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Microbial quality of organic and conventional vegetables from Polish farms

Magdalena Szczech[a,*], Beata Kowalska[a], Urszula Smolińska[a], Robert Maciorowski[b], Michał Oskiera[a], Anna Michalska[a]

[a] Research Institute of Horticulture, Department of Microbiology, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
[b] Research Institute of Horticulture, Economics and Statistics Section, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

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ABSTRACT

Microbiological analyses of lettuce, radish, carrot and beetroot were conducted to determine the effect of production system (organic and conventional) on the microbial quality of vegetables in Poland. During 2010–2014 growing seasons, 600 organic and 372 conventional samples were collected from certified farms. The vegetables were analyzed for aerobic mesophilic bacteria, yeasts and molds, *Enterobacteriaceae*, coliforms and *Escherichia coli* according to Polish standards. The farmer's survey was conducted to collect information on farm management practices. The index (from 0 – no risk to 4 – high risk) of potential contamination of the produce by human pathogens, related to fertilization system was developed.

The mesophilic bacteria, yeasts and molds, coliforms and *Enterobacteriaceae* numbers for the radish and carrot were similar for organic and conventional cultivation systems (mesophilic bacteria 7.0 $\log_{10}$ cfu g^{-1} and 6.6 $\log_{10}$ cfu g^{-1}; yeasts and molds 5.1 and 4.8; coliforms 1.3 and 1.5; *Enterobacteriaceae* 2.1 and 2.3 for radish and carrot respectively). Organic lettuce harbored significantly more bacteria than conventional (mesophilic 6.7 $\log_{10}$ cfu g^{-1} and 6.4 $\log_{10}$ cfu g^{-1}, coliforms 1.8 and 1.4; *Enterobacteriaceae* 2.5 and 1.9 for organic and conventional respectively). Organic beetroot contained higher number of yeasts and molds (5.1 $\log_{10}$ cfu g^{-1}) and *Enterobacteriaceae* (2.9 $\log_{10}$ cfu g^{-1}) than conventional (4.9 and 2.5 $\log_{10}$ cfu g^{-1}). The vegetables from organic farms showed significantly higher load of *E. coli* (on average 0.42 $\log_{10}$ cfu g^{-1}) than conventionally cultivated vegetables (in average 0.05 $\log_{10}$ cfu g^{-1}).

The index 0–4 of potential risk of produce contamination by human pathogens was created according to fertilization practices in both farm types. Its value increased with enhanced contribution of manures and other animal wastes. In organic production the main fertilization practice was application of animal manures, composted and not composted. A popular practice was also top dressing of growing plants with fermented plant extracts, sometimes enriched with dungwater. In conventional farming system mineral fertilization was the main source of the vegetable nutrition. Therefore, organic produce indicated higher index of contamination risk (2–4) than conventional vegetables (1–2). High indexes were positively associated with higher number of *E. coli*. It was found that fertilization system practiced in organic farms may deteriorate sanitary quality of the produce.

1. Introduction

In recent years, an enhanced consumption of fresh and ready-to-eat produce is one of the reasons for increased number of foodborne outbreaks (Ding et al., 2013; Ethelberg et al., 2010; Friesema et al., 2008; Lynch et al., 2009; Matthews, 2014; Slayton et al., 2013). Usually the outbreaks related to these products are associated with contamination of fruits or vegetables with pathogens such as *Salmonella*, *Listeria monocytogenes* or pathogenic *Escherichia coli* (Jones and Heaton, 2008). The last large outbreak in Europe was reported in May 2011 when the emerging aggregative EHEC O104:H4 strain caused high disease incidence in Germany, and sprouted seeds were identified as a source of the infection (Tzschoppe et al., 2012). However, outbreaks are more likely to be identified than sporadic cases, which number is unknown. Screening of fresh produce for the presence of enteric pathogens in several countries revealed that the risk of hazardous contaminations is real, especially for leafy vegetables (Cerna-Cortes et al., 2015; Delbeke et al., 2015; Herman et al., 2015; Skočková et al., 2013; Ssemanda et al., 2017).

Numerous human bacterial pathogens are well adapted for survival in soil and water, and can persist in the plant spermosphere, rhizosphere and phyllosphere. These bacteria form biofilms adhering to

* Corresponding author.
E-mail address: magdalena.szczech@inhort.pl (M. Szczech).

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plant surfaces or may migrate through the root system to other parts of the plants (Mandrell, 2009; Ryser et al., 2009; Yaron, 2014). Therefore, washing is only marginally effective in reducing populations of pathogenic and spoilage microorganisms, because attached and internal bacteria are difficult to wash off (Jones and Heaton, 2008).

Contamination of vegetables may take place during growing, harvesting or handling and distribution (Mandrell, 2009). Preharvest contamination is mostly caused by application of manures and composts as fertilizers to soil, and by irrigation water containing fecal bacteria (Gerba, 2009; Jiang and Shepherd, 2009). Animal manures are considered as a major reservoir for foodborne pathogens, and their inappropriate use in agriculture contributes a risk to consumer health (Cote and Quessy, 2005; Guan and Holley, 2003). Diez-Gonzalez and Mukherjee (2009) and Jiang and Shepherd (2009) in their reviews described the results of many studies, demonstrating that human pathogens are able to persist for extended periods in manures and manure-amended soil.

