



Differences in microbiological quality of leafy green vegetables

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Abstract

Objective. The aim of the study was to determine the presence and concentration of indicator microorganisms on leafy green vegetables available in Poland.

Material and methods. Microbial analyses of 122 samples of leafy green vegetables: rocket, lamb's lettuce, iceberg lettuce, chive, spinach, celery, dill and parsley, sold in Polish supermarkets in 2018 and 2019, were conducted. The vegetables were analyzed for aerobic mesophilic bacteria, yeasts and moulds, *Enterobacteriaceae*, *Enterococcus*, coliforms and *Escherichia coli* number according to Polish standards.

Results. The most microbiologically contaminated vegetables occurred to be parsley, rocket and spinach, and the least contaminated was iceberg lettuce. The presence of total mesophilic bacteria was found in all samples. The highest average number of mesophilic bacteria was found for lamb's lettuce, rocket, parsley and spinach. Numerous yeasts were presented in almost all leafy green samples while rocket and parsley were the most contaminated with moulds. *Enterobacteriaceae* were isolated from all samples of parsley. High populations of these bacteria were found in rocket and celery samples. Of the 122 vegetable samples, 95 (78%) were positive for coliforms, especially all samples of parsley were inhabited by these bacteria. 24% of all samples were contaminated by *Enterococcus*, mainly parsley, spinach and chive. The presence of *E. coli* was found in only one sample of spinach.

Conclusions. The levels of aerobic mesophilic bacteria, yeasts and moulds, *Enterobacteriaceae*, *Enterococcus* and coliforms in leafy green vegetables sold in Poland could be classified as moderate or low. The abundant presence of the studied microorganisms may pose a risk for some categories of people consuming leafy green vegetables, mostly immunocompromised persons.

Key words

bacteria, fungi, prevalence, vegetables, microbiological contamination

INTRODUCTION AND OBJECTIVE

Vegetables are considered as important components of a healthy and balanced diet, and recognized as a main source of nutrients, vitamins and fibre. Thus, their consumption, especially when fresh, is encouraged in many countries by governmental health agencies, to prevent many of civilisation's diseases. Consumption of fresh produce has increased significantly (by 30%) in the past few decades [1].

However, vegetables, which have been considered a tool for healthier living, have also been frequently linked to outbreaks of foodborne diseases [2]. The available research evidence indicates, that the burden of foodborne illness due to contaminated produce has increased in recent decades. In the United States, from 1996–2010, 23% of outbreaks of total foodborne illness were produce related [3]. In Europe, from 2007–2011, produce was linked with 10% of outbreaks, 35% of hospitalizations and 46% of deaths [4, 5]. Vegetables, especially leafy greens, have been mostly implicated in these outbreaks [6]. These incidents have caused growers, shippers, fresh-cut produce processors, distributors, retailers, importers, and government public health officials to re-evaluate the risk of contracting foodborne illness from the consumption of fresh

fruits and vegetables, and re-evaluate current production and handling practices. As a consequence of the situation, more and more consumers pay greater attention to the quality and safety of these foods sold in markets.

The risk of microbiological contamination of vegetables, especially of leafy greens, e.g. lettuce, wild rocket, chive, spinach, is concerning. Many studies show that raw, leafy vegetables are considerably contaminated microbiologically [7, 8, 9, 10, 11, 12]. This may create a health risk for consumers due to the possibility of occurrence of harmful microorganisms. The routine procedure for assessment of the microbiological contamination of vegetables includes determination of the levels of total mesophilic aerobic bacteria and Gram-negative bacteria of the *Enterobacteriaceae* family, with special relevance to faecal coliform bacteria as general indicators of pollution. *E. coli*, *Enterobacteriaceae* and coliforms are frequently used indicators for the assessment of the quality of fresh vegetables and hygienic conditions present in their production and handling environments [12, 13]. Enterococci are commonly found in the digestive tract of humans and animals, but they also enter into the environment and are easily colonised due to their high adaptability. The prevalence of *Enterococcus* spp. in food, e.g. leafy greens, could be a threat to human health, especially for immune-compromised persons [14].

