Risk management in fruits and vegetables production and supply: Constructing a control chart for Enterobacteriaceae

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Abstract

In Sardinia (Italy), Enterobacteriaceae are commonly quantified in raw fruits and vegetables, but their guide value for official controls remains undefined. Enterobacteriaceae include environmental species that are often present at high levels in the production process of fresh produce. While not necessarily pathogenic to humans, Enterobacteriaceae are potentially pathogenic to immunocompromised individuals, and so establishing appropriate guide values is crucial. Lax values endanger food security, while overly restrictive ones pose unrealistic targets for food operators without safety benefits for consumers. The aim of this study was to demonstrate the utility of introducing a control chart for Enterobacteriaceae in HACCP (hazard analysis and critical control points) systems for risk management within fresh produce production and supply processes and promote its widespread adoption to define acceptable contamination levels based on empirical data. We developed a control chart for Enterobacteriaceae to assess the acceptability of the supply process and food safety of iceberg salad in a canteen. Using the 2023 data, we set warning and action levels that helped food business operators to (i) understand, control, and improve the supply processes; (ii) promptly detect anomalies in these processes and act accordingly; and (iii) verify the effectiveness of any actions taken. The large-scale use of this approach in HACCP systems could aid defining realistic and safe levels for Enterobacteria in fresh produce production and supply.

Keywords: Enterobacteriaceae; X-mR control chart; food control chart; HACCP; process control systems; fresh produce

Introduction

Fresh vegetables and fruits are considered fundamental components of a healthy and balanced diet by the World Health Organization (WHO) (World Health Organisation, 2004; World Health Organization, 2020). WHO recommends a daily consumption of at least 400 g of fruits and vegetables to fulfill the requirements of various micronutrients and prevent several diseases (World Health Organization, 2003, 2019, 2020). Adequate consumption of fruits and vegetables contributes, together with other factors, to reducing the risk of cardiovascular disease, cancer, and all-cause mortality (Aune *et al*., 2017; Hartley *et al*., 2013). Fruits and vegetables are a very important source of dietary fiber, vitamins, and minerals such as calcium, iron, and magnesium. In addition to being rich in antioxidants, they also contain a wide variety of biologically active nonnutritive compounds, known as phytochemicals, capable of conferring additional health benefits (Essa *et al*., 2023; Kumar *et al*., 2021;

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Temple, 2022). However, consuming raw produce poses health risks due to the potential contamination by foodborne pathogens (Beuchat, 1996; Olaimat and Holley, 2012). Several factors contribute to the increase in foodborne diseases, including (i) world trade expansion allowing wide availability of all types of fresh produce throughout the year; (ii) advanced diagnostic methods enabling identification of fresh produce as a source of foodborne diseases (Altekruse *et al*., 1997); and (iii) climate change, which through several climate-sensitive pathways impacts the existence and persistence of microorganisms and vectors increasing the pathogenic microbial contamination of water and food (Awad *et al*., 2024).

According to the European Union One Health 2022 Zoonoses Report (European Food Safety Authority [EFSA] and European Centre for Disease Prevention and Control [ECDC], 2023), the European Union's consumption of contaminated vegetables and other plants was found to be associated with a wide variety of pathogens such as bacterial agents—*Listeria monocytogenes* (Murray *et al*., 1926; Pirie, 1940), *Salmonella* (Lignieres, 1900), Shiga toxin-producing *Escherichia coli* (STEC) (Migula 1895; Castellani and Chalmers, 1919), *Yersinia enterocolitica* (Schleifstein and Coleman, 1939; Frederiksen 1964)), bacterial toxins, protozoa *Cryptosporidium parvum* (Tyzzer, 1912), and viruses (norovirus).

In the US, 21% of foodborne illness cases between 1990 and 2005 were linked to vegetables and other plants (DeWaal and Bhuiya, 2007). According to RASFF (Rapid Alert System for Food and Feed) notifications (European Commission, 2022) in 2022, a total of 4339 were sent to the European Community, and 3888 were related to human consumption. The main hazard in 2022 was the presence of pesticide residues (1011 notifications), followed by pathogenic microorganisms (786 notifications), and mycotoxins (495 notifications). The highest number of non-compliances in member states affected fruits and vegetables (821). Pesticide residues and pathogenic bacteria represent the greatest risk of contamination in vegetables that are predominantly consumed raw or semi-processed. Such contaminations can occur during cultivation, harvesting, and transportation (Ng *et al*., 2005). The use of pesticides in agriculture is aimed at protecting plants from potential pest attacks and increase yield; however, it has a significant environmental impact as residues can reach water, soil, and air and persist for a long time (Alaoui *et al*., 2024; El-Sheikh *et al*., 2022). In particular, the misuse of pesticides due to inadequate management of good hygiene and agricultural practices can affect the soil's self-purification capacity. Some microorganisms can degrade pesticides, indicating a symbiotic association and creating conditions that increase health risks for consumers due to the simultaneous presence of pesticides and microbial contaminants in fresh fruits and vegetables (Kapeleka *et al*., 2020). In this context, it is crucial to closely monitor and control bacterial contamination to ensure food safety and protect public health.

