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COMPREHENSIVE REVIEW

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A review of conditions influencing fate of Shiga toxin-producing *Escherichia coli* O157:H7 in leafy greens

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Abstract

The accuracy of predictive microbial models used in quantitative microbial risk assessment (QMRA) relies on the relevancy of conditions influencing growth or inactivation. The continued use of log-linear models in studies remains widespread, despite evidence that they fail to accurately account for biphasic kinetics or include parameters to account for the effect of environmental conditions within the model equation. Although many experimental studies detail conditions of interest, studies that do not do so lead to uncertainty in QMRA modeling because the applicability of the predictive microbial models to the conditions in the risk scenarios is questionable or must be extrapolated. The current study systematically reviewed 65 articles that provided quantitative data and documented the conditions influencing the inactivation or growth of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in leafy greens. The conditions were identified and categorized as environmental, biological, chemical, and/or processing. Our study found that temperature ($n = 37$ studies) and sanitizing and washing procedures ($n = 12$ studies) were the most studied conditions in the farm-to-table continuum of leafy greens. In addition, relative humidity was also established to affect growth and inactivation in more than one stage in the continuum. This study proposes the evaluation of the interactive effects of multiple conditions in processing and storage stages from controlled experiments as they relate to the fate of STEC O157:H7 in leafy greens for future quantitative analysis.

KEYWORDS
conditions, farm-to-table, growth, inactivation, lettuce, STEC O157:H7

1 | INTRODUCTION

Increased vegetable consumption is generally a healthy practice recommended by the World Health Organization and the US Dietary Guidelines for Americans (Cámara et al., 2021; Kalmpourtzidou et al., 2020). The World Health Organization recommends a vegetable intake of ≥240 g/day among the adult population (Kalmpourtzidou et al., 2020) and the US Dietary Guidelines for Americans recommends 2.5 cups per day of vegetable intake among adults (Cámara et al., 2021). However, the consumption of raw vegetables presents exposure routes for pathogenic microorganisms. The increasing consumption of raw vegetables such as leafy greens in high-income

countries has been accompanied by an increase in the cases of foodborne disease outbreaks (Söderqvist et al., 2017). It is estimated that fresh produce is the second leading food implicated in foodborne outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) globally, accounting for 15% of the cases (FAO/WHO, 2019). The global statistics estimate associated with annual cases of STEC is 1 million illnesses and 13,000 disability-adjusted life years (DALYs). Total foodborne STEC O157:H7 outbreaks reported to the Centers for Disease Control and Prevention (CDC) in the United States stood at 7565 between 2009 and 2020 with a hospitalization rate of 19.6% (CDC, 2022). Between 2012 and 2022, the country had a total of 5 multistate outbreaks of STEC O157:H7 due to consumption of contaminated Romaine lettuce, with the most severe outbreak being in 2018, which had 210 cases and five deaths across 36 states (CDC, 2022). Contamination of leafy greens can occur from farm to fork (Luna-Guevara et al., 2019), with sources including animal fecal deposits, irrigation water, agricultural runoff (Jeamsripong et al., 2019; Pang et al., 2017), soil, and handling (Luna-Guevara et al., 2019; Pang et al., 2017). Moreover, currently, there are no postharvest treatment equivalents to thermal processes (cooking) with respect to reducing populations of pathogens and associated risks in leafy greens consumed raw.

Quantitative microbial risk assessments (QMRAs) are used to model pathogen behavior in leafy greens to provide estimated public health risks that may result from the intake of contaminated produce (Hamilton et al., 2006; Ottoson et al., 2011; Pang et al., 2017). QMRAs quantify pathogens across the pre- and postharvest environment, through processing, storage, transport, and distribution in order to predict the contamination level at the point of consumption and the associated health risks. While widely utilized and established for supporting food safety goals, the utility and adequacy of a QMRA rely on the sufficiency and quality of the data (accounting for both variability and uncertainty) that are used in the modeling (Seidu et al., 2013). The survival of pathogens across the farm-to-fork exposure pathway is a leading source of uncertainty in QMRA models. Though experimentation to collect microbial growth/inactivation kinetics data seeks to address these uncertainties, consistency among the environmental conditions present during the experimental studies needs to be well documented in order to elucidate how those conditions affect growth and/or inactivation so that only the most relevant rates are applied in QMRAs. The inclusion or exclusion of these conditions may affect die-off or growth rates of STEC O157:H7 predicted through modeling (Belias et al., 2020; Seidu et al., 2013). The exponential model is an example of monophasic inactivation that assumes a constant die-off rate (Dean & Mitchell, 2022) and does not account for survival patterns of shouldering, tailing, and sigmoidal curves (Mitchell & Akram, 2017). Fitting a biphasic inactivation pattern with such a model will initially provide estimates that are equal to or overestimate pathogen concentrations and will subsequently underestimate after a certain point (Brouwer et al., 2017).

This systematic review examined the qualitative identification of conditions influencing the growth and inactivation of STEC O157:H7 in leafy greens intended for raw consumption. The study was a preliminary step for quantitative modeling that elucidated the effect of these conditions on the fate of STEC O157:H7 in leafy greens. Thus, the study explored the conditions for explaining survival trends to determine whether log-linear or nonlinear models would best depict the kinetics of the observed growth or inactivation. A diversity of leafy greens can be incorporated into raw salads (Kintz et al., 2019), and the cultivation, handling, and processing of those differ (Coulombe et al., 2020; IFPA et al., 2006). It is with this understanding that the defined scope of vegetables covered by the review was only limited to leafy greens including spinach and lettuce that are consumed raw, including in salads (Coulombe et al., 2020). The review addressed two research questions: (1) what are the conditions that influence growth and inactivation of STEC O157:H7 in leafy greens? (2) What are the differences and the effects of these conditions influencing the fate of STEC O157:H7 in different stages of the farm–fork continuum of leafy greens? Modelers often use data from the peer-reviewed literature when specific experimental studies are not available to develop a QMRA (Franz & van Bruggen, 2008; Pang et al., 2017). In this case, they rely upon the adequacy of the documented methodology and findings of other researchers to generate reliable models. The output of the review can be used by food microbiologists and risk modelers in evaluating data quality and reducing the uncertainties in QMRA for leafy greens intended for consumption in raw form. Moreover, the study maps out conditions in the farm–table continuum of leafy greens that would be of interest in setting up experimental studies on growth and inactivation.

2 | METHODS

2.1 | Searching for the studies in databases

The combination of key words (("*Escherichia coli*" AND O157:H7) OR ("*E. coli*" AND O157:H7) OR (Ec.O157:H7) OR ((Shiga AND Toxin AND Producing) OR (enterohemorrhagic) OR (Verocytoxin AND Producing) AND (*Escherichia* AND *coli*) OR (EHEC OR STEC OR VTEC OR Pathogen OR Bacteria) AND (Growth OR Decay

OR Kinetics OR Surviv* OR Fate OR Persistence) AND ((Leafy OR Green) AND Vegetables) OR "Salad Green" OR Lettuce OR Romaine OR "*Lactuca* AND *sativa*" OR Spinach OR "Spinacia oleracea" OR Iceberg)) as shown in Table S1 was searched through the literature databases including Scopus and PubMed. The search was conducted between April 1, 2022, and June 30, 2022.

2.2 | Screening of the generated articles

The scope of the literature search centered on studies that provided experimental trials evaluating the kinetics of STEC O157:H7 in leafy greens consumed raw. Articles that solely focused on surrogates without studying STEC O157:H7 were excluded from this review. Studies that reported other fresh vegetables or leafy greens commingled with other ingredients such as meat (IFPA et al., 2006) were judged to be out of the scope of this study. Studies that had mixes of leafy greens were only included if and only if the models or microbial kinetic data for the specific leafy greens were distinctively identified. Moreover, studies on nonfood substrates or purely extracts from the vegetables were also excluded from further evaluation in the review.