Usually the outbreaks are associated with the contamination of vegetables with human pathogens, such as

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Salmonella, *Listeria monocytogenes*, pathogenic *Escherichia coli*, *Shigella* or *Yersinia enterocolitica* [15, 16, 17, 18, 19, 20, 21, 22]. Leafy green vegetables can be also contaminated by pathogenic organisms the mycelium of which grows over the surface of a leaf. The hazard of fungi is linked to the production of mycotoxins, which are particularly dangerous for human health and bioaerosols [23, 24]. The most important mould toxins are aflatoxin, ochratoxin A, zearalenone, trichothecenes and fumonisins. Mycotoxins are characterized by multidirectional action: mutagenic, neurotoxin, immunosuppressive and carcinogenic. In this respect, the most relevant threats to human are filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Cladosporium* [25, 26]. Among identified genera on leafy greens the most common are *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Botrytis*, *Colletotrichum* [27, 28, 29].

Overall, microbial hazards are significant for producers and consumers of fresh leafy green vegetables. Therefore, ways to reduce sources of contamination and deeper understanding of pathogen survival and growth on fresh produce are required to reduce the risk to human health and the associated economic consequences. It should be emphasized that the effect of foodborne diseases affects not only the sick person but also has considerable economic repercussions. There are costs related to the medical care of the sick person and absence from work, as well as the expenses incurred in research into the outbreak, the loss of income due to the closure of food companies, legal expenses for mitigation related to diseases, and expenses incurred by public medical services.

Contamination of vegetable by microorganisms can occur anywhere, from the farm to the table, including contamination of seed stocks and crops during cultivation, harvesting, postharvest handling, storage, processing, transport and retail distribution [3, 30, 31]. The following agents have been identified as potential vehicles for the contamination of vegetables: water and soil [32, 33, 34], animal manures [33, 35, 36], and people working with the produce [30, 33]. There are several processing steps in the fresh vegetable production chain, in each of which many points exist for potential microbial contamination. Thus, processing (cleaning, washing, cutting, packaging) and storage conditions lead to enhanced decay and loss of quality of fresh vegetables [31, 37].

Because of the difficulties in reducing microbiological contamination in fresh produce, the use of high quality

raw materials and efficient temperature control during manufacture, distribution and retailing are key factors for maintaining the microbiological quality and safety of minimally processed and packed vegetables [10]. However the quality of raw materials and the maintenance of the cold chain are difficult to be implemented and controlled. In fact, knowledge about the potential risk caused by the presence of potentially pathogenic bacteria and fungi on the surface of vegetables sold in Poland is insufficient. Accordingly, the aim of the present study was to determine the presence and concentration of indicator microorganisms: total mesophilic bacteria, yeast and moulds, *Enterobacteriaceae*, *Enterococcus*, coliforms and *Escherichia coli* in leafy greens, sold on the retail market in Poland.

MATERIALS AND METHOD

Origin of samples. Plant material for the study was collected in 2018–2019. A total of 122 vegetable samples representing the main leafy greens sold in Poland were purchased from chain supermarkets. The leafy greens included: rocket (*Diplotaxis tenuifolia*) – 17 samples, lamb's lettuce (*Valerianella locusta* L.) – 17 samples, iceberg lettuce (*Lactuca sativa* L.) – 14 samples, chive (*Allium cepa* L.) – 15 samples, spinach (*Spinacia oleracea* L.) – 15 samples, celery (*Apium graveolens* var. *dulce* Mill.) – 17 samples, dill (*Anethum graveolens*) – 14 samples and parsley (*Petroselinum crispum*) – 13 samples. All samples were taken in triplicate. The countries of origin of the samples are presented in Table 1. The vegetables were bought and transported in original packagings, mostly foil. All vegetables had a valid consumption date. In addition, attention was paid that samples of the same vegetables taken for one analysis and bought on a single day, should come from different producers or distributors. The samples were taken to a laboratory and kept at 4 °C until analyzed, but for no longer than 24 h.

Microbiological analyses. One sample of each vegetable was analyzed in three replications. Microbial analyses were conducted according to described Polish standard methodologies (Tab. 2). Twenty-five grams of plant material were transferred into 225 ml of peptone water in 400 ml sterile stomacher filter bags. The samples were homogenized in a stomacher BagMixer® 400 P with fixed speed 8 stroke/s for 10 min. Further decimal dilutions were prepared with the same diluent and inoculated onto the media to

Table 1. Origin and number of collected fresh leafy green samples

Country of origin	Number of samples from different countries								Total
	Rocket	Lamb's lettuce	Iceberg lettuce	Chive	Spinach	Celery	Dill	Parsley	
Poland	8	0	7	13	4	2	10	9	53
Italy	9	12	0	1	7	0	1	1	31
Spain	0	0	7	0	4	13	2	2	28
France	0	3	0	0	0	0	0	0	3
Unknown EU country	0	2	0	0	0	0	0	0	2
Iraq	0	0	0	0	0	0	0	1	1
India	0	0	0	1	0	0	0	0	1
No information	0	0	0	0	0	2	1	0	3
Total	17	17	14	15	15	17	14	13	122