The normal flora of many fruits and vegetables consists of bacteria belonging to the Enterobacteriaceae family, whose presence can result from non-composted natural fertilizers and contamination from soil and irrigation water, exacerbated by the changing climate patterns. While *E. coli* (Migula 1895; Castellani and Chalmers, 1919) is commonly found in humans and animals' intestines, other genera such as *Klebsiella* (Trevisan, 1885), *Enterobacter* (Hormaeche and Edwards, 1960 [Approved Lists 1980] emend. Brady et al. 2013), *Citrobacter* (Werkman and Gillen, 1932), and *Serratia* (Bizio, 1823) are environmental saprophytes. Enterobacteriaceae regularly colonize raw materials that provide a favorable substrate for their survival (Lenzi, Marvasi and Baldi, 2021), and have replaced coliforms as an indicator of food processing and preservation quality due to their greater environmental resistance (Batt and Tortorello, 2014). They help avoid the non-detection of slow fermenting lactose species and behave just like the pathogens regarding technological treatments that impair the viability of pathogens, making Enterobacteriaceae particularly useful as a process indicator in cases where food has been subjected to heat treatments, prolonged freezing, and fermentation (Tiecco, 1997). Although their presence in food does not necessarily imply fecal contamination or the presence of pathogens, they can pose health risks, especially to debilitated and immunocompromised individuals (Berg *et al*., 2014) where Enterobacteriaceae could access critical sites in the body and originate infections (Symmers, 1965). The pathogenicity of these microorganisms can have serious implications for the patients' conditions (Al-Kharousi *et al*., 2016; Falomir *et al*., 2010; Koneman, 2019), making it essential to take necessary precautions to prevent their spread. Many Enterobacteriaceae have acquired antibiotic resistance, posing significant public health concerns (Olaimat and Holley, 2012). They can also influence resistance gene transfer in natural habitats, such as the human colon (Al-Kharousi *et al*., 2016).

Determining the microbiological quality of fresh produce marketed and served is crucial, especially in a hospital environment to prevent the transmission of opportunistic pathogens to immunocompromised patients and their spread in the hospital setting.

The regulation EC Reg. 2073/05 and amendments "On microbiological criteria for foodstuffs" (European Commission, 2005, 2007) defines (i) precut ready-to-eat vegetables and fruit; and (ii) *Salmonella* spp. (Lignieres, 1900), and *L. monocytogenes* (Murray *et al*., 1926; Pirie, 1940) as food safety criteria. The EU Reg. 209/13 (European Commission, 2013), following the *E. coli*

O104:H4 outbreak in 2011, defines *E. coli* STEC (Migula 1895; Castellani and Chalmers, 1919) as a safety criterion for sprouted seeds. In addition, Reg. 2073/05 and amendments define *E. coli* (Migula 1895; Castellani and Chalmers, 1919) as a process hygiene criterion while Enterobacteriaceae (Rahn, 1937), which are mostly of environmental origin, are not taken into account. Nevertheless, the Enterobacteriaceae parameter is quantified as part of the official controls carried out by the competent authorities at the local level (Autonomous Region of Sardinia, 2017). The maximum concentration value for Enterobacteriaceae is assumed to be equal to the one set for *E. coli* (Migula 1895; Castellani and Chalmers, 1919), an indicator of fecal contamination and a potential pathogen (Autonomous Region of Sardinia, 2017).

Guide values represent a valuable tool for controlling and preventing foodborne illnesses; however, *E. coli* (Migula 1895; Castellani and Chalmers, 1919) value (two of the five samples collected can show a concentration between 10^2 and 10^3 UFC/g [units formed per gram] when the other three are under 10^2) is too restrictive for Enterobacteriaceae, which include environmental species that are often present at naturally high levels in the production cycle of fruits and vegetables and are not necessarily pathogenic for humans. In this complex scenario, a guide value that is too lax endangers food security, while one that is too restrictive poses unrealistic targets for food business operators and offers no safety benefits to consumers. An unnecessary strict value is challenging to comply with by food business operators and increases the likelihood of using excessively high levels of disinfectants. The challenge here is to balance these aspects, guaranteeing the microbiological quality of fresh produce marketed and served, especially in healthcare settings.

The aim of this study was twofold: first, to illustrate our on-field experience using a control chart for Enterobacteriaceae in managing risks within fresh produce production and supply processes; and second, to promote the widespread adoption of control charts to provide a reliable framework for defining acceptable official control values of Enterobacteriaceae. This approach would support food business operators in maintaining high standards and help regulatory authorities set realistic and safe guidelines for bacterial contamination in fresh produce, complementing Commission Regulation (EC) No. 2073/2005 and amendments (European Commission, 2005, 2007) and local regulations (Autonomous Region of Sardinia, 2017). For these purposes, collaborating with a food business operator, we developed an Enterobacteriaceae Individual-Moving Range (X-mR) control chart, helpful in setting warning and action levels to promptly detect anomalies in the production and supply processes and act accordingly. Control charts are easy-to-build and easy-to-use tools that have great potential for the control of processes' variance. These graphical tools help quickly identify changes in processes' outputs. While control charts have been a common practice in monitoring industrial processes since their development in the1920s (Shewhart, 1940), their use is not consistently integrated into the food industry and the HACCP (hazard analysis and critical control points) systems (Augustin and Minvielle, 2008). With its proactive, systematic approach, the aims of HACCP are to prevent, remove, or minimize risks to acceptable levels to guarantee food safety to consumers by identifying, assessing, and managing potential hazards. With the integration of microbial count control charts in fresh produce, HACCP plans might enhance the monitoring and verification processes, providing a more robust framework for managing food safety risks and a statistically reliable data history.

Materials and Methods

From January to December 2023, we collected 232 samples of fresh produce. Of these, 142 samples were of processed end products, consisting of fresh salads, fennel, and pot herbs such as parsley, from collective catering (e.g., school and university canteens, nursing homes) in central-southern Sardinia (Table 1). The remaining 90 samples were sourced from local retailers and consisted of IV gamma salads and fruits (Table 1).

All products were minimally processed, that is, hulled, cut, washed, and packed (IV gamma fruits and vegetables). Samples were taken at regular intervals so that a control chart could be set up to assess the quality of the production and supply process.

All samples were transported in dedicated refrigerated bags, ensuring a temperature between 1°C and 8°C, to the Food Hygiene Laboratory of Cagliari University, Department of Medical Sciences and Public Health, which operates according to the UNI EN CEI ISO 17025:2018. The samples were analyzed within 24 hours (ISO 7218:2007/Amd1:2013) and the following microbiological parameters were determined: (i) Enterobacteriaceae and β-glucoronidasi positive *E. coli* (Migula 1895; Castellani and Chalmers, 1919) as process parameters; and (ii) some primary pathogens such as *Salmonella* (Lignieres, 1900) to gain further knowledge on the hygienic and sanitary situation of the products considered.

