ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 23(3), 3-13
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2024.5351

ORIGINAL PAPER

Received: 12.02.2024
Accepted: 29.04.2024
Issue published: 28.06.2024

ASSESSMENT OF THE MICROBIAL QUALITY OF READY-TO-EAT VEGETABLE SALADS AND BERRY FRUIT AVAILABLE ON POLISH MARKET

Beata Kowalska ✉, Magdalena Szczech

Department of Microbiology and Rhizosphere, The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

ABSTRACT

The consumption and sale of ready-to-eat products, i.e., fresh-cut vegetable salads and fruit, have been growing tremendously in the present time. Therefore, the microbial safety of such products is of great concern. In the current study, a survey of general microbiological contamination (mesophilic bacteria, yeasts, molds, *Enterobacteriaceae*, coli bacteria, *Enterococcus*, *Lactobacillus*, and *Escherichia coli*) of fresh-cut vegetable salads and berry-packed fruits available in Polish supermarket was undertaken.

In ready-to-eat salads, the high number of mesophilic bacteria ranged from 6.43 to 8.56 $\log_{10}$ cfu g^{-1}, and also *Enterobacteriaceae* with mean value from 3.45 to 4.08 $\log_{10}$ cfu g^{-1} was detected. Mesophilic bacteria, yeast, and *Enterobacteriaceae* were detected in all salad samples. The molds were detected in 45% of salad samples, and their mean number ranged from 0.63 $\log_{10}$ cfu g^{-1} in salads with carrot or beetroot to 1.80 $\log_{10}$ cfu g^{-1} in salads with rocket.

Berry fruit was also heavily microbiologically contaminated. In particular, the means of mesophilic bacteria, molds, and yeasts were high. The number of molds detected in fruit samples, especially in raspberry samples, could be alarming. *Enterobacteriaceae*, *Enterococcus*, and coli bacteria were detected in a few samples of berry fruit. None of the ready-to-eat salads and fruit samples were contaminated by *Escherichia coli*.

Based on the tests that were conducted, it was found that the microbiological quality was not satisfactory. The findings suggest that following hygienic measures during processing and handling, the microbiological quality of vegetable salads and berry fruit available in Polish markets should be improved.

Keywords: food safety, microbiological safety, microbiological contamination, bacteria, fungi, mold, ready-to-eat

INTRODUCTION

Consumers perceive fresh fruit and vegetables as healthful and nutritious because of the numerous scientifically substantiated and documented health benefits. The demand for ready-to-eat (RTE) products, including salads and fruit packed in small portions, is significantly increasing. The products are easy to consume; they do not need any preparatory work before consumption. Therefore, they are also called "convenience food", which has become an increasingly common alternative to traditionally prepared meals. The consumption of these products increases, especially during winter when fresh products are less available. The fruit and vegetables are often imported from other areas, affecting the products' quality and durability and increasing the risk of cross-contamination.

Recent outbreaks of foodborne diseases worldwide have been increasingly connected epidemiologically to the consumption of fresh fruit and vegetables [Caponigro et al. 2010, Mir et al. 2018, Arienzo et al. 2020, Kłapeć et al. 2022, EFSA 2023, Thomas et al.

✉ beata.kowalska@inhort.pl

2024]. These cases have caused growers, fresh produce processors, distributors, retailers, importers, and government public health officials to take note of the considerable risk of contracting foodborne diseases from consumption of fresh fruit and vegetables and to evaluate current production and practices [Machado-Moreira et al. 2019].

Vegetable salads and fruit are eaten raw, without being cooked, and often without being washed and peeled, increasing the risk of food poisoning [Kumar et al. 2022, Łepecka et al. 2022]. There are various sources of microbiological contamination of RTE products. Microorganisms can contaminate vegetables and fruit by growing, harvesting, and transporting them during production. Therefore, healthy food quality depends on the conditions and ways of obtaining raw materials, pre-treatment, storage, transport, and marketing practices. Commercially, vegetables used to prepare RTE salads are washed before use. However, it should be highlighted that washing does not remove all microorganisms. Moreover, cutting leaves causes tissue juice leakage and can stimulate the multiplication of microorganisms in the product. Moreover, cross-contamination may occur during processing when equipment in contact with potentially contaminated products is not regularly sanitized and cleaned [Berthold-Pluta et al. 2017, Alegbeleye et al. 2018, Mir et al. 2018, Alegbeleye et al. 2022, Finger et al. 2023].

Fruit and vegetables can become contaminated with spoilage and pathogenic microorganisms at several points from the field through the time they are consumed. Given sufficient time at the right temperature, microorganisms can grow on products to populations more than 10^7 colony-forming units per gram of plant material (cfu g^{-1}), causing an increased risk of human infections. These microorganisms may pose a risk due to the activity of undesired pathogens, the producers of toxins, bacteria-carrying antibiotic resistance genes, and potential spoilage activity [Szczech et al. 2018, Zhang et al. 2020, Kowalska and Szczech 2022, Kumar et al. 2022, Palumbo et al. 2022, Sobiczewski and Iakimova 2022, Murali et al. 2023, Thomas et al. 2024].

Fruits containing high sugars and other nutrients and high water activity are an ideal matrix for microbial growth. Their low pH makes them particularly susceptible to fungal spoilage due to a big part of the bacterial competition being eliminated since most bacteria prefer near-neutral pH. Soft fruits, in particular, are very delicate and susceptible to microbial contamination [Ortiz-Solá et al. 2020]. The fungi, e.g., *Botrytis* sp., *Alternaria* sp., *Cladosporium* sp., and *Penicillium* sp., grow very fast on fruit tissues [Williamson et al. 2007, Kłapeć et al. 2022, Bi et al. 2023]. Moreover, the shelf life of soft fruit is limited to a few days postharvest. Fruit loses its firmness, and its susceptibility to rot is very high if not appropriately preserved. Individual spoilt fruit in packed containers is not uncommon when shopping in supermarkets, particularly for raspberries and strawberries. Shop staff must make a great effort to systematically check the condition of the fruit in the containers to ensure that the fruit is in a healthy condition [Tournas and Katsoudas 2005, Ortiz-Solá et al. 2020].

