Food Control 29 (2013) 226–230

Contents lists available at SciVerse ScienceDirect

Food Control

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

Microbiological quality of organic and conventional vegetables sold in Brazil☆

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ARTICLE INFO

Article history:
Received 1 February 2012
Received in revised form
31 May 2012
Accepted 5 June 2012

Keywords:
Vegetables
Food safety
Organic and conventional farming
Indicator microorganisms
Salmonella spp.

ABSTRACT

While searching for healthier diets, people became more attentive to organic produce. Yet, organic foods may be more susceptible to microbiological contamination because of the use of organic fertilizers, a possible source of pathogenic bacteria. In this study, 130 samples of different organic and conventional vegetable varieties sold in Brazil were analyzed for mesophilic aerobic bacteria, yeasts and molds, total coliforms, *Escherichia coli* and *Salmonella* spp. Most of the mesophilic aerobic bacteria counts in organic and conventional vegetables ranged from 6 to 7 log10 CFU/g; most of the yeasts and molds counts ranged from 5 to 6 log10 CFU/g and most of the total coliforms counts ranged from 4 to 5 log10 CFU/g. *E. coli* was found in 41.5% of the organic and 40.0% of the conventional vegetables, and most samples had counts ranging from 1 to 2 log10 CFU/g. *Salmonella* spp. was not found in any sample. Comparative analyses of the microbial counts of organic and conventional vegetables showed that some organic varieties have greater counts. However, the global results show that this is not a trend. These results indicate the need of good farming practices, and proper sanitization before consumption, to ensure food quality and safety.

1. Introduction

Vegetables are important dietary components because they provide essential nutrients, such as vitamins, minerals and fibers, and many health benefits. Regular consumption of vegetables is highly recommended since it reduces the risk of certain diseases, namely cardiovascular diseases, obesity, cancer, among others (Ignarro, Balestrieri, & Napoli, 2007; Liu, 2003).

Despite the health benefits, the risk of microbiological contamination on leafy greens is concerning. Many foodborne illness outbreaks in numerous countries have been associated with consumption of contaminated fresh vegetables (Beuchat, 2002; FAO/WHO, 2008). Hence, many consumers question the quality and safety of these foods.

During the last decades, alternative cropping systems have been developed because of society's increasing concern with sustainability and the safety and quality of conventional produce because conventional farming uses large amounts of chemical fertilizers. Organic farming has attracted the attention of the entire food production sector around the globe since it revives ecoagricultural principles that contemplate soil, water and air quality and horticulture, respecting the environment and social, economic and cultural relations (Bettiol, Ghini, Galvão, & Siloto, 2004; Gomiero, Pimentel, & Paoletti, 2011).

Regrettably, organic produce is more exposed to microbiological contamination than conventional produce, since organic fertilizers often consist of manure, and manure may harbor pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Johannessen et al., 2004; McMahon & Wilson, 2001). Of all organic foods, vegetables stand out as important sources of foodborne illness.

Despite the various studies in the literature assessing the microbiological quality of vegetables produced in Brazil (Nascimento et al., 2005; Oliveira, Souza, Bergamini, & Martinis, 2011; Simoes et al., 2001; Takayanagui et al., 2006) and other countries (Aycicek, Oguz, & Karci, 2006; Oliveira et al., 2010; Quiroz-Santiago et al., 2009; Rincon, Ginestre, Romero, Castellano, & Avila, 2010), the number of studies comparing the microbiological quality of organic and conventional produce is low.

The objective of this study was to compare the microbiological quality of different organic and conventional vegetable varieties sold in Brazil.

☆ Individual contributions for this study: (i) conception of the study: M. P. L. M Catanozi and N. F. A. Silveira, (ii) collection, microbiological analysis, statistical analysis, interpretation of data and writing of the text: D. F. Maffei and M. P. L. M. Catanozi.

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http://dx.doi.org/10.1016/j.foodcont.2012.06.013

Table 1
Variety and number of organic and conventional vegetables analyzed by the present study.

Samples	Conventional (n)[a]	Organic (n)[a]
Looseleaf lettuce	10	10
Butterhead lettuce	10	10
Romaine lettuce	10	10
Red looseleaf lettuce	10	10
Chicory	7	7
Catalogna	6	6
Collard greens	6	6
Arugula	6	6
Total	65	65

[a] Number of samples analyzed.