Manures and other animal wastes are widely used in organic farming. Therefore, potentially, microbial contamination of organically grown plants may be higher than in conventional cultivation, where chemical treatments may reduce the microbial loading of the raw products (Maffei et al., 2013; Oliveira et al., 2010). The organic cropping system has been developed during the last decades as an alternative to conventional produce, because of society's increasing concern of environment safety and food quality. Consumers frequently consider that organic fruits and vegetables are better because they have less pesticide residues, but they ignore the microbiological safety issues resulting from organic production. There are reports that the potential for microbial contamination is higher for organic produce than for conventionally grown crops (Diez-Gonzalez and Mukherjee, 2009; Denis et al., 2016; Mukherjee et al., 2007). There are also reports that consumption of organically produced vegetables does not represent an increasing risk of foodborne diseases (Jones and Heaton, 2008; Maffei et al., 2013; Tango et al., 2014). However, there is still too little published evidence to support those concerns, and in consequence the microbiological quality of organic vegetables is under question (Maffei et al., 2016).

In Poland a substantial increase in the number of growers and land under organic management took place partly due to the economic support scheme, and it is growing continuously. Organic production is controlled mostly on the account of chemicals prevention or on the use of proper seed and plant propagation material. But microbial and sanitary condition of fresh products are not under control. Therefore, the objective of this study was to determine the effect of production system (conventional or organic) on the microbial quality of various vegetable species in Poland.

2. Materials and methods

2.1. Origin of samples

Plant material for the studies was collected in the years 2010–2014. It included: lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* var. *sativus*) collected during spring, carrot (*Daucus carota* L.) and beetroot (*Beta vulgaris* L.) collected during autumn. The vegetables were obtained from organic and conventional farms, that were located in central Poland (2010), south-west (2011), north-west (2012), north-east (2013) and south-east parts of Poland (2014). In the following years (2010–2014): 31, 32, 43, 44 and 28 farms were visited. In total we visited 85 organic and 93 conventional farms. All organic farms, from where the samples were collected, were certified by competent national authorities. The farms were chosen with the aid of Agricultural Advisory Centers in Poland.

2.2. Sampling and samples preparation for microbial analyses

The samples for both production systems, conventional and organic, were collected directly from the farm fields. All vegetables were sampled in triplicate from different locations on the field. The total number of the samples collected in the years 2010–2014 was as follow: organic lettuce 159, conventional lettuce 99, organic radish 135, conventional radish 66, organic carrot 165, conventional carrot 117, organic beetroot 141, conventional beetroot 90. Samples were put in sterile plastic ziplock bags without being washed and were properly marked by recording farm identity, sample number and date of collection. The bags were placed in electric coolers with extra ice-packs (4–8 °C) and were transported to the laboratory. Samples were stored at 4 °C for 24–48 h until tested.

The sample amounts varied from one produce type to another. For lettuce three heads were collected for one replicate, for radish 12 plants, for beetroot 10 roots and for carrot 10 roots were collected as well. Sample preparation for microbial analysis depended on the type of vegetable. In the case of lettuce the outer leaves (dry and rotten) were removed and discarded, and the remaining leaves were hand cut in pieces with a disinfected knife. For radish, carrot and beetroot the roots were shaken of loosely adhered soil and were peeled off with a disinfected knife. The peelings were used to analyze.

2.3. Microbiological analyses

Microbial analyses were conducted according to Polish standard methodologies described in Table 1. Twenty five grams of plant material were transferred into 225 ml of peptone water in sterile stomacher filter bags 400 ml. The samples were homogenized in a stomacher BagMixer® 400 P with fixed speed 8 stroke/s for 10 min. Further decimal dilutions were made with the same diluent and analyzed for: aerobic mesophilic bacteria, yeasts and molds, *Enterobacteriaceae*, coliforms and *Escherichia coli*. Mesophilic aerobic bacteria were enumerated on plate count agar (PCA), yeasts and molds on yeast extract glucose chloramphenicol agar (YGC agar), *Enterobacteriaceae* on VRBG agar, coliforms on violet red bile lactose (VRBL agar) and *E. coli* on selective Chromacoult® TBX agar. All agar media were purchased from Merck (Germany). The results were expressed as colony-forming units per gram of plant material (cfu g^{-1}). For statistical analysis the data were transformed to logarithm. For *E. coli* the values below detection limit were calculated as 0 $\log_{10}$.

Table 1
List of methodologies used to determine microbial quality.

Determined microorganisms	Methodology	Description
Aerobic mesophilic bacteria	PN-EN ISO 4833:2004 PN-EN ISO 4833-1:2013-12	Microbiology of food and animal feeding stuff – Horizontal methods for the enumeration of microorganisms. Colony count technique at 30 °C
Yeasts and molds	PN-ISO 7954	General guidance for enumeration of yeasts and molds – Colony count technique at 25 °C
Enterobacteriaceae	PN-ISO 21258-2	Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method
Coliforms	PN-ISO 4832	Horizontal method for the enumeration of coliforms – colony-count technique
Escherichia coli	PN-ISO 16649-2	Horizontal method for the enumeration of β-glucoronidase-positive *Escherichia coli*