Due to numerous cases of food poisoning caused by microorganisms and toxins found in food, research and the control of food products are essential [Kumar et al. 2022, Łepecka et al. 2022]. There are legal regulations concerning food, defining microbiological criteria and limits for the occurrence of microorganisms in food. However, these criteria apply to selected food groups and regulate the selected acceptable limits of microorganisms [Commission Regulation 2005, Commission Regulation 2007].

Limited information is available about the incidence and survival of microorganisms in fresh, minimally processed vegetable salads and berry fruit sold in Polish markets. Therefore, the study aimed to assess the microbiological quality of ready-to-eat leafy vegetable salads and packed blueberries, raspberries, and strawberries available in Polish supermarkets.

MATERIAL AND METHODS

Sampling and sample preparation. The research was conducted for three years, in 2021–2023. Plant material for the study was collected from some supermarket chains operating on the city's territory in Skierniewice (Province Łódź, central Poland). The studied material comprised leafy vegetable salad (80 samples) and packed berry fruit (75 samples). The RTE vegetable salads were a mixture of several ingredients, mainly lettuces mixed with other leafy vegetables (spinach, rocket, Lamb's lettuce) and fresh-cut vegetables (carrot, beetroot). The mixes did not contain any sauces, herbs, or spices. The salads were packed in original plastic packing units (primarily bags), containing from 250 g to 500 g of the product. The products were collected before the expiration date according to the information on the label. To the laboratory, they were transported in thermal bags and kept in a refrigerator (4–8 °C) until use, but not longer than 12 hours. For each product designed for testing, three packs from the same batch were purchased simultaneously and used as three replications of the product. Because the compositions of the salads were different, they were divided into five groups: 1 – mixed lettuces (n = 24); 2 – lettuce with rocket (n = 20); 3 – lettuce with Lamb's lettuce (n = 14); 4 – lettuce with spinach (n = 8); 5 – lettuce with fresh cut carrot or beetroot (n = 14).

Fresh berries, blueberries, raspberries, and strawberries were tested. All the berries were initially packed in plastic containers with lids containing 125 g, 250 g, or 500 g of the fruits. The number of tested samples for each kind was 25. The fruit was bought in original plastic containers, immediately transported to the laboratory, and kept at 4 °C until analyzed, but for 12 h. All samples were taken in triplicate.

Microbiological analyses. Polish standard methodologies described in Table 1 were used to conduct microbiological analyses. Plant material (25 g) with peptone water (225 cm^{-3}) was transferred into sterile stomacher filter bags 400 cm^{-3}. The samples were homogenized in a stomacher BagMixer® 400P, Interscience, France (8 stroke/s, 10 min). The serial dilution method and inoculation of selective media were used to determine the abundance and enumeration of the following microorganisms: aerobic mesophilic bacteria (plate count agar – PCA), yeasts and molds (yeast extract glucose chloramphenicol agar – YGC), *Lactobacillus* (MRS agar according to DeMan, Rogosa and Sharpe), *Enterobacteriaceae* (violet red bile glucose agar – VRBG), *Enterococcus* (Slanetza medium), coliforms (violet red bile lactose agar – VRBL) and *Escherichia coli* (Chromacoult® TBX agar). All used media were purchased from the Merck company (Germany). The results were expressed as colony-forming units per gram of plant material (cfu g^{-1}). The data were transformed into logarithms for statistical analysis. Representative colonies of filamentous fungi were observed under an Olympus BX 41 microscope (Olympus Corporation, Japan) based on morphological characteristics of the mycelium (e.g., size, color, shape, presence or absence of septa, presence of structures for sexual reproduction). They were classified into genera according to the systematic key [Dugan 2006].

Statistical analyses. The number of selected microorganisms was analyzed in a log scale ($\log_{10}$ cfu g^{-1}). Different distributions characterized the dependent variables from the experiments that were conducted. The number of microorganisms was normally distributed (estimated Lilliefors test); hence, the classical linear model was used, and an analysis of variance was performed. The Tukey-Kramer procedure made comparisons of the means at $p = 0.05$. The number of contaminated samples in berry fruit was analyzed through a generalized linear model (GLM)

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 moulds	PN-ISO 7954 General guidance for enumeration of yeasts and moulds – 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*
Enterococcus	PN-EN 15788: 2009E Method for the detection and enumeration of *Enterococcus* spp.

with a logistic link function because they had a binomial distribution. Type I likelihood ratio statistics (LRT test) were used to test the global hypothesis. When the tested effect was significant, a Wald test at $p = 0.05$ was used to assess differences among species. Calculations and analyses were made using Statistica v. 13 (Dell Inc., 2016).

RESULTS

The data obtained from the microbiological analysis provided evidence that numerous natural microorganisms inhabited ready-to-eat vegetable salads and packed berry fruit, but they also contained microorganisms that may adversely affect the consumers' health and safety.

The results obtained in microbiological analyses of RTE salads were compared in groups depending on the composition: 1) mix lettuce, 2) salads with rocket, 3) salads with Lamb's lettuce, 4) salads with spinach, 5) salads with cut carrot or beetroot (Tables 2 and 3). The most dominant microbial groups were mesophilic bacteria and yeast. The bacteria number was the highest in salads with carrot or beetroot and ranged from 7.04 to 8.56 $\log_{10}$ cfu g^{-1}; the mean was 8.03 $\log_{10}$ cfu g^{-1} (Table 2). Mesophilic bacteria less contaminated the other salads but the means were also high (7.63–7.71 $\log_{10}$ cfu g^{-1}). The yeast number was also the highest

Table. 2. Microbiological contamination (mesophilic bacteria, molds, yeasts) of the tested vegetable salads.

