COMPARISON OF GROWTH RATE DISTRIBUTIONS FOR GROWTH OF *ESCHERICHIA COLI* (A), *SALMONELLA* (B), VARIOUS PATHOGENS IN MELONS AND RELATED PRODUCTS (C), AND TOTAL PLATE COUNT IN CALABAZA, *CURCUBITA MOSCHATA* (D)

Data for *E. coli* and *Salmonella* were obtained from the Common Database of Predictive Microbiology Information (ComBase) <http://wyndmoor.arserrc.gov/combase/> for growth conditions appropriate for calabaza.

room temperature for an amount of time such that less than a 1 log₁₀ CFU increase in pathogen concentration would occur. The problem then becomes one of deciding which growth rate estimate to use.

If the absolute worst case situation were chosen from Fig. 2 this would be a growth rate of 0.9 Log₁₀ CFU/h (*E. coli*, Panel A). Based on this growth rate, the product should be stored at room temperature for 0.9⁻¹ h or 1.1 h. A second option would be to choose the worst case option from the published data on related products (Panel C), for an estimated growth rate of 0.4 Log₁₀ CFU/h or 2.5 h at

room temperature. A third would be to choose the median (most common) growth rate value from ComBase dataset. This would be 0.3 Log₁₀ CFU/h or 3.3 h at room temperature. Another but by no means final option would be to choose the median value from the total plate count data (0.15 Log₁₀ CFU/h, Panel D) for a storage time of 6 h at room temperature.

It is clear from the discussion above that, in the absence of definitive data, some assumptions and expert judgment must be used to determine an appropriate room temperature storage time. Further research is required to determine the effect of temperature on growth of pathogens and spoilage microbes in cut calabaza, but published research on melons and related fruits indicates that refrigeration temperatures should prevent pathogen growth in such foods (Golden *et al.* 1993; Del Rosario and Beuchat 1995).

REFERENCES

- BEUCHAT, L.R. and BRACKETT, R.E. 1987. Vegetables and related products. In *Food and Beverage Mycology*, (L.R. Beuchat, ed.) pp. 129, Van Nostrand Reinhold, New York.
- COOPER, A., POIRIER, S., MURPHY, M., OSWALD, M.J. and PROCISE, C. 1995. South Florida tropics: Calabaza. Fact Sheet HE 8518. University of Florida Cooperative Extension Service. <http://www.uog.edu/cals/PEOPLE/PUBS/SQUASH/He8518.pdf>.
- CUNIEWSKI, P., CARTER, A., MANGAN, F. and HAZZARD, R. 1998. Introducing Calabaza: a new crop for Massachusetts growers. Fact sheet Veg SF-5-98. University of Massachusetts Extension. http://www.umassvegetable.org/soil_crop_pest_mgt/specialty_crops/growing_calabaza.html.
- DEL ROSARIO, B.A. and BEUCHAT, L.R. 1995. Survival and growth of enterohemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. *J. Food Prot.* 58, 105-107.
- DUFFY, S. and SCHAFFNER, D.W. 2001. Modeling the survival of *Escherichia coli* O157:H7 in apple cider using probability distribution functions for quantitative risk assessment. *J. Food Prot.* 64, 599-605.
- ESCARTIN, E.F., AYALA, A.C. and LOZANO, J.S. 1989. Survival and Growth of *Salmonella* and *Shigella* on Sliced Fresh Fruit. *J. Food Prot.* 52, 471-472.
- FDA. 1998. *Bacteriological Analytical Manual*. AOAC International, Gaithersburg, MD.
- GOLDEN, D.A., RHODEHAMEL, E.J. and KAUTTER, D.A. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon and honeydew melons. *J. Food Prot.* 56, 194-196.
- Institute of Food Technologists. 2001. Evaluation and definition of potentially hazardous foods. IFT/FDA Contract No. 223-98-2333 Task Order No. 4.

- JARVIS, B. 1989. *Statistical Aspects of the Microbiological Analysis of Foods*. Elsevier, New York.
- KANEKO, K., HAYASHIDANI, H., OHTOMO, Y., KOSUGE, J., KATO, M., TAKAHASHI, K., SHIRAKI, Y. and OGAWA, M. 1999. Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot.* 62, 644-649.
- MAYNARD, D.N., ELMSTROM, G.W., TALCOTT, S.T. and CARLE, R.B. 2002. 'El Dorado' and 'La Estrella': compact plant tropical pumpkin hybrids. *HortScience* 37, 831-833.
- PARK, C.M. and BEUCHAT, L.R. 1999. Evaluation of sanitizers for killing *Escherichia coli* O157:H7, *Salmonella*, and naturally occurring microorganisms on cantaloupes, honeydew melons, and asparagus. *Dairy Food Envir. Sanit.* 19, 842-847.
- TAUXE, R., KRUSE, H., HEDBERG, C., POTTER, M., MADDEN, J. and WACHSMUTH, K. 1997. Microbial hazards and emerging issues associated with produce - A preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *J. Food Prot.* 60, 1400-1408.
- THUNBERG, R.L., TRAN, T.T., BENNETT, R.W., MATTHEWS, R.N. and BELAY, N. 2002. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot.* 65, 677-682.
- ZWIETERING, M.H., JONGENBURGER, I., ROMBOUTS, F.M. and VAN'T RIET, K. 1990. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* 56, 1875-1881.