Food Control 85 (2018) 350–358

Contents lists available at ScienceDirect

Food Control

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

Assessment of pesticide residues and microbial contamination in raw leafy green vegetables marketed in Italy

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ARTICLE INFO

Article history:
Received 10 July 2017
Received in revised form
15 September 2017
Accepted 27 September 2017
Available online 28 September 2017

Keywords:
Leafy green vegetables
Ready-to-eat
Pesticides
Salmonella
Enteropathogenic Escherichia coli
Hepatitis E virus

Chemical compounds studied in this article:
Boscalid (PubChem CID: 213013)
Propamocarb (PubChem CID: 32490)
Imidacloprid (PubChem CID: 86418)
Pyraclostrobin (PubChem CID: 6422843)
Metalaxyl (PubChem CID: 42586)
Cyprodinil (PubChem CID: 86367)
Thiamethoxam (PubChem CID: 107646)
Dimethomorph (PubChem CID: 5889665)
Deltamethrin (PubChem CID: 40585)
Iprodione (PubChem CID: 37517)

ABSTRACT

Vegetables that can be eaten raw in salads are considered at higher risk for contamination by foodborne pathogens and pesticide residues. In this study fresh leafy green vegetables, both pre-cut and uncut, were sampled at retail in all 20 Italian regions and tested for pathogens and pesticide residues contamination.

Both in pre-cut and in uncut vegetables 1.00% of samples tested positive for pathogens investigated. Pesticide residues were detected in 53.33% of samples, pre-cut vegetables were more often contaminated (59.06%) than uncut vegetables (47.68%). Samples tested positive from one (monoresidual samples, 27.33%) to seven chemical compounds (multiresidual samples, 26.00%). Maximum Residual Levels (MRL) established by European legislation were exceeded in two multiresidual samples of uncut romaine lettuce (overall non-compliant prevalence 0.67%).

A few samples resulted non-compliant for MRL or contaminated by pathogens. Nevertheless in more than half of the samples analysed pesticide residues were found; furthermore vegetables encompassed in this survey are eaten raw and process does not include any effective microbial elimination step. These findings can suggest that public health risk remains, especially regarding pre-cut vegetables.

1. Introduction

Fruit and vegetables are an important component of a healthy diet: (FAO/WHO, 2004) regular daily consumption of these foods in sufficient amounts can help prevent major diseases such as cardiovascular diseases and certain cancers (La Vecchia, Altieri, & Tavani, 2001; Link & Potter, 2004; WHO, 2003).

Global vegetable production and consumption have grown significantly since 1980, however in many countries per capita vegetables intake is below recommendations (European Commission, 2007) and there are international programs promoting vegetables consumption (FAO/WHO, 2004).

Therefore, vegetables production and consumption are expected to continue to increase in the future and Italy follows the global trend of continuous rise in demand of vegetables (CSO, 2016; Ferrante, 2013; ISMEA Mercati, 2013).

A drawback to the health benefits is that fresh produce may be contaminated by pathogenic microorganisms and pesticide residues (Keikotlhaile & Spanoghe, 2011). Vegetables at harvest are normally colonized by a variety of microorganisms, mostly non-

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[1] The Authors would like to dedicate this paper to the memory of Vincenza Annunziata Prencipe and Antonella D'Agostino who intensely worked on the present survey and passed away before the submission of this manuscript.

https://doi.org/10.1016/j.foodcont.2017.09.035
0956-7135/

pathogenic, but during any of the steps from farm to consumer (growth, harvest, processing, packaging, transport, handling, retail) further contamination can occur from different sources (environmental, animal or human) (FAO/WHO, 2008; Hou, Fink, Radtke, Sadowsky, & Diez-Gonzalez, 2013; Lindow & Brandl, 2003; Park et al., 2012; Yaron & Römling, 2014).

Since vegetables are attacked by pests and diseases during production and storage, pesticides are used in order to reduce the loss and maintain the quality; however the use of pesticides during production often leads to the presence of residues in vegetables after harvest (Keikotlhaile & Spanoghe, 2011). The presence of chemical residues is a concern because pesticides are known to have potentially harmful effects to other non-targeted organisms, causing cancer and asthma and interfering with the reproductive systems and foetal development (Gilden, Huffling, & Sattler, 2010).

Raw leafy vegetables consumption has been also correlated to some foodborne disease outbreaks worldwide (Beuchat, 1996; Berger et al., 2010; Mercanoglu Taban & Halkman, 2011). Recent data from European Union reveal that the incidence of foodborne outbreaks linked to food category "vegetables and juices" have increased during last years (EFSA, 2015).