Salad type	Total mesophilic bacteria ($\log_{10}$ cfu g^{-1})		Molds ($\log_{10}$ cfu g^{-1})		Yeasts ($\log_{10}$ cfu g^{-1})	
	mean ±SD (range)	median	mean ±SD (range)	median	mean ±SD (range)	median
Mixed lettuce	7.67 ±0.29a (7.21–8.24)	7.74	1.2 ±1.74a (0.00–5.26)	0.00	6.37 ±0.46a (1.20–4.66)	6.35
Rocket	7.71 ±0.35a (6.92–8.12)	7.85	1.80 ±1.98a (0.00–5.07)	1.16	6.20 ±0.66a (4.38–7.14)	6.26
Spinach	7.63 ±0.47a (6.67–8.03)	7.79	1.62 ±1.83a (0.00–4.41)	1.22	5.82 ±0.65a (4.80–6.62)	5.71
Lamb's lettuce	7.63 ±0.53a (6.43–8.34)	7.72	1.61 ±1.84a (0.00–4.96)	1.33	6.13 ±0.45a (0.83–4.31)	6.19
Lettuce with carrot or beetroot	8.03 ±0.36a (7.04–8.56)	8.12	0.63 ±1.21a (0.00–3.98)	0.00	6.50 ±0.71a (4.79–7.70)	6.39

Means followed by the same letter in the column do not differ significantly at $p = 0.05$ according to Tukey-Kramer test.

Table. 3. Microbiological contamination (*Enterobacteriaceae*, coli, and *Lactobacillus* bacteria) of the tested vegetable salads

Salad type	*Enterobacteriaceae* ($\log_{10}$ cfu g^{-1})		Coli bacteria ($\log_{10}$ cfu g^{-1})		*Lactobacillus* ($\log_{10}$ cfu g^{-1})	
	mean ±SD (range)	median	mean ±SD (range)	median	mean ±SD (range)	median
Mixed lettuce	3.78a ±0.91a (1.20–4.62)	4.08	3.41a ±1.11a (0.00–4.21)	3.82	2.71 ±1.12 ab (0.00–3.67)	3.20
Rocket	3.46a ±0.84a (1.08–5.04)	3.49	3.42a ±0.74a (1.86–4.94)	3.58	1.98 ±1.13 b (0.00–3.85)	2.29
Spinach	3.48a ±1.08a (0.95–4.54)	3.74	3.45a ±0.74a (2.28–4.37)	3.61	1.56 ±1.09 b (0.00–2.84)	1.71
Lamb's lettuce	3.21a ±1.10a (0.83–4.31)	3.45	2.98a ±1.31a (0.95–4.42)	3.52	2.80 ±0.84 ab (1.48–3.99)	2.78
Lettuce with carrot or beetroot	3.24a ±1.35a (0.00–4.49)	3.87	3.50a ±0.90a (0.83–4.26)	3.78	3.30 ±1.44 a (0.00–4.21)	3.97

Means followed by the same letter in the column do not differ significantly at $p = 0.05$ according to Tukey-Kramer test.

in carrot or beetroot salads – 6.50 log_{10} cfu g^{-1}, and yeasts were present in all studied samples. The number of mesophilic bacteria and yeast compared among different salads was not statistically significant.

Molds were detected in 45% of all salad samples (Table 4). The contamination of RTE salads with molds was low. The mold mean number ranged from 0.63 to 1.80 log_{10} cfu g^{-1}. These values indicate that the most contaminated with molds were salads with rocket, spinach, and Lamb's lettuce. Salads with rocket obtained the highest mean value – 1.80 log_{10} cfu g^{-1}. However, differences among the studied kinds of salads were not significant (Table 2).

Bacteria from the *Enterobacteriaceae* family were detected in all salad samples (Table 4). Mean values for these bacteria numbers were very close in all types of salads and ranged from 3.21 in Lamb's lettuce salads to 3.78 log_{10} cfu g^{-1} for mixed lettuce salads (Table 3). Similar results were obtained for coli bacteria, ranged from 2.98 in Lamb's lettuce salads to 3.50 log_{10} cfu g^{-1} for lettuce with carrot or beetroot. Salads had no significant differences, and the average number of coli bacteria was 3.35 log_{10} cfu g^{-1}. *Lactobacillus* bacteria were detected in 88% of salad samples (Table 4). Their number was significantly higher in salads with carrot or beetroot (the mean value 3.30 log_{10} cfu g^{-1}) than in rocket and spinach salads. The *Lactobacillus* range in the salads with carrot and beetroot was wide and estimated from 0.00 to 4.21 log_{10} cfu g^{-1} (Table 3).