2. Materials and methods

2.1. Sampling

A total of 130 vegetable samples, 65 organic (certified by competent national authority) and 65 conventional, were purchased in a farmers' market in the city of Araraquara, São Paulo, located in the southeast region of Brazil. The samples included: lettuces (*Lactuca sativa* L.) of the following varieties: looseleaf, butterhead, Romaine and red looseleaf; chicory and catalogna (*Cichorium intybus* L.); collard greens (*Brassica oleracea* L.) and arugula (*Eruca sativa* L.) (Table 1). These represent the main leafy greens sold in the region. All samples were taken to the laboratory in sterile plastic bags and kept under refrigeration until tested.

2.2. Microbiological analysis

Fifty grams of each sample were placed inside a sterile plastic bag with 450 mL of 0.1% buffered peptone water (BPW) (Difco, France), and gently hand-rubbed for 1 min.

Mesophilic aerobic bacteria and yeasts and molds were enumerated using traditional methods (Beuchat & Cousin, 2001; Morton, 2001) and total coliforms and *E. coli* were enumerated using the fast PetrifilmTM method (Kornacki & Johnson, 2001), and the results were expressed as colony-forming units per gram (CFU/g). To isolate *Salmonella* spp., the rinsate was used as pre-enrichment and the methods were carried out according to ISO 6579:2002 (2007), with the results expressed as presence or absence of *Salmonella* spp.

2.3. Statistical analysis

Results expressed as CFU/g were converted to decimal logs and treated by the Student's *t*-test and Mann–Whitney test, depending on variable distribution, to determine whether the levels of contamination of conventional and organic vegetables differed significantly ($p \leq 0.05$). The software Sigma Stat version 3.11 (Systat Software Inc., USA) was used for the statistical treatments.

3. Results and discussion

The microbiological quality of organic and conventional vegetables was determined by analysis of *Salmonella* spp. and enumeration of the following microorganisms: mesophilic aerobic bacteria, yeasts and molds, total coliforms and *E. coli*.

This study did not find *Salmonella* spp. in any of the samples, which is in agreement with the Brazilian legislation (Brasil, 2001, pp. 45–53), since the presence of this pathogen in foods is unacceptable because of its serious health hazard. Nascimento et al. (2005) and Machado et al. (2006) did not find *Salmonella* in fresh vegetables produced in Brazil either. However, this pathogen has been found in vegetables produced in some countries, namely Mexico (Quiroz-Santiago et al., 2009), Turkey (Aytac, Ben, Cengiz, & Taban, 2010), Canada (Arthur, Jones, Fabri, & Odumeruz, 2007) and Brazil (Simoes et al., 2001; Takayanagui et al., 2006).

Table 2 shows the counts of the following microorganisms: mesophilic aerobic bacteria, yeasts and molds, total coliforms and *E. coli* in samples of organic and conventional vegetables. For mesophilic aerobic bacteria, the results varied from 5 to >7 log10 CFU/g for organic vegetables and 3 to >7 log10 CFU/g for conventional vegetables. Most samples had a count ranging from 6 to 7 log10 CFU/g. Generally, mesophilic aerobic counts are useful for indicating the shelf-life duration and microbial quality of foods (Pianetti et al., 2008). Since these vegetables are farmed on soil and exposed to all kinds of environmental conditions, they reflect the conditions in which they were farmed, and their counts can be as high as 7 log10 CFU/g (Brackett & Splittstoesser, 1992).

Yeasts and molds were present in smaller counts than mesophilic aerobic bacteria, ranging from 4 to >7 log10 CFU/g in organic and conventional vegetables. The counts of most samples varied from 5 to 6 log10 CFU/g. Oliveira et al. (2010) obtained similar results for samples of vegetables produced in Spain, with yeasts and molds present in smaller counts than bacteria. Although this microbial group is associated with food spoilage, high counts may also be a health hazard because of the mycotoxins produced by molds. The diseases caused by mycotoxins vary greatly, including carcinogen and immunosuppressive effects, among others (Kovács, 2004).

