Lettuce Salad as a Carrier of Microorganisms of Public Health Significance

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ABSTRACT

Culture, distribution, and preparation of lettuce for salad offers opportunities for contamination with and growth of microorganisms. Protection and preservation methods, even when appropriate, may likewise be favorable for the contaminants. Fresh lettuce, as commercially available, was studied to determine the magnitude of contamination and the nature of representative contaminants. Specific contaminants of public health interest were added to test portions to determine their fate during storage of lettuce as a salad at room temperature. Storage of lettuce in bowls on ice resulted in very little cooling of most of the lettuce. Microbial plate counts on fresh lettuce commonly were over $10^5$ and the diversity of the microflora indicated a generally favorable microenvironment for many types of bacteria. Inocula of Salmonella typhimurium, Escherichia coli, and Staphylococcus aureus fared well on lettuce salad and were able to grow at room temperature. Commercial “whitener” added to lettuce to preserve freshness reduced the total microflora and indicator organisms of public health significance.

Food preparation for consumption outside the home is a rapidly growing segment of the food industry, and most reported foodborne illnesses are attributed to this segment (1). The growing complexity of mass production of meals away from home presents new challenges in food protection (16). Lettuce is an example of food products undergoing changes in preparation and storage methods. There is increasing use of central processing to provide ready-to-serve forms of lettuce. Under any system, lettuce is a fresh raw vegetable, which is subjected to a wide spectrum of exposure before reaching the food preparation area. The common regimen of production has inherent opportunities for contamination from such sources as manure for fertilizer, contaminated irrigation water, wild animals, and personal contact in the harvesting process. However, Dunlop and Wang (2) found surprisingly few pathogens on lettuce irrigated with sewage. At the time of harvesting, lettuce is cooled for its protection which arrests growth but provides protection for contaminants.

When lettuce reaches the food preparation area, there are various opportunities for contamination through mechanical equipment and human contact. Scheduling to maximize use of labor may involve holding lettuce or salads at room temperature, thereby allowing growth of pathogenic organisms if they are present.

Lettuce as prepared for salads by traditional methods and evaluated immediately may carry a total microbial load of millions per gram (3-6). Some of these organisms may grow during storage under refrigeration (4). More rapid growth would be expected with lettuce in warmer conditions up to room temperature.

Preparation of lettuce for serving involves creation of new surfaces and a new, unstudied microenvironment which may be more favorable for microbial growth (14). An integral step in preparation is washing, which provides residual moisture for growth of contaminating organisms. Furthermore, the physical nature of lettuce provides a high surface area to unit weight and ample protection of the microbial contaminants.

Where there is human contact there is a possibility of contamination with pathogens of human origin. Hall and Hauser (7) found 6.4% of healthy workers to be carriers of enteropathogenic Escherichia coli.

The purpose of this work was to determine the magnitude and nature of the microflora of lettuce and to obtain more information on lettuce as a carrier of pathogens.

METHODS

Cultures

For observations on the fate of pure cultures, Salmonella typhimurium, Staphylococcus aureus, and E. coli were used. They were propagated in m-Plate Count Broth (PCB; Difco) at 25°C for approximately 48 h and used in this state or held at 5°C for further propagation and use. Cultures were diluted in phosphate buffer (9), for the contamination experiments.

Evaluation of microflora

The general procedures were those outlined in Recommended Methods for the Microbiological Examination of Foods (15) and Standard Methods for the Examination of Dairy Products (8). Total aerobic plate counts at 32°C were made on Plate Count Agar (PCA; Difco). Coli-

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form counts were made by plating with Violet Red Bile Agar (VRBA; Difco). Staphylococcal counts were made on Staphylococcus Medium 110 (S110; Difco). Salmonella typhimurium counts were made on Brilliant Green Agar (BGA; Difco).

To study the nature of the microflora occurring naturally on commercial lettuce, 10 colonies were picked by random design from the countable plates used to determine the total microbial count. The colonies were inoculated into litmus milk and streaked on PCA containing skim milk. After growth, isolates were further observed for gram reaction, morphology, spore formation, proteolysis of casein, catalase, oxidase, appearance on EMG Agar, and gas production in Brilliant Green Lactose Bile Broth. The organisms were then grouped according to major factors of interest to the food industry (12,13).