Pre-cut products are more perishable than whole produce: although remaining in a fresh state, they are physically altered during processing operations and have living tissues characterised by an accelerated metabolism. These products respond to the demand of the consumer to reduce the meal preparation time as well as the volume of the kitchen waste, but they are at the same time very fragile because their processing does not result in a biological stabilization and presents, in fact, a shelf-life limited to few days (Arienzo et al., 2013; da Cruz, Cenci, & Maia, 2008). The preliminary operations to which pre-cut products are subjected cause some mechanical and physiological damages which are responsible of the induction and speeding of chemical and enzymatic reactions (Arienzo et al., 2013). Furthermore these operations can lead to cross contamination; this risk is of big concern, since these products are intended to be eaten raw without any treatment for microbial elimination. In order to guarantee the safety of pre-cut leafy vegetables, food industry must implement a wide range of measures. These measures are intended not only to control the main process aimed to reduce microbial contamination, e.g. the washing step, but also to monitor all production steps, implementing an assurance quality systems from primary production to processing, packaging and distribution (Castro-Ibañez, Lòpez-Gàlvez, Gil, & Allende, 2016; da Cruz, Cenci, & Maia, 2006a; da Cruz, Cenci, & Maia, 2006b; da Cruz at al., 2008; De Giusti et al., 2010; Gil, Marín, Andujar, & Allende, 2016).

Since leafy green vegetables can be considered vehicles in the consumption of pesticide residues and in the transmission of foodborne pathogens, a number of laboratory surveys have been carried out worldwide to assess the prevalence of chemical residues and of bacterial, protozoan and viral pathogens in vegetables, usually reporting low frequency of contamination particularly in more developed countries (Castro-Ibañez et al., 2016; Park et al., 2016).

Regarding Italy, some data already exist on chemical and microbial safety of raw leafy green vegetables: surveys have usually encompassed only regions or areas, while studies carried out on the whole national territory considered only microbiological contamination or were part of official controls, that are not performed according to *ad hoc* designed plans and include a broad variety of produce (Arienzo et al., 2013; De Giusti et al., 2010; Losio et al., 2015; Ministero della Salute, 2016; Tesauro et al., 2013).

The aim of the present study is to evaluate the presence of foodborne pathogens and pesticide residues in raw leafy green vegetables, both uncut and pre-cut (ready-to-eat), sampled in the whole Italian territory. In order to get data that could be directly used for the evaluation of the exposure of consumers in the framework of risk analysis studies, all the samples included in the survey have been collected at retail. Since fresh leafy vegetables that can be eaten raw in salads are considered at higher risk for consumers (FAO/WHO, 2008), only these types of produce have been encompassed in the survey. In detail, prevalence and levels of contamination had been evaluated for more than 200 pesticide residues and for *Salmonella* spp., thermotolerant *Campylobacter*, *Listeria monocytogenes*, Enteropathogenic and Shiga toxin-producing *E. coli* (hereafter named, respectively, with the acronyms EPEC and STEC), Hepatitis A and Hepatitis E viruses (hereafter named with the acronyms HAV and HEV), Norovirus genotypes GI and GII, *Cryptosporidium* spp. and *Cyclospora cayetanensis.*

2. Materials and methods

2.1. Sampling

Two categories of fresh leafy green vegetables were included in the study: uncut vegetables (selected, washed and cut by the consumers) and other ready to eat pre-cut vegetables (selected, washed and cut by the producers).

As the market share for large retailers operating in food sales in Italy at the time of the survey was over 70% (ISMEA-Nielsen, 2010), all samples were taken from large retailers. No more than one sample for each vegetable category was purchased from each retailer.

Sampling was carried out during a 22-month period (from September 2013 to June 2015) in all 20 Italian regions. A sampling programme was designed taking into account data on Italian population and vegetable market. The number of samples to be acquired on the national territory has been stratified proportionally to provincial population (ISTAT, 2013) and to regional data on sales of the two considered vegetable categories (pre-cut and uncut) (ISMEA Mercati, 2013).

A total of 602 samples, 301 pre-cut and 301 uncut vegetables, were taken from 374 retailers (one sample in 146 points of sale, two samples —one pre-cut and one uncut vegetable-in 228 points of sale). Sample size was designed to provide, supposing an expected prevalence of 1%, a desired accuracy of 1% and a level of confidence of 95%.

Data related to samples were gathered into the National Veterinary Information System for Food Safety (SINVSA).

2.2. Laboratory analyses

Microbiological tests were carried out on all 602 samples, while pesticide residues were investigated on 300 samples (149 pre-cut and 151 uncut vegetables); details on samples analysed (number of samples for each vegetable type) are described in Table 1.

Rapid and sensitive screening molecular methods were used. From each sample, portions of 25 g were cultured in enrichment broths specific for *Salmonella* spp., *Listeria monocytogenes*, thermotolerant *Campylobacter* and *E. coli*, then tested with commercially available PCR based kits, respectively BAX® System PCR Assay for *Salmonella* spp., BAX® System PCR Assay for *Listeria monocytogenes*, BAX® System Real Time PCR Assay for *Campylobacter jejuni/coli/lari* (DuPont Nutrition & Health, Wilmington, DE, USA) and TaqMan®STEC Screening Assay ISO (Applied Biosystems™, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Positive results obtained with the above mentioned kits were then confirmed according to ISO detection methods for *Salmonella* spp. - ISO 6579:2002 Corr.1:2004 - (ISO, 2002), *Listeria monocytogenes* - ISO 11290-1:1996 Amd.1:2004 — (ISO, 1996),