Table 2. 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 moulds	PN-ISO 7954 General guidance for enumeration of yeasts and moulds – colony count technique at 25°C.
<i>Enterobacteriaceae</i>	PN-ISO 21258-2 Horizontal method for the detection and enumeration of <i>Enterobacteriaceae</i> – Part 2: colony-count method.
Coliforms	PN-ISO 4832 Horizontal method for the enumeration of coliforms – colony-count technique
<i>Escherichia coli</i>	PN-ISO 16649-2 Horizontal method for the enumeration of β -glucuronidase-positive <i>Escherichia coli</i>
<i>Enterococcus</i>	PN-EN 15788: 2009E Method for the detection and enumeration of <i>Enterococcus</i> spp.

enumerate: aerobic mesophilic bacteria, yeasts and moulds, *Enterobacteriaceae*, *Enterococcus*, coliforms and *Escherichia coli*. Mesophilic aerobic bacteria were cultured on plate count agar (PCA), yeasts and moulds on yeast extract glucose chloramphenicol agar (YGC agar), *Enterobacteriaceae* on violet red bile glucose agar (VRBG agar), coliforms on violet red bile lactose (VRBL agar), *Enterococcus* on Slanetza medium 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. Representative colonies of filamentous fungi were identified under Olympus BX 41 microscope on the basis of morphological characteristics of the mycelium.

Statistical analysis. Microbial counts were analyzed in log scale (log₁₀ cfu g⁻¹). The results were statistically analyzed using one-way analysis of variance with the Tukey test ($\alpha = 0.05$), using the statistical programme Statistica 13.1. Data not significantly different from each other were marked with the same letters.

RESULTS

The data presented provide evidence that leafy green vegetable sold in Polish markets are numerously inhabited by natural microorganisms, but are also contaminated by microorganisms which may adversely affect the consumers' health and safety (Tab. 3 and 4).

Table 3. Number (log₁₀ cfu g⁻¹) of microorganisms (mesophilic bacteria, moulds, yeasts) isolated from fresh green leafy produce obtained at retail in Poland

Produce type	Mean±SD (range)	Mediana	Mean±SD (range)	Mediana	Mean±SD (range)	Mediana
	Total mesophilic bacteria		Moulds		Yeasts	
Parsley	7.44 ± 0.48 bcd (6.26-8.00)	7.56	4.61 ± 0.30 bc (4.24-5.01)	4.58	5.63 ± 0.51 bc (4.87-6.63)	5.53
Rocket	7.48 ± 0.8 cd (5.47-8.39)	7.68	4.99 ± 0.81 c (3.52-5.95)	5.40	5.62 ± 0.92 bc (3.45-6.81)	5.83
Spinach	7.25 ± 0.59 bcd (5.66-7.71)	7.43	3.38 ± 1.98 abc (0.00-5.04)	4.12	5.55 ± 0.85 bc (4.03-6.68)	5.56
Chive	6.62 ± 1.70 abc (1.83-8.19)	6.9	2.89 ± 1.83 ab (0.00-4.67)	3.11	4.46 ± 2.04 ab (0.00-6.90)	5.18
Celery	7.07 ± 0.46 bcd (5.99-7.87)	7.07	2.66 ± 1.51 a (0.00-4.44)	2.77	4.94 ± 0.64 bc (3.51-5.80)	5.13
Dill	6.37 ± 0.69 ab (5.24-7.68)	6.25	3.34 ± 1.20 abc (0.00-4.41)	3.90	4.86 ± 0.50 bc (3.63-5.64)	4.93
Lamb's lettuce	7.73 ± 0.33 d (7.37-8.26)	7.66	2.27 ± 1.77 a (0.00-4.97)	1.83	5.94 ± 0.52 c (4.66-6.74)	6.09
Iceberg lettuce	5.55 ± 1.53 a (2.08-7.16)	6.03	1.67 ± 1.40 a (0.00-3.67)	1.83	3.41 ± 1.89 a (0.00-5.59)	3.96

Similar letters above the means in the same column indicate significant difference at $p < 0.05$ by ANOVA and Tukey test

Table 4. Number (log₁₀ cfu g⁻¹) of microorganisms (coliforms, *Enterobacteriaceae*, *Enterococcus*) isolated from fresh leafy produce obtained at retail in Poland

