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Review

Bacteriological safety of sprouts: A brief review

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ABSTRACT

The germination process causes changes in the chemical composition of seeds that improves the nutritional value of sprouts, while decreasing their microbiological safety, since the germination conditions are ideal for bacterial growth as well. This review explores the bacteriological safety of sprouts and their involvement in foodborne illness outbreaks, worldwide. Additionally, approaches to improve the shelf-life and microbiological safety of sprouts are discussed. According to the literature, sprout consumption is associated with more than 60 outbreaks of foodborne illness worldwide, since 1988. Alfalfa sprouts were most commonly involved in outbreaks and the most commonly implicated pathogens were *Salmonella* and pathogenic *Escherichia coli* (especially, Shiga toxin producing *E. coli*). In the pre-harvest stage, the implementation of good agricultural practices is an important tool for producing high-quality seeds. In the post-harvest stage, several methods of seed decontamination are used commercially, or have been investigated by researchers. After germination, seedlings should be kept under refrigeration and, if possible, cooked before consumption. Finally, microbiological analyses should be performed at all stages to monitor the hygiene of the sprout production process.

1. Introduction

Sprouts are of interest as a food owing to several factors, including rapid and easy cultivation, including more sustainable production, a consumer perception of values such as freshness and health, in addition to the high concentration of bioactive compounds (Reed et al., 2018). Sprouts are historical food, especially in Asian countries, where seedlings are traditionally consumed as an important component of cooking (Benincasa et al., 2019). Consumption of sprouts has also increased in popularity in other countries as a result of consumer demand for healthier and more exotic foods (Benincasa et al., 2019). In addition, consumption of sprouts has become popular throughout the world in the last decades, owing to consumer preferences for foods with high nutrient content and less processing (Peñas and Martínez-Villaluenga, 2020).

Sprouts are the product obtained from germination of seeds in water or other media, harvested before the leaves develop and intended to be consumed as a whole, including the seeds (EFSA, 2011). Sprouts are produced by first immersing viable seeds into water and then placing them in a warm, humid environment for an average of 3 to 7 days to encourage germination and sprout growth (NACMCF, 1999). Germinated seeds are usually sprouted in flasks, silos or rotating drums without light, often with recirculating water (Turner et al., 2020). Growing conditions depend on the type of sprout; however, temperature during sprouting is typically around 21–26 °C (NACMCF, 1999).

In recent years, sprouting has been identified as a promising strategy to improve the nutritional value of seeds (Peñas and Martínez-Villaluenga, 2020). Germination activates seed metabolism, leading to catabolism and degradation of macronutrients and antinutritional compounds, as well as biosynthesis of secondary metabolites with potential health benefits (Peñas and Martínez-Villaluenga, 2020).

On the other hand, consumption of raw sprouts has been identified to be an important risk factor for the occurrence of foodborne diseases (Proctor et al., 2001). In comparison with other fresh products, sprouts pose a challenge in terms of food safety, since the production of sprouts involves a process of seed germination that can promote the growth of pathogens (Ding et al., 2013). Contamination of sprouts by microbial pathogens is a major concern and presents a challenge because the ideal conditions for sprouting are also ideal for bacterial proliferation (Ding et al., 2013).

Considering that sprout consumption is gaining interest not only in the field of gastronomy or specialized nutrition, but also in the food industry, knowledge of the risks posed by foodborne pathogens and ways to reduce those risks are important tools for product safety. This review explores the bacteriological safety of sprouts and their

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association with foodborne diseases around the world. Additionally, methods to prevent bacterial proliferation in sprouts are discussed. Although sprouts can have biological contamination from bacteria, viruses, and fungi, the present review focuses specifically on bacterial contamination.

2. Bacterial pathogens and sprouts

The survey of foodborne diseases around the world allows an understanding of which foods and bacteria are most involved in outbreaks and how to prevent them. Therefore, estimates of foodborne disease are useful for policy makers in prioritizing resources to confront the problem of these diseases (Scharff, 2012). The consumption of fresh products is part of a healthy diet; however, contamination of these products with pathogens has resulted in serious consequences for public health (Scharff, 2012). Considering that fresh products are often consumed raw, without any cooking to eliminate harmful bacteria, there is an increased risk of exposure to foodborne pathogens (Carstens et al., 2019). Food outbreaks have been related to tomatoes, spinach, lettuce, and sprouts (Jung et al., 2014). Specifically sprouts have the potential to serve as vehicles for transmission of foodborne bacterial pathogens (Cui et al., 2017).

2.1. Outbreaks of bacterial foodborne diseases associated with sprouts worldwide

The Centers for Disease Control and Prevention (CDC, 2020a) defines a foodborne disease outbreak as two or more people contracting the same illness from the same contaminated food or drink. Carstens et al. (2019) summarized outbreaks of foodborne bacterial infection in the US, from 2010 to 2017, which were associated with different fresh products. The authors reported that the most frequently identified food vehicles within the vegetable category were sprouts (27.6%) (Carstens et al., 2019). Table 1 summarizes foodborne outbreaks related to sprout consumption in the world from 1988 to 2020. The outbreaks totaled 14,739 cases, with about 214 hospitalizations and 58 deaths. There were at least 64 outbreaks related to sprouts in that period, and alfalfa was involved in more than 55% of them, followed by beans and clover sprouts. Table 1 also shows that the most frequently isolated pathogens in these outbreaks were *Salmonella* spp. and pathogenic *Escherichia coli* (*E. coli*).

In 2011, a sprout-related outbreak with 3842 cases of human infection with *Escherichia coli* O104: H4 occurred in Germany (Beutin and Martin, 2012). The outbreak was caused by one strain bearing the virulence factors of two different diarrheagenic *E. coli* pathotypes, enteroaggregative *E. coli* (EAEC) and Shiga toxin-producing *E. coli* (STEC) (Santos et al., 2020). The World Health Organization has reported cases involving this outbreak in Denmark, United Kingdom, United States, Sweden, France, Austria, Switzerland, Norway, Spain, and the Netherlands; most of the people affected had travelled to Germany before the onset of their disease (WHO, 2011). More than 800 patients developed hemolytic-uremic syndrome, which is a complication of *E. coli* infection that destroys red blood cells and leads to kidney damage (Beutin and Martin, 2012). Fifty-three deaths were attributed to this outbreak (Beutin and Martin, 2012).

An outbreak of *E. coli* O157:H7 (STEC) occurred in Japan in 1996 and had the highest number of confirmed cases of illness linked to sprout to date. Most of the patients were children who had had lunch in primary schools in the target city (Watanabe et al., 1999). Epidemiological research identified white radish sprouts, a popular food in Japan, as the food with the greatest association with outbreak cases (Watanabe et al., 1999). The sprouts in question had been produced on a specific farm. In this outbreak, more than 6000 cases were reported, with 398 hospitalizations and 3 deaths (Taormina et al., 1999). At least two additional outbreaks of *E. coli* have occurred in Japan (Taormina et al., 1999).

Another outbreak with a high number of cases (552 patients) was reported in Canada in 2005 and was related to *Salmonella* contamination in mung bean sprouts (Nesbitt et al., 2012). In addition to *E. coli* and *Salmonella*, *Listeria monocytogenes* has been involved in outbreaks of illnesses associated with sprouts, such as the two cases that occurred in the United States in 2008 and 2014 involving alfalfa and bean sprouts, respectively (Garner and Kathariou, 2016).

2.2. Bacteriological contamination of sprouts

Microbiological contamination of sprouts can be attributed to many sources of pre- and post-harvest contamination, which include seeds, germination medium, and soaking water, as well as transport, handling, and storage of seedlings. Sources of contamination before harvest include irrigation water, soil, human and animal faeces, the presence of insects, and human manipulation (Iwu and Okoh, 2019). After harvesting, sources of contamination include equipment, tools and containers used for harvesting, water for washing harvested products, ice, vehicles, and handlers (Olaimat and Holley, 2012). According to the U.S. Food and Drug Administration, most sprout-related outbreaks were caused by seeds contaminated with bacterial pathogens before the beginning of the germination process (NACMCF, 1999). Many microorganisms can survive for months under the dry conditions used for seed storage (NACMCF, 1999). However, the distribution of pathogenic microorganisms on the surface of seeds is low and heterogenous, making them difficult to detect by routine testing (Cui et al., 2017).

The contamination of sprout seeds is likely to occur in the field (FDA, 2015). Once contaminated, sprouts present an ideal environment for the replication of bacteria, such as *Salmonella* (Fahey et al., 2006). During the germination process, increased humidity, warm temperatures and high availability of nutrients in the seeds themselves result in rapid proliferation of *Salmonella* (Brankatschk et al., 2014). If bacterial contamination is present in seeds or production equipment, or introduced by insects or farm workers, rapid bacterial growth will occur owing to high water activity during the germination process, which results in contamination of the entire lot (Turner et al., 2020).

The isolation of *Salmonella* spp. from sprouts and their seeds suggests that enteric pathogens can multiply and persist for long periods of time during sprout production (Brankatschk et al., 2014). Only minimal levels of *Salmonella* spp. are required for contamination, as the pathogens can rapidly multiply during the sprouting manufacturing processes (Brankatschk et al., 2014).

Numerous factors may affect bacterial interactions with seed surfaces. Cui et al. (2017) investigated the ability of four *S. enterica* strains, three *E. coli* O157:H7 strains, and one *E. coli O104:H4* strain to attach to four different types of plant seeds (alfalfa, fenugreek, tomato, and lettuce). The study showed that, on average, *S. enterica* had higher attachment ratios than *E. coli*. The *E. coli* O104:H4 used in this study had the lowest attachment potential. The numbers of bacterial cells attached to plant seeds were generally higher than those recovered from seed wash water. This suggests that more cells of these two bacteria were weakly associated with the surface of the seeds and more easily washed away (Cui et al., 2017).

Another study aimed to observe the physiological behavior of four *Salmonella* strains (*S.* Baildon, *S.* Cubana, *S.* Montevideo, and *S.* Stanley), three *E. coli* O157:H7 strains and one *E. coli* O104:H4 strain that were isolated from fresh produce-associated outbreaks of human gastrointestinal infections. *Salmonella* and *E. coli* cells were artificially internalized in plant seeds during the germination process. Surface decontaminated seeds of alfalfa, fenugreek, lettuce, and tomato were infiltrated in a vacuum with *Salmonella* or *E. coli*. The artificially internalized *Salmonella* and *E. coli* in plant seeds caused the contamination of different sprout/seed tissues, and the pathogenic growth in the germination seeds is dependent on bacterial species and type of plant seeds. The average *Salmonella* populations recovered were larger than the *E. coli* populations. It should be noted that a discussion based on the *E. coli* O157:H7 and *E. coli* O104:H4 strains would allow better interpretation of the results. The authors warned that sprouts, often

Table 1
Foodborne outbreaks related to sprout consumption in the world from 1988 to 2020.

Date	Location	Organism	Food carrier	Total no. of ill	Number of people hospitalized	Number of deaths	References
2020	USA	*E. coli* O103 (STEC)	Clover sprouts	51	3	0	(CDC, 2020b)
2017–2018	USA	*Salmonella* Montevideo	Raw sprouts	10	0	0	(CDC, 2018)
2016	USA	*Salmonella* serotype Reading and *Salmonella* serotype Abony	Alfalfa sprouts	36	7	0	(CDC, 2016a)
2016	USA	*E. coli* O157 (STEC)	Alfalfa sprouts	11	2	0	(CDC, 2016b)
2015–2016	USA	*Salmonella* Muenchen and *Salmonella* Kentucky	Alfalfa sprouts	26	8	0	(CDC, 2016c)
2015	USA	*Salmonella* Paratyphi B variant L(+) tartrate (+)	Raw brand sprouted nut butter spreads	13	0	0	(CDC, 2015a)
2014	USA	*Salmonella* Enteritidis	Bean sprouts	115	28	0	(CDC, 2015b)
2014	USA	*Listeria monocytogenes*	Mung bean sprouts	5	5	2	(Garner and Kathariou, 2016)
2014	USA	*Salmonella* Newport, *Salmonella* Hartford and *Salmonella* Oranienburg	Organic sprouted chia powder	31	5	0	(CDC, 2014a)
2014	USA	*E. coli* O121 (STEC)	Raw clover sprouts	19	7	0	(CDC, 2014b)
2011	USA	*E. coli* O26 (STEC)	Raw clover sprouts	29	7	0	(CDC, 2012)
2011	France	*E. coli* O104:H4 (EAEC/STEC)	Sprouts	16	8	0	(EFSA, 2011)
2011	USA	*Salmonella* Enteritidis	Alfalfa and spicy sprouts	25	3	0	(CDC, 2011a)
2011	Germany	*E. coli* O104:H4 (EAEC/STEC)	Sprouts	3842	Unknown	53	(Beutin and Martin, 2012)
2010	USA	*Salmonella*	Alfalfa sprouts	140	Unknown	0	(CDC, 2011b)
2010	United Kingdom	*Salmonella* Bareilly	Bean sprouts	190	Unknown	Unknown	(Yang et al., 2013)
2010	USA	*Salmonella* Newport	Alfalfa sprouts	44	7	0	(CDC, 2010)
2009	Finland	*Salmonella* Bovismorbificans	Alfalfa sprouts	42	Unknown	Unknown	(Yang et al., 2013)
2009	Canada	*Salmonella* Cubana	Onion sprouts	20	Unknown	Unknown	(Kozak et al., 2013)
2009	USA	*Salmonella* Saintpaul	Alfalfa sprouts	235	8	0	(CDC, 2009)
2008	USA	*Salmonella* Typhimurium	Alfalfa sprouts	24	Unknown	Unknown	(Yang et al., 2013)
2008	USA	*Listeria monocytogenes*	Sprouts	20	16	0	(Garner and Kathariou, 2016)
2007	Norway, Denmark and Finland	*Salmonella* Weltevreden	Alfalfa sprouts	45	Unknown	Unknown	(Emberland et al., 2007)
2007	Sweden	*Salmonella* Stanley	Alfalfa sprouts	51	Unknown	Unknown	(Werner et al., 2007)
2007	USA	*Salmonella* Montevideo	Bean sprouts	24	3	0	(Dechet et al., 2014)
2006	Sweden	*Salmonella* Bareilly and *Salmonella* Virchow	Mungo beans sprouts	115	Unknown	Unknown	(de Jong et al., 2007)
2006	USA	*Salmonella* Braenderup	Bean sprouts	4	0	0	(CDC, 2006)
2005	Canada	*Salmonella*	Mung bean sprouts	552	Unknown	Unknown	(Nesbitt et al., 2012)
2004	USA	*Salmonella* Bovismorbifans	Alfalfa sprouts	35	5	Unknown	(CDC, 2004)
2004	USA	*E. coli* O157:NM	Alfalfa sprouts	2	Unknown	Unknown	(CDC, 2004)
2003	USA	*Salmonella* Chester	Alfalfa sprouts	26	Unknown	Unknown	(CDC, 2003)
2003	USA	*E. coli* O157:NM	Alfalfa sprouts	13	1	0	(Ferguson et al., 2005)
2003	USA	*Salmonella* Saintpaul	Alfalfa sprouts	16	Unknown	Unknown	(CDC, 2003)
2003	USA	*E. coli* O157	Alfalfa sprouts	7	2	0	(Ferguson et al., 2005)
2002	USA	*E. coli* O157:H7	Alfalfa sprouts	15	Unknown	Unknown	(Yang et al., 2013)
2002	USA	*Salmonella* Enteritidis	Mung bean sprouts	15	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2001	USA	*Salmonella* Kottbus	Alfalfa sprouts	31	3	0	(Mohle-Boetani et al., 2001)
2001	USA	*Salmonella* Enteritidis	Mung bean sprouts	33	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2001	Canada	*Salmonella* Enteritidis	Mung bean sprouts	84	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2001	USA	*Salmonella* Enteritidis	Mung bean sprouts	22	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2000	The Netherlands	*Salmonella* Enteritidis	Mung bean sprouts	27	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2000	Canada	*Salmonella* Enteritidis	Mung bean sprouts	10	Unknown	Unknown	(Mohle-Boetani et al., 2009)
2000	USA	*Salmonella* Enteritidis	Mung bean sprouts	75	Unknown	Unknown	(Mohle-Boetani et al., 2009)
1999	USA	*Salmonella*	Alfalfa sprouts	34	Unknown	Unknown	(CDC, 1999)

(*continued on next page*)

Table 1 (*continued*)

Date	Location	Organism	Food carrier	Total no. of ill	Number of people hospitalized	Number of deaths	References
1999	Canada	*Salmonella*	Alfalfa sprouts	51	Unknown	Unknown	(Stratton et al., 2001)
1999	USA	*Salmonella* Muenchen	Alfalfa sprouts	157	6	Unknown	(Proctor et al., 2001)
1999	USA	*Salmonella* Saintpaul	Clover sprouts	36	2	0	(CDC, 1999)
1999	USA	*Salmonella* Mbandaka	Alfalfa sprouts	87	Unknown	0	(Gill et al., 2003)
1999	USA	*Salmonella* Typhimurium	Clover sprouts	112	3	Unknown	(Brooks et al., 2001)
1998	USA	*E. coli* O157:NM (STEC)	Alfalfa and clover mixed sprouts	8	2	0	(Mohle-Boetani et al., 2001)
1998	USA	*Salmonella* Havana	Alfalfa sprouts	18	0	0	(Mohle-Boetani et al., 2001)
1998	USA	*Salmonella* Cubana	Alfalfa sprouts	22	22	1	(Mohle-Boetani et al., 2001)
1997	Canada	*Salmonella* Meleagridis	Alfalfa sprouts	78	Unknown	Unknown	(Taormina et al., 1999)
1997–1998	USA	*Salmonella* Senftenberg	Alfalfa-clover mixed sprouts	60	1	0	(Mohle-Boetani et al., 2001)
1997	USA	*E. coli* O157:H7	Alfalfa sprouts	85	Unknown	Unknown	(Taormina et al., 1999)
1997	USA	*Salmonella* serotypes Infantis and Anatum	Alfalfa sprouts	109	Unknown	Unknown	(Taormina et al., 1999)
1997	Japan	*E. coli* O157:H7 (EHEC)	White radish sprouts	126	Unknown	Unknown	(Taormina et al., 1999)
1996	Japan	*E. coli* O157:H7 (STEC)	White radish sprouts	6000	Unknown	Unknown	(Taormina et al., 1999)
1996	USA	*Salmonella* Meleagridis	Alfalfa sprouts, clover sprouts, alfalfa-clover mixed sprouts	75	7	0	(Mohle-Boetani et al., 2001)
1996	USA	*Salmonella* Montevideo	Alfalfa sprouts	417	10	1	(Mohle-Boetani et al., 2001)
1995	USA and Finland	*Salmonella* Stanley	Alfalfa sprouts	242	Unknown	Unknown	(Mahon et al., 1997)
1995	USA and Denmark	*Salmonella* Newport	Alfalfa sprouts	133	Unknown	Unknown	(Taormina et al., 1999)
1994	Sweden and Finland	*Salmonella* Bovismorbificans	Alfalfa sprouts	595	Unknown	Unknown	(Ponka et al., 1995)
1988	United Kingdom	*Salmonella* Saintpaul	Mungo sprouts	143	Unknown	Unknown	(Taormina et al., 1999)

consumed raw, have a potential health risk for consumers (Liu et al., 2018). Thus, people who are at greater risk of complications, such as young children, the elderly, as well as immunocompromised and chronically ill people should avoid eating sprouts without further treatment (Gill et al., 2003; van Duynhoven et al., 2002).

3. Prevention of sprout contamination

3.1. Implementation of good practice programs

The primary step to prevent the occurrence of bacteriological contamination is to respect the preventive measures, including Good Agricultural Practices, on-Farm Food Safety Management Practices, Good Manufacturing Practices and Sanitation Standard Operating Procedures in primary production, postharvest handling and processing (EFSA, 2011). As is the case with other green leaf and salad products, more assessments based on risk factors and the probability of hazards occurring should be carried out (Soon et al., 2013).

Fig. 1 shows a summary of safety measures to reduce seed contamination used for germination, as well as sprouts. According to Fig. 1, many procedures must be performed adequately to prevent sprout contamination, including pre-harvest, post-harvest, and germination process activities. At pre-harvest, the procedures are related to seed quality and crop hygiene procedures. The United States Department of Agriculture (USDA, 2011) has established Good Agricultural Practices to reduce the risk of contamination of fresh produce. These recommendations include irrigation water quality, manure management, and also health and hygiene of farm workers. At post-harvest, seeds will need good handling and sanitization procedures, as discussed below. It is recommended that at least two seed disinfection procedures should be chosen. After disinfection, seeds should be stored in a dry, insect-free and hygienic place. During the germination process, good handling practices need to be implemented. Pre-germination soaking may also cause cross contamination (EFSA, 2011). Use of potable water for the production of sprouts is important. After germination, sprouts should be kept refrigerated to prevent the growth of pathogenic bacteria during the storage of sprouts (EFSA, 2011). Sprouts are highly perishable owing to the high respiratory rate, which can be controlled by low storage temperature. Temperature control is a critical point in the distribution and handling of sprouts, since exposure (30 min) to a temperature of 20 °C can reduce shelf life by up to 50% (Warriner and Smal, 2014). According to Rediers et al. (2009), fresh produce should be kept at temperatures below 5 °C to reduce the proliferation of spoilage microorganisms and human pathogens. However, defining the optimal storage temperature is not so simple and depends on the cold sensitivity of each species (Benincasa et al., 2019).

Cooking sprouts is highly effective at eliminating pathogens; however, sprouts are often consumed raw, and the sensory changes resulting from cooking maybe unappealing to consumers (Gill et al., 2003). A recent review approached the use of sprouts in the development of food products (Miyahira et al., 2021). The authors noted that most studies used sprout flours to replace wheat flour in bakery products to enhance the nutritional value of food products. The authors also concluded that the acceptance of these products depended on the type of sprout used and the amount of traditional flour that was substituted.

If cooking is not an option, sprouts can be sanitized before