Total coliforms counts varied from 2 to 7 log10 CFU/g for organic vegetables and 1 to 7 log10 CFU/g for conventional vegetables. Most samples had counts ranging from 4 to 5 log10 CFU/g. These microorganisms are widely distributed in nature and commonly found in raw vegetables; hence, they are not associated with fecal contamination (Brackett & Splittsesser, 1992; Doyle & Erickson, 2006).

Table 2
Prevalence of microbial counts in organic and conventional vegetables.

Count interval[a]	Mesophilic aerobic bacteria (%)		Yeasts and molds (%)		Total coliforms (%)		*Escherichia coli* (%)	
	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic
10^1–10^2	0.0	0.0	0.0	0.0	6.2	0.0	35.4	33.8
10^2–10^3	0.0	0.0	0.0	0.0	26.1	24.6	4.6	3.1
10^3–10^4	1.5	0.0	0.0	0.0	26.1	29.3	0.0	4.6
10^4–10^5	1.5	0.0	15.3	20.0	30.8	40.0	0.0	0.0
10^5–10^6	23.1	7.7	50.8	41.5	7.7	4.6	0.0	0.0
10^6–10^7	41.5	55.4	27.7	37.0	3.1	1.5	0.0	0.0
$>10^7$	32.4	36.9	6.2	1.5	0.0	0.0	0.0	0.0

[a] Range in CFU/g.

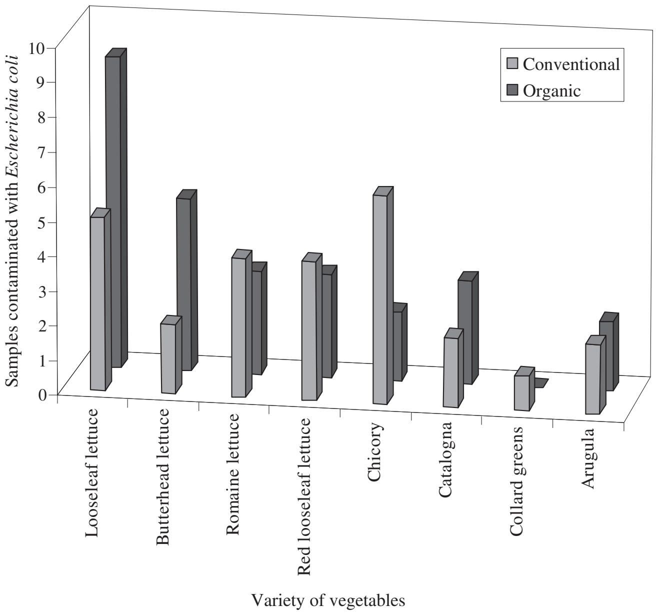

Fig. 1. Presence of *Escherichia coli* in organic and conventional vegetables according to cropping systems.

E. coli is the best indicator of fecal contamination because they are found exclusively in the digestive tract of men and animals and is frequently used for monitoring the sanitary quality of foods (Doyle & Erickson, 2006; Jay, Loessner, & Golden, 2005, p. 790). Furthermore, some strains are pathogenic to men, such as *E. coli* O157:H7, which has been associated with many foodborne illness outbreaks (Cieslik & Bartoszcze, 2011; Delaquis, Bach, & Dinu, 2007).

In this study, *E. coli* was found in 41.5% of organic and 40.0% of conventional vegetables, and most samples had counts ranging from 1 to 2 log10 CFU/g (Table 2). The vegetable with the highest incidence of *E. coli* was organic looseleaf lettuce: of the 10 studied samples, 9 were positive. On the other hand, collard greens presented the lowest incidence of contamination, with *E. coli* being found in only one conventional sample (Fig. 1). Mukherjee, Speh, Dyck, and Diez-Gonzales (2004) and Oliveira et al. (2010) found similar results, with a greater prevalence of *E. coli* in samples of organic than conventional lettuce. The high incidence of *E. coli* in samples of looseleaf lettuce is reason for concern, since this is one of the most consumed leafy greens (Agrianual, 2010, p. 520), exposing the population to contamination.

