Survey SF10
Microbiological quality of lettuce used in catering premises

Summary report
November 2006

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Food Standards Sub-Committee

Survey SF10: Microbiological quality of lettuce used in catering premises

Summary

84 samples of commercially pre-washed prepared salad leaves, seven samples of unwashed prepared salad leaves and 48 samples of raw whole lettuce were taken from catering premises and submitted for examination.

None of the specific pathogens for which the samples were examined were found in any of the samples.

Consideration of the results for indicator organisms did not reveal any firm relationship between the degree of washing/processing and microbiological quality.

Background

Previous surveys raised concerns over the microbiological quality of salads provided by catering premises. This concern was exacerbated by suggestions that lettuce may have been a vehicle of infection in some food poisoning outbreaks.

The survey aimed to focus on the microbiological quality of lettuce and to establish whether there is any difference in the microbiological quality of whole lettuce and bagged, cut and/or washed lettuce.

Sampling

Sampling was conducted during a 12 month period commencing in September 2005. 134 samples were submitted.

Results

Using other data input to FSSNet, an attempt was made to identify the true nature of samples and allow subsequent collation.

77 of the 84 samples of salad leaves submitted appear to have been supplied as washed and ready to eat. 25 of these samples were reported as not being satisfactory when compared with the PHLS guidelines for ready to eat foods.

There are no recognised microbiological criteria for raw salad leaves. However, five of the seven unwashed samples were compared against the PHLS guidance for ready to eat foods – four samples fulfilled the “satisfactory” criteria.

Pathogenic organisms

Table 1 outlines the samples examined for a number of specific organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of samples examined</th>
<th>Number of samples where organism detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>116</td>
<td>0</td>
</tr>
<tr>
<td>E coli O157</td>
<td>112</td>
<td>0</td>
</tr>
<tr>
<td>Listeria monocytogonnes</td>
<td>128</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella</td>
<td>130</td>
<td>0</td>
</tr>
</tbody>
</table>
Staphylococcus aureus* 9 0
Clostridium perfringens* 3 0

Table 1 - Samples examined for presence a variety of organisms

No samples showed the presence of any of the listed pathogens. The organisms marked “*” were not listed in the survey protocol and only a limited number of samples were examined for their presence.

Indicator Organisms

One sample of 128 tested showed the presence of Listeria species. This was a sample of washed prepared salad and was classified as “unsatisfactory” when compared to the PHLS guidelines.

Table 2 outlines the E coli results for the samples examined. All results are expressed in terms of the PHLS guidelines for ready to eat foods.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Satisfactory</th>
<th>Acceptable</th>
<th>Unsatisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed prepared</td>
<td>72</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unwashed leaves</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Unwashed whole</td>
<td>47</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>lettuce</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 – E coli results compared to PHLS guidelines for ready to eat foods

Although not specified in the survey protocol as required examinations, a number of samples were examined for aerobic colony count (ACC) and/or Enterobacteriaceae.

Table 3 outlines the ACC results for the samples examined. All results are expressed in terms of the PHLS guidelines for ready to eat foods.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Satisfactory</th>
<th>Acceptable</th>
<th>Unsatisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed prepared</td>
<td>25</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unwashed leaves</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Unwashed whole</td>
<td>24</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>lettuce</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 – ACC results compared to PHLS guidelines for ready to eat foods

Six samples were tested for Enterobacteriaceae; none revealed the presence of any of this group of bacteria.

Discussion

Sampling officers were unable to collect a significant number of samples of unwashed salad leaves. This may reflect the dominance in the market for bagged salad leaves of washed “ready to eat” products. However, four (57%) of the seven samples were satisfactory when compared to the PHLS guidelines for ready to eat foods.

The largest group of samples taken were washed ready to eat salad leaves. The overall evaluation of these samples indicated that 52 (67%) of the 77 samples were satisfactory.

Examining the results for E coli it can be seen that when compared to the guidelines for ready to eat foods 94% of washed leaves and 86% of unwashed leaves were satisfactory. 98% of unwashed whole lettuce samples also satisfied this criteria.
None of the samples, raw or ready to eat, revealed the presence of any pathogenic organisms.

**Conclusion**

The low number of samples of unwashed prepared leaves submitted makes it impossible to draw any firm conclusions from the survey but the results do not support any suggestion that washed prepared salad leaves are of a significantly better microbiological quality than either unwashed prepared leaves or, indeed, raw whole lettuce.

The survey results do not address the possible reasons for the apparent lack of improved microbiological quality which commercial preparation and washing might have been expected to offer. Two possible explanations are a lack of cleaning efficacy or the effects of prolonged storage and distribution after processing.

**Recommendations**

- Environmental Health Departments enforcing food safety law in premises preparing and washing salad leaves should continue to consider the efficacy of the washing and packing process as part of their routine food hygiene inspections.
- Liaison Groups and individual local authorities should continue to consider the sampling of salad leaves in catering premises as part of their sampling plans.
- FSSC should give consideration to further survey work aimed at examining the cleaning efficacy and the effects of prolonged storage and distribution after processing of commercially prepared and washed salad leaves.
**Scottish Food Enforcement Liaison Committee**  
**Food Standards Sub-Committee**  
**Survey SF10 – Microbiological quality of lettuce used in catering premises**  
**Final Protocol – August 2005 (Rev 2B, September 2005)**

**Background**

Previous surveys have raised concerns over the microbiological quality of salads provided by catering premises. This concern is exacerbated by suggestions that lettuce may have been a vehicle of infection in some food poisoning outbreaks.

This survey aims to focus on the microbiological quality of lettuce and to establish whether there is any difference in the microbiological quality of whole lettuce and bagged, cut and/or washed lettuce.