Detection and counting of Enterobacteriaceae

The presence and concentration of Enterobacteriaceae were measured (ISO 21528-2:2017) as follows: 90 mL of Buffer Pepton Water was added to 10 g of the sample

Table 1. Samples details.

Type	no
Catering total	**142**
Salads (iceberg, radicchio)	36
Iceberg salads	53
Fennel	15
Grated carrots	15
Tomato, corn, carrots	5
Grated parsley	18
IV Range total	**90**
Mix salads (iceberg, curly salad, radicchio)	16
Mix salads with carrots	10
Iceberg	16
Arugula	12
Pineapple	12
Fruit salad	12
Melon	12

to obtain the stock suspension; then 1 mL was transferred to the bottom of a sterile Petri dish; and18 mL of the specific medium Violet Red Bile Glucose (VRBG) agar was added and cooled to a temperature between 47°C and 50°C. Once the medium solidified, the plates were incubated for 24 ± 2 hours in a thermostat at 37°C. Characteristic colonies (pink to red to purple, with or without precipitation halos) were isolated on NUA (Nutrient Agar medium) and the latter incubated at 37°C for 24 hours (Figure 2). After 24 hours, biochemical confirmation of the typical colonies was performed. It included the oxidase test and the glucose fermentation test on the colonies that tested negative for oxidase. After incubation at 37°C for 24 hours, if the color of the medium turned yellow, it confirmed the presence of Enterobacteria. Miniaturized biochemical tests were used to type microorganisms, specifically API 20 E galleries (Biomerieux).

Detection and counting of E. coli

The presence and concentration of *E. coli* (Migula 1895; Castellani and Chalmers, 1919) were measured (UNI ISO 16649-2:2015) as follows: from the stock suspension, 1 mL was transferred to the bottom of a sterile Petri dish. Subsequently, 18 mL of the specific TBX agar medium, cooled to between 47°C and 50°C, was added. Once the medium solidified, the plates were incubated for 18–24 hours in a thermostat at 44°C. Typical colonies are green in color due to the use of the chromogenic substrate contained in the seeding medium.

Detection of Salmonella spp.

The presence of *Salmonella* spp. (Lignieres, 1900) was measured (UNI EN ISO 6579-1:2017/Amd1:2020) as follows: 225 g Buffer Peptone Water was added to 25 g of the sample; the resulting solution was incubated at 37°C for 18–20 hours (pre-enrichment); 1 mL was taken from this solution and added to 10 mL of MKttn (Mueller Kauffmann Tetrathionate), and 0.1 mL was taken and added to 10 mL of Rappaport Vassiliadis. The MKttn was incubated at 37°C and the Rappaport Vassiliadis at 41.5°C (enrichment). After 24 hours, using a ring loop, the two broths were sown on the first-choice medium XLD (Xylose Lesine Desoxycholate agar) and the second-choice medium SS (Salmonella and Shigella agar). After incubation at 37°C for 24 hours, the presumptive colonies were subjected to biochemical confirmation: isolation on NUA (Nutrient Agar medium), fermentation test of the three sugars on TSI (Tryptose Sugar Iron), indole test and biochemical identification by API (Analytical Profile Index) 20E tunnel, and finally serological confirmation by the omnivalent serum.

Construction of the control chart

For the construction of the control chart, we focused on the concentration of Enterobacteriaceae in iceberg lettuce salad collected from a selected canteen. We took 53 samples, about one per week during 2023. The construction of the control chart (Shewhart, 1940) required the identification of a central line (average value), a range that indicates the normal functioning of the process, namely values that indicate functioning at the limits of acceptability (upper and lower control values). The values were determined using a diagram of the results obtained (at least 10) with the measurement (CFU/g [colony-forming units per gram] in our case) as the y-axis and the number of samplings on the x-axis. The data was not normally distributed as shown in the Q-Q plot (see Supplemental Materials Figure 1) and by Shapiro-Wilk test ($p < 0.001$). We performed the analysis with JASP (Jeffrey's Amazing Statistics Program) (JASP Team, 2024; JASP Version 0.19.3 [computer software]). We used the X-mR (Individuals-Moving Range) chart that can be used to monitor variation of data with several types of distribution. The individual values, collected at regular intervals, were plotted on the X chart to monitor the mean and shifts in the process. The moving range between consecutive individual values was plotted on the mR (moving range) chart to monitor the process variation tracking the absolute difference between each measurement to its previous measurement. Combining the two charts provided a complete picture of process behavior.

The values of the X-mR chart are defined as:

- "Central line" CFU/g indicates the arithmetic mean of the measurements.
- "Upper control limit" (UCL) and "Lower control limit" (LCL) CFU/g define the normal variation of the process. We could consider the LCL and UCL as "Action Limits". Values exceeding the UCL require immediate action to remove the underlying causes. Constructing an X-chart with microbiological data, we should consider that LCL are rarely of any use (the count goes from zero upwards; no growth = 0).
- "Warning limit" (WL)—Values approaching the UCL and over the WL are still considered within normal fluctuation but require specific investigations.

We obtained these values and constructed the control chart through statistical analysis of Enterobacteriaceae count results. We first calculated the average value of microbial counts ($\bar{x}$), then the moving average range (mR) and set the CLs for each chart.

The process standard deviation can be estimated as

$$\sigma\bar{x} = \frac{mR}{d_2}$$

where d_2 is a correction constant that depends on the sample size. For n = 2 observations in each subgroup (differences between successive pairs of data), $d_2 \approx 1.128$.