The mean microbial counts of the different organic and conventional vegetable varieties were compared to verify whether they differed significantly ($p \leq 0.05$) (Table 3). Organic samples of looseleaf lettuce and chicory presented higher mesophilic aerobic bacteria counts than conventional samples ($p = 0.01$ and 0.02, respectively); organic arugula also had higher yeasts and molds counts than conventional arugula ($p = 0.01$) and organic collard greens had higher total coliform counts than conventional collard greens ($p = 0.005$).

The mean counts of each microbial group were also compared, regardless of vegetable variety, considering only the cropping system, that is, organic *versus* conventional (Fig. 2). All mean counts were similar, except those of mesophilic aerobic bacteria, which were significantly higher in the organic varieties ($p = 0.04$). *Outliers* were not excluded from the analysis because they are part of the sampling and may stem from variations in vegetable microbiota. These variations are determined by time of harvest, environmental conditions and handling.

Although the only significant difference observed in this study was the higher microbial count of organic vegetables compared with conventional vegetables, this fact does not indicate a trend, since this was not observed for all microbial groups and varieties of vegetables analyzed. Additionally, many other studies would be necessary to confirm this observation. Moreover, other variables, such as environmental and handling conditions, should also be considered, since they may impact the microbial profile of organic and conventional vegetables.

Vegetable microbiota is very diverse and vegetable quality and safety depend on many factors, including soil, fertilizer, irrigation water, presence of animals on the field and good practices during handling and merchandizing. Additionally, leaf shapes can further contribute to the differences in the contamination levels of different vegetable varieties. Hence, it is difficult to control contamination with pathogenic and food-spoilage microorganisms (Beuchat, 2002; Oliveira et al., 2011).

The composting process, characteristic of organic farming, is an excellent alternative to manage organic wastes and provides several benefits to plants when applied to the soil. However, can also affect microbial proliferation when done incorrectly (inadequate composting duration and temperature) (Suárez-Estrella, Vargas-García, Elorrieta, López, & Moreno, 2003). This is one of the factors that may explain the high microbial counts found in organic vegetables when compared with conventional vegetables, for some varieties of vegetables analyzed in this study.

In conclusion, the results of this study show that organic and conventional vegetables sold in the city of Araraquara, SP, Brazil, do not contain *Salmonella* spp. but contain considerable levels of the others microorganisms analyzed, indicating the need of hygiene practices during farming, transport and merchandizing of vegetables, as well as of making the population aware of the importance of

Table 3
Microbial counts of mesophilic aerobic bacteria, yeasts and molds, total coliforms and *Escherichia coli* in different varieties of organic and conventional vegetables.

Samples	Mesophilic aerobic bacteria		Yeasts and molds		Total coliforms		*Escherichia coli*	
	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic
Looseleaf lettuce	6.50 ± 0.59*	7.14 ± 0.26*	5.88 ± 0.32	5.55 ± 0.60	4.69 ± 0.89	4.62 ± 0.92	1.38 ± 0.61	1.93 ± 0.98
Butterhead lettuce	6.07 ± 0.93	6.73 ± 0.59	5.33 ± 0.38	5.22 ± 0.69	3.11 ± 1.48	3.37 ± 0.79	1.30 ± 0.00	1.59 ± 0.93
Romaine lettuce	6.50 ± 0.73	6.81 ± 0.34	5.24 ± 0.47	5.41 ± 0.50	3.23 ± 1.06	3.50 ± 1.00	1.58 ± 0.44	1.48 ± 0.00
Red looseleaf lettuce	6.66 ± 0.75	6.69 ± 0.65	6.61 ± 0.73	6.31 ± 0.61	4.04 ± 0.78	3.18 ± 0.73	1.23 ± 0.29	1.16 ± 0.28
Chicory	6.57 ± 0.59*	7.19 ± 0.22*	6.03 ± 0.41	6.10 ± 0.79	4.28 ± 0.50	4.64 ± 0.32	1.49 ± 0.32	1.45 ± 0.21
Catalogna	7.48 ± 0.46	6.67 ± 0.90	6.01 ± 0.78	6.02 ± 0.46	5.05 ± 0.97	4.69 ± 0.56	1.50 ± 0.71	1.49 ± 0.85
Collard greens	6.24 ± 0.35	6.33 ± 0.57	5.48 ± 0.22	5.43 ± 0.61	2.94 ± 0.51*	4.22 ± 0.35*	1.48 ± 0.00	<1.00 ± 0.00
Arugula	6.25 ± 1.27	6.85 ± 0.88	5.09 ± 2.02*	6.36 ± 0.17*	2.93 ± 0.73	2.99 ± 0.98	1.30 ± 0.43	2.09 ± 0.55

Results expressed as mean ± SD (log10 CFU/g).
Statistical differences between the groups were determined by the Mann–Whitney test ($p \leq 0.05$)
*Significant differences.