The studied berry fruit samples also contained high microbiological contamination. The results obtained for the species of berry fruit were compared with those of other species. The overall microbiological contamination of tested blueberry, raspberry, and strawberry

Table. 4. 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	Molds	Yeasts	*Entero-bacteriaceae*	Coli bacteria	*Lactobacillus*
Mix lettuce	100 (24/24)	42 (10/24)	100 (24/24)	100 (24/24)	96 (23/24)	92 (22/24)
Rocket	100 (20/20)	50 (10/20)	100 (20/20)	100 (20/20)	100 (20/20)	80 (16/20)
Spinach	100 (8/8)	50 (4/8)	100 (8/8)	100 (8/8)	100 (8/8)	75 (6/8)
Lamb's lettuce	100 (14/14)	57 (8/14)	100 (14/14)	100 (14/14)	100 (14/14)	100 (14/14)
Lettuce with carrot or beetroot	100 (14/14)	29 (4/14)	100 (14/14)	100 (14/14)	100 (14/14)	86 (12/14)
All samples	100 (80/80)	45 (36/80)	100 (80/80)	100 (80/80)	99 (79/80)	88 (70/80)

Table 5. Microbiological contamination of the tested berry fruit by mesophilic bacteria, molds, and yeasts

Fruit type	Total mesophilic bacteria (log_{10} cfu g^{-1})		Molds (log_{10} cfu g^{-1})		Yeasts (log_{10} cfu g^{-1})	
	mean ± SD (range)	median	mean ±SD (range)	median	mean ±SD (range)	median
Blueberry	4.35 ±1.19a (1.16–6.55)	4.95	3.39 ±1.27b (0.00–5.60)	5.00	3.95 ±1.47a (0.00–5.80)	4.11
Raspberry	4.75 ±0.77a (2.54–6.25)	4.95	4.89 ±0.37a (4.03–5.41)	5.00	3.49 ±1.72a (0.00–5.84)	4.11
Strawberry	4.81 ±0.56a (3.00–5.72)	4.82	4.55 ±0.87a (1.16–5.40)	4.81	4.03 ±1.55a (0.00–5.69)	4.50

Means followed by the same letter in the column do not differ significantly at $p = 0.05$ according to Tukey-Kramer test.

Table 6. Microbiological contamination of the tested berry fruit by *Enterobacteriaceae*, coli, *Enterococcus*, and *Lactobacillus* bacteria.

Fruit type	*Enterobacteriaceae*		Coli bacteria		*Enterococcus*		*Lactobacillus*	
	average concentration in positive samples ±SD ($\log_{10}$ cfu g^{-1})	rate of detection [%] and number of contaminated samples	average concentration in positive samples ±SD ($\log_{10}$ cfu g^{-1})	rate of detection [%] and number of contaminated samples	average concentration in positive samples ±SD ($\log_{10}$ cfu g^{-1})	rate of detection [%] and number of contaminated samples	average concentration in positive samples ±SD ($\log_{10}$ cfu g^{-1})	rate of detection [%] and number of contaminated samples
Blueberry	1.44 ±1.16a	16 (4)b	1.16 ±0.90a	16 (4)a	2.90a	4 (1)a	3.50 ±1.03a	20 (5)a
Raspberry	1.25 ±0.76a	32 (8)ab	1.23 ±0.83a	20 (5)a	0.62a	4 (1)a	2.24 ±1.15a	44 (11)a
Strawberry	1.89 ±0.85a	48 (12)a	2.13 ±0.96a	24 (6)a	1.20a	8 (2)a	1.62 ±0.38a	28 (7)a

In the average concentration, means followed by the same letter in column do not differ significantly at $p = 0.05$ according to Tukey-Kramer test. In the rate of detection, means followed by the same letter in column do not differ significantly at $p = 0.05$ according to the Wald test.

samples is summarized in Tables 5 and 6. All samples contained mesophilic bacteria; their average concentration was 4.35, 4.75, and 4.81 $\log_{10}$ cfu g^{-1} for blueberry, raspberry, and strawberry, respectively. Molds contaminated all raspberry samples, and their mean concentration (4.89 $\log_{10}$ cfu g^{-1}) was higher than in blueberry (3.39 $\log_{10}$ cfu g^{-1}) and strawberry (4.55 $\log_{10}$ cfu g^{-1}) samples. Yeasts were detected in almost all samples, and their average concentration was 3.49, 3.95, and 4.03 $\log_{10}$ cfu g^{-1} for raspberry, blueberry, and strawberry, respectively. Bacteria from the *Enterobacteriaceae* family were present in 16%, 32%, and 48% of blueberry, raspberry, and strawberry samples, respectively (Table 6). The data significantly differed between blueberry and strawberry. Coli bacteria were detected in a few studied samples (Table 6). *Lactobacillus* bacteria were detected in 20%, 28%, and 44% of blueberry, strawberry, and raspberry samples, respectively. The highest number of these bacteria was obtained in blueberries at 3.50 $\log_{10}$ cfu g^{-1}, and the lowest was in strawberries at 1.62 $\log_{10}$ cfu g^{-1}; however, the differences were not significant (Table 6).

Escherichia coli was not detected in any ready-to-eat vegetable samples analyzed ($n = 80$) or fruit samples ($n = 75$). *Enterococcus* bacteria were detected only in 4 samples of fruit (Table 6) and not in any samples of salads.

Molds obtained from studied vegetable and fruit materials were subjected to microscopic observations. The following genera were dominated: *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium*, and *Phialophora*.

DISCUSSION

Food safety is a vital issue that affects public health around the world. Eating food contaminated with foodborne pathogens and spoilage bacteria or fungi can cause serious diseases [Łepecka et al. 2022, Finger et al. 2023]. Consumers view ready-to-eat products as being ready for direct consumption without additional processing. In addition, on the packaging, the producers of vegetable salads often declare that the product is "washed and safe to consume" or "washed and ready to eat". The information is misunderstood because the consumers believe the products are safe, and their alertness remains dormant. This means they often skip washing the products before consumption [Balali et al. 2020, Kowalska and Szczech 2022, Łepecka et al. 2022].

Our studies showed that microorganisms highly inhabit RTE products. In the case of fresh-cut vegetable salads, there are bacteria and yeasts, but molds and bacteria are the dominant groups for berry fruit. Apart from the hazard related to food poisoning, high microbiological contamination of cut RTE salads harms sensory qualities, like texture, odor, appearance, and taste [Balali et al. 2020]. It should be highlighted that consumers buy more fresh, unprocessed products such as fruit and vegetables. On the other hand, with people paying particular attention to food safety and quality, consumers are demanding that fresh food should have minor processing and most natural preservatives [Mendoza et al. 2022]. However, they should be aware of the risks associated with consuming such products.