X-chart UCL and LCL were calculated as $\bar{\bar{x}} \pm 3 \times \sigma\bar{x}$

WL was calculated as $\bar{\bar{x}} + 2 \times \sigma\bar{x}$

UCL value of the mR-chart was calculated as: mR d_3. where d_3 is a constant equal to 3.2665, based on the number of observations in each subgroup to calculate mR as per d_2.

The software used to perform statistical analysis and generate the X-mR chart is Microsoft Excel (Microsoft Corporation, 2018).

Results

Enterobacteriaceae were consistently detected in all 241 samples examined, highlighting their ubiquitous presence in both salads served in canteens and nursing homes as well as in the IV range fruits and vegetables (Table 2). The genus *Pantoea* (Gavini *et al.*, 1989) was the most frequently isolated species (Figures 1 and 2). Despite the widespread presence of Enterobacteriaceae, *E. coli* (Migula 1895; Castellani and Chalmers, 1919) levels remained below the detection threshold, with no *Salmonella* (Lignieres, 1900) detected, suggesting that while environmental contamination is common, the presence of specific pathogens was minimal.

Figure 3 shows the control chart for Enterobacteriaceae concentration (CFU/g) in samples of iceberg lettuce salad served in a selected canteen during 2023.

Table 2. Average values for Enterobacteriaceae in all types of samples.

	UFC/g		
Type	**Enterobacteriaceae**	***Escherichia coli***	***Salmonella* spp.**
Catering samples			
Iceberg salad	2.4×10^4	<10	n.d.
Mix salad	1.9×10^5	<10	n.d.
Tomato salad	2.8×10^1	<10	n.d.
Grated carrots	1.9×10^1	<10	n.d.
Fennels	8.9×10^1	<10	n.d.
Grated parsley	5.5×10^5	<10	n.d.
IV Range samples			
Melon	3×10^4	<10	n.d.
Fruit salad	9.3×10^5	<10	n.d.
Pineapple	9×10^2	<10	n.d.
Iceberg lettuce (inner part)	2.4×10^4	<10	n.d.
Rocket salad	2.1×10^4	<10	n.d.
Mix salad with carrots	8.8×10^3	<10	n.d.
Mix salad	4.4×10^4	<10	n.d.

n.d. = not detected.

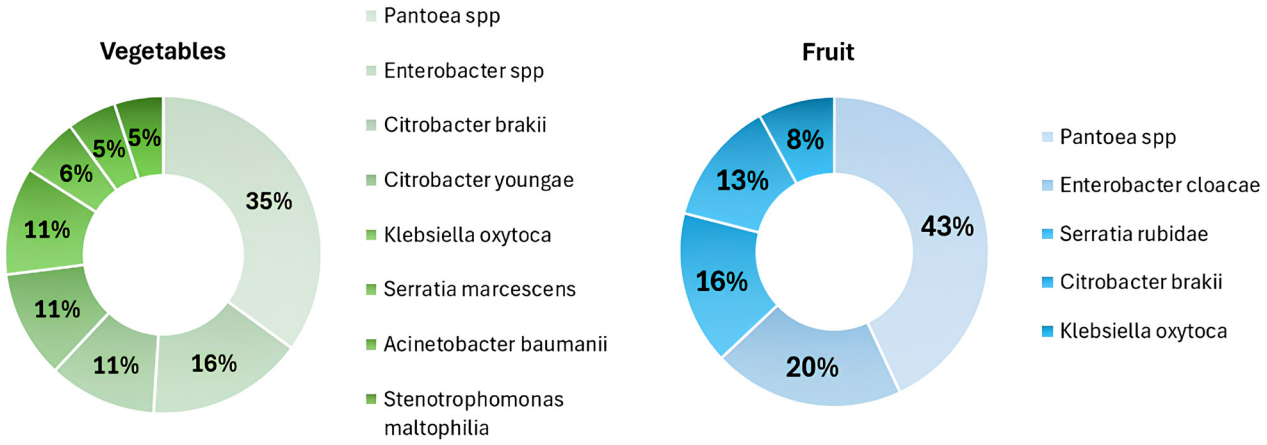

Figure 1. Catering samples: Percentages of frequencies of Enterobacteriaceae species in vegetables and fruits samples from collective catering—*Pantoea* spp (Gavini *et al.*, 1989), *Enterobacter cloacae* (Jordan 1890; Hormaeche and Edwards, 1960), *Enterobacter* spp (Hormaeche and Edwards, 1960), *Serratia rubidae* (Stapp 1940; Grimont *et al.*, 1978), *S. Marcescens* (Bizio, 1823), *Citrobacter brakii* and *C. youngae* (Brenner *et al.*, 1993), *K. oxytoca* (Flügge, 1886; Lautrop, 1956), *Acinetobacter baumanii* (Bouvet and Grimont, 1986), and *Stenotrophomona malthophila* (Palleroni & Bradbury, 1993).

