Food Microbiology 27 (2010) 679–684

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Microbiological quality of fresh lettuce from organic and conventional production

M. Oliveira [a], J. Usall [b], I. Viñas [a], M. Anguera [b], F. Gatius [c], M. Abadias [b,*]

[a] University of Lleida, UDL-IRTA Centre, XaRTA-Postharvest, Rovira Roure 191, 25198-Lleida, Spain
[b] IRTA, UdL-IRTA Centre, XaRTA-Postharvest, Rovira Roure 191, 25198-Lleida, Spain
[c] University of Lleida, Chemistry Department, Rovira Roure 191, 25198-Lleida, Spain

ARTICLE INFO

Article history:
Received 14 January 2010
Received in revised form
10 March 2010
Accepted 13 March 2010
Available online 25 March 2010

Keywords:
Organic production
Conventional production
Foodborne pathogens prevalence
Foodborne pathogens incidence
Produce safety
Listeria monocytogenes
Salmonella
Escherichia coli

ABSTRACT

Previously there was no available information on the levels of indicator bacteria and the prevalence of pathogens in fresh lettuce grown in organic and conventional farms in Spain. A total of 72 lettuce samples (18 farms for 4 repetitions each) for each type of the agriculture were examined in order to assess the bacteriological quality of the lettuces, in particular the prevalence of selected pathogens. The lettuce samples were analyzed for the presence of aerobic mesophilic, psychrotrophic microorganisms, yeasts and moulds, *Enterobacteriaceae*, mesophilic lactic acid bacteria, *Pseudomonas* spp. and presumptive *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*. The mean aerobic mesophilic counts (AM) were 6.35 ± 0.69 $\log_{10}$ cfu g^{-1} and 5.67 ± 0.80 $\log_{10}$ cfu g^{-1} from organic and conventional lettuce, respectively. The mean counts of psychrotrophic microorganisms were 5.82 ± 1.01 $\log_{10}$ cfu g^{-1} and 5.41 ± 0.92 $\log_{10}$ cfu g^{-1} from organic and conventional lettuce, respectively. Yeasts and moulds (YM) mean counts were 4.74 ± 0.83 $\log_{10}$ cfu g^{-1} and 4.21 ± 0.96 $\log_{10}$ cfu g^{-1} from organic and conventional lettuce, respectively. Lactic acid bacteria (LAB) were present in low numbers and the mean counts were 2.41 ± 1.10 $\log_{10}$ cfu g^{-1} and 1.99 ± 0.91 $\log_{10}$ cfu g^{-1} from organic and conventional lettuce, respectively. *Pseudomonas* spp. mean counts were 5.49 ± 1.37 $\log_{10}$ cfu g^{-1} and 4.98 ± 1.26 $\log_{10}$ cfu g^{-1} in organic and conventional lettuce, respectively. The mean counts for *Enterobacteriaceae* were 5.16 ± 1.01 $\log_{10}$ cfu g^{-1} and 3.80 ± 1.53 $\log_{10}$ cfu g^{-1} in organic and conventional lettuce, respectively. *E. coli* was detected in 22.2% (16 samples) of organic lettuce and in 12.5% (9 samples) of conventional lettuce. None of the lettuce samples was positive for *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. From the samples analyzed by principal component analysis (PCA) a pattern with two different groups (conventional and organic) can be observed, being the highest difference between both kinds of samples the *Enterobacteriaceae* count.

1. Introduction

In recent years, an increasing number of gastrointestinal disease outbreaks have been linked to the consumption of fresh fruits and vegetables. Most reports indicated that raw vegetables may harbor potential foodborne pathogens. Some outbreaks associated with consumption of lettuce contaminated with pathogens such as *Listeria monocytogenes* (Francis et al., 1999; Sagoo et al., 2003), *Salmonella* (Ercolani, 1976; Garcia-Villanova Ruiz et al., 1987; FDA, 2001; Sagoo et al., 2003) and *Escherichia coli* O157:H7 (Ackers et al., 1998; Hilborn et al., 1999; Friesema et al., 2007) have been reported. Vegetables can become contaminated with such pathogenic organisms while growing or during harvesting, postharvest handling, or during distribution. Pre-harvest contamination of vegetables can occur directly or indirectly via animals, insects, water, soil, dirty equipment, and human handling. However, the most important considerations are the application of manure or compost as fertilizer to fields where crops are grown and the fecal contamination of irrigation water.

Manure and other animal wastes are widely used in agriculture, both organic and conventional. The use of manure as fertilizer, whether in organic or conventional agriculture, gives rise to concern about the possible contamination of produce with microbial pathogens (IFST, 1999). Some reports demonstrated that pathogens like *E. coli* O157, *Salmonella enterica* and *L. monocytogenes* are able to survive for extended periods (up to months) in manure (Franz et al., 2005; Scott et al., 2006) and manure-amended soil (Franz et al., 2008; Watkins and Sleath, 1981).

Organic production has been considered to represent an increased risk to public health than conventional production, due to the method of cultivation and processing, where natural fertilizers such as animal manure are used, and where no chemical

* Corresponding author. Tel.: +34 973003430; fax: +34 973238301.
E-mail address: isabel.abadias@irta.cat (M. Abadias).

0740-0020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2010.03.008

treatments are employed to reduce the microbiological loading of the raw product, but there is little scientific evidence to support this suggestion (McMahon and Wilson, 2001).

In Spain, from 1996 to 2003 a substantial increase in the number of growers and land under organic management took place, partly due to the agri-environmental economic support scheme for organic farming. The number of organic farms increased by more than 15% from the year 2001, from 15 609 to 18 226 organic farms in 2007. The area of organically managed land increased from 485 079 ha to 988 323, and thus nearly doubled. Although the size of the organic processing industry in Spain is still small, it has been growing continuously in the last five years at an annual rate of 10%–22% (MAPA, 2007).