Produce type	Mean±SD (range)	Mediana	Mean±SD (range)	Mediana	Mean±SD (range)	Mediana
	Coliforms		<i>Enterobacteriaceae</i>		<i>Enterococcus</i>	
Parsley	3.68 ± 0.57 c (2.85-4.87)	3.75	3.89 ± 0.74 b (2.48-4.97)	4.08	0.88 ± 1.17 a (0.00-2.78)	0
Rocket	2.69 ± 1.55 bc (0.00-4.16)	3.09	3.42 ± 0.92 b (0.00-5.43)	3.88	0.39 ± 0.92 a (0.00-2.71)	0
Spinach	3.42 ± 1.09 c (0.00-4.50)	3.56	3.41 ± 1.50 b (0.00-5.35)	4.00	0.78 ± 1.11 a (0.00-2.62)	0
Chive	3.13 ± 1.35 c (0.00-4.50)	3.54	3.04 ± 1.51 ab (0.00-4.18)	3.68	0.33 ± 0.61 a (0.00-1.99)	0
Celery	2.50 ± 1.00 bc (0.00-3.85)	2.74	2.65 ± 1.07 ab (0.00-3.84)	2.83	0.44 ± 0.83 a (0.00-2.32)	0
Dill	2.23 ± 1.60 bc (0.00-3.80)	2.63	2.42 ± 2.02 ab (0.00-4.90)	3.38	0.28 ± 0.62 a (0.00-1.96)	0
Lamb's lettuce	0.48 ± 1.00 a (0.00-2.82)	0.00	2.35 ± 1.37 ab (0.00-3.92)	3.01	0.36 ± 0.87 a (0.00-2.99)	0
Iceberg lettuce	1.44 ± 1.40 ab (0.00-4.03)	1.23	1.49 ± 1.57 a (0.00-4.10)	0.99	0.11 ± 0.40 a (0.00-1.48)	0

Similar letters above the means in the same column indicate significant difference at $p < 0.05$ by ANOVA and Tukey test

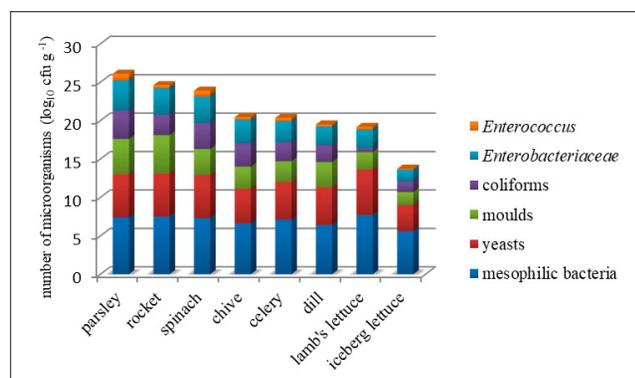


Figure 1. Number of microorganisms isolated from fresh green leafy produce

The results showed that the highest microbial load was indicated by parsley, rocket and spinach, while iceberg lettuce had the lowest number of microorganisms (Fig. 1). The mesophilic bacteria was the most dominated microbial group found in all 122 tested vegetable samples. Their number ranged from 1.83 for chive to 8.39 \log_{10} cfu g⁻¹ for rocket. The highest average number of mesophilic bacteria was found for lamb's lettuce, followed by rocket, parsley and spinach, values – 7.73, 7.48, 7.44 and 7.25 \log_{10} cfu g⁻¹, respectively (Tab. 3). The data were statistically different. The lowest density of bacterial population was found in iceberg lettuce where the mean value was 5.55 \log_{10} cfu g⁻¹ and the median value – 6.03 \log_{10} cfu g⁻¹. However, in the case of iceberg lettuce and chive, large variations between samples were observed, the values ranged from 2.08–7.16 \log_{10} cfu g⁻¹ for iceberg lettuce, and from 1.83–8.19 \log_{10} cfu g⁻¹ for chive.

The second dominant group of microorganisms in the studied vegetables were yeasts and moulds. Nearly all samples were found to contain yeasts, with the exception of two iceberg lettuce samples and one chive sample (Tab. 5). The

highest average number of yeasts was recorded for lamb's lettuce, parsley and rocket – 5.94; 5.63 and 5.62 \log_{10} cfu g⁻¹, respectively (Tab. 3). The highest medians were also reported for these vegetables – 6.09, 5.53 and 5.83 \log_{10} cfu g⁻¹, respectively. The lowest, statistically relevant mean number and the lowest median value of yeasts, were recorded for iceberg lettuce – 3.41 and 3.96 \log_{10} cfu g⁻¹. It is worth noting that yeasts predominated over moulds in all kind of studied vegetables.