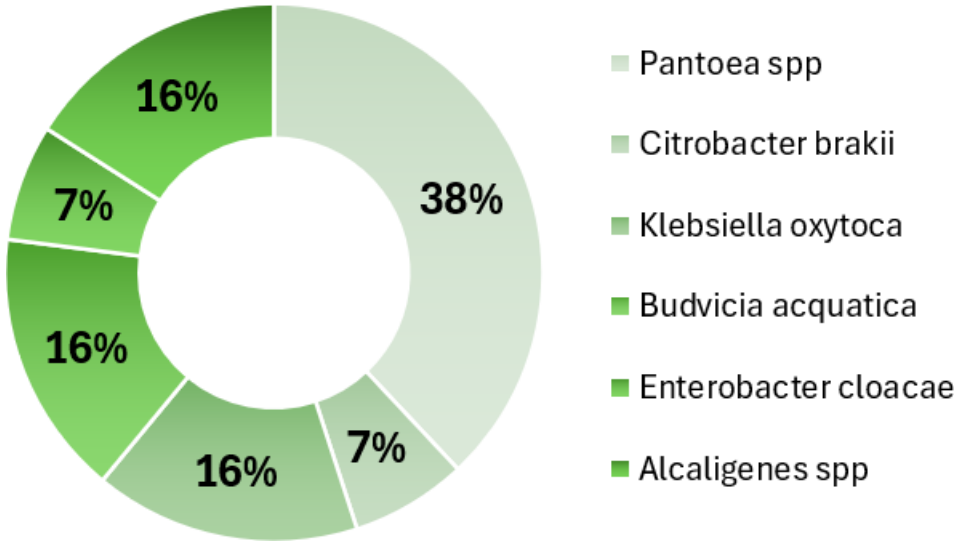

Figure 2. IV Range samples: Percentages of frequencies of Enterobacteriaceae species in vegetables and fruit samples from local retailers—*Pantoea* spp (Gavini *et al.*, 1989), *C. brakii* (Brenner *et al.*, 1993), *K. oxytoca* (Flügge, 1886; Lautrop, 1956), *Budvicia acquatica* (Bouvet and Grimont, *et al.*, 1985), *E. cloacae* (Jordan 1890; Hormaeche and Edwards, 1960), and *Alcaligenes* spp (Castellani and Chalmers, 1919).

The X-control chart revealed that Enterobacteriaceae levels remained within acceptable limits throughout the study period, with significant deviations observed at certain points. It highlighted an upward trend of Enterobacteriaceae values from end June to July 2023 (samples 24, 25, 26). This upward trend culminated with deviations (sample 25: 140,000 CFU/g; sample 26: 130,000 CFU/g; sample 27: 560,000 CFU/g) from the range of values considered as normal variations of the Enterobacteriaceae concentration (CFU/g) exceeding the action value of the UCL (Figure 3). The spikes falling outside the UCL signaled the presence of a special cause of variation, a potential contamination event, or temporary lapses in sanitation procedures that needed additional action to determine the nature of the problem and eliminate it. The mR-control chart represents the variation between the consecutive values measured; most of the variations between samples fall withing the control limits, indicating that the process was generally stable. However, as in the X-chart, some points approached and exceeded the UCL, signaling the presence of special causes of variation. Following the laboratory alert and

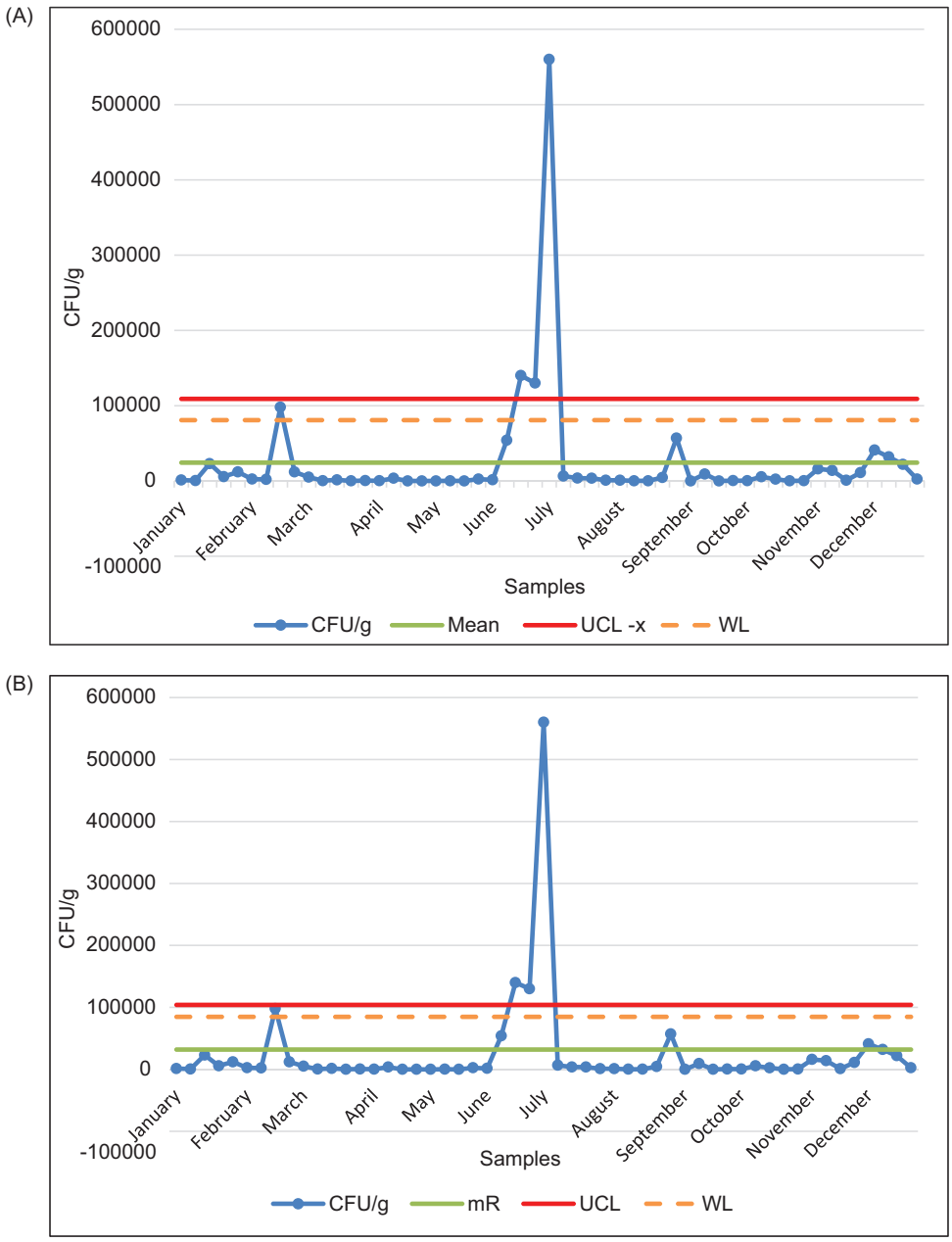

Figure 3. Enterobacteriaceae Individual (X)-Moving Range (mR) Control Chart: Iceberg lettuce salad collected in 2023 in a selected canteen. Panel A—X-chart: CL, central line (mean: 24,371 CFU/g); UCL, upper control limit (109,060 CFU/g); LCL, lower control limit; WL, warning limit (80,822 CFU/g); blue dots represent the 53 samples collected in 2023 (see the x-axis for months details). Panel B—mR-Chart: MR-bar, average moving range (31,838); UCL, upper control limit (104,047 CFU/g).

supplier's intervention, Enterobacteriaceae concentration values returned to normal ranges, indicating that potential contamination events were promptly addressed.