2.3 | Evaluation of included studies

Quality assessment of the studies focused on the experimental approaches and the microbial count data collected. Articles selected for this study had to document at least four time points for time-series microbial data to facilitate the fitting of biphasic models, two- and three-parameter, as alternatives to the exponential model (Dean & Mitchell, 2022). Articles that used secondary data were only included in the review if the data used had not been published in any manuscript and adequate information on experimental conditions was provided. The selected articles that documented field or greenhouse production of the vegetables had to state the temperature and other growth conditions (Coulombe et al., 2020). For postharvest processing of lettuce, washing with sanitizers or other technologies applied during processing had to include the concentration of the treatments used.

2.4 | Extraction and synthesis of the data

The generated list of articles was loaded into the Mendeley Reference Management Software (Parabhoi, 2017) for screening and removal of any duplicates. The full texts of the screened articles were evaluated, and a Microsoft Excel spreadsheet summarizing the eligibility was prepared. A data summary of the bibliographic information, experimental design, treatments, and stage of the value chain of all the eligible articles was prepared. However, data on the limit of detection (LOD) of the experiments was limited, as only 25 articles explicitly specified a value. An additional five articles that specified the concentration of the dilutions were used to estimate the LOD for culture techniques, assuming <30 CFU for plates (O'Toole, 2016). Another intrinsic characteristic that was least studied was culture conditions. For instance, the specifics of whether the cells were grown to the exponential phase or evaluated for the injured fraction were less detailed on their impact on inactivation. Only a single study (Uzeh & Adepoju, 2013) did not grow the culture in a new media before inoculation onto produce. In this paper, only the descriptive summary of the articles and experimental approaches was presented.

3 | RESULTS

3.1 | Studies selected for the review

The systematic review was done following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines as shown in Figure 1. The review included 65 articles. Table 1 provides a descriptive analysis of the reviewed papers based on the stage of the value chain, produce, method of enumeration or quantification of microbial growth, and the experimental designs deployed in the study. In the subsequent sections of this paper, a descriptive analysis of the articles that details the conditions that influenced the growth and inactivation of the microbial cells has been presented. Only the effects of conditions investigated in the articles were highlighted.

3.2 | Primary production

Twenty-three of the reviewed articles focused on the growth/inactivation of STEC O157:H7 in leafy greens during primary production. The primary production stage encompasses activities that result in raw materials including tillage (Chase et al., 2019), seed treatment (Dixit et al., 2021), weeding and fertilizer application on the growing plants (Chase et al., 2017), irrigation (Markland et al., 2013), and harvesting of the produce (Moyne et al., 2020). The two most studied processes in primary production were planting (21 studies) and irrigation (seven studies). The studies detailed the conditions affecting microbial growth and inactivation on leafy greens as environmental such as temperature and relative humidity (RH), weather conditions, agronomic practices such as irrigation and

TABLE 1 Descriptive analysis of the reviewed articles.

Source	Stage in the value chain	Type of leafy greens	Enumeration method	Condition	Experimental design
Abdul-Raouf et al., 1993	Retail or consumer storage	Iceberg lettuce	Plate count technique	Packaging	Laboratory controlled experiment
Abnavi et al., 2021	Processing	Iceberg lettuce	Most probable number	Sanitization (chlorine)	Laboratory randomized experiment
Bayacioglu et al., 2013	Retail or consumer storage	Spinach Romaine lettuce Leaf lettuce	Plate count technique	Packaging Temperature Bacteriophage	Laboratory controlled experiment
Belias et al., 2020	Primary production	Lettuce Spinach	Plate count technique	Weather conditions Irrigation	Outdoor randomized studies
Bezanson et al., 2012	Primary production	Romaine lettuce	Plate count technique	Soil conditions Weather conditions	Outdoor experiment
Chang & Fang, 2007	Retail storage	Iceberg lettuce	Plate count technique	Sanitization (organic acids)	Laboratory controlled experiment
Chase et al., 2017	Primary production	Romaine lettuce	Plate count technique	Agronomic practices	Outdoor randomized trials
Chase et al., 2019	Primary production	Romaine lettuce	Plate count technique	Irrigation Seasons	Outdoor randomized trials
Choi et al., 2011	Retail or consumer storage	Spinach	Plate count technique	Temperature Relative humidity Inoculum level	Laboratory controlled experiment
Cui et al., 2018	Retail or consumer storage	Lettuce	Plate count technique	Sanitization (cold nitrogen plasma)	Laboratory controlled experiment
Davidson et al., 2014	Processing	Iceberg lettuce	Plate count technique	Sanitization (chlorine)	Simulation study
Davidson et al., 2017	Processing	Iceberg lettuce	Plate count technique	Sanitization (organic acids)	Laboratory randomized trials
Delbeke et al., 2015	Retail and consumer storage	Spinach Butterhead lettuce Baby leaves Iceberg lettuce	Plate count technique	Temperature Package	Laboratory randomized experiment
Diaz & Hotchkiss, 1996	Retail and consumer storage	Iceberg lettuce	Plate count technique	Temperature Modified atmosphere	Laboratory randomized experiment
Ding et al., 2009	Retail storage	Iceberg lettuce	Plate count technique	Temperature Sanitization (alkaline electrolyzed water)	Laboratory randomized experiment
Dinu & Bach, 2011	Primary production	Iceberg lettuce	Plate count technique	Temperature	Greenhouse randomized trials
Dixit et al., 2021	Primary production	Red romaine lettuce	Plate count technique	Washing Sanitization	Laboratory multifactorial experiment

(Continues)

TABLE 1 (Continued)

Source	Stage in the value chain	Type of leafy greens	Enumeration method	Condition	Experimental design
Doering et al., 2009	Primary production to processing	Iceberg lettuce Spinach	Plate count technique	Temperature Sanitization (chlorine)	Laboratory randomized experiment
Ergönül et al., 2011	Retail or consumer storage	Lettuce	Plate count technique	Temperature	Laboratory controlled experiment
Erickson et al., 2010	Primary production	Leaf lettuce Spinach	Plate count technique	Irrigation	Outdoor randomized block study
Francis & O'Beirne, 2001	Retail storage	Iceberg lettuce	Plate count technique	Plant characteristics Temperature Package	Laboratory randomized experiment
Gleeson & O'Beirne, 2005	Processing	Butterhead lettuce	Plate count technique	Type of processing	Laboratory randomized experiment
Ibekwe et al., 2007	Primary production	Romaine lettuce	Real-time qPCR	Soil conditions	Randomized experiment—growth chamber
Ibekwe et al., 2009	Primary production	Romaine lettuce	Plate count technique	Plant characteristics	Randomized experiment—growth chamber
Ingram et al., 2011	Primary production	Baby spinach	Most probable number	Irrigation	Controlled experiment—growth chamber
Işık et al., 2020	Primary production	Leaf lettuce Radish green	Plate count technique	Soil conditions	Laboratory randomized experiment
Islam et al., 2004	Primary production	Leaf lettuce	Plate count technique	Soil conditions Irrigation water	Outdoor split-plot block design
Khalil, 2016	Retail storage	Spinach Lettuce Cabbage	Plate count technique	Temperature	Laboratory controlled experiment
Kim et al., 2013	Processing	Spinach Iceberg lettuce	Plate count technique	Temperature	Model simulation
Koseki & Isobe, 2005	Retail and consumer storage	Iceberg lettuce	Plate count technique	Temperature	Model simulation
Lee & Baek, 2008	Retail and consumer storage	Spinach	Plate count technique	Package Sanitization (chlorine)	Laboratory controlled experiment
Li et al., 2001	Processing	Iceberg lettuce	Plate count technique	Temperature Sanitization (chlorine)	Laboratory randomized experiment
Likotrafiti et al., 2013	Retail and consumer storage	Romaine lettuce	Plate count technique	Temperature	Laboratory randomized experiment