Organic farming was officially regulated in Spain in 1989, with the National regulation of Generic Denomination 'Organic Agriculture', which was applied until the EU Regulation 2092/91 on Organic Agriculture came into force. A Spanish regulation, RD 1852/1993 established a new regulation for organic farming, based on EU Regulation (CEE) 2092/91, and at the same time the Spanish regions assumed official responsibility in the monitoring of organic production. Under the same law (RD 1852/1993) the Advisory Group CRAE of the National Ministry for Agriculture, Fisheries and Food (MAPA) was created. This group includes organic stakeholders, regional and central authorities as well as the directors of the regional public certification bodies (Gonzálvez, 2007). The EC regulation N°2092/91 was repealed in the 1 January 2009 by the Council Regulation (EC) No 834/2007 of 28 June 2007.

The aim of this study was to determine the effect of production system (conventional or organic) on the microbiological quality of fresh lettuce produced in Spain.

2. Material and methods

2.1. Origin of samples

Farmers that grew lettuces were invited by telephone or personal contact to participate in this study. The samples were obtained from organic and conventional farms that were located in the northeast of Spain. Organic lettuces were produced according the EU Regulation (CEE) 2092/91, as samples were analyzed before the 1 January 2009. All organic fields were certified by competent national authorities. Farmers were asked about the use of organic and inorganic fertilizers and chemical treatments. Livestock manure (sheep or cattle) were only applied in four organic fields. The others were fertilized with composted farmyard or plant manure. Conventional producers did not report the use of livestock manure.

2.2. Sampling and preparation of lettuce

A total of 72 lettuce (*Lactuca sativa L.*) samples for each type of the agriculture were collected directly from the farm fields and were transported to the laboratory without being washed and immediately analyzed. For each type of the agriculture, 18 farms were analyzed and four samples of each were collected. Different lettuce cultivars belonging to two different groups were used: *L. sativa* var. *longifolia* (Romaine lettuce) and *L. sativa* var. *capitata* (Batavia, 'Trocadero', Iceberg and 'Maravella' lettuce). The outer leaves and core of the lettuce were removed and discarded. The remaining leaves were hand cut on pieces with a disinfected sharp knife.

2.3. Microbiological analyses

Microbial analyses were carried out using the standard methodologies described in Table 1. Twenty-five grams of lettuce were transferred in 225 mL of saline peptone solution (SP, 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone), in sterile stomacher bags. The samples were homogenized in a Stomacher 400 (Seward, London, UK) set at 230 rpm for 2 min. Further decimal dilutions were made with the same diluent and analyzed for aerobic mesophilic, psychrotrophic microorganisms, yeasts and moulds, *Enterobacteriaceae*, mesophilic lactic acid bacteria, *Pseudomonas* spp. and presumptive *E. coli*. Another 25 g were diluted in 225 mL of buffered peptone water (Oxoid, CM1049) for the enumeration of *L. monocytogenes* and detection of *Salmonella*.

Table 1
List of methodologies used to determine microbial quality.

Determination	Methodology	Description
Aerobic mesophilic count (AM)	ISO 4833:2003	Microbiology of food and animal feeding stuffs – Horizontal methods for the enumeration of microorganisms. Colony-count technique at 30 °C.
Psychrotrophic microorganisms	ISO 17410:2001	Microbiology of food and animal feeding stuffs – Horizontal methods for the enumeration of psychrotrophic microorganisms.
Yeasts and moulds	ISO 7954:1987	Microbiology – General guidance for enumeration of yeasts and moulds – Colony-count technique at 25 °C.
Lactic acid bacteria	ISO 15214:1998	Microbiology of food and animal feeding stuffs – Horizontal methods for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 °C.
Enterobacteriaceae	ISO 21528-2:2004	Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method.
Pseudomonas spp.	ISO 13720:1995	Meat and meat products – Enumeration of *Pseudomonas* spp. (used for vegetable products).
Presumptive *E. coli*[a]	ISO 7251:2005	Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of presumptive *Escherichia coli* – Most probable number technique.
Salmonella spp.	ISO 6579:2002	Microbiology of food and animal feeding stuffs – Horizontal methods for the detection of *Salmonella* spp.
L. monocytogenes	ISO 11290-2:1998	Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *L. monocytogenes*. Part 2: Enumeration method.

[a] Presumptive *E. coli* strains isolated were subsequently plated in Tergitol BCIG agar and Sorbitol MacConkey Agar and incubated at 44 ± 1 °C for the detection of β-glucuronidase and sorbitol positive strains, respectively.

The pathogenicity of *E. coli* strains was analyzed by the "Servicio de Bacteriología, Centro Nacional Microbiología, Instituto de Salud Carlos III" (Majadahonda, Madrid, Spain). The following tests were carried out: verotoxin gene Type 1; verotoxin gene Type 2; Intimin (gene eae); Enterohemolysin gene; adhesin (gene bfp); CVD432 plasmid; ipaH gene; heat stable toxins (st gene) and heat-labil toxin (lt gene).

2.4. Statistical analyses

To provide a general overview of the samples, a principal component analysis (PCA) was developed. Samples, coded as ORG (organically produce lettuce) and CON (conventional produce lettuce), were characterized by the microbial content, labelled as AM, PSI, YM, LAB, ENT and PSE, referring to aerobic mesophilic, psychrotrophic, yeast and moulds, lactic acid bacteria, *Enterobacteriaceae* and *Pseudomonas* spp., respectively. Raw data was used in the PCA