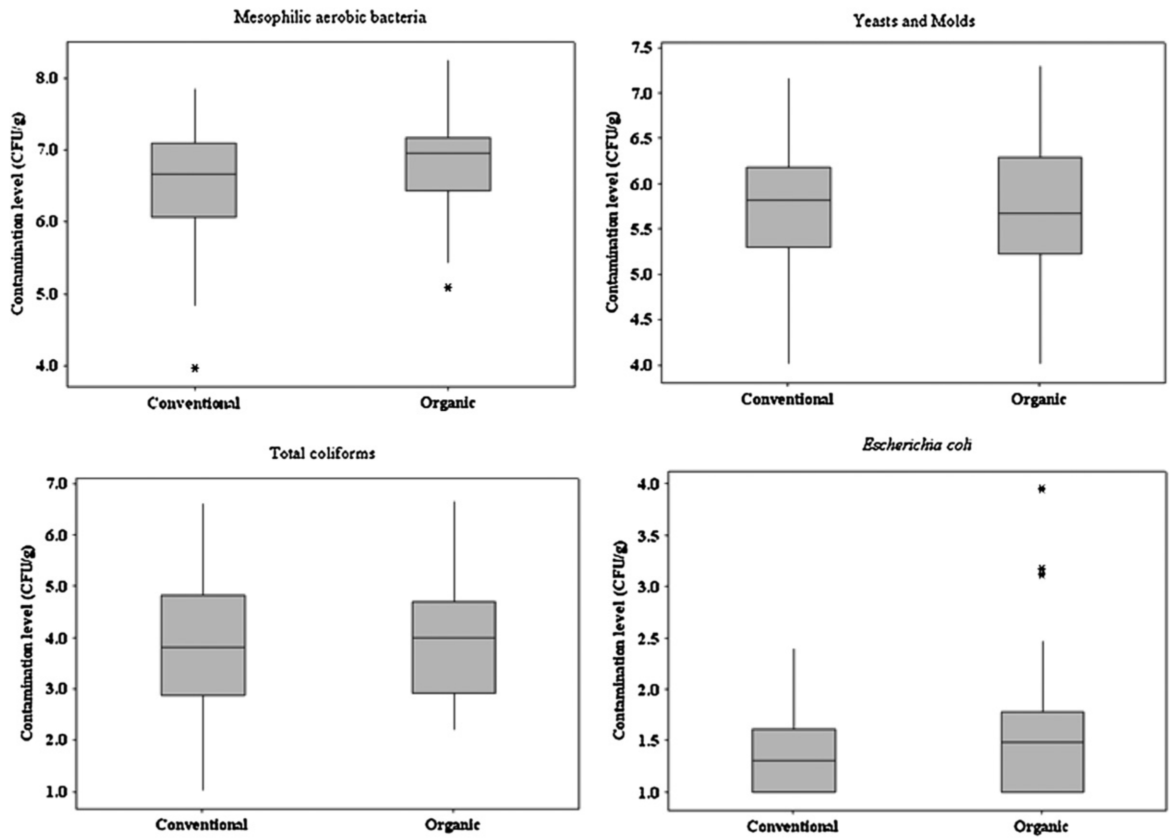

Fig. 2. Data distribution *boxplots* according to microbial groups in organic and conventional vegetables. *outliers. The Student's *t*-test was used to compare the differences between yeasts and molds counts in organic and conventional vegetables. The Mann–Whitney test was used for all other microbial groups ($p \leq 0.05$).

vegetable sanitization before consumption to minimize risk of contamination and foodborne diseases.

Acknowledgments

The authors thank Programa de Apoio ao Desenvolvimento Científico (PADC/UNESP) (Project 2010/20-I) for financial support and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master's degree grant given to the first author.

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