(Continues)

TABLE 1 (Continued)

Source	Stage in the value chain	Type of leafy greens	Enumeration method	Condition	Experimental design
Luo et al., 2009	Processing Retail and consumer storage	Baby spinach	Plate count technique	Temperature	Laboratory randomized experiment
Luo et al., 2010	Retail and consumer storage	Romaine lettuce Iceberg lettuce	Plate count technique	Temperature	Randomized block experiment
Madamba et al., 2022a	Primary production to processing	Romaine lettuce	Not applicable	Temperature Sanitization (chlorine)	Simulation study
Madamba et al., 2022b	Primary production to processing	Romaine lettuce	Not applicable	Temperature Sanitization (chlorination)	Simulation study
Mahmoud & Linton, 2008	Retail and consumer storage	Iceberg lettuce	Most probable number	Temperature Sanitization (chlorine)	Laboratory randomized experiment
Markland et al., 2013	Primary production	Lettuce	Most probable number	Plant characteristics Irrigation	Greenhouse randomized trials
McKellar et al., 2012	Distribution	Lettuce	Secondary data	Temperature	Secondary data
McKellar et al., 2014	Primary production	Lettuce	Secondary data	Plant characteristics	Secondary data
Min et al., 2017	Processing	Romaine lettuce	Plate count technique	Atmospheric cold plasma	Laboratory randomized experiment
Moyne et al., 2011	Primary production	Romaine lettuce	Plate count technique	Irrigation Plant characteristics Season	Outdoor randomized trials
Moyne et al., 2013	Processing	Romaine lettuce	Plate count technique	Season Growing environment	Laboratory controlled experiment Outdoor randomized trials
Moyne et al., 2020	Primary production	Romaine lettuce	Plate count technique	Plant characteristics Time of contamination	Outdoor randomized trials
Mukhopadhyay et al., 2021	Processing	Romaine lettuce	Plate count technique	Packaging Pulsed light treatment	Laboratory controlled experiment
Munther et al., 2020	Primary production	Romaine lettuce	Plate count technique	Temperature Relative humidity	Randomized trials—plant chamber
Neal et al., 2008	Processing	Baby spinach	Plate count technique	Sanitization (irradiation)	Laboratory randomized trials
Nou et al., 2011	Processing	Iceberg lettuce Baby spinach	Plate count technique	Sanitization (chlorine)	Laboratory randomized trials
Oliveira et al., 2010	Retail and consumer storage	Romaine lettuce	Plate count technique	Modified atmosphere Temperature	Laboratory controlled experiment
Ottoson et al., 2011	Primary production	Lettuce	Plate count technique	Light intensity Temperature	Growth chamber
Posada-Izquierdo et al., 2013	Processing Retail and consumer storage	Iceberg lettuce	Plate count technique	Sanitization (chlorine) Modified atmosphere	Laboratory randomized experiment

(Continues)

TABLE 1 (Continued)

Source	Stage in the value chain	Type of leafy greens	Enumeration method	Condition	Experimental design
Posada-Izquierdo et al., 2014	Retail and consumer storage	Lettuce	Plate count technique	Sanitization (neutralized electrolyzed water) Modified atmosphere	Laboratory controlled experiment
Puerta-Gomez et al., 2013	Retail and consumer storage	Baby spinach	Plate count technique	Temperature	Laboratory randomized experiment
Seidu et al., 2013	Primary production	Lettuce Cabbage	Real-time qPCR	Plant characteristics	Outdoor randomized trials
Sharma et al., 2011	Retail and consumer storage	Iceberg lettuce	Plate count technique	Packaging Temperature	Laboratory randomized experiment
Song et al., 2019	Retail and consumer storage	Spinach	Plate count technique	Packaging Temperature Sanitization (chlorine)	Laboratory controlled experiment
Solomon et al., 2003	Primary production	Romaine lettuce	Plate count technique	Irrigation water	Outdoor randomized trials
Theofel & Harris, 2009	Retail and consumer storage	Romaine lettuce	Plate count technique	Temperature	Greenhouse randomized experiment
Tyagi et al., 2019	Primary production-Harvesting	Romaine lettuce	Plate count technique	Season Relative humidity	Laboratory randomized experiment
Uzeh & Adepoju, 2013	Consumer storage	Lettuce Cabbage	Plate count technique	Temperature	Laboratory randomized experiment
Veys et al., 2016	Retail and consumer storage	Lettuce	Plate count technique	Temperature	Laboratory randomized experiment
Wang et al., 2012	Retail and consumer storage	Lettuce	Plate count technique	Temperature Relative humidity	Laboratory controlled experiment
Zeng et al., 2014	Transportation and retail storage	Romaine lettuce	Plate count technique	Temperature	Field surveillance study
Zhang et al., 2009	Primary production	Iceberg, romaine, and leaf lettuce	Plate count technique	Soil conditions	Laboratory controlled experiment

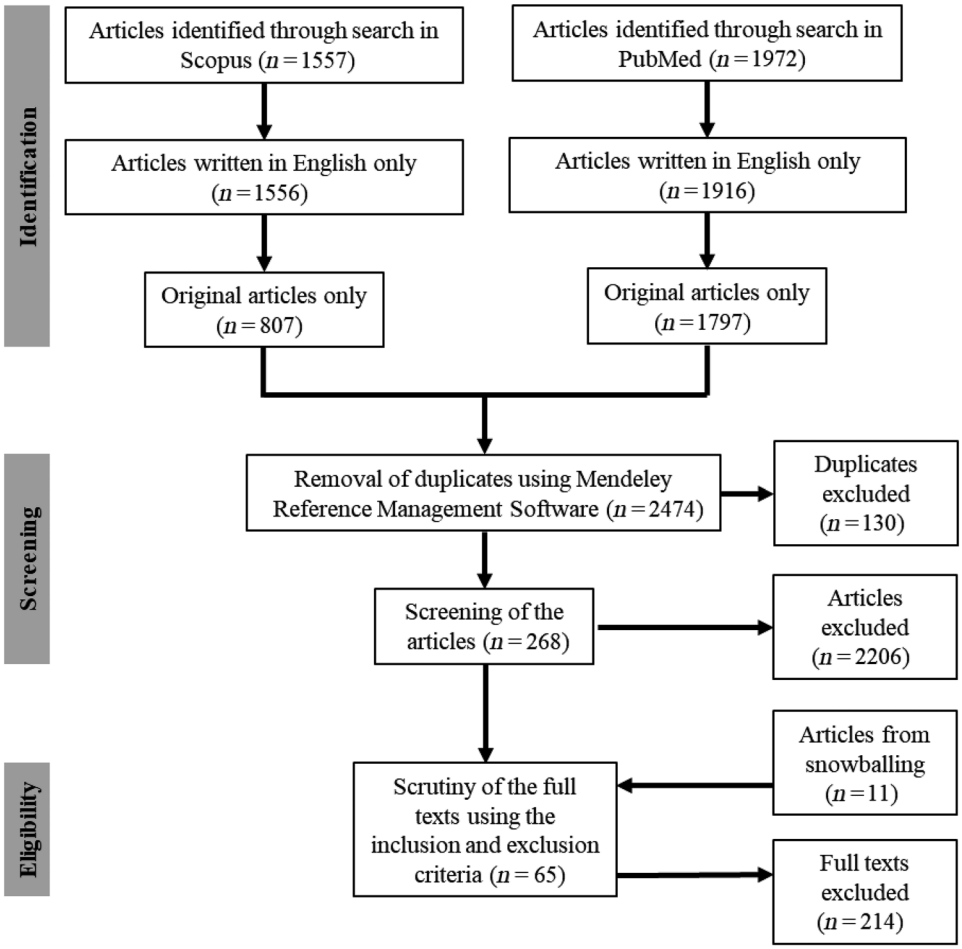

FIGURE 1 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flow chart of the systematic review of literature sources (Page et al., 2021).

application of manure and fertilizers, and the type of leafy green (Table 2).

