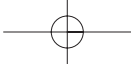


**SCOTTISH FOOD CO-ORDINATING COMMITTEE**

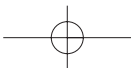
**REPORT OF THE FOOD  
STANDARDS SUB-COMMITTEE**

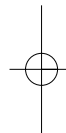
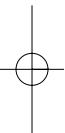
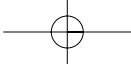
**SURVEY OF  
MICROBIOLOGICAL AND CHEMICAL  
QUALITY OF FRESH LETTUCE AND  
SPINACH**

Report Prepared by:  
D. J. Waddell of Glasgow Scientific Services  
*June 1998*



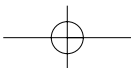
**SURVEY OF  
MICROBIOLOGICAL AND CHEMICAL  
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## 1. Introduction

THE SCOTTISH FOOD CO-ORDINATING COMMITTEE was set up in 1983 with the purpose of co-ordinating specific issues relevant to the enforcement of the Food & Drugs (Scotland) Act 1956, and its successor, the Food Safety Act 1990.

The Committee provides a forum for discussion of topics relevant to current problems and may initiate surveys or projects, or co-ordinate specific investigations initiated by the Local Food Liaisons Groups which have been set up in five Scottish geographical areas.

Membership comprises Head of Environmental Health, Public Analysts, Microbiologists, Representatives from the Society of Directors and Environmental Health, Royal Environmental Health Institute of Scotland and the Society of Chief Officers of Trading Standards in Scotland, and panel members of the Local Authorities Co-ordinating Body on Food and Trading Standards. In addition, the Scottish Centre for Infection and Environmental Health, the Scottish Office Agriculture, Environment and Fisheries Department, the Convention of Scottish Local Authorities and the Local Authorities Co-ordinating Body on Food and Trading Standards have observer status.

The Committee acts under the following terms of reference:

1. Liaison with LACOTS on food matters affecting Scotland.
2. Consideration of matters of interest submitted by Local Liaison Groups.
3. Assessment of the adequacy of existing food legislation applying in Scotland.
4. Advice to the Scottish Office Agriculture, Environment and Fisheries Department, Local Authorities and other bodies on food matters affecting Scotland.
5. Co-ordination of food enforcement in Scotland.

Two standing sub-committees form the Food Standards Sub-committee and the Food Safety Sub-committee. The Food Standards Sub-committee, considers matters relating to food labelling and compositional standards and is responsible for the publication of this report.

## 2. Survey

Following the introduction of new legislation the nitrate levels and bacteriological quality of 40 samples of lettuce and 14 samples of spinach on retail sale were tested. The results indicated that 15% of samples had excessive levels of nitrate, 5% of lettuce samples and 65% of spinach samples showed evidence of faecal contamination.

### 3. Purpose

The Conaminants in Food Regulations, which came into force in 1997, prescribe maximum levels for the nitrate content of lettuce and spinach. The primary purpose of this survey was to establish if the nitrate concentration in lettuce and spinach on general sale in Scotland during the late summer of 1997 was complying with the recently introduced legislation.

In the summer of 1994 an outbreak of dysentery occurred in Scotland affecting a total of 102 people. The outbreak was thought to be associated with consumption of imported Iceberg lettuce. A similar outbreak in Sweden had previously been linked to imported lettuce consumption. By the time the UK outbreak had been recognised, no samples were available for examination as supply had switched from Southern Europe suppliers to UK produced lettuce.

The opportunity to include an assessment of the microbiological quality of spinach and lettuce was recognised. Consequently the original aim of the proposed Nitrate Survey was extended to include microbiological examination for Total Viable Count and Faecal Indicator Organisms.

### 4. Sampling

Samples were taken over a twelve week period from August to October 1997. During this period, 54 samples were collected by Environmental Health Departments within the 5 food liaison groups in Scotland.

The sample protocol (Appendix A) specified that each sample submitted should be accompanied by a sample submission form (Appendix B) on which details of the sample taken would be collated.

### 5. Examination and Analysis

Each sample submitted was examined and analysed for:

- A. TVC 30°C
- B. E.coli
- C. Clostridium perfringens
- D. Nitrate

### 6. Methods Used

The methods used for the survey are detailed in Appendix C.

## 7. Results

The results of the lettuce and spinach samples submitted during the survey are shown in Tables 1 and 2 respectively.