In the studied RTE salads consisting of mixtures of lettuce with other leafy or fresh-cut root vegetables, mesophilic bacterial load was about 8 $\log_{10}$ cfu g^{-1} (Table 2) and *Enterobacteriaceae* plate counts up to 5.04 $\log_{10}$ cfu g^{-1} for the salad with rocket (Table 3). The high number of mesophilic and coli bacteria, and *Enterobacteriaceae* carries an increased risk of food poisoning. Moreover, the presence of coliforms in almost all examined samples indicated deficiencies in production hygiene. The results agree with those conducted by other researchers [Xylia et al. 2021, Uhlig et al. 2022], who also studied microbiological contamination of ready-to-eat salads throughout their shelf-life. In these studies, mesophilic bacteria ranged from 5.8 to 7.7 $\log_{10}$ cfu g^{-1} and *Enterobacteriaceae* – from 2.0 to 5.0 $\log_{10}$ cfu g^{-1}. The researchers suggest that the shelf-life time should be shorter than the present recommendations. On the other hand, because of differences between products, further studies are needed to define these products' correct shelf-life to ensure a safe product even at the end of the shelf-life period. In studies by Berthold-Pluta et al. [2017], total aerobic mesophilic bacteria in RTE lettuce mixed ranged from 5.6 to 7.6 $\log_{10}$ cfu g^{-1}. In the presented studies, the amount was higher. It ranged from 6.43 $\log_{10}$ cfu g^{-1} to 8.56 $\log_{10}$ cfu g^{-1} (Table 2). The count of mesophilic bacteria is an indicator of only the overall microbiological quality of a food product, and there are no binding standards for the microbiological quality of products of this type. Good hygiene practices must be introduced by producers in order to exclude bacterial contamination.

The contamination of berry fruit by mesophilic bacteria was also high, and average values were 4.35, 4.75, and 4.81 $\log_{10}$ cfu g^{-1} for blueberry, raspberry, and strawberry, respectively (Table 5). The lowest contamination was estimated for blueberries. Since these fruits have smooth, hard skin, they are probably impermeable to colonization by microorganisms. Moreover, the fruits with limited contact with the soil are harvested from branches at a certain distance above the ground. On the other hand, raspberries and strawberries are significantly thinner and more susceptible to injury and breakage of the epidermis with numerous indentations and fiber-like protuberances, where the various microorganisms can easily attach and invade the inner tissues of the fruit. Berry fruits are also more susceptible to damage during harvest and transport and are more vulnerable to microbial multiplication. Notably, most of the berry fruit samples tested in the present study were imported, which may explain the high colonization by mesophilic bacteria in these samples.

The microorganisms on plant products are primarily of natural origin. However, the high number of bacterial cells may indicate the contamination of a product and the degree of its deterioration. According to FAO/WHO [2003], leafy vegetables (spinach, lettuce, cabbage) and fresh herbs have a very high microbiological risk. Leafy vegetables, used in RTE fresh products, cannot be thermally treated, and deactivation of microorganisms is problematic. Therefore, hygienic cultivation of raw materials and cleaning of leaves are crucial stages in the production process. All stages of RTE salad production should be performed in highly hygienic conditions [Olaimat and Holley 2012, Xylia et al. 2019, Mendoza et al. 2022].

The European Union has no established microbiological criteria for ready-to-eat food; the only applicable regulation is the Commission Regulation [2007]. However, it does not include the food category, only its components. For fruit and vegetables, the limit is established for bacteria *E. coli* (1000 cfu g^{-1} of product), and pre-cut ready-to-eat fruit and vegetables are limited to *Salmonella* (absence in 25 g of product). In the microbiological criteria defined in Commission Regulation 2073/2005 [2005], *E. coli* is controlled in pre-cut vegetables. In our study, *E. coli* was not detected in any of the analyzed samples. However, in many other studies conducted in different countries the bacterium was detected in leafy vegetable salads also served as ready-to-eat products [Nousiainen et al.

2016, Toe et al. 2017, Mir et al. 2018, Luna-Guevara et al. 2019, Bhullar et al. 2021, Łepecka et al. 2022, Uhlig et al. 2022, Al-Musawi et al. 2023, Habib et al. 2023]. In the presented study, the products were not tested for *Salmonella*. However, it is worth noting that *Salmonella* bacteria can contaminate plant products and pose a risk to human health [Kowalska 2022].

Lactobacillus bacteria were isolated from 88% of salad samples. Their presence is a positive phenomenon because the bacteria can act as a biocontrol agent through the production of antimicrobial compounds, reactive oxygen species, and bacteriocins by excluding pathogens by pre-emptively colonizing plant tissues vulnerable to infection [Agriopoulou et al. 2020, Ibrahim et al. 2021, Alegbeleye et al. 2023].