3.2.1 | Atmospheric and environmental conditions

In documenting the effect of atmospheric and environmental conditions on the fate of STEC O157:H7, the articles narrowed down on weather data, temperature, RH, and light intensity. The effect of temperature and RH was investigated both in the outdoors and in plant growth chambers including greenhouses (Chase et al., 2017; Tyagi et al., 2019). Of the 10 studies that were reviewed, five investigated the effect of atmospheric/environmental conditions outdoors. Other conditions that were studied included light intensity and seasonal climatic effects (Chase et al., 2017, 2019; Ottoson et al., 2011).

Atmospheric/environmental temperature influences the growth and inactivation of STEC O157:H7 during the production of leafy greens. An initial inoculum level of ~8 log CFU/g of microbial cells on lettuce had a more rapid decline at 8°C compared to 16°C. Ottoson et al. observed that temperature and light intensity had an interaction in influencing the fate of STEC O157:H7 on lettuce grown in a climate chamber (Ottoson et al., 2011). With increasing light intensity (0 to 400 and 600 mml/m^2/s), the microbial inactivation increased at higher temperatures. Moyne et al. showed similar findings, showing that within a 2-h period, inoculation in the morning had a 2-log reduction compared to 0.5 log over 8–10 h in field-inoculated lettuce at night (Moyne et al., 2020). The higher survival during the night was also explained by the high RH. Stressful low temperatures of 8°C reduced the culturable STEC O157:H7 while increasing the transition to a persistent state (Dinu & Bach, 2011). The multistrain experiment established that STEC O157:H7 in lettuce attained a nonculturable state (viable but nonculturable [VBNC]) within 6 days postinoculation at 8°C temperature, compared to 12 days at 16°C. However, the study established that the switch

TABLE 2 Factors influencing the fate of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 on leafy greens during primary production.

Factors	Effect on STEC O157:H7 on leafy greens	Sources
Temperature	Affected the microbial growth and inactivation	Ottoson et al., 2011
	Low temperature increased transition to nonculturability (VBNC)	Dinu & Bach, 2011
Relative humidity	High humidity enhanced survivability	Moyne et al., 2020
	Dry conditions increased persister formation	Munther et al., 2020
	Affected inactivation rates	Belias et al., 2020
Light intensity	Affected the microbial growth and inactivation	Ottoson et al., 2011
Seasonal changes	Affected the growth and inactivation rates	Tyagi et al., 2019
Irrigation	Overhead irrigation increased contamination	Markland et al., 2013
	Contaminated water enhanced survival	Ingram et al., 2011; Islam, Morgan, et al., 2004; Solomon et al., 2003
	Continuous use of contaminated water increased produce contamination	Solomon et al., 2003
Soil conditions	Application of fecal slurry increased the microbial population.	Chase et al., 2017, 2019
	Application of manure increased survival on soil and produce	Islam, Doyle, et al., 2004
	Soil sanitization reduced pathogen survival	Ibekwe et al., 2007
Characteristics of the microbial cells	Influenced the inactivation rates	McKellar et al., 2014
	Influenced survival	Bezanson et al., 2012; Moyne et al., 2013
Vegetable species	Affected the contamination	Işık et al., 2020
	Affected the inactivation and growth rate	Belias et al., 2020; Seidu et al., 2013
	Contamination differed in different plant parts	Dixit et al., 2021; Erickson et al., 2010; Ibekwe et al., 2009; Zhang et al., 2009
Maturity of the vegetables	Affected survivability	Moyne et al., 2020
Occurrence of contamination event	Contamination event near harvesting increased survivability	Moyne et al., 2011

Abbreviation: VBNC, viable but nonculturable.

rate to the VBNC state was not influenced by the inoculum size.

RH affected the survival and growth/inactivation rate of the microbial pathogens on the phyllosphere of the plant (Belias et al., 2020; Moyne et al., 2020). An evaluation of a biphasic segmented log-linear model of the time-to-harvest interval of STEC O157:H7 on lettuce and spinach found that a higher RH resulted in a higher die-off rate in segment 1 and an earlier breakpoint for transition to segment 2 (Belias et al., 2020). The factorial experiment by Tyagi et al. (2019) combined the effect of RH and seasonal temperature and light exposure. Although the effect of RH was pronounced in the harvest season of June, a limited effect was noted in the harvest season of March. On the other hand, low RH (dry conditions) enhances the formation of the persister fraction of STEC O157:H7 on the lettuce phyllosphere (Munther et al., 2020). Under low RH, the formation of the persister fraction of STEC O157:H7 on the lettuce increased 49.6-fold to 0.221% of the culturable cells at 48 h.

3.2.2 | Type of irrigation

The type of irrigation and the quality of water used influenced the levels of contamination of leafy greens. Survival of STEC O157:H7 on leaves irrigated on the abaxial side (2.14 log CFU/g) was higher than those irrigated on the adaxial (0.44 log CFU/g) (Erickson et al., 2010). Contaminated irrigation water also enhances the survival of STEC O157:H7 in the produce. STEC O157:H7 was detected on the harvested lettuce 77 days post-irrigation (Islam, Morgan, et al., 2004). When uncontaminated water was used in either drip or overhead irrigation, there were no differences in the fate of STEC O157:H7 on the phyllosphere (Moyne et al., 2011). Similar findings were reported by Ingram et al. (2011) and Solomon et al. (2003) in irrigation water with low STEC O157:H7 counts (2 log CFU/mL). Moyne et al. (2011), however, point out that even under low levels of contamination, there was increased survival of STEC O157:H7 on the vegetables

(4 weeks postinoculation). On the other hand, the use of overhead irrigation with water contaminated with 4 log CFU/g STEC O157:H7 increased the contamination of the lettuce to 5 log CFU/g. Harvesting shortly after irrigation aggravated the risk of high contamination levels of the produce (Chase et al., 2019).

3.2.3 | Soil conditions

The interest in investigating the effect of the soil conditions on the microbial kinetics of STEC O157:H7 in leafy greens is due to differences in survival in different soils (Dixit et al., 2021; Ibekwe et al., 2007; Islam, Doyle, et al., 2004). The application of the fecal slurry in the soil introduced STEC O157:H7 for potential transfer to the phyllosphere of leafy greens (Chase et al., 2019). The use of inadequately composted manure is another agent of STEC O157:H7 contamination of leafy greens. In investigating the effect of the varied composition of compost manure on the persistence of STEC O157:H7 on the harvested lettuce and parsley, Islam, Doyle, et al. (2004) reported no difference; however, the overall effect of the use of any manure showed increased survival of up to 77–177 and 154–217 days on the produce and soil, respectively. To address the risk of long survival of pathogens in the soils, Ibekwe et al. (2007) fumigated the soils with methyl bromide and methyl iodide, which resulted in undetectable levels in the soil within 50 days. STEC O157:H7 in the nonfumigated soils survived for up to 90 days.