Moulds were detected in 106 (87%) samples of the vegetables tested (Tab. 5), with the highest recorded median for isolated colonies – 5.40 \log_{10} cfu g⁻¹ for rocket (Tab. 3). Among the tested samples of vegetables, rocket and parsley proved to be the most contaminated with moulds (Tab. 3). Filamentous fungi were present in all samples of these plants (Tab. 5). The average number of moulds was 4.99 and 4.61 \log_{10} cfu g⁻¹ for rocket and parsley, maximum values – 5.95 and 5.01 \log_{10} cfu g⁻¹, respectively (Tab. 3). Iceberg lettuce, lamb's lettuce and celery proved to be the least affected by moulds, ranging from 0–4.97 \log_{10} cfu g⁻¹. The number of moulds expressed as the mean values were 1.67 \log_{10} cfu g⁻¹ for iceberg lettuce, 2.27 \log_{10} cfu g⁻¹ for lamb's lettuce and 2.66 \log_{10} cfu g⁻¹ for celery. The data were statistically different compared to rocket and parsley samples.

Microscopic observations allowed to distinguish the following types of moulds inhabiting examined leafy greens: *Cladosporium*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Alternaria*, *Gliocladium*, *Botrytis*, *Fusarium*, *Mucor*, *Pythium*, *Trichoderma* (Tab. 6). The most encountered fungal types were *Cladosporium*, *Penicillium*, and *Aspergillus*. Less frequently were isolated *Rhizopus* and *Alternaria*. The other listed fungi were isolated occasionally. Parsley and rocket were outstanding not only for the high amount of the fungi, but also for their diversity, compared to other leafy greens (Tab. 6), followed by spinach. In contrast, the smallest

Table 5. Rate of detection in percent (%) of microorganism groups by produce type and number of positive samples/number of all examined samples

Produce type	Total mesophilic bacteria	Moulds	Yeasts	Coliforms	Enterobacteriaceae	Enterococcus
Parsley	100 (13/13)	100 (13/13)	100 (13/13)	100 (13/13)	100 (13/13)	39 (5/13)
Rocket	100 (17/17)	100 (17/17)	100 (17/17)	82 (14/17)	94 (16/17)	18 (3/17)
Spinach	100 (15/15)	80 (12/15)	100 (15/15)	93 (14/15)	93 (14/15)	33 (5/14)
Chive	100 (15/15)	80 (12/15)	93 (14/15)	93 (14/15)	87 (13/15)	33 (5/15)
Celery	100 (17/17)	88 (15/17)	100 (17/17)	94 (16/17)	94 (16/17)	24 (4/17)
Dill	100 (14/14)	93 (13/14)	100 (14/14)	71 (10/14)	64 (9/14)	21 (3/14)
Lamb's lettuce	100 (17/17)	82 (14/17)	100 (17/17)	24 (4/17)	82 (14/17)	18 (3/17)
Iceberg lettuce	100 (14/14)	71 (10/14)	86 (12/14)	71 (10/14)	57 (8/14)	7 (1/14)
Average	100 (122/122)	87 (106/122)	98 (119/122)	78 (95/122)	84 (103/122)	24 (29/122)

Table 6. Fungus genera isolated from fresh leafy vegetables obtained at retail in Poland

Produce type	Identified filamentous fungi
Parsley	<i>Cladosporium</i> > <i>Aspergillus</i> > <i>Penicillium</i> > <i>Alternaria</i> > <i>Botrytis</i> > <i>Pythium</i> > <i>Fusarium</i> > <i>Rhizopus</i>
Rocket	<i>Cladosporium</i> > <i>Penicillium</i> > <i>Aspergillus</i> > <i>Pythium</i> > <i>Gliocladium</i> > <i>Botrytis</i> > <i>Mucor</i> > <i>Fusarium</i> > <i>Rhizopus</i> > <i>Trichoderma</i>
Spinach	<i>Cladosporium</i> > <i>Rhizopus</i> > <i>Gliocladium</i> > <i>Penicillium</i> > <i>Aspergillus</i> > <i>Botrytis</i>
Chive	<i>Cladosporium</i> > <i>Penicillium</i> > <i>Aspergillus</i> > <i>Rhizopus</i>
Celery	<i>Rhizopus</i> > <i>Cladosporium</i> > <i>Penicillium</i> > <i>Botrytis</i>
Dill	<i>Penicillium</i> > <i>Rhizopus</i> > <i>Cladosporium</i> > <i>Mucor</i> > <i>Gliocladium</i>
Lamb's lettuce	<i>Cladosporium</i> > <i>Aspergillus</i> > <i>Penicillium</i>
Iceberg lettuce	<i>Penicillium</i> > <i>Cladosporium</i> > <i>Aspergillus</i> > <i>Alternaria</i>