Discussion

Preventing contamination and keeping it at the lowest possible levels is crucial for controlling food-borne diseases in fresh products. In this study, no pathogenic bacteria, such as *E. coli* and *Salmonella* spp (Lignieres, 1900), were found, but opportunistic microorganisms, such as *Pantoea* spp (Gavini *et al.*, 1989), *S. marcescens* (Bizio, 1823), *K. pneumoniae* (Trevisan, 1887), *E. cloacae* (Jordan, 1890), *Enterobacter* spp (Hormaeche and Edwards, 1960), and *A. baumannii* (Bouvet and Grimont, 1986) were detected. These microorganisms may represent epiphytic flora or originate from soil contamination

from animals or humans and pose risks to vulnerable groups such as older people, children, and immunocompromised subjects. In this study, we developed a strategy to complement the Commission Regulation (EC) No. 2073/2005 and amendments and local regulations (Autonomous Region of Sardinia, 2017) about Enterobacteriaceae levels in processed fruits and vegetables, and help manufacturers detect hygiene procedure breaks more quickly and efficiently. The control chart for Enterobacteriaceae helps address overly restrictive maximum concentrations while ensuring microbial quality and food safety to all consumers.

In some cases, during official controls, Enterobacteriaceae concentrations of 10^4 UFC/g are often deemed too high, while a reference value of < 10^3 UFC/g, similar to *E.coli*, is too restrictive. In fact, the presence of *E. coli* (Migula 1895; Castellani and Chalmers, 1919) can be traced back to fecal contamination, but the Enterobacteriaceae family also includes species of environmental origin that can stick to plant surfaces and internalize in plant tissue, especially in protective niches created by leaves' conformation. Washing, whether with or without disinfectants, can be ineffective and can even create stress for microbial cells, leading to the formation of biofilms. To remove the residual microbial load after washing, fresh produce processes often require sanitizers and disinfectants (Joshi *et al*., 2013). Sodium hypochlorite, a cost-effective antibacterial and antimicrobial agent, is often used, but as its reaction with other organic compounds could cause toxicity concerns, the levels should be strictly monitored. While a branch of research seeks new nonhazardous disinfectants (Joshi *et al*., 2013), our study proposes a control strategy for contamination risk management considering guidelines, nature of the microorganism, data history, and local context. This strategy could be considered a first step in defining realistic and safe levels for Enterobacteriaceae in processed fruits and vegetables production and supply at the local level. It would be desirable that competent authorities base guide values on local average levels (safe range of variation), considering that Enterobacteriaceae also include environmental species that only pose a risk for human consumption at high concentrations and under specific conditions. The main purpose of constructing a control chart for Enterobacteriaceae is to prevent contamination by identifying undesirable trends early in the production process. Literature reports average mesophilic counts in raw materials like fruits and vegetables reaching 10^7 CFU/g and Enterobacteriaceae values between 10^4 and 10^6 CFU/g (Coroneo *et al.*, 2014; Halablab *et al.*, 2011; Oliveira *et al.*, 2010;Galli Volonterio, 2009). The reduction achieved in the analyzed product may not be sufficient to meet the established limits, and our control chart provided a clear view of the situation over time in the canteen selected for the analysis.

Overall, the findings underscore the importance of continuous monitoring and timely interventions to manage microbial contamination in fresh produce. Our results showed an upward trend affecting the last iceberg lettuce salad samples of June and July 1, 2023, with a considerable shift of three points of the trend, spiking over the UCL signaling potential contamination events or temporary lapses in sanitation procedures. We responded quickly, alerting the food business operator of the situation and provided targeted advice on what steps to follow for the "food flow" to solve the problem. We suggested an immediate check of the critical control points, specifically of procedures which when not done correctly could be a source of contamination. The subject responsible implemented the corrective action established as part of the HACCP plan. Through the use of the control chart, we verified and monitored over time the efficacy of these measures. The use of the Enterobacteriaceae control chart proved to be valuable in identifying and addressing potential contamination events, thereby enhancing the safety and quality of the food supply. The consistent integration of control charts within HACCP systems presents several practical implications for preventing microbiological contamination. By plotting microbial counts, anomalies can be detected in near real-time, enabling food safety managers to maintain control over critical points in the production process. Early detection of microbial trends facilitates timely interventions before contamination becomes a significant issue. Swift corrective actions ensure that potential contamination is addressed promptly, thereby reducing the risk of foodborne diseases. Furthermore, control charts provide documented evidence of the ongoing monitoring and control activities, essential for compliance with food safety regulations. Utilizing control charts would allow food business operators to adopt a proactive approach to microbiological risk management, ensuring food safety and quality and fostering consumer trust. Moreover, control charts save costs by reducing waste and preventing major issues like recalls. They also would push for continuous improvement by providing feedback to competent authorities for refining food safety protocols.

Conclusions

The adoption of control charts within the HACCP systems has several practical implications for preventing Enterobacteriaceae contamination, and also is a proactive approach to monitoring microbial trends and maintain real-time process control. By conducting periodic analyses on processed products, food business operators can create control charts and set warning and action levels for the most frequently identified microorganisms. This would help identify undesirable trends in the production process, enabling timely corrective actions to prevent

risks and verify the effectiveness of these measures. Furthermore, using control charts would ensure that all process controls and corrective actions are documented and validated during official controls. This data are essential for future research aimed at revising guide values and promoting a bottom-up approach based on local data to support regulatory updates.