3.2.4 | Vegetable species

The type of plant and the intrinsic properties such as the chemical compounds and defense response influence the capacity of STEC O157:H7 to colonize, grow, and internalize in the edible portion. STEC O157:H7 was undetected on spinach, even by enrichment, 3 days postinoculation, whereas 14–17 days postinoculation, it was isolated from lettuce (Markland et al., 2013). In another study, Seidu et al. (2013) reported a decline of 3.2 logs of STEC O157:H7 on lettuce in a 10-day trial, which was higher than the 2.5 logs reported on cabbage over the same period. Poor adherence of the microorganisms to the phyllosphere of different plants has been corroborated as a factor that influences attachment. A study related this interspecies variation to different inactivation rates. Belias et al. (2020) reported that spinach showed faster (odds 1.77) biphasic segmented log-linear die-off rates in the first segment than lettuce in the fields.

Produce contamination in the fields also differs by plant parts. The inedible portion (plant parts 2 cm above growth media and below) where the microorganisms had better attachment had higher microbial counts in both the lettuce (2.85–5.38 log CFU/g) than the edible portions (plant parts beyond 2 cm above growth media; <1.00–1.83 log CFU/g) (Işık et al., 2020). Dixit et al. (2021) also reported a higher contamination of STEC O157:H7 on the roots of lettuce (∼5–7 log CFU/g) than on the leaves (∼6–9 log CFU/g). Another randomized experimental study in a growth chamber found that STEC O157:H7 in the root region of the plant survived for 21 days, whereas on the leaves, it survived only 7 days postinoculation (Ibekwe et al., 2009). Contamination on the leaves was also found to have marked differences; the abaxial (lower) side of the leaves exhibited higher microbial cell survival than the adaxial, upper (Erickson et al., 2010; Zhang et al., 2009).

One of the plant properties investigated by Moyne et al. (2020) was the maturity of the plants whereby it was established that STEC O157:H7 had better survivability in more mature leafy greens. Survival of microbial cells in 4-week-old plants 7 days postinoculation was only detectable by enrichment, whereas a population of 1.44–2.55 log CFU/plant was found in 6-week-old plants. It is noteworthy that this study controlled the effect of irrigation water.

3.2.5 | Inherent properties of microbial cells

One of the critical factors influencing the survival of microbial cells is the initial inoculum size and their physiological state (Bezanson et al., 2012; McKellar et al., 2014). The rate of inactivation of the STEC O157:H7 was found to be positively correlated with the initial inoculum size, with larger inoculum sizes leading to a higher rate of die-off (McKellar et al., 2014). However, it is noteworthy that the level of contamination investigated from the secondary data in this study may not be replicated under commercial production, where contamination levels are likely to be lower. While traditional techniques for enumerating microbial cells indicated a 100- to 1000-fold decline in cell numbers 24 h after inoculation, real-time quantitative polymerase chain reaction (qPCR) analysis revealed that this decline was primarily due to a transition to a nonculturable state rather than complete inactivation (Moyne et al., 2013). In enumerating the nonculturable fraction (difference between the live and culturable cells), live cells are distinguished from dead cells by using propidium monoazide (PMA; Biotum). PMA completely inhibits the amplification of deoxyribonucleic acid (DNA) from dead cells, allowing for accurate quantification of live cells. This change in physiological state is a critical factor contributing to the enhanced survival of these cells. Bezason et al., in finding that the die-off rates did not

significantly differ across different experimental sites with varying weather and soil conditions, elaborated that intrinsic factors and changes in physiological states enhanced cell survival (Bezanson et al., 2012).

3.3 | Processing

The second stage of the supply chain that was studied was processing, with a total of 20 studies. The documented processes included cutting or shredding (Gleeson & O'Beirne, 2005) and sanitizing and washing procedures (Davidson et al., 2017; Gleeson & O'Beirne, 2005). Processing of leafy greens can either result in growth or inactivation and cross-contamination, which affects microbial kinetics (Madamba et al., 2022b; Nou et al., 2011). Despite a number of studies treating the sanitization process such as chlorine treatment and washing as a single step (Madamba et al., 2022a, 2022b), this paper separately discusses antimicrobial treatments and washing in order to highlight the distinct conditions in the latter that influence microbial inactivation.

3.3.1 | Cutting or shredding and handling

Among various processing methods, such as the shredding of produce, certain practices pose a risk for microbial growth in leafy greens. Specifically, when produce is shredded using blunt knives, it can lead to increased susceptibility to microbial contamination. Research conducted by Gleeson and O'Beirne (2005) demonstrated that lettuce subjected to shredding with blunt knives exhibited a higher survival of STEC O157:H7. In contrast, produce that was cut using sharp razors maintained microbial counts at approximately 4.5 log CFU/g or lower, and these counts tended to decrease over time. Conversely, lettuce cut with blunt knives exhibited microbial counts exceeding 4.5 log CFU/g, with no observed decline.

3.3.2 | Washing

Although the washing of leafy greens can be done before or together with the application of sanitizers, it stands out as a separate process. Washing that excludes antimicrobial treatments increases the chances of cross-contamination of produce. In instances where the washing is combined with antimicrobial treatment, the dose rate of the free chlorine (FC) and chemical oxygen demand(COD) is also an important factor in determining a contamination event (Madamba et al., 2022a). Washing in chlorine solution has been shown to reduce pathogen concentration, including in cases of continuous use (Nou et al., 2011). Madamba et al. (2022b) reported contamination of 10% of the processed lettuce when washed with chlorine (6–21 ppm of free chlorine [FC]) compared to 80%–100% contamination levels when no chlorine was used. The simulation study established that >5 ppm of FC concentration induced inactivation of STEC O157:H7 in the lettuce, but <5 ppm of FC concentration increased the likelihood of contamination of the produce by the wash water.

3.3.3 | Antimicrobial treatments

Out of all the processes studied, sanitizing and washing procedures received the most attention, with a total of 16 articles. Among the antimicrobials identified in these studies, chlorine and its compounds were the focus of 12 articles (Abnavi et al., 2021; Davidson et al., 2014; Doering et al., 2009; Lee & Baek, 2008; Li et al., 2001; Madamba et al., 2022a, 2022b; Mahmoud & Linton, 2008; Nou et al., 2011; Posada-Izquierdo et al., 2014; Song et al., 2019; Tyagi et al., 2019), while organic acids were examined in two articles (Chang & Fang, 2007; Davidson et al., 2017). Additionally, nonconventional techniques such as irradiation, electrolyzed water, pulsed light treatment, and cold plasma were explored in four articles (Cui et al., 2018; Ding et al., 2009; Mukhopadhyay et al., 2021; Neal et al., 2008). Table 3 presents a summary of the antimicrobials that have been presented in the reviewed articles.

Use of chlorine in wash water

The inactivation of STEC O157:H7 on leafy greens during washing with chlorine is a function of time, concentration, pH of wash water, and organic load (Abnavi et al., 2021; Davidson et al., 2014; Posada-Izquierdo et al., 2013). Chlorine treatment (log-6 reduction of STEC O157:H7) of the fresh lettuce was achieved within 2 min for wash solutions with low initial chlorine concentrations (C_0) (≥0.25 ppm of FC) (Abnavi et al., 2021). Higher concentrations of C_0 (≥0.5 ppm of FC) achieved 99.9% inactivation of STEC O157:H7 in 10 s compared to 0.12 ppm that took 1 min. When washing lettuce with chlorine, higher organic loads result in less efficacy; Davidson et al. (2014) reported STEC O157:H7 reductions of >5 and <3.7 log CFU/mL for organic loads of 0% and 10%, respectively. In addition, temperature (13–14°C) of the wash water was found to be insignificant in increasing the efficiency of inactivation with chlorine. Li et al. (2001) showed that there was no significant difference in the reduction of microbial population treated with 20 ppm chlorine at 20 and 50°C wash water temperature.