number and variability was observed among fungi from iceberg lettuce, which was mostly colonized by *Penicillium* and *Cladosporium*. In the presented study, the number of these microorganisms for parsley, rocket and spinach was higher and ranged from 5.5–5.9 \log_{10} cfu g^{-1} , but for the other leafy greens ranged from 4.5–5 \log_{10} cfu g^{-1} . It is also significant, that moulds comprised a large proportion of this group of microorganisms – yeasts and moulds (Fig. 1).

It was also found that parsley, spinach and rocket harboured the highest number of coliforms, *Enterobacteriaceae* and *Enterococcus*, followed by chive which indicated a similar load of coliforms and *Enterobacteriaceae*. Of the 122 samples, 95 (78%) were positive for total coliforms (Tab. 5) which were detected in all parsley samples, mean number and median value – 3.68 and 3.75 \log_{10} cfu g^{-1} , respectively. Coliforms were also detected at a high number in spinach and chive – mean value 3.42 and 3.13 \log_{10} cfu g^{-1} , respectively (Tab. 4). The least coliform contaminated vegetable was lamb's lettuce. Only 24% of samples were inhabited by these bacteria and the mean number of colonies was 0.48 \log_{10} cfu g^{-1} (Tab. 4 and 5). The number of coliforms in lamb's lettuce was statistically different compared to other vegetables tested. Iceberg lettuce samples also had a low contamination by these microorganisms, mean and median value – 1.44 and 1.23 \log_{10} cfu g^{-1} .

A high contamination level of studied vegetables was also observed for the *Enterobacteriaceae* family. Of the 122 samples, 103 (84%) were positive for these bacteria (Tab. 5). They were isolated from all samples of parsley and the average amount of population was 3.89 \log_{10} cfu g^{-1} (Tab. 4). High populations were also found in rocket and spinach, 94 and 93% of the samples were inhabited by these bacteria at the level of 3.44 and 3.41 \log_{10} cfu g^{-1} , respectively (Tab. 4 and 5). In the case of celery, the number of *Enterobacteriaceae* exceeded 3.00 \log_{10} cfu g^{-1} in seven samples (data not presented), mean and median values – 2.65 and 2.83 \log_{10} cfu g^{-1} (Tab. 4). Chive, dill and lamb's lettuce were less inhabited by *Enterobacteriaceae*, and significantly the lowest load was found for iceberg lettuce (Tab. 4).

The other bacterial group, considered as a food sanitary indicator, is *Enterococcus*. In the current study, 29 samples (24%) were positive for the presence of these bacteria (Tab. 5), mean number was low – 0.11–0.88 \log_{10} cfu g^{-1} (Tab. 4). The least contaminated vegetable by *Enterococcus* was iceberg lettuce, where only one sample contained these bacteria and the population size was 1.48 \log_{10} cfu g^{-1} . The highest load of *Enterococcus* was indicated for parsley (0.88 \log_{10} cfu g^{-1}) and spinach (0.78 \log_{10} cfu g^{-1}). The other leafy greens were less contaminated by these bacteria, and in all cases the differences were not statistically significant (Tab. 4).

As regards the presence of *E. coli* in the studied samples of leafy greens, these bacteria were found in only one sample of spinach, population size – 2.36 \log_{10} cfu g^{-1} .

DISCUSSION

Tests conducted on 122 samples of leafy green vegetables (rocket, lamb's lettuce, iceberg lettuce, chive, spinach, celery, dill and parsley), purchased in Polish supermarkets indicate that these vegetables are contaminated to varying degrees by mesophilic bacteria, yeasts, moulds, *Enterobacteriaceae*, *Enterococcus* and coliforms.