The sample protocol required samples to be collected in pairs so that microbiological examination and chemical analysis could occur independently. Some authorities submitted pairs of samples to laboratories with individual reference numbers, whilst other authorities used the same reference number for each of the pair. For clarity, both sample numbers are displayed in columns 2 and 3 of the table. Due to an administrative error, two of the 54 samples submitted for microbiological examination were not examined.

Ten samples showed the presence of *E.coli* at levels between 40-27,000 cfu (colony forming units)/gm.

Two samples showed the presence of *C.perfringens*. Of these two samples, one had a low level of *C.perfringens* (20 cfu/gm). This was the sample which gave the highest recovery of *E.coli*.

The other sample was contaminated with a high level of *C.perfringens* (>1500 cfu/gm), but was not found to be contaminated with *E.coli*.

The TVC30 of the 52 samples examined, ranged from 800 – >200,000,000 cfu/gm. There is no evidence of any correlation between a high TVC and the presence of *E.coli* or *C.perfringens*.

The 54 samples submitted were analysed for nitrate and gave results which ranged from 630 – 4323 ppm.

## 8. Discussion

### 8.1 Chemical Analysis

The maximum nitrate level permitted under the Contaminants in Food Regulations 1997 varies according to the time of harvesting. Spinach harvested from April to October should not exceed 2,500 milligrams per kilogram (mg/kg) and therefore all samples of spinach submitted during the course of this survey should meet this standard.

Lettuce harvested in August should not exceed 2,500 mg/kg nitrate, in September should not exceed 3,500 mg/kg nitrate and in October should not exceed 4,500 mg/kg. For the purpose of this survey the purchase date is taken to be the date of harvesting.

**Of the 54 samples analysed for nitrate, 5 spinach and 3 lettuce samples exceeded the appropriate maximum level prescribed by the Contaminants in**

**Food Regulations 1997. The nitrate results which exceed the set standards are highlighted in Tables 1 and 2.**

## **8.2 Microbiological Examination**

The presence of E.coli and/or C.perfringens indicates that the food has been subject to faecal contamination. In certain circumstances these organisms can themselves be the cause of food poisoning symptoms although it is conventional to regard them as indicator organisms.

The presence of faecal indicator organisms in food suggests that the food has been contaminated with faeces, either through sewage contamination, animal excreta or poor hygiene practices. The presence of indicator organisms also means that there is a risk from more significant pathogens such as Salmonella, Shigella, Campylobacter and toxigenic strains of E.coli. There is also a risk from enteroviruses and enteric parasites.

PHLS Guidelines for ready-to-eat foods such as vegetables suggest that the presence of either E.coli or C.perfringens in excess of 100 cfu/gm is unsatisfactory, whilst the presence of these organisms at levels in excess of 10,000 cfu/gm is unacceptable and that the food might present a hazard to public health.

Although spinach is usually cooked prior to consumption and is not normally considered as a Ready-To-Eat food, it can be incorporated into salads as a raw ingredient. On this basis, eight of the spinach samples would be regarded as unsatisfactory due to the E.coli count exceeding 100cfu/gm. None of the spinach samples would be considered unsatisfactory with regard to C.perfringens.

Lettuce is regarded as a Ready-To-Eat food and it is appropriate to criticise samples using the PHLS Guidelines For Food Examiners. **On this basis, two of the lettuce samples examined would be unsatisfactory. One due to the E.coli count exceeding 100cfu/gm and another due to the C.perfringens count exceeding 100cfu/gm. These results are highlighted in Table 1.**

The PHLS guidelines for food examiners suggests that it is inappropriate to set standards for TVC30 for fresh vegetables due to natural contamination and therefore no level is considered to be unsatisfactory. These results are shown for information only.

## **9. Conclusions**

1. The results of this survey show that approximately 15% of the samples examined did not comply with the statutory requirements for nitrate. However, it should be borne in mind that these are results on an individual lettuce, and that the sampling of the lettuce according to the regulations requires that a laboratory sample consists of a minimum of ten units.

2. 5% of the lettuce samples examined showed evidence of faecal contamination and would be considered as unsatisfactory with regard to the PHLS Guidelines for Food Examiners.
3. Although spinach is more often cooked prior to consumption, it can be included as a raw ingredient in salads. Considering spinach as a Ready-To-Eat Food, 57% of the samples examined showed evidence of faecal contamination at a level which would be regarded as unsatisfactory with regard to the PHLS Guidelines. Even where it is intended that spinach will be subjected to a cooking process which will destroy pathogens, there is still a potential health risk relating to the introduction of foodstuffs bearing faecal contamination into the kitchen environment as this can provide an opportunity for cross-contamination to occur.