Chlorination has its shortfalls, including the oxidation of produce (Nou et al., 2011) and the development of chlorine tolerance in STEC O157:H7, due to the upregulation

TABLE 3 Summary of studies of decontamination agents of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 on leafy greens.

Sanitizer	Mode of application	Concentration	Measurement of the parameters of the decontamination process	Sources
Chlorine-based compounds	Sodium hypochlorite in a batch process at 4°C	26 ppm (FC) for 30 s at pH 6.5	Every 2 min for 30 min	Abnavi et al., 2021
	Sodium hypochlorite in a continuous process at 4°C	12 (FC) ppm for 30 s at pH 6.5	Every 2 min for 30 min	Abnavi et al., 2021
	Acidified sodium hypochlorite (XY-12) in a flume washer at 12–15°C	50 ppm (TC) and 0.5 ppm (FC) for 90 s at pH 7.85 and 6.5	After every 10 min	Davidson et al., 2014
	Chlorine water (unspecified compound) in batch washing	50 ppm (FC) for 1 min at pH 7.0–9.5	Before wash cycle	Doering et al., 2009
	Sodium hypochlorite at elevated temperatures (20 and 50°C) in a batch process	20 ppm (FC) for 90 s at pH 7.0	Before wash cycle	Li et al., 2001
	Sodium hypochlorite at 22°C in a batch process	100 ppm (TC) for 5 min at pH 6.20–6.62	After the cycle	Lee & Baek, 2008
	Chlorine dioxide gas at 22°C	100 ppm for 5 min	After the cycle	Lee & Baek, 2008
	Chlorine water (unspecified compound) in a flume washer[a]	6–21 ppm (FC) for 1 min	Unspecified	Madamba et al., 2022a
	Chlorine dioxide gas at 22°C in a treatment chamber	5.0 ppm for 14.5 min	Every 2 min	Mahmoud & Linton, 2008
	6% sodium hypochlorite in a batch process at	20 ppm (FC) for 60 s at pH 6.5	Before and after wash cycle	Nou et al., 2011
	Sodium hypochlorite in a batch washing at 4°C	150 ppm (TC) for 30 s at pH 6.5	Before and after wash cycle	Posada-Izquierdo et al., 2013
	Sodium hypochlorite solution in a batch washing[a]	100 ppm (TC) for 1–2 s	After wash cycle	Song et al., 2019
	Sodium hypochlorite in a batch process[a]	40 ppm (FC) for 2 min at pH 6.93	Before wash cycle	Tyagi et al., 2019
Organic acids	Peroxyacetic acid in a flume washer at 12–15°C	50 ppm for 90 s	Before wash cycle	Davidson et al., 2017
	Peracid in a flume washer at 12–15°C		Before wash cycle	
	Rice vinegar (acetic acid) in a batch process at 25°C	0.05%–5% acetic acid (pH 4.09–3.0) for 5 min	Before wash cycle	Chang & Fang, 2007

(Continues)

TABLE 3 (Continued)

Sanitizer	Mode of application	Concentration	Measurement of the parameters of the decontamination process	Sources
Pulsed light treatment	Direct and in-package application at 22°C	1 s (Fluence ~1.05 J/cm^2) to 60 s (63 J/cm^2) at 21°C	Controlled during experiment	Mukhopadhyay et al., 2021
Irradiation	Direct application[a]	0.4–2.49 kGy	Controlled during experiment	Neal et al., 2008
Cold nitrogen plasma	Direct application[a]	400–600 W for 120 s	Before wash cycle	Cui et al., 2018
Electrolyzed water	Alkaline electrolyzed water produced from 0.1% sodium chloride using flow-type electrolysis generator[a]	2 L for 3 min	Controlled during experiment	Ding et al., 2009
	Neutral electrolyzed water at 4°C	3.5 g in 40 L for 30 s	Before wash cycle	Posada-Izquierdo et al., 2014

Abbreviations: FC, free chlorine; TC, total chlorine.

[a]Temperature during the treatment process was not specified, leading to the assumption that the experiment was conducted under ambient conditions.

of genes contributing to osmotic and oxidative stress resistance (Tyagi et al., 2019). Other than the chlorine-based solution, experimental trials have also explored the use of chlorine dioxide gas (ClO_2) for pathogen inactivation in lettuce and spinach (Lee & Baek, 2008; Mahmoud & Linton, 2008). Treatment of the produce with ClO_2 was done in a chamber with a regulated flow and fitted with a fan for continuous mixing. A *D*-value of 2.97 min at 5.0 ppm of ClO_2 gas and a *Z*-value of 16.2 ppm were determined for sanitization of lettuce contaminated with STEC O157:H7 (Mahmoud & Linton, 2008).

As opposed to other sanitization processes, the buildup of organic matter due to increasing wash cycles affects the efficacy of chlorine. Four studies had more than one point measurement of chlorine concentration for a wash cycle (Table 3). In order to avoid reducing the efficacy of the removal of STEC O157:H7, studies continuously monitored and replenished the FC concentrations (Abnavi et al., 2021; Davidson et al., 2014; Mahmoud & Linton, 2008). The batch experiments conducted in the studies were at a small scale of 20 L or less of chlorine water with continuous replenishing after every run (Abnavi et al., 2021; Davidson et al., 2014). Measuring the reduction of FC before and after a wash cycle showed declining efficacy in the decontamination process (Nou et al., 2011; Posada-Izquierdo et al., 2013). Abnavi et al. (2021) showed that an initial FC concentration of 0.12–0.5 ppm is completely depleted by the third cycle of washing lettuce with an initial inoculum size of 5–6 log CFU/g. Although Nou et al. (2011) included chlorine stabilizer (T-128) to reduce the depletion, the accumulation of 2% lettuce extract (organic load) still depleted chlorine after a wash cycle.

Organic acids and other nonconventional techniques

While the use of chlorine and organic acids is the most common in the antimicrobial treatment of leafy greens, there are other nonconventional techniques of irradiation, such as pulsed light treatment, electrolyzed water, and cold nitrogen plasma (Cui et al., 2018; Mukhopadhyay et al., 2021; Neal et al., 2008; Posada-Izquierdo et al., 2014). Washing lettuce in a flume with peroxyacetic acid and peracid for 90 s resulted in 0.97–1.74 and 1.35 log CFU/g reduction of STEC O157:H7, respectively (Davidson et al., 2017). Chang and Fang (2007) also treated shredded iceberg lettuce with rice vinegar (5% acetic acid) for 5 min, achieving a 3-log reduction of STEC O157:H7. The efficacy of the rice vinegar though was lost at a concentration of <0.5%, which achieved <1-log reduction of STEC O157:H7. The organic load of wash water did not affect the efficacy of the organic acids in the sanitization process. Sanitizer concentration in the experiment by Davidson et al. (2017) was monitored and adjusted every 10 min in an 890-L flume washer to undo any dilution, whereas Chang and Fang

(2007) used batch experiments, with the ratio of produce to wash solution being 1:4 (w/v).

Direct treatment of the lettuce with a pulsed light treatment at a dose of 31.5 J/cm^2 resulted in a 3.16-log reduction in the STEC O157:H7 in leafy greens (Mukhopadhyay et al., 2021). Increasing the dose of the pulsed light treatment had no proportional increase in the die-off rate of STEC O157:H7. The use of radiation was tested by Neal et al. (2008), who found that >0.40 kGy resulted in a >3.7-log reduction of STEC O157:H7. The two techniques—irradiation and pulse light treatment—must be performed with care to avoid any deleterious effects on the produce. Posada-Izquierdo et al. (2014) showed that neutral electrolyzed water was efficient in sanitizing produce, as it achieved longer lag times (temporary period of nonreplication of STEC O157:H7 cells in new media; 19.4 days) compared to chlorine treatment (5.9 days) at 8°C. On the other hand, alkaline electrolyzed water was shown to be ineffective in the sanitization of produce as the microbial population decreased by 0.5–0.6 logs after treatment (Ding et al., 2009).