Additionally, developing methods to prevent biofilm formation on fresh produce and nonhazardous disinfectants is essential as well as implementing good agricultural and processing practices across the entire production chain. Future steps should aim to develop comprehensive monitoring systems that include control charts to track microbial contamination from the field to distribution, strengthening "field-to-fork" monitoring and ensuring greater food safety.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Author Contributions

Conceptualization: A.S. and V.C.; methodology, A.S. and V.C.; software, V.C. and S.M.P.; validation, A.S. and V.C.; formal analysis, S.M.P.; investigation, A.S and V.C..; resources, V.C..; data curation, A.S., S.M.P., and V.C.; writing—original draft preparation, A.S., S.M.P., and V.C.; writing—review and editing, A.S., S.M.P., and V.C.; visualization, S.M.P.; supervision, V.C.; project administration, V.C.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

Al-Kharousi, Z.S., Guizani, N., Al-Sadi, A.M., et al., 2016. Hiding in fresh fruits and vegetables: Opportunistic pathogens may cross geographical barriers. International Journal of Microbiology 2016: 4292417. https://doi.org/10.1155/2016/4292417

Alaoui, A., Christ, F., Abrantes, N., et al., 2024. Assessing pesticide residue occurrence and risks in the environment across Europe and Argentina. Environmental Pollution 363: 125056. https://doi.org/10.1016/j.envpol.2024.125056

Altekruse, S.F., Cohen, M.L., Swerdlow, D.L., 1997. Emerging foodborne diseases. Emerging Infectious Diseases 3(3): 285–293. https://doi.org/10.3201/eid0303.970304

Augustin, J.C., Minvielle, B., 2008. Design of control charts to monitor the microbiological contamination of pork meat cuts. Food Control 19(1): 82–97. https://doi.org/10.1016/j.foodcont.2007.02.007

Aune, D., Giovannucci, E., Boffetta, P., et al., 2017. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality – A systematic review and dose-response meta-analysis of prospective studies. International Journal of Epidemiology 46(3): 1029–1056. https://doi.org/10.1093/ije/dyw319

Autonomous Region of Sardinia, 2017. Official Controls Guidelines under regulations (EU) 882/2004 E 854/2004. Available at: https://www.regione.sardegna.it/documenti/1_274_20170512103100.pdf. Accessed 26 March 2025.

Awad, D.A., Masoud, H.A. and Hamad, A., 2024. Climate changes and food-borne pathogens: The impact on human health and mitigation strategy. Climatic Change 177(6): 92. https://doi.org/10.1007/s10584-024-03748-9

Batt, C.A., Tortorello, M.L., 2014. Encyclopedia of Food Microbiology: Second Edition.

Berg, G., Erlacher, A., Smalla, K., et al., 2014. Vegetable microbiomes: Is there a connection among opportunistic infections, human health and our "gut feeling"? Microbial Biotechnology 7: 487–495. https://doi.org/10.1111/1751-7915.12159

Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection 59(2):204–216. https://doi.org/10.4315/0362-028X-59.2.204

Coroneo, V., Dessì, S., Carraro, V., et al., 2014. Qualità microbiologica del processo di produzione di vegetali di i gamma (lattuga). Industrie Alimentari 53: 5–10. Available at: https://iris.unica.it/handle/11584/58680.6. Accessed 26 March 2025.

DeWaal, C.S., Bhuiya, F.A.R.I.D.A., 2007. Outbreaks by the numbers: Fruits and vegetables. International Association for Food Protection 94th Annual Meeting.

El-Sheikh, E.S.A., Ramadan, M.M.R., El-Sobki, A.E., et al., 2022. Pesticide residues in vegetables and fruits from farmer markets and associated dietary risks. Molecules 27(22). https://doi.org/10.3390/molecules27228072

Essa, M.M., Bishir, M., Abid Bhat, A., et al., 2023. Functional foods and their impact on health. Journal of Food Science and Technology. 60(3):820–834. https://doi.org/10.1007/s13197-021-05193-3

European Commission, 2005. Regulation (EC) 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union.

European Commission, 2007. Commission regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union. Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:EN:PDF. Accessed 26 March 2025.

European Commission, 2013. Commission Regulation (EU) No 209/2013 of 11 March 2013 amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcases and fresh poultry meat (Text with EEA relevance). Official Journal of the European Union. Available at: https://eur-lex.europa.eu/eli/reg/2013/209/oj/eng. Accessed 26 March 2025.

European Commission, 2022. Rapid Alert System for Food and Feed (RASFF) Window. Available at: https://webgate.ec.europa.eu/rasff-window/screen/search?searchQueries=eyJkYXRlIjp7InN0YXJ0UmFuZ2UiOiIyMDIxLTEyLTMxVDIzOjAwOjAwLjAwMFoiLCJlbmRSYW5nZSI6IjIwMjItMTItMzBUMjM6MDA6MDAuMDAwWiJ9LCJub3RpZmljYXRpb25TdGF0dXMiOnsibm90aWZpY2F0aW9uU3RhdHVzIjpbWzFdXX19. Accessed 26 March 2025.

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2023. The European Union One Health 2022 Zoonoses Report. European Food Safety Authority Journal 21(12): e8442. https://doi.org/10.2903/j.efsa.2023.8442

Falomir, M., Gozalbo, D., Rico, H., 2010. Coliform bacteria in fresh vegetables: From cultivated lands to consumers. Current research, technology and education topics in applied microbiology and microbial biotechnology. Available at: https://www.researchgate.net/profile/Hortensia-Rico/publication/265940318_Coliform_bacteria_in_fresh_vegetables_From_cultivated_lands_to_consumers/links/54b912bf0cf2c27adc4911ee/Coliform-bacteria-in-fresh-vegetables-From-cultivated-lands-to-consumers.pdf. Accessed 26 March 2025.