**Sample allocation**

West of Scotland Food Liaison Group will submit a minimum of 80 samples. Each other Food Liaison Group will submit a minimum of 40 samples. LAs are asked to strive to take roughly equal numbers of whole and bagged lettuce samples.

Samples will be taken during the period 1st September 2005 – 31st August 2006. Liaison Groups are asked, as far as possible, to ensure that samples are submitted throughout the year.

**Sample recording**

No proforma is necessary, as all of the required information will be captured via FSS.

*In order to allow for rapid collation of data all samples must be submitted via the FSS,* ensuring the following input as well as noting all label/coding information available.

- **Reason for sample taken:** Surveillance/monitoring
- **Sample was taken as part of a survey**
- **Survey Body:** SFELC
- **Survey Ref:** SF10

- **Category tree:**
  - 10.03.01 Fresh veg (raw, whole lettuce)
  - 10.03.03.01 Prepared salad (bagged lettuce)

- **Additional category information:** For prepared salad – specify “washed” or “unwashed”

Sampling officers will wish to ensure that sufficient detail from any available labels or documents is recorded to allow the source of the lettuce to be established if necessary.
**Sampling**

**Initial Procedures**
1. Pre-select premises from which samples are to be taken.
2. Ensure that the following equipment is available
   - Coolbox with ice-packs and bottle of water, or a suitable datalogger.
   - Labels
   - Sterile Bottles, Containers or Plastic Food Bags
   - Plastic Disposable Gloves
   - Calibrated thermometer

**Sample Collection Procedures**
1. Samples should be collected from any type of catering premises
2. Each sample should consist of at least 150 g of salad leaves or one whole lettuce head. Samples may be placed into either sterile jars or food bags. If only part of a bag of leaves is sampled an inverted bag should be used to take the sample.
3. The sample container should be labelled in accordance with the requirements of the laboratory being used.
4. Samples should be placed in a coolbox and delivered to the laboratory as soon as possible, and preferably, under normal circumstances, no later that 6 hours after sampling.

**Transportation of Samples**
Samples should be transported in an insulated coolbox capable of operating at a temperature of 1-8°C.

1. Pre-cool the insulated box in a refrigerator for at least 1 hour (preferably overnight). Place a small screw-capped bottle of water, or a datalogger, within the coolbox.
2. Load with sufficient ice packs to ensure adequate cooling.
3. Once samples are delivered to the laboratory, the internal temperature of the coolbox should be measured by placing the thermometer in the bottle of water. The temperature should be recorded on the sample form before the samples are handed over. This step is not required if a datalogger is used.
4. Where samples are delivered to the laboratory by a third party, the coolbox should be clearly marked to indicate that the temperature should be recorded on site by laboratory personnel, and that the coolbox should not be opened by office staff. Again, this step is not required if a datalogger is used.
**Microbiology Laboratory Protocol**

**Sample Preparation**

Using a suitable sterile implement, aseptically weigh a representative portion of the sample into a sterile stomacher bag or other equivalent sterile homogenisation system. Add by weight (to the nearest gram), sufficient sterile diluent to create a 1:10 suspension.

The homogenate prepared in sterile diluent is the primary \((10^{-1})\) sample suspension. A dilution should be prepared by adding 1ml of the homogenised primary sample solution to a bottle containing 9ml of sterile diluent. This is the secondary dilution \((10^{-2})\). This process should be continued until sufficient dilution of the sample has taken place. Use the primary decimal dilution and/or further decimal dilutions thereof to inoculate the appropriate media as described in the following enumeration methods.

**TVC 30**

Samples should be examined using the spread plate method described in ISO 4833/1991. This method requires the preparation of a series of spread plates using decimal dilutions of the sample. Plate Count Agar should be used and plates should be incubated at 30°C for 3 days and then counted. Alternatively any equivalent method may be used. Whichever method is used it is important to use a procedure which will allow counts to be made in the range \(10^2\) – \(10^8\)/gm.

**E.coli**

Samples should be examined in accordance with the method described in BS 5763: Part 13 (1995) (Enumeration of E.coli using membranes). Alternatively any equivalent accredited method may be used. Whichever method is used it is important to use a procedure which will allow counts to be made in the range \(10\) - \(10^4\)/gm.

**Listeria monocytogenes and Listeria spp**

Samples should be examined in accordance with the methods described in BS ISO 11290 Parts 1 and 2 or equivalent accredited method. All isolates of *Listeria monocytogenes* should be sent to the Food Safety Microbiology Reference Laboratory at Colindale for further typing. Whichever method is used it is important to use a procedure which will allow counts to be made in the range 10-10³/gm.

**E.coli 0157**

Samples should be examined in accordance with the method described in BS EN ISO 166544 (2001) or equivalent accredited method. All isolates should be sent to the Scottish E.coli Reference Laboratory for typing.

**Salmonella spp**

Samples should be examined using the method described in BS EN 12824 : 1998, or a suitable UKAS accredited method. All isolates of Salmonella should be sent to the Scottish Salmonella reference laboratory for typing.

**Campylobacter spp**

Samples should be examined in accordance with the method described in BS 5763 Part 17:1996 and ISO10272:1995 or any equivalent accredited method.
All isolates of Campylobacter spp. should be sent without delay to the Laboratory of Enteric Pathogens (LEP) at the HPA Specialist and Reference Microbiology Division (SRMD) Colindale, for confirmation, antibiotic sensitivity and typing as appropriate AND to Dr Ken Forbes, Medical Microbiology, Aberdeen University, Polwarth Building, Medical School, Foresterhill, Aberdeen AB25 2ZD for MLST typing as requested by FSA Scotland.