As regards molds, their number in salads ranged from 0.63 for lettuce with carrot or beetroot to 1.80 for lettuce with rocket (Table 2) and in berry fruit – from 3.39 in blueberry to 4.89 $\log_{10}$ cfu g^{-1} for raspberry fruit (Table 5). As no widely accepted values exist for permissible concentrations of molds in vegetables, fruits, or both, the levels of the microorganisms found and assessed in this study were compared with the results obtained in other investigations. The data obtained by Kłapeć et al. [2022] for strawberries and raspberries were lower and amounted to 3.08 and 3.49 $\log_{10}$ cfu g^{-1}, respectively. Ortiz-Solá et al. [2020] obtained similar results, detecting fungal populations in strawberries ranging between 2.10 and 5.86 $\log_{10}$ cfu g^{-1}. The presence of molds in salads is a common phenomenon, and its range is vast [Arienzo et al. 2020, Younus et al. 2020, Kłapeć et al. 2021, Łepecka et al. 2022]. Therefore, our studies agree with those of other researchers regarding the identified fungi genera. According to Tournas and Katsoudas [2005] and Kłapeć et al. [2022], the most common fungi found in berry fruit are *Botrytis cinerea, Alternaria, Cladosporium*, *Penicillium*, *Fusarium* and *Rhizopus*. In the presented studies, the most common fungi found in berries were *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Penicillium*, *and Rhizopus*. Mainly, *B. cinerea* is a dangerous pathogen; it grows rapidly and consumes the fruit, producing an unsightly watery rot accompanied by an off odor. Devastating losses of berry fruit have been reported in the past [Hua et al. 2018]. Although the development of a pathogenic fungus often starts from one or two berries, it quickly spreads over the neighboring healthy ones and consumes the whole sample within 3–4 days. It is also crucial that packed soft fruit in supermarkets is not stored in refrigerators but on the shelf at room temperature. These conditions are very suitable for microorganism development. It is worth mentioning that molds in fruits and vegetable salads may cause chronic human diseases. The microorganisms produce mycotoxins (e.g., aflatoxins, ochratoxins, patulin, trichothecenes, fumonisins, zearalenone), secondary metabolites that can elicit severe systemic diseases, e.g., carcinomas, systems dysfunctions and immunosuppressed states [Kłapeć et al. 2022].

CONCLUSIONS

The obtained results suggest that the microbiological quality of the evaluated ready-to-eat salads and berry fruit was not satisfactory enough from a safety point of view. Bacterial and yeast contamination was observed in the salads, while in berry fruit molds and mesophilic bacteria dominated. The number of molds in raspberry fruits is alarming. The obtained data indicate that such products have a high risk of contamination with microorganisms. Notably, although the products appear edible according to visual inspection, they may contain microorganisms that cause product spoilage or human diseases.

Considering the limitation of the study, which was the lack of research on the presence of *Salmonella* sp. and *Listeria monocytogenes* in the studied products, future investigation should be expanded. Such data would provide valuable information and significantly interest researchers, producers, and consumers of ready-to-eat salads and berry fruits.

The research presented concludes that preventive measures in the fruit and vegetable production chain must be considered to avoid possible foodborne diseases related to the microbiological quality of the RTE salads and fruit. Implementing Good Agricultural Practices (GAP) during primary production and Global Handling Practices (GHP) during postharvest stages and processing seems to be an excellent solution to mitigate microbial contamination while producing RTE vegetables and fruit. Moreover, the application of Hazard Analysis Critical Control Point (HACCP) principles and adherence to the International Organization for Standardization (ISO) 22000 standard can ensure the quality and safety of these products [Raffo et al. 2022, Wajahat 2023, Thomas et al. 2024].

Given consumers' increasing demand for convenience food, the market share of pre-packaged fresh-cut leafy salad vegetables on supermarket shelves has increased lately. Considering the direct impact of microbiological contamination of vegetable salads and berry fruit on consumer safety, it is highly recommended that the cultural level and health awareness of producers of these products, workers at each stage of production, and, first of all, consumers be raised.

SOURCE OF FUNDING

This work was financed by the Polish Ministry of Education and Science, task ZM/2/2018 (2018–2023): "Development methods limiting the occurrence of microbiological contamination of ready-to-eat vegetables and fruit".

REFERENCES

Agriopoulou, S., Stamatelopoulou, E., Sachadyn-Król, M., Varzakas, T. (2020). Lactic acid bacteria as antibacterial agents to extend the shelf life of fresh and minimally processed fruits and vegetables: quality and safety aspects. Microorganisms 8(6), 952. https://doi.org/10.3390/microorganisms8060952

Alegbeleye, O., Alegbeleye, I., Oroyinka, M.O., Daramola, O.B., Ajibola, A.B., Alegbeleye, W.O., Adetunji, A.T., Afolabi, W.A., Oyedeji, O., Awe, A., Badmus, A., Oyeboade, J.T. (2023). Microbiological quality of ready to eat coleslaw marketed in Ibadan, Oyo-State, Nigeria. Int. J. Food Prop. 26(1), 666–682. https://doi.org/10.1080/10942912.2023.2173775

Alegbeleye, O.O., Odeyemi, O.A., Strateva, M., Stratev, D. (2022). Microbial spoilage of vegetables, fruits and cereals. Appl. Food Res. 2(1), 100122. https://doi.org/10.1016/j.afres.2022.100122

Alegbeleye, O.O., Singleton, I., Sant'Ana, A.S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. Food Microbiol. 73, 177–208. https://doi.org/10.1016/j.fm.2018.01.003

Al-Musawi, A.T., Abu-Almaaly, R.A., Kereem, H.S. (2023). Fecal coliform bacteria in vegetable salads prepared in Baghdad restaurants. J. Pure Appl. Microbiol., 17(2), 1214–1220. https://doi.org/10.22207/JPAM.17.2.51

Arienzo, A., Murgia, L., Fraudentali, I., Gallo, V., Angelini, R., Antonini, G. (2020). Microbial quality of ready-to-eat leafy green salads during shelf-life and home-refrigeration. Foods 9(10), 1421. https://doi.org/10.3390/foods9101421

Balali, G.I., Yar, D.D., Dela, V.G.A., Adjei-Kusi, P. (2020). Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. Intern. J. Microbiol. 2020, 3029295. https://doi.org/10.1155/2020/3029295

Berthold-Pluta, A., Garbowska, M., Stefańska, I., Pluta, A. (2017). Microbiological quality of selected ready-to-eat leaf vegetables, sprouts and non-pasteurized fresh fruit-vegetable juices including the presence of Cronobacter spp. Food Microbiol. 65, 221–230. https://doi.org/10.1016/j.fm.2017.03.005