Mesophilic bacteria were present in all tested samples with mean values ranging from 5.55–7.73 \log_{10} cfu g^{-1} for iceberg lettuce and lamb's lettuce. Badosa et al. [38] found that in examined fresh leafy vegetables, aerobic bacteria counts were mostly in the range from 7–9 \log_{10} cfu g^{-1} . Korir et al. [39] also obtained similar results in the study of total aerobic bacteria on the surface of spinach and lettuce. They found average counts of these bacteria at 8.02 and 7.76 \log_{10} cfu g^{-1} for spinach and lettuce, respectively. They also found that most of the tested samples of these vegetables contained >5 \log_{10} cfu g^{-1} total aerobic bacteria. In the studies by Kłapeć et al. [40], the number of mesophilic bacteria in fresh lettuce was 5.27 \log_{10} cfu g^{-1} and in dill – 4.64 \log_{10} cfu g^{-1} . Wójcik-Stopczyńska et al. [41] observed that the population of mesophilic bacteria in spinach, immediately after harvesting, ranged from 4.7–5.2 \log_{10} cfu g^{-1} . In the present study, the mesophilic bacterial count in spinach was higher – mean value 7.25 \log_{10} cfu g^{-1} , but it should be noted that the spinach samples were not fresh. It is obvious that the bacterial counts may increase as the product is stored. It is therefore very important to ensure the highest possible microbiological purity of the product intended for short-term storage.

The high number of the mesophilic bacteria in the analyzed produce may be due to the fact that the leafy vegetables have large surface areas for microbial attachment [7, 42]. Moreover, technological processes, such as rinsing and packaging, are often ineffective in reducing contamination, and may undergo cross-contamination [10, 41]. Badosa et al. [38] observed that products which were processed (washing, trimming, etc.) before packaging showed higher bacterial counts than non-processed vegetables. This results could be explained by the fact that processing delivers also nutrients that provide an opportunity for subsequent bacterial multiplication.

Yeasts and moulds contaminated almost all tested products (Tab. 5). Yeasts were detected in 119 samples, while moulds were detected in 106. Merlini et al. [43] reported that the population of yeasts and moulds in leafy vegetables (lettuce, iceberg lettuce, chicory, parsley) was about 4 log cfu g^{-1} . In the presented study, the average mean of yeasts ranged from 3.41 log cfu g^{-1} for iceberg lettuce and 5.94 log cfu g^{-1} for lamb's lettuce, while the average mean of moulds ranged from 1.67 log cfu g^{-1} iceberg lettuce and 4.99 log cfu g^{-1} for rocket. Marinelli et al. [27] found that fresh rocket was contaminated by moulds at the range of <1.0–7.18 \log_{10} cfu g^{-1} and by yeasts at range 6.70–8.36 \log_{10} cfu g^{-1} . They also observed an increase for yeasts and moulds during the shelf-life of fresh vegetables. In the current study, yeasts predominated over moulds which was in agreement with the findings of Caponigro et al. [44].

As regards the identified species of filamentous fungi inhabiting the tested vegetables, the results obtained in the current study are confirmed by other studies which report that the most common fungi contaminated vegetables purchased in supermarkets are *Penicillium*, *Cladosporium* and *Aspergillus* [24, 28, 29]. Although fungi are a natural part of plant epiphytic microflora, an excessive load may be harmful to both the product and health of the consumer [45]. Many of these fungi, e.g. genera *Aspergillus*, *Penicillium*, *Alternaria* or *Fusarium* produce mycotoxins, cause chronic intoxication of the human body [23, 46]. Locally formed mycotoxins may migrate into the plant tissue, and structural damages promote their diffusion [23]. The mean number of both yeasts and moulds indicated for organic or conventionally grown vegetables was about 5 \log_{10} cfu g^{-1} [12]. The presence

of filamentous fungi on the vegetable surface may create a risk for mycotoxin contamination or bioaerosol formation in working places and market environments [24], especially for fresh produce of rocket and spinach, prepared and consumed in larger quantities than parsley.

Coliforms were another group of microorganisms studied. They were detected in 95 (78%) samples of the vegetables tested, including all parsley samples and almost all spinach, chive, and celery samples. Similarly, Bartz et al. [47] conducted analyses of fresh vegetables and found that coliforms were present in over 93% of all samples. The results obtained in the current study on contamination of parsley leaves by coliforms, show a quite high contamination of this vegetable compared to the literature data. The study conducted by Korir et al. [39] in USA, shows that out of 414 samples of vegetables, such as parsley, spinach, lettuce and basil, only 38.65% of samples were positive for coliforms. Moreover, mean and median values for these bacteria in these vegetables were less than the limit of detection (\log_{10} cfu g^{-1}). However, the maximum content for coliforms observed among the fresh produce analyzed, ranged from 3.00 for lettuce to 4.88 \log_{10} cfu g^{-1} for parsley [39], which is similar to the findings in the presented study (4.03 for iceberg lettuce and 4.87 \log_{10} cfu g^{-1} for parsley). A low contamination of leafy vegetables by coliforms (1.08–1.96 \log_{10} cfu g^{-1}) was also reported by Merlini et al. [43]. Similarly, in the study conducted by Kłapeć et al. [40], the median concentrations of faecal coliforms were 1.0 \log_{10} cfu g^{-1} in fresh vegetable obtained immediately after harvest, and 0.00 in vegetables sold at retail market.