**Lettuce**

Head lettuce was obtained fresh as needed from local supermarkets. Lettuce for institutional use was prepared in a central operation and distributed to a restaurant where it was obtained for this work. An 11-g sample was blended in 99 ml of phosphate buffer (pH 6.8) in a Waring blender for 1 min after which serial dilutions were made in phosphate buffer for plating.

Various portions of lettuce heads were sampled to determine the general magnitude of contamination. First, the entire edible portion of head lettuce was torn by hand into serving-size pieces, mixed and an 11-g sample was removed for plate counts. To determine the location of contamination, the aesthetically unacceptable leaves were removed and discarded. Then one to three leaves were taken for a microbial sample of extreme outer portion, and the results were noted as "outside leaves." For counts on the "inside," leaves were taken from the very center of the head.

**Contamination of lettuce**

Fresh edible leaves of lettuce were washed in tap water to provide representative test samples of 30 g each. The culture to be studied as a contaminant was diluted 1:1000 in phosphate buffer. With disposable single-service gloves, hands were submerged to the second knuckle, removed and shaken. With the contaminated gloved hands a 11-g sample was removed for plate counts. To determine the location of contamination, the aesthetically unacceptable leaves were removed and discarded. Then one to three leaves were taken for a microbial sample of extreme outer portion, and the results were noted as "outside leaves." For counts on the "inside," leaves were taken from the very center of the head.

**Chemical treatment of lettuce**

A commercial product "Tater White" (L. K. Baker and Co., Columbus, Ohio) containing sodium meta-bisulfite, sodium citrate, and sodium erythorbate was used. Lettuce was treated according to directions with 15 g/gal of wash water and a treatment contact time of 1 1/2 min.

**Temperature measurements**

Localized temperature and heat transfer in salad bowls was measured with a Honeywell recording potentiometer with a copper-constantan thermocouple. Dimensions, of the salad bowls were:

(a) wood (14.5 cm diameter, 3.5 cm deep), (b) plastic (12.5 cm diameter, 5 cm deep), (c) glass (12.5 cm diameter, 5 cm deep).

**RESULTS**

**Microflora of commercial lettuce**

The magnitude of the total microbial load and counts on selective media, based on an average of at least three trials with duplicate plating, are shown in Table 1. It was apparent that most of the microorganisms were on the outer leaves, as counts on the inner parts were very low. The extreme outer leaves, the total blend of the heads, and the institutional prepared products contained approximately the same magnitude of microbial load. Apparently blending and disruption of colonies offset the very low count on the inner parts of the lettuce head.

<table>
<thead>
<tr>
<th>Fraction observer</th>
<th>PCA</th>
<th>S110</th>
<th>VRBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head lettuce</td>
<td>1.6 x 10^4</td>
<td>6.3 x 10^3</td>
<td>5.6 x 10^3</td>
</tr>
<tr>
<td>Outside leaves</td>
<td>6.3 x 10^4</td>
<td>5.0 x 10^2</td>
<td>1.3 x 10^3</td>
</tr>
<tr>
<td>Inside leaves</td>
<td>3.2 x 10^2</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Institutional</td>
<td>4.2 x 10^2</td>
<td>2.9 x 10^2</td>
<td>8.3 x 10^2</td>
</tr>
</tbody>
</table>

Also, the microenvironment of the extreme outer leaves may be less favorable than further into the head. Results on total blended heads of lettuce were in agreement with previous reports by Ercolani (3) and Fowler and Foster (4). The presence of indicator organisms as shown by the counts on VRBA and S110 indicate lettuce is an acceptable environment for microorganisms of public health significance.

The nature of the microflora of head lettuce and prepared institutional lettuce was studied by making isolates from the above countable plates. The results (Table 2) indicated there was a broad spectrum of microorganisms, which further indicated that the microenvironment would support many types of contaminants of public health significance. A total of 480 isolates from various parts of lettuce were studied and the results indicated those from head lettuce and institutional lettuce were similar.