3.4 | Transportation and distribution

The fate of STEC O157:H7 on leafy greens during this stage of the supply chain was only documented by four articles in the review. This stage accounts for transportation from the field to processing (Zeng et al., 2014), processing to retail (Doering et al., 2009; Koseki & Isobe, 2005; McKellar et al., 2012), and retail to consumer (McKellar et al., 2012; Zeng et al., 2014). The temperature of romaine lettuce in transit had a mean distribution of 2–8°C, a mode of 4°C, and a time of 0–45 h (Zeng et al., 2014). A study in Japan that analyzed the temperatures in the distribution chain of lettuce established a range of 3–15°C (Koseki & Isobe, 2005). Refrigerated storage of lettuce (<8°C) resulted in arrested growth of STEC O157:H7 during simulated transportation (Zeng et al., 2014). In their evaluation of microbial populations in leafy greens at the retail level, Doering et al. (2009) concluded that transportation temperature (whether 4 or 12°C) was not a significant factor.

3.5 | Consumer and retail storage

In this review, it was difficult to distinguish the studies as either consumer or retail storage due to limitations in the methods described by the authors, thus the two stages of the value chain have been presented together. A total of 34 articles focused on the fate of STEC O157:H7 on leafy greens in storage. Of these studies, only three were outrightly specifying the storage as either retail or consumer. Storage trials focused on conditions that have been broadly classified as storage, packaging, and sanitization processes in this review as shown in Table 4.

3.5.1 | Storage conditions

The storage conditions that were studied in the reviewed articles included temperature and RH (Choi et al., 2011; Delbeke et al., 2015; Likotrafiti et al., 2013). Thirty-one of the articles focused on the effect of temperature on the fate of STEC O157:H7 in leafy greens during storage. The models tested by Wang et al. (2012) showed that storage temperature had a greater influence on the fate of STEC O157:H7 on lettuce than RH. Storage of lettuce and other leafy greens at refrigerated temperatures (2–8°C) resulted in a log reduction of STEC O157:H7 (Abdul-Raouf et al., 1993; Delbeke et al., 2015; Ergönül, 2011; Mahmoud & Linton, 2008; Theofel & Harris, 2009). Abdul-Raouf et al. (1993) found >1-log reduction of STEC O157:H7 on lettuce that was stored at 5°C over 14 days notwithstanding the packaging technique. With increasing temperatures, from refrigerated storage to cold (8–15°C) and room temperature (25°C), STEC O157:H7 in the lettuce grew (Choi et al., 2011; Cui et al., 2018; Kim et al., 2013; Li et al., 2001; Noor et al., 2015; Puerta-Gomez et al., 2013; Theofel & Harris, 2009). However, the effect of temperature is dependent on RH, antimicrobial treatments, and type of packaging (Choi et al., 2011; Posada-Izquierdo et al., 2013; Zeng et al., 2014). Although Choi et al. (2011) registered a growth of STEC O157:H7 on spinach stored at 12 and 25°C temperatures and 100% RH, there was a decline in population for the same temperatures at 43% RH. At temperatures >25°C and RH >75%, the predictive models by Wang et al. (2012) established specific growth rates of 0.538–0.959 per hour, higher than 0.099 per hour reported at temperature 15°C and RH 75%.

Increasing RH favored the growth of STEC O157:H7 on leafy greens (Choi et al., 2011; Wang et al., 2012). Choi et al. (2011) found a population growth of 1-log cycle over 24 h of inoculation of lettuce stored at 100% RH and room temperature. For similar temperature conditions (30°C), higher specific growth rates were reported at 80% RH (0.870 per hour) than at 60% RH (0.705 per hour) (Wang et al., 2012).

3.5.2 | Packaging of processed products

Packaging has been used to influence the composition of the atmosphere surrounding the lettuce, which in turn affects the growth and inactivation of STEC O157:H7 (Lee & Baek, 2008; Luo et al., 2010). Use of packaging films with permeability of 3500 cm^3/m^2/day/atm at 23°C for both O_2 and CO_2 rather than 1100 cm^3/m^2/day/atm at 23°C resulted in a >1-log increase in STEC O157:H7 counts

TABLE 4 Factors influencing the fate of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 on leafy greens in storage.

Condition	Category of the effect	Classification of conditions	Sources
Temperature	Inactivation	Storage at refrigerated temperatures (1–5°C)	Abdul-Raouf et al., 1993; Ergönül, 2011; Luo et al., 2009; Mahmoud & Linton, 2008; McKellar et al., 2012; Song et al., 2019; Theofel & Harris, 2009
		Storage at cold temperatures (8–15°C) with low RH (<55%)	Choi et al., 2011; Khalil, 2016
		Storage at room temperature combined with antimicrobial treatments	Chang & Fang, 2007; Ding et al., 2009
		Storage at room temperature in acidic pH	Uzeh & Adepoju, 2013
	Growth	Storage at room temperature	Choi et al., 2011; Delbeke et al., 2015; Kim et al., 2013; Li et al., 2001; Likotrafiti et al., 2013; Noor et al., 2015; Puerta-Gomez et al., 2013; Theofel & Harris, 2009
		Storage at cold temperatures (8–15°C) combined with high RH (>85%)	Choi et al., 2011; Kim et al., 2013; Wang et al., 2017
		Packaged in permeable film	Oliveira et al., 2010
Sanitization processes	Inactivation	Treated leafy greens stored at refrigerated temperatures (2–8°C)	Doering et al., 2009; Posada-Izquierdo et al., 2013
		Treated leafy greens stored at room temperature	Cui et al., 2018; Doering et al., 2009
	Increase in the lag time	Treated leafy greens stored at cold temperatures (8–15°C)	Posada-Izquierdo et al., 2014; Zeng et al., 2014
Packaging (modified packaging)	Inactivation	Storage at refrigerated temperatures (2–8°C)	Francis & O'Beirne, 2001; Sharma et al., 2011
	Growth	Storage at cold temperatures (8–15°C)	Francis & O'Beirne, 2001; Gleeson & O'Beirne, 2005; Luo et al., 2010
	Reduced growth rate	Storage at cold temperatures (13°C)	Diaz & Hotchkiss, 1996
Cross-contamination	Decreased contamination	Washing with chlorine	Madamba et al., 2022a

Abbreviation: RH, relative humidity.

at room temperature in the first 24 h (Oliveira et al., 2010). Three different studies established that packaging on its own may not be of greater influence in the presence of other factors such as temperature and decontamination (Francis & O'Beirne, 2001; Gleeson & O'Beirne, 2005; Oliveira et al., 2010). Gleeson and O'Beirne (2005) found that the combination of modified atmosphere packaging and low-temperature storage resulted in a 2-log reduction in STEC O157:H7 counts on the produce over 10 days. A comparative analysis of refrigerated and room temperature storage of lettuce found that the former had a 1-log reduction and the latter had an increase of 4-log cycles (Oliveira et al., 2010).

3.5.3 | Use of sanitizers and other antimicrobial treatments

Building on the prior discussion in this review, using sanitizers such as chlorine and organic acids in adequate concentrations during processing leads to the inactivation or arrest of growth of STEC O157:H7 on leafy greens during storage (Posada-Izquierdo et al., 2013, 2014). The antimicrobial treatments that were evaluated in postprocessing storage trials included chlorination (Doering et al., 2009; Mahmoud & Linton, 2008), organic acids (Chang & Fang, 2007), and electrolyzed water (Posada-Izquierdo et al., 2014). Inefficient sanitization led to continued growth in storage at ≥8°C (Posada-Izquierdo et al., 2013). STEC O157:H7 counts in untreated spinach were 4.89–4.98 log CFU/g and continued to increase in storage, whereas treatment with chlorine dioxide resulted in an initial decline of 2.72–2.86 logs and declined further when stored in a modified atmosphere at 7 ± 2°C (Lee & Baek, 2008). The added effect of sanitization with neutral electrolyzed water on STEC O157:H7 in lettuce was an increase in the lagging time to 19 days above the untreated in storage (Posada-Izquierdo et al., 2014).