Enterobacteriaceae bacteria were isolated from 103 (84%) samples of the tested vegetable samples. The lowest population was in the case of iceberg lettuce, mean value – 1.49 \log_{10} cfu g^{-1} , while the highest population was enumerated in parsley samples, mean value – 3.89 \log_{10} cfu g^{-1} . On the contrary, Kłapeć et al. [40] reported that the concentration of *Enterobacteriaceae* in lettuce sold in retail ranged from 3.82–4.17 \log_{10} cfu g^{-1} , and for dill – 3.98–4.20 \log_{10} cfu g^{-1} . Ssemamanda et al. [11] observed that mean *Enterobacteriaceae* counts ranged from 5.6–6.9 \log_{10} cfu g^{-1} for fresh vegetables purchased at the market. The data were higher than those obtained from Polish products.

With regard to *Enterococcus*, these results show a lower contamination of vegetables bought on the Polish market, in contrast to the data presented by Quansah et al. [48], who studied vegetables sold in Ghana. They observed that the mean *Enterococcus* count ranged from 3.87–4.86 \log_{10} cfu g^{-1} , depending on type of vegetables. In the presented study, the mean number of *Enterococcus* ranged from 0.11 \log_{10} cfu g^{-1} for iceberg lettuce to 0.88 \log_{10} cfu g^{-1} for parsley. The data are promising because bacteria of the genus *Enterococcus* are ubiquitous microorganisms, but have a predominant habitat in the gastrointestinal tract of humans and animals. Their appearance on the surface of vegetables may be indicative of the faecal contamination of produce.

The presence of *E. coli* has been found in only one spinach sample among the 122 studied vegetable samples. *E. coli* is one of the most common indicators of food contamination, and its acceptable hygienic limit is 100 cfu g^{-1} [49, 50]. The obtained result indicates the good sanitary condition of the examined produce. In vegetables, various researchers have reported differing prevalences for *E. coli* pathogen. Szczech et al. [12] found that the average number of *E. coli* isolated from organic vegetables was significantly higher (0.42 \log_{10} cfu g^{-1}) than

for conventionally cultivated vegetables (0.05 \log_{10} cfu g^{-1}), as well as the incidence of serious contamination. Microbial risk differs from one locality and season to another, and is also greatly influenced by level of implementation of GAPs on vegetable farms. For example, *E. coli* was reported at 6.1% (6/99) in Rwanda [34], 10.65% (44/414) in USA [39] and 0% (0/36) in Brazil [51] of samples of examined vegetables.

In summary, it has been found that the least microbiologically contaminated vegetable turned out to be iceberg lettuce, which was found to have a significantly lower average number load of mesophilic bacteria, moulds, yeasts and coliforms. Only 7% of the studied iceberg lettuce samples contained *Enterococcus* and 57% – *Enterobacteriaceae* bacteria. On the contrary, the most microbiological contaminated vegetable species proved to be parsley, rocket and spinach, which were inhabited by a high population of bacteria and moulds which create a potential risk for consumer health, and may markedly influence the sensory and commercial quality of the product.

This study provided valuable information on the microbiological quality of fresh produce leafy greens sold in Poland. The results of this study can be used for further studies in the area to support the development of risk management policies in Poland.

CONCLUSIONS

In conclusion, the results of this study show that leafy green vegetables sold in Poland are contaminated by different numbers of mesophilic bacteria, yeasts, moulds, *Enterobacteriaceae*, *Enterococcus* and coliforms.

Considering the increasing interest of consuming these vegetables, it is strongly recommended in order to assess the human health risk. Given the lack of information about potential microbial contamination of these vegetables in Poland, this is required in order to establish further specific methods for guaranteeing the safety of consuming leafy vegetables. Particular attention should be paid to the maintenance of appropriate sanitary conditions during the cultivation, harvesting, transport and storage of these vegetables. The abundant presence of the studied microorganisms may pose a risk for some categories of people consuming leafy green vegetables, mostly immunocompromised persons.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the research reported in this paper.

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