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Percentage</th>
<th>Head lettuce</th>
<th>Prepared institutional lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPOREFORMERS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram +, catalase + asporogenous, non-proteolytic rods</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Gram +, catalase -, rods</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gram -, rods, proteolytic</td>
<td>18</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Gram +, rods, non-proteolytic</td>
<td>47</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>COLIFORMS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram + cocci, catalase negative</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gram + cocci, catalase positive</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coccobacilli, catalase positive</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Gram +, rods, catalase +, proteolytic</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>100%</th>
<th>100%</th>
</tr>
</thead>
</table>

**Fate of contaminants of public health significance on lettuce**

Test cultures of E. coli, S. typhimurium, and S. aureus individually were added to lettuce and stored uncovered at 23-25 C. The numbers were determined by periodic plating on VRBA, BGA, and S110, respectively. Counting of colonies on the selective media was based on known appearance of inoculum added for study. Results of an average of three trials are shown in Fig. 1. These data indicated there was a lag of approximately 2 h after which there was some growth. Triplicate experiments also were done with one change in procedure, which included covering the lettuce with thin polyethylene film. The results were essentially the same as those with uncovered lettuce.
Temperature of lettuce as a salad presented in a cafeteria-like environment

Salad bowls of wood, plastic, or glass were filled with torn lettuce and placed on finely crushed ice and held at room temperature. Temperature measurements within a bowl were made in three locations: (a) within 2 mm of the bottom center with a lettuce leaf between the bottom and the thermocouple, (b) near the side and approximately halfway between the bottom and rim of the bowl, and (c) top center with a lettuce leaf covering the thermocouple. The temperature pattern over a 6-h period in a glass bowl is shown in Fig. 2. Temperatures indicated by the thermocouples on the side and top were essentially the same and these data were combined for presentation in Fig. 2. Construction material of the salad bowl or minor changes in configurations as exemplified by measurements of these bowls had no apparent effect on the temperature of the lettuce during the period of study. Covering the lettuce and bowl with a thin polyethylene film did not have a significant effect on the temperature profile. The data indicate that most of the lettuce is far warmer than might be implied by the temperature of storage on ice.

Effect of “whitener” on the microflora of lettuce

A commercial source of whitener was used to treat lettuce leaves according to the recommended procedure in prepared systems for preserving fresh appearance. The results in Table 3 show a comparison of treated and untreated halves of the same head of lettuce after 48 h at 5°C. Immediately after treatment, the counts were lower than on the untreated lettuce and the difference was even greater after 48 h. Thus the chemical treatment preserved the appearance of the lettuce and reduced microbial activity.

DISCUSSION

There are numerous microorganisms on fresh lettuce as indicated in this work, as well as reports by others. More important, however, than numbers is the great diversity of types proliferating on lettuce. The microenvironment is favorable for microorganisms with the same requirements as common microorganisms of public health significance.

Since the microenvironment is favorable for microorganisms of public health significance, contamination should be avoided. Microorganisms can grow on lettuce as it is prepared and presented for selection in institutional feeding systems. The regimen of preparation and service should not allow such practices for banquets as place setting of salads at room temperature long before consumption. The motivation is full labor utilization, but the restraint is overwhelmingly in favor of public health protection.

Covering of lettuce base salads for presentation in a cafeteria-type service has aesthetic appeal, affords some protection against airborne contamination but has little effect on maintaining properly refrigerated conditions. This equilibrium of cooling and heating is quite different from the protective effect provided by cooling hot foods
where water vaporization is a major factor (12). Warm lettuce at room temperature, though in a bowl on ice, showed little temperature change in hours. Presentation of bowls on ice is mostly aesthetic as the patron feels the cold bowl, but cooling is insignificant for most of the lettuce.

The practice of using whiteners, which possess a preservative, not only preserve the fresh appearance but reduce the numbers of microorganisms. There is also a residual effect up to 48 h as observed in this work. The merits or acceptability of such a practice are beyond the scope of this work, which was solely to observe the effect on total load and some microorganisms of public health significance. Further and more detailed work, however, should be done to determine which fraction of the microflora is suppressed and the significance of outgrowth of those remaining.

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REFERENCES