Bhullar, M., Perry, B., Monge, A., Nabwiire, L., Shaw, A. (2021). *Escherichia coli* survival on strawberries and unpacked romaine lettuce washed using contaminated water. Foods 10(6), 1390. https://doi.org/10.3390/foods10061390

Bi, K., Liang, Y., Mengiste, T., Sharon, A. (2023). Killing softly: a roadmap of *Botrytis cinerea* pathogenicity. Trends Plant Sci. 28(2), 211–222. https://doi.org/10.1016/j.tplants.2022.08.024

Caponigro, V., Ventura, M., Chiancone, I., Amato, L., Parente, E., Piro, F. (2010). Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer. Food Microbiol. 27(8), 1071–1077. https://doi.org/10.1016/j.fm.2010.07.011

Commission Regulation (2005). Commission Regulation (EC) No 2073/2005 of November 2005 on Microbiological Criteria for Foodstuffs. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02005R2073-20140601&from=DA [date of access: 2.12.2021].

Commission Regulation (2007). Commission Regulation (EC) No 1441/2007 of 5 December 2007 Amending Regulation (EC) No 2073/2005 on Microbiological Criteria Foodstuffs. Available: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:EN:PDF [date of access: 2.12.2021].

Dugan, F.M. (2006). The identification of fungi. An illustrated introduction with keys, glossary, and guide to literature. APS Press, USA.

EFSA (European Food Safety Authority). 2023. Annual report of the Scientific Network on Microbiological Risk Assessment. EFSA supporting publication 2023:EN-8451. Pp. 11. https://doi.org/10.2903/sp.efsa.2023.EN-8451

Finger, J.A.F.F., Santos, I.M., Silva, G.A., Bernardino, M.C., Pinto, U.M., Maffei, D.F. (2023). Minimally processed vegetables in Brazil: an overview of marketing, processing, and microbiological aspects. Foods 12(11), 2259. https://doi.org/10.3390/foods12112259

FAO/WHO (2003). Code of hygienic practice for fresh fruits and vegetables. CXC 53-2003. Codex Aliment.

Habib, I., Al-Rifai, R.H., Mohamed, M.I., Ghazawi, A., Abdalla, A., Lakshmi, G., Agamy, N., Khan, M. (2023). Contamination level and phenotypic and genomic characterization of antimicrobial resistance in *Escherichia coli* isolated from fresh salad vegetables in the United Arab Emirates. Trop. Med. Infect. Dis. 8(6), 294. https://doi.org/10.3390/tropicalmed8060294

Hua, L., Yong, C., Zhanquan, Z., Boqiang, L., Guozheng, Q., Shiping, T. (2018). Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. Food Qual. Saf. 2(3), 111–119. https://doi.org/10.1093/fqsafe/fyy016

Ibrahim, S.A., Ayivi, R.D., Zimmerman, T., Siddiqui, S.A., Altemimi, A.B., Fidan, H., Esatbeyoglu, T., Bakhshayesh, R.V. (2021). Lactic acid bacteria as antimicrobial agents: food safety and microbial food spoilage prevention. Foods 10(12), 3131. https://doi.org/10.3390/foods10123131

Kłapeć, T., Wójcik-Fatla, A., Farian, E., Kowalczyk, K., Cholewa, G., Cholewa, A., Dutkiewicz, J. (2021). Levels of filamentous fungi and selected mycotoxins in leafy and fruit vegetables and analysis of their potential health risk for consumer. Ann. Agric. Environ. Med. 28(4), 585–594. https://doi.org/10.26444/aaem/143031

Kłapeć, T., Wójcik-Fatla, A., Farian, E., Kowalczyk, K., Cholewa, G., Cholewa, A., Dutkiewicz, J. (2022). Mycobiota and mycotoxins in various kinds of vegetables and fruits as potential health risk factors for consumer – summary of multiyear study. Ann. Agric. Environ. Med. 29(2), 316–320. https://doi.org/10.26444/aaem/150522

Kowalska, B., (2023). Fresh vegetables and fruit as a source of *Salmonella* bacteria. Ann. Agric. Environ. Med., 30(1), 9–14. https://doi.org/10.26444/aaem/156765

Kowalska, B., Szczech, M. (2022). Differences in microbiological quality of leafy green vegetables. Ann. Agric. Environ. Med., 29(2), 238–245. https://doi.org/10.26444/aaem/149963

Kumar, S., Yadav, M., Devi, A., Uniyal, M., Kumar, V., Sehrawat, N., Singh, R. (2022). Assessment of pathogenic microorganisms associated with vegetable salads. Asian J. Biol. Life Sci., 11(1), 1–7. https://doi.org/10.5530/ajbls.2022.11.1

Luna-Guevara, J.J., Arenas-Hernandez, M.M.P., Martinez de la Peňa, C., Silva, J. L., Luna-Guevara, M.L. (2019). The role of pathogenic *E. coli* in fresh vegetables: behavior, contamination factors, and preventive measures. Intern. J. Microbiol., 2894328. https://doi.org/10.1155/2019/2894328

Łepecka, A., Zielińska, D., Szymański, P., Buras, I., Kołożyn-Krajewska, D. (2022) Assessment of the microbial quality of ready-to-eat salads – are there any reasons for concern about public health? Int. J. Environ. Res. Public Health 19(3), 1582. https://doi.org/10.3390/ijerph19031582

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., Burgess, C.M.J. (2019). Microbial contamination of fresh produce: what, where, and how? Comp. Rev. Food Sci. Food Saf. 18(6), 1727–1750. https://doi.org/10.1111/1541-4337.12487