Though not a conventional treatment, the presence of bacteriophages affected the growth or inactivation of STEC

O157:H7 in leafy greens. Although it was detailed earlier that at ≥8°C, microbial growth was reported in leafy greens, Boyacioglu et al. (2013) reported a 1.99- to 3.99-log reduction in leafy greens, on which a cocktail of bacteriophages was added and stored at 10°C. The addition of STEC O157:H7 phages in lettuce reduced the microbial population from ~6 log CFU/g to nondetectable levels after 3 days of storage at 4–25°C (Cui et al., 2018). However, the studied phages were specific to STEC O157:H7 (Boyacioglu et al., 2013; Cui et al., 2018), limiting their industrial application to disinfecting leafy greens only against this specific pathogen and not other foodborne pathogens.

4 | SYNTHESIS OF THE RESULTS

The farm-to-table fate factors of STEC O157:H7 in leafy greens that have been studied in the reviewed articles can be categorized as follows: environmental, chemical, biological, and processing conditions.

4.1 | Environmental and atmospheric conditions

The environmental conditions are further reclassified into natural conditions where, for instance, there is an interaction among atmospheric conditions and indoor (controlled) environment where individual conditions can be investigated (Chase et al., 2017; Dinu & Bach, 2011; Munther et al., 2020). The agreement in the literature is that the effect of temperature cuts across all the stages of the value chain as it induces the proliferation or reduction of STEC O157:H7 in lettuce (Zeng et al., 2014). In the postharvest stage, dynamic temperature above the refrigerated conditions (>8°C) led to the growth of the pathogen, with the reverse causing inactivation (Abdul-Raouf et al., 1993; McKellar et al., 2012). Another common environmental condition that affected the inactivation or growth of STEC O157:H7 was RH, as in ambient conditions, increasing RH favored proliferation (Choi et al., 2011). Of all the stages, it is in storage that fluctuation of RH showed the greatest impact on the proliferation of STEC O157:H7 on leafy greens. Other conditions such as weather including rainfall were only documented and indicated in experiments conducted in the outdoor environment (Chase et al., 2017). The effects of weather for instance were not studied as standalone variables but as combined effects on fate. The combined effect of the weather on the inactivation of STEC O157:H7 was best observed by the biphasic model as the log-linear model underestimated the die-off rates (Belias et al., 2020). The environmental conditions influenced the switch rate of the biphasic segmented log-linear model from one segment to the other, indicative of a change in the die-off rates.

Initial contamination levels in the environment influenced the inactivation/growth rates of STEC O157:H7 in green vegetables (Ibekwe et al., 2007; Moyne et al., 2020). Although the contamination of the irrigation water was found to influence the transfer to leafy greens, the use of uncontaminated water did not aid the transfer (Solomon et al., 2003). At the production level, the use of known fomites such as fecal slurry or improperly composted animal manure is known to aggravate the risk of contamination. Reduction of the level of contamination in the environment reduces the proliferation of the microorganism in leafy greens (Ibekwe et al., 2007).

Interaction of the environmental conditions was shown to influence the fate of STEC O157:H7, in that the pronounced effects of one may be masked by the other. Wang et al. (2012) found that changes in temperature rather than RH at ambient conditions had a greater influence on the growth of STEC O157:H7 in lettuce during production. Some of the studies used biphasic models to observe the effect of the interactive factors on the kinetics of STEC O157:H7 on leafy greens (Belias et al., 2020; Seidu et al., 2013).

4.2 | Chemical conditions

Processing parameters including concentration and duration of application of antimicrobial treatments affected the growth or inactivation of the pathogen (Doering et al., 2009; Posada-Izquierdo et al., 2013). The effect of antimicrobial treatments on the fate of STEC O157:H7 is pronounced in processing, postprocessing transportation and distribution, and storage (Abnavi et al., 2021; Posada-Izquierdo et al., 2014). Chlorination was the most studied sanitization process and treated produce had lower counts of STEC O157:H7 during storage and distribution (Posada-Izquierdo et al., 2013). Other nonconventional treatments such as electrolyzed water and irradiation have also been evaluated with varying efficiencies in reducing STEC O157:H7 (Neal et al., 2008; Posada-Izquierdo et al., 2014). The sanitization processes are not without negative effects on the quality of the produce; thus, it is essential to get the concentration and duration of the treatment right for the efficiency of the microbial removal from leafy greens.

The inactivation models of STEC O157:H7 in leafy greens that showed the effect of the antimicrobials only were mainly first-order kinetics (Neal et al., 2008; Posada-Izquierdo et al., 2013). However, the nonlinear models were found to account for variability due to changes in the physiological state of microorganisms (Posada-Izquierdo

et al., 2013). The first-order log-linear models did not account for this variation due to stress-related transition to a persister state; thus, the biphasic models would best describe the inactivation due to the decontamination processes.

4.3 | Biological conditions

The biological conditions, in this case, included species of leafy greens and their intrinsic properties (Belias et al., 2020), characteristics of the microbial cells, and the presence of bacteriophages. At the primary production level, the interspecies variation influenced the survival and proliferation of STEC O157:H7 on leafy greens. The reviewed articles only investigated the effect of interspecies variation at the production level, and this was not pursued further to the processing and storage stages. The biphasic models used to explain the fate of STEC O157:H7 on different species of leafy greens showed better fitting of the cells on the outer leaves than the inner leaves of cabbage and lettuce (Seidu et al., 2013). In modeling the inactivation, a similar model that accounted for the physiological state, the biphasic segmented log-linear model, best described the changes in the kinetics due to interspecies variation (Belias et al., 2020).

5 | CONCLUSION

The review provides insights into the conditions that experimental and modeling studies must consider to better account for the accuracy of the generated inactivation/growth rates. The most studied conditions were temperature and chlorination, with the former affecting microbial kinetics from production to storage. From the detailed analysis of the 65 published articles with sufficient data to be modeled, the two stood out as having the greatest effects on the kinetics of STEC O157:H7 on leafy greens. The study points out the two conditions to have a multistage effect in the supply chain of green vegetables. The analysis also found that the study of the interactive effects of multiple conditions would best detail the fate modeling rather than the individual conditions that tended to adopt more of the log-linear models. The natural experiments detailed the interactive effects in the modeling as compared to the laboratory-scale experiments that rather controlled for the effects of other conditions. The most understudied area, however, is incorporating changes due to the heterogeneity of STEC O157:H7 in the kinetics. From this review, we propose the study of the known conditions, such as temperature and sanitization process, and the possible stressors that influence the composition of the microbial populations and switch rates, to enhance the accuracy of the inactivation and growth rates. Furthermore, the effect of the interactions on the switch rates of the microorganism to a nonculturable state and modeling of growth should be explored by future studies. Since pathogen survival varies among different types of leafy greens, future research should investigate the fate of pathogens in various types of produce.

AUTHOR CONTRIBUTIONS

Joshua Ombaka Owade: Investigation; writing—original draft; data curation; formal analysis; writing—review and editing. **Teresa Bergholz**: Funding acquisition; conceptualization; writing—review and editing; project administration. **Jade Mitchell**: Conceptualization; funding acquisition; writing—review and editing; methodology; supervision; formal analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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