TABLE 1—LETTUCE RESULTS

Date Sampled	Sample Reference Chemical	Sample Reference Numbers	Sample	Origin	Retailer	Nitrate ppm	TVC30°C cfu/gm	E.coli cfu/gm	C.perfringens cfu/gm
19.8.97	IN/FD/127/I/1997/X	IN/FD/127/I/1997/M	Lettuce	England	Fruit Shop	747	2100000	<10	<10
23.9.97	109/9805675	110/97005066	Lettuce	UK	Greengrocers	570	950000	<10	<10
23.9.97	476A 6111	476A 6111	Lettuce	Scottish	Supermarket	1875	200000	<10	<10
23.9.97	111/9805676	112/97005067	Lettuce	UK	Supermarket	755	780000	<10	<10
27.8.97	97004524	97991234	Lettuce	Local	Veg packers	755	38000	<10	<10
27.8.97	97004525	97991233	Lettuce	Local	Veg packers	630	100000	<10	<10
22.9.97	WR4B/5941	WR4B/5941	Lettuce	Scottish	Supermarket	3060	2020000	<10	<10
2.9.97	FK/FD/267/S/1997/X	FK/FD/268/S/1997/M	Lettuce	UK	Supermarket	748	71000	<10	<10
2.9.97	97004657	97991286	Lettuce	Local	Farm	1265	410000	<10	<10
22.9.97	WR7B/5947	WR7B/5947	Lettuce	UK	Supermarket	<b>4175</b>	1190000	<b>9700</b>	<10
23.9.97	NC448	NC448	Lettuce	UK	Fruit Shop	1221	6400	<10	<10
22.9.97	WR1A/B/5935	WR1A/B/5935	Lettuce	UK	Supermarket	<b>4275</b>	108000	<10	<10
15.9.97	97004834	97991373	Lettuce	Scottish	Supermarket	650	170000	<10	<10
27.10.97	AIF6397	AIF6397	Lettuce	English	Supermarket	4323	1000000	<10	<10
9.9.97	DC/L1/97	DC/L1/97	Lettuce	UK	Supermarket	1423	900	<10	<10
27.10.97	ACF6297	ACF6297	Lettuce	Local	Wholesaler	950	5000000	<10	<10
4.9.97	RM139	RM139	Lettuce	Local	Farm Gate	640	250000	<10	<10
4.9.97	RM140	RM140	Lettuce	Scottish	Supermarket	1294	800	<10	<10
23.9.97	NC450	NC450	Lettuce	UK	Supermarket	2037	3800000	<10	<10
23.9.97	NC452	NC452	Lettuce	Scottish	Supermarket	917	270000	<10	<10
23.9.97	NC451	NC451	Lettuce	UK	Supermarket	1096	5400000	<10	<10
15.9.97	97004837	97991372	Lettuce	UK	Supermarket	1515	1700000	<10	<10
2.9.97	97004656	97991287	Lettuce	Local	Farm	1780	200000	<10	<10