Galli Volonterio, A., 2009. Analisi microbiologica degli alimenti. In Microbiologia degli alimenti. Ambrosiana: Milano, 2009. 205, 279 pp.

Halablab, M.A., Sheet, I.H. and Holail, H.M., 2011. Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon. American Journal of Food Technology 6: 129–139. https://doi.org/10.3923/ajft.2011.129.139

Hartley, L., Igbinedion, E., Holmes, J., et al., 2013. Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases. Cochrane Database of Systematic Reviews 2013(6):CD009874. https://doi.org/10.1002/14651858.CD009874.pub2

Joshi, K., Mahendran, R., Alagusundaramet, K., et al., 2013. Novel disinfectants for fresh produce. Trends in Food Science & Technology 34(1): 54–61. https://doi.org/10.1016/j.tifs.2013.08.008

Kapeleka, J.A., Sauli, E., Omowunmi Sadik, O., et al., 2020. Co-exposure risks of pesticides residues and bacterial contamination in fresh fruits and vegetables under smallholder horticultural production systems in Tanzania. PLoS ONE 15(7):e0235345. https://doi.org/10.1371/journal.pone.0235345

Koneman, W.E. Enterobatteriaceae. In *Testo Atlante di Microbiologia Diagnostica*. Antonio Delfino editore. Jones & Bartlett Learning: Burlington, MA, USA, 2019. 211–303 pp.

Kumar, M., Ved Prakash Giri, V.D., Pandey, S., et al., 2021. Plant-growth-promoting rhizobacteria emerging as an effective bioinoculant to improve the growth, production and stress tolerance of vegetable crops. International Journal of Molecular Sciences 22(22): 12245. https://doi.org/10.3390/ijms222212245

Lenzi, A., Marvasi, M., Baldi, A., 2021. Agronomic practices to limit pre- and post-harvest contamination and proliferation of human pathogenic Enterobacteriaceae in vegetable produce. Food Control. 119: 107486. https://doi.org/10.1016/j.foodcont.2020.107486

Ng, P.J., Fleet, G.H., Heard, G.M., 2005. Pesticides as a source of microbial contamination of salad vegetables. 101(2): 237–250. https://doi.org/10.1016/j.ijfoodmicro.2004.11.009

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

Oliveira, M., Usall, J., I Viñas I., et al., 2010. Microbiological quality of fresh lettuce from organic and conventional production. Food Microbiology (5):679–84. https://doi.org/10.1016/j.fm.2010.03.008

Shewhart, W.A., 1940. Statistical method from the viewpoint of quality control. Supplement to the Journal of the Royal Statistical Society 7(1), 86–87 pp. https://doi.org/10.2307/2983634

Symmers, W.S.C., 1965. Opportunistic Infections: The concept of "Opportunistic Infections." Journal of the Royal Society of Medicine 93(1): 39–40. https://doi.org/10.1177/003591576505800521

Temple, N.J., 2022. AA rational definition for functional foods: A perspective. Frontiers in Nutrition 29(9): 957516. https://doi.org/10.3389/fnut.2022.957516

Tiecco, G., 1997. Metodi per la valutazione delle contaminazioni microbiche. In Igiene e Tecnologia Alimentare. Calderini Edagricole: Bologna, Italy, 1997. 283 p.

World Health Organization, 2003. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO expert consultation. WHO technical report series no. 916., WHO technical report series. Available at: https://iris.who.int/bitstream/handle/10665/42665/WHO_TRS_916.pdf?sequence=1. Accessed 26 March 2025.

World Health Organisation, 2004. Global strategy on diet, physical activity and health. In: Fifty-seventh World Health Assembly,Geneva, 17–22 May 2004. In Resolutions and decisions, annexes. Geneva, World Health Organisation. Available at: https://apps.who.int/gb/ebwha/pdf_files/wha57/a57_r17-en.pdf. Accessed 25 March 2025.

World Health Organization, 2019. Increasing fruit and vegetable consumption to reduce the risk of noncommunicable diseases, e-Library of Evidence for Nutrition Actions (eLENA). Available at: https://www.who.int/tools/elena/interventions/fruit-vegetables-ncds. Accessed on 26 March 2025.

World Health Organization (2020) Healthy diet. Available at: https://www.who.int/news-room/fact-sheets/detail/healthy-diet. Accessed 26 March 2025.

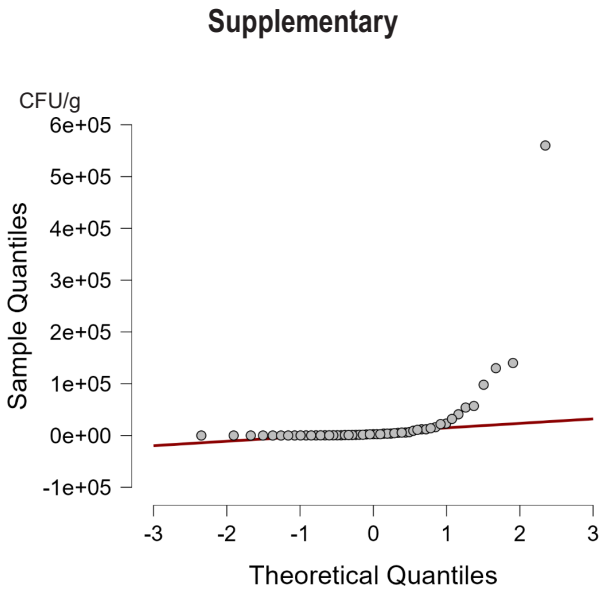

Figure S1. Q-Q plot. Visual assessment of the distribution of the sample data. The deviations from the reference line, particularly in the tails, indicate that the sample data is not normally distributed.