Mendoza, I.C., Luna, E.O., Pozo, M.D., Vásquez, M.V., Montoya, D.C., Moran, G.C., Romero, L.G., Yépez, X., Salazar, R., Romero-Peňa, M., León, J.C. (2022). Conventional and non-conventional disinfection methods to prevent microbial contamination in minimally processed fruits and vegetables. LWT 165, 113714. https://doi.org/10.1016/j.lwt.2022.113714

Mir, S.A., Shah, M.A., Mir, M.M., Dar, B.N., Greiner, R., Roohinejad, S. (2018). Microbial contamination of ready-to-eat vegetables salads in developing countries and potential solutions in the supply chain to control microbial pathogens. Food Control, 85, 235–244. https://doi.org//10.1016/j.foodcont.2017.10.006

Murali, A.P., Trząskowska, M., Trafialek, J. (2023). Microorganisms in organic food-issues to be addressed. Microorganisms, 11(6), 1557. https://doi.org/10.3390/microorganisms11061557

Nousiainen, L.L., Joutsen, S., Lunden, J., Hanninen, M.L., Fredriksson-Ahomaa, M. (2016). Bacterial quality and safety of packaged fresh leafy vegetables at the retail level in Finland. Int. J. Food Microbiol., 232, 73–79. https://doi.org/10.1016/j.ijfoodmicro.2016.05.020

Olaimat, A.N., Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. Food Microbiol., 32(1), 1–19. https://doi.org/10.1016/j.fm.2012.04.016

Ortiz-Solá, J., Viñas, I., Colás-Medá, P., Anguera, M., Abadias, M. (2020). Occurrence of selected viral and bacterial pathogens and microbiological quality of fresh and frozen strawberries sold in Spain. Int. J. Food Microbiol., 314, 108392. https://doi.org/10.1016/j.ijfoodmicro.2019.108392

Palumbo, M., Attolico, G., Capozzi, V., Cozzolino, R., Corvino, A., de Chiara, M.L.V., Pace, B., Pelosi, S., Ricci, I., Romaniello, R., Cefola, M. (2022). Emerging postharvest technologies to enhance the shelf-life of fruit and vegetables: an overview. Foods 11(23), 3925. https://doi.org/10.3390/foods11233925

Raffo, A., Paoletti, F. (2022). Fresh-cut vegetables processing: environmental sustainability and food safety issues in a comprehensive perspective. Front. Sustain. Food Syst., 5, 681459. https://doi.org/10.3389/fsufs.2021.681459

Sobiczewski, P., Iakimova, E.T. (2022). Plant and human pathogenic bacteria exchanging their primary host environments. J. Hortic. Res. 30(1), 11–30. https://doi.org/10.2478/johr-2022-0009

Szczech, M., Kowalska, B., Smolińska, U., Maciorowski, R., Oskiera, M., Michalska, A. (2018). Microbial quality of organic and conventional vegetables from Polish farms. Int. J. Food Microbiol., 286, 155–161. https://doi.org/10.1016/j.ijfoodmicro.2018.08.018

Toe, E., Dadie, A., Dako, E., Loukou, G. (2017). Bacteriological quality and risk factors for contamination of raw mixed vegetable salads served in collective catering in Abidjan (Ivory Coast). Adv. Microbiol., 7(6), 405–419. https://doi.org/10.4236/aim.2017.76033

Thomas, G.A., Gil, T.P., Müller, C.T., Rogers, H.J., Berger, C.N. (2024). From field to plate: how do bacterial enteric pathogens interact with ready-to-eat and vegetables, causing disease outbreaks? Food Microbiol., 117, 104389. https://doi.org/10.1016/j.fm.2023.104389

Tournas, V.H., Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. Int. J. Food Microbiol., 105(1), 11–17. https://doi.org/10.1016/j.ijfoodmicro.2005.05.002

Uhlig, E., Kjellström, A., Oscarsson, E., Nurminen, N., Nabila, Y., Paulsson, J., Lupan, T., Velpuri, N.S.B.P., Molin, G., Håkansson, Å. (2022). The live bacterial load and microbiota composition of prepacked "ready-to-eat" leafy greens during household conditions, with special reference to *E. coli*. Int. J. Food Microbiol., 377, 109786. https://doi.org/10.1016/j.ijfoodmicro.2022.109786

Wajahat, S.S. (2023). Emerging trends and advancements in the biopreservation of fruits. J. Hortic. Res., 31(1), 1–24. https://doi.org/10.2478/johr-2023-0006

Williamson, B., Tudzynski, B., Tudzynski, P., Van Kan, J.A.L. (2007). Botrytis cinerea: the cause of grey mould disease. Mol. Plant Pathol., 8(5), 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x

Xylia, P., Botsaris, G., Chrysargyris, A., Skandamis, P., Tzortzakis, N. (2019). Variation of microbial load and biochemical activity of ready-to-eat salads in Cyprus as affected by vegetables type, season, and producer. Food Microbiol., 83, 200–210. https://doi.org/10.1016/j.fm.2019.05.013

Xylia, P., Botsaris, G., Skandamis, P., Tzortzakis, N. (2021). Expiration date of ready-to-eat salads: effects on microbial load and biochemical attributes. Foods 10(5), 941. https://doi.org/10.3390/foods10050941

Younus, M.I., Sabuj, A.A.M., Haque, Z.F., Sayem, S.M., Majumder, S., Parvin, M.S. Islam, M.A., Saha, S. (2020). Microbial risk assessment of ready-to-eat mixed vegetable salads from different restaurants of Bangladesh Agricultural University campus. J. Adv. Vet. Anim. Res., 7(1), 34–41. https://doi.org/10.5455/javar.2020.g390

Zhang, H., Yamamoto, E., Murphy, J., Locas, A. (2020). Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. Int. J. Food Microbiol., 335, 108855. https://doi.org/10.1016/j.ijfoodmicro.2020.108855