TABLE 1—LETTUCE RESULTS—Continued

Date Sampled	Sample Reference Numbers Chemical	Microbiological	Sample	Origin	Retailer	Nitrate ppm	TYC30°C cfu/gm	E.coli cfu/gm	Campylobacter cfu/gm
20.8.97	GC/FD/410/INW/1997/X	GC/FD/437/INW/1997/M	Lettuce	Scottish	Supermarket	1093	170000	<10	<10
16.9.97	RE/FD/551/E/1997/X	RE/FD/561/E/1997/M	Lettuce	Scotland	Supermarket	788	170000	<10	<10
16.9.97	ED/FD/071/FG/1997/X	ED/FD/421/FG/1997/M	Lettuce	Scotland	Supermarket	1282	610000	<10	<10
16.9.97	NL/FD/193/ISW/1997/X	NL/FD/691/ISW/1997/M	Lettuce	England	Fruit Shop	1021	2000000	<10	<10
16.9.97	NL/FD/202/ICE/1997/X	NL/FD/202/ICE/1997/M	Lettuce	UK	Greengrocers	750	1600000	<10	<10
16.9.97	SA/FD/799/S/EH/1997/X	SA/FD/798/S/EH/1997/M	Lettuce	England	Fruit Shop	<b>3605</b>	370000	<10	<10
16.9.97	EA/FD/113/P/ND/1997/X	EA/FD/112/P/ND/1997/M	Lettuce	England	Supermarket	1492	930000	<10	<10
2.9.97	CL/FD/101/S/1997/X	CL/FD/100/S/1997/M	Lettuce	Local	Fruit Shop	3457	ND	ND	ND
2.9.97	CL/FD/99/S/1997/X	CL/FD/98/S/1997/M	Lettuce	Local	Fruit Shop	896	ND	ND	ND
16.9.97	WD/FD/74/1997/X	WD/FD/262/X/CB/1997/M	Lettuce	UK	Fruit Shop	1357	850000	<10	<10
19.8.97	DG/FD/84/P/KX/1997/X	DG/FD/83/P/KX/1997/M	Lettuce	Scottish	Supermarket	502	6600	<10	<10
9.9.97	DC/L2/97	DC/L2/97	Lettuce	English	Supermarket	1306	640000	<10	<10
2.9.97	FK/FD/269/S/1997/X	FK/FD/270/S/1997/M	Lettuce	UK	Greengrocers	1139	4000000	<10	<10
2.9.97	FK/FD/265/S/1997/X	FK/FD/266/S/1997/M	Lettuce	Scotland	Supermarket	1270	3000000	<10	<10
2.9.97	FK/FD/261/S/1997/X	FK/FD/262/S/1997/M	Lettuce	English	Supermarket	1053	3000000	<10	<10
1.9.97	SG/FD/133/S/1997/X	SG/FD/134/S/1997/M	Lettuce	UK	Deli	1715	1400000	<10	<10
16.9.97	NA/FD/271/I/1997/X	NA/FD/272/I/1997/M	Lettuce	UK	Supermarket	1962	1200000	<10	<10

ND=NOT DETERMINED

TABLE 2—SPINACH RESULTS

Date Sampled	Sample Reference Numbers Chemical	Microbiological	Sample	Origin	Retailer	Nitrate ppm	TYC30°C cfu/gm	E.coli cfu/gm	C.perfringens cfu/gm
19.8.97	GC/FD/391/PS/1997/X	GC/FD/373/PSC/1997/M	Spinach	Dutch	Supermarket	3015	>2000000000	<10	<10
2.9.97	FK/FD/263/S/1997/X	FK/FD/264/S/1997/M	Spinach	UK	Supermarket	2230	93000000	270	<10
27.10.97	AIF6497	AIF6497	Spinach	English	Supermarket	2530	2000000	<10	<10
19.8.97	GC/FD/252/ICC/1997/X	GC/FD/368/ICC/1997/M	Spinach	UK	Supermarket	3195	670000000	200	<10
22.9.97	WR6B/5945	WR6B/5945	Spinach	UK	Supermarket	2920	55000000	27000	20
23.9.97	NC453	NC453	Spinach	UK	Supermarket	1856	530000000	40	<10
16.9.97	NL/FD/203/ICE/1997/X	NL/FD/203/ICE/1997/M	Spinach	UK	Supermarket	1392	560000	<10	<10
1.8.97	SG/FD/131/S/1997/X	SG/FD/132/S/1997/M	Spinach	UK	Supermarket	3715	87000000	320	<10
23.9.97	NC449	NC449	Spinach	UK	Fruit Shop	1780	120000000	<10	<10
22.9.97	473A 6107	473A 6107	Spinach	English	Supermarket	2300	53000000	3200	<10
22.9.97	WR8B/5949	WR8B/5949	Spinach	UK	Supermarket	2085	58000000	20000	<10
22.9.97	WR3A/B/5939	WR3A/B/5939	Spinach	UK	Supermarket	2455	3200000	450	<10
22.9.97	WR2A/B/5937	WR2A/B/5937	Spinach	UK	Supermarket	2260	65000000	3600	<10
27.10.97	ACF6397	ACF6397	Spinach	English	Supermarket	1637	9000000	<10	<10

## Appendix A

# SAMPLING PROTOCOL

### Initial Procedures

1. Preselect premises from which samples are to be taken.
2. Ensure that the following equipment is available:—
  - Coolbox with icepacks and bottle of water
  - Copies of sample submission form (Appendix B)
  - Labels
  - Plastic Food Bags
  - Plastic Gloves
  - Calibrated thermometer

### Sample Collection Procedures

1. Samples should be collected from retail outlets.
2. Each samples should consist of either a matched pair of either fresh lettuce or spinach.  
Samples should be selected according to the following order of priority:—
  - Local produce
  - Scottish produce
  - UK produce
  - Foreign produce

3. If not already prepacked, each pair of samples should be transferred aseptically into two separate plastic bags using plastic gloves. The separation into two bags is required to facilitate the processing of the samples for chemical and bacteriological analysis. The bags should be labelled in accordance with the requirements of the laboratory being used (i.e. with either the same sample reference number or two different numbers).

If two different numbers are used then these should be cross referenced with each other so that the relationship of the two samples with each other is unambiguous.

4. Samples should be placed in a coolbox and delivered to the laboratory as soon as possible, and preferably, under normal circumstances, no later than 6 hours after sampling.

### Transportation of Samples

1. Samples should be transported in an insulated coolbox capable of operating at a temperature of 1-5°C.

2. Pre-cool the insulated box in a refrigerator for at least 1 hour (preferably overnight). Place a small screw-capped bottle of water within the coolbox.
3. Load with sufficient ice packs to ensure adequate cooling.
4. 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 sample form and samples are handed over.

**Appendix B**  
**SAMPLES SUBMISSION FORM**

Name of Authority .....

Liaison Group.....

Sample Number(s) .....

Date Submitted.....

Type of Sample (Lettuce or Spinach) .....

Origin of Samples (Local, Scottish, UK, Foreign) .....

Nature of Premises Sample Collected From .....

Date Collected .....

Time Collected .....

Coolbox Temperature on Arrival at Laboratory .....

Sampling Officer .....

---

**RESULTS (This Section for Laboratory Use Only)**

Nitrate (mg/kg) :

Total Count (cfu/g) :

E.coli (cfu/g) :

C.perfringens (cfu/g) :

Completed forms should be returned to:

J. Waddell  
Glasgow Scientific Services  
64 Everard Drive  
Glasgow  
G21 1XG

## Appendix C

# LABORATORY PROTOCOL

### *Sample Preparation for Microbiological Examination*

Wear disposable gloves whilst handling or preparing samples. Remove any leaves from the lettuce which might normally be discarded by the consumer due to physical damage or lack of freshness. Using suitable sterile implements (knife, spoon or fork) aseptically remove representative portions of the sample of lettuce or spinach. Sampling implements must be sterilised before use either by autoclaving or by immersion in 70% ethanol solution followed by ignition and flaming.

Weigh approximately 25 grams 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 (e.g. 24.5 gm + 220.5 gms = 245 gms).

Stomach/homogenise sample and diluent for a 1 minute period. The homogenate is the primary ( $10^{-1}$ ) sample suspension. Dilutions should be prepared by adding 1 ml of the homogenised primary sample solution to a bottle containing 9 ml 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 methods.

### *Total Count at 30°*

Samples should be examined using the spread plate method described in BS 5763: Part 5 (1994). This method requires the preparation of a series of spread plates (0.5 ml on Plate Count Agar) using decimal dilutions of the sample. Plates are incubated at 30°C for 3 days and then counted.

Fresh vegetables can have very high TVC<sub>30°C</sub> values ( $>10^7/\text{gm}$ ) so it is important to prepare plates using a sequence of decimal dilutions to reach a limit of detection in excess of  $10^8/\text{gm}$ .

Examination using a Spiral Plater is an acceptable alternative. In this case, a reduced range of dilutions can be selected for inoculation on to the agar media but a 0.5 ml spread plate of the  $10^{-1}$  dilution should be used to give the same lower limit of detection.

*E.coli*

Samples should be examined in accordance with the method described in BS 5763: Part 13 (1995) (Enumeration of *E.coli* using membranes). Plates should be inoculated with 1 ml from each decimal dilution from  $10^{-1}$ - $10^{-4}$ .

Alternatively samples should be examined using a spread plate method similar to the one described above but substituting Tryptone Bile Glucuronide Agar (LabM) for Plate Count Agar. 2 x 0.5 ml spread plates should be inoculated from each decimal dilution ( $10^{-1}$ - $10^{-4}$ ).

Plates should be incubated at 30°C for 4hrs followed by 18hrs at 44°C. The confirmation procedures described in BS 5763: Part 13 should be followed.

*Clostridium perfringens*

Samples should be examined in accordance with the method described in BS 5763: Part 9 (1992). Pour plates should be prepared by inoculation with 1 ml from each decimal dilution from  $10^{-1}$ - $10^{-4}$ . It is essential that the addition of an overlayer as described in the method is carried out and that the plates are incubated in an anaerobic atmosphere. The confirmation procedures described in the method should be followed.

*Nitrate*

Samples should be analysed for nitrate content using a validated HPLC procedure. Results should be expressed as mg NO<sub>3</sub>/kg.