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Bacteriological quality of organically grown leaf lettuce in Norway

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ABSTRACT

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Aim: To investigate bacteriological quality in organically grown leaf lettuce, including the presence of selected pathogenic bacteria, and to obtain information about organic lettuce production, including fertilizing regimes.

Methods and Results: Altogether 179 samples of Norwegian organically grown lettuce were collected from 12 producers. *Escherichia coli* was isolated from 16 of the lettuce samples, but in 12 of these contamination was sufficiently low (<100 CFU g^{-1}) that they would be considered to be of acceptable bacteriological quality. *Escherichia coli* O157 and *Salmonella* were not detected in any of the samples. *Listeria monocytogenes* serogroups 1 and 4 were isolated from two samples.

Conclusions: Organic lettuce produced in Norway was generally of acceptable bacteriological quality, but the results show that contamination of organic lettuce with *E. coli* and *L. monocytogenes* do occasionally occur.

Significance and Impact of the Study: These results suggest that organically grown lettuce may be contaminated with *E. coli* and *L. monocytogenes* during cultivation.

Keywords: *Escherichia coli*, *E. coli* O157:H7, faecal indicators, *Listeria monocytogenes*, organic lettuce, *Salmonella* spp.

INTRODUCTION

Production of organic foods has been significantly increasing worldwide; annual increases in production are between 8 and 20% and consumption between 20 and 30% (Organic-Europe 2004; Willer and Yussefi 2004). A goal of the Norwegian Government is that by 2010, 10% of the agricultural land should be managed organically. The area of organically converted farmland in Norway expanded by about 30% annually during the years 1994–97 and by about 15% annually during 1997–2000 (Debio 2003). Organic products are grown, processed and packaged in accordance with Norwegian standards for organic agriculture and food processing, which is based on European Community Council Regulation 2092/91.

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Organic vegetable production in Norway, excluding potatoes, is small, totalling 180 hectares (ha) (Debio 2003). Altogether 29 producers of organically grown lettuce were registered in 2001, accounting for 2·4 ha (Debio, personal communication).

In organic agriculture, manure is widely used as fertilizer. The use of manure has led to concern about the potential for contamination of vegetables with human pathogens such as *Escherichia coli* O157, *Salmonella* spp. and *Listeria monocytogenes*. *Escherichia coli* O157 and *Salmonella* may be present in the intestinal tract of animals and thus in animal manure used as fertilizer. *Listeria monocytogenes* are ubiquitous bacteria, often found in decaying plants, soil and animal manure, and as a consequence may contaminate vegetables growing in the field.

Concern about pathogens in vegetables has risen because of increasing numbers of outbreaks of food-borne illnesses caused by consumption of fresh, whole, cut and minimally processed vegetables (De Roever 1999; Beuchat 2002). A few

outbreaks associated with consumption of conventionally grown lettuce contaminated with pathogens such as *E. coli* O157:H7 and *Salmonella* spp. have been reported (Ackers *et al.* 1998; Hilborn *et al.* 1999; Sagoo *et al.* 2003).

To the best of our knowledge, there are few studies providing information on occurrence of pathogenic bacteria in organically grown lettuce at retail, and these do not report the presence of *E. coli* O157, *Salmonella* spp. and/or *L. monocytogenes* (McMahon and Wilson 2001; Sagoo *et al.* 2001). Currently, there are no data available that support or refute whether organically grown vegetables are more or less bacteriologically safer than conventionally grown vegetables.

The present study was performed in order to obtain information on the bacteriological quality of organically grown leaf lettuce produced in Norway. Another objective was to obtain general information about production of organic lettuce in Norway, including fertilization regimes.

MATERIALS AND METHODS

Sample collection

Altogether 179 samples of domestic organically grown leaf lettuce were obtained from 12 farm producers, with production areas ranging between 0·85 and 0·01 ha (covering both large and small producers), representing 41% of producers and 67·5% of the production area in Norway. All producers, except one, cultivated the lettuce outside. Samples of postharvest, unwashed, ready-for-sale lettuce were collected twice from each producer, at 1- to 7-week intervals during July to October 2001. Each consignment of lettuce contained between five and eight heads of lettuce randomly collected by the producers.

All samples were packed in separate PVC bags and transported to the laboratory immediately after sampling or overnight (max 15 h) together with ice in cardboard boxes. The samples were analysed either immediately after arrival in the laboratory or following overnight storage in the refrigerator at 5 ± 3°C.

Bacterial analyses

Each sample comprised one head of lettuce. The lettuce head was halved and one half was cut into smaller pieces, mixed and transferred to stomacher bags for further analysis. The samples of lettuce were examined for thermotolerant coliform bacteria (TCB) and *E. coli*, *E. coli* O157, *Salmonella* spp. and *L. monocytogenes*. For the enumeration of TCB and *E. coli* a 10-g sample was used, whereas for the detection of the pathogens, a 25-g sample was used for each parameter. TCB were enumerated according to Nordic Committee on Food Analysis (NMKL 1996) no. 125 which includes further identification of *E. coli*.

Automated enzyme-linked fluorescent immunoassay VIDAS (bioMérieux, Mercy-l'Etoile, France) was used for rapid screening for the pathogens. VIDAS ECO Assay and VIDAS SLM Assay were used according to the manufacturer's instruction for detection of *E. coli* O157 and *Salmonella* spp. respectively. For detection of *L. monocytogenes*, VIDAS LMO Assay was used. Presumptive positive samples were confirmed according to NMKL (1999) no. 136. *Listeria monocytogenes* isolates were serotyped with Listeria O Antiserum types 1 and 4 (Difco laboratories, Detroit, MI, USA).

Production information

Farm producers were supplied with a questionnaire for recording information on lettuce samples including production, growth period, fertilizer application, irrigation and marketing.

RESULTS

Bacterial analysis of organic lettuce showed that 16 of 179 (8·9%) of the samples harboured TCB, which were all further identified as *E. coli*. Eight of the producers (67%) had one or more positive samples on one of the sampling occasions. One producer had five positive samples, one had three positive samples, two had two positive samples, and four had one positive sample.

In 12 of the samples from which *E. coli* was isolated, the numbers were below 100 CFU g^{-1}, while the remaining four positive samples had levels above 100 CFU g^{-1} (100, 120, 1700 and 5000 CFU g^{-1} respectively). The two samples with the highest number of *E. coli* come from the same producer, who had three positive samples.

Listeria monocytogenes was isolated from two samples from two different producers. Serotyping of these isolates showed that one belonged to serogroup 1 and one to serogroup 4. *Escherichia coli* O157 and *Salmonella* spp. were not detected in any samples.

Producer information

Six of 12 farm producers (50%) answered the questionnaire. These farmers produced between 30 and 4000 heads of lettuce in 2001. All the producers also cultivated other organic vegetables. Five farmers propagated their own seedlings and fertilized the seedlings with either chicken or cattle manure, compost, straw or peat. Four of the producers used different types of animal manure as fertilizer on the lettuce; three used compost and one used slurry. The remaining two used green manure (soil incorporation of field or forage crop while green) or had not applied fertilizer this season. Application of fertilizer typically took place between

two months and 1 week prior to transplanting. Compost was usually made of cattle manure, but unfortunately producers did not record composting time. The amount of manure used was between 18 and 80 tonnes per ha. The growth period of the lettuce was approx. 9 weeks and three of the producers irrigated their plants. The producers delivered their lettuce to shops, hotels or sold them locally on the greenmarket.

Escherichia coli was detected in lettuce from two of the six producers who answered the questionnaire and who used different types of animal manure as fertilizer (compost from cattle and horse manure; slurry from small ruminants). Another two of the producers who responded to the questionnaire and had *E. coli*-positive samples, had either used no fertilizer or had used green manure in this growth season.

DISCUSSION

Escherichia coli was isolated from 8·9% of the samples of Norwegian organically grown lettuce. Although not directly comparable, a previous study of conventionally produced lettuce in Norway demonstrated presence of TCB (not further identified) in 1·5% of the samples (Johannessen *et al.* 2002) indicating that the prevalence of such bacteria was low. Other studies assessing the bacteriological quality of organic vegetables (McMahon and Wilson 2001; Sagoo *et al.* 2001; Johannessen *et al.* 2004) also found that the numbers of *E. coli* present in lettuce were usually low. Mukherjee *et al.* (2004) reported that 22·4% of organic lettuce was positive for *E. coli*, whereas in certified organic lettuce the prevalence was 4·3%. However, when Mukherjee *et al.* (2004) compared the prevalence of *E. coli* in certified-organic and conventionally produced lettuce, the difference was not statistically significant.

An interesting observation from the present study is that *E. coli* was isolated from samples from eight different producers on only one of the sampling occasions, which may indicate that the contamination was relatively random. However, as one of the producers had five positive samples at one sampling occasion, but none at the second, sporadic faecal contamination may occur. A likely source of such sporadic contamination is irrigation water. The lettuce could also have been contaminated in the field by birds, rodents or insects or splashes from the soil, at harvest or postharvest by different equipment.

Microbiological guidelines (The Norwegian Food Safety Authority 2002) in Norway do not stipulate maximum levels for *E. coli* in raw vegetables. In UK, PHLS guidelines for assessment of the microbiological quality of ready-to-eat foods (sampled at point of sale) require that there are <100 CFU g^{-1} of *E. coli* for the produce to be considered to be of acceptable quality (PHLS – Public Health Laboratory Service 2000). In the present study, four of the samples exceeded these stipulated levels for *E. coli*, and would therefore have been considered unacceptable by these criteria. This suggests that the bacteriological quality of the produce sampled in this study was generally good, but that faecal contamination may have occurred.

In the present study, all the TCB were identified as *E. coli*. As TCB other than *E. coli*, such as *Enterobacter* spp. and *Klebsiella* spp., are normally part of the native microflora of fresh vegetables, *E. coli* is considered a more appropriate indicator of faecal contamination.

Other studies that have assessed the microbiological quality of vegetables have also suggested using *E. coli* as the indicator of faecal contamination (De Roever 1999; Little *et al.* 1999; Johannessen *et al.* 2002).

The apparent lack of *E. coli* O157 and *Salmonella* spp. in domestically produced lettuce concurs with the results of previous studies on Norwegian lettuce (Johannessen *et al.* 2002, 2004) and may be explained by the low occurrence of these pathogens in Norwegian livestock (Vold *et al.* 1998, 2001; Hofshagen *et al.* 2004). Solomon *et al.* (2002) showed in an experimental study that manure contaminated with high numbers of *E. coli* O157:H7 could be a source of transmission of this pathogen to the plant tissue. Johannessen *et al.* (2004), however, reported the presence of *E. coli* O157:H7 in manure and slurry used as fertilizer in a field trial, but not in organic lettuce harvested from the contaminated soil. Similarly, in an experimental study, *E. coli* O157:H7 could not be isolated from lettuce when low numbers of this pathogen were in the manure (Johannessen *et al.* 2005).

Sporadic contamination with *L. monocytogenes* of organic lettuce was observed (two positive samples from two different producers). In Norway, the microbiological guidelines (The Norwegian Food Safety Authority 2002) require that *E. coli* O157 and *Salmonella* spp. are absent from raw vegetables, but there are no regulations for *L. monocytogenes*. In UK, PHLS guidelines for assessment of the microbiological quality of ready-to-eat foods (sampled at point of sale) require that there are <100 CFU g^{-1} of *L. monocytogenes* (PHLS – Public Health Laboratory Service 2000). Absence or very low incidence of *L. monocytogenes* in both, organically and conventionally grown lettuce has been previously reported (Little *et al.* 1999; McMahon and Wilson 2001; Sagoo *et al.* 2001; Johannessen *et al.* 2002). The ubiquitous nature of *Listeria* spp. and its occasional detection in cattle faeces (Unnerstad *et al.* 2000) used in fertilizer may generate concerns about the potential for contamination of organically grown lettuce with *L. monocytogenes*. It is also important to keep in mind that *L. monocytogenes* is often isolated from the environment, and that strains of environmental origin might be of more importance than faecal strains when considering sources of *L. monocytogenes* during production of vegetables.

The information acquired by the questionnaire about production of organically grown lettuce in Norway was limited, but showed that different forms of animal manure are commonly used. Farmyard manure may be suspected to be a major source of faecal contamination of lettuce and it could be speculated the use of animal manure as fertilizer might influence the occurrence of faecal indicators in fresh produce. However, in the present study, of the producers that answered the questionnaire, two which had *E. coli*-positive samples had either not fertilized or used green manure as fertilizer, and another two with *E. coli*-positive samples had used different types of animal manure. Due to the limited data set, conclusions on the relationships between fertilizer use and the number of *E. coli*-positive samples cannot be made. The results from a field study by Johannessen *et al.* (2004) indicated that different *E. coli* counts in soil fertilized with different fertilizers did not influence the hygienic quality of lettuce. In a recent survey of *E. coli* in organic lettuce in the US, the age of the manure had a significant effect on the prevalence of *E. coli*, while application of manure in the spring or fall was not related to an increased number of *E. coli*-positive samples (Mukherjee *et al.* 2004).

In conclusion, the results from the present study suggest that the bacteriological quality of organically produced lettuce in Norway is generally good, but that contamination with faecal indicators and pathogenic bacteria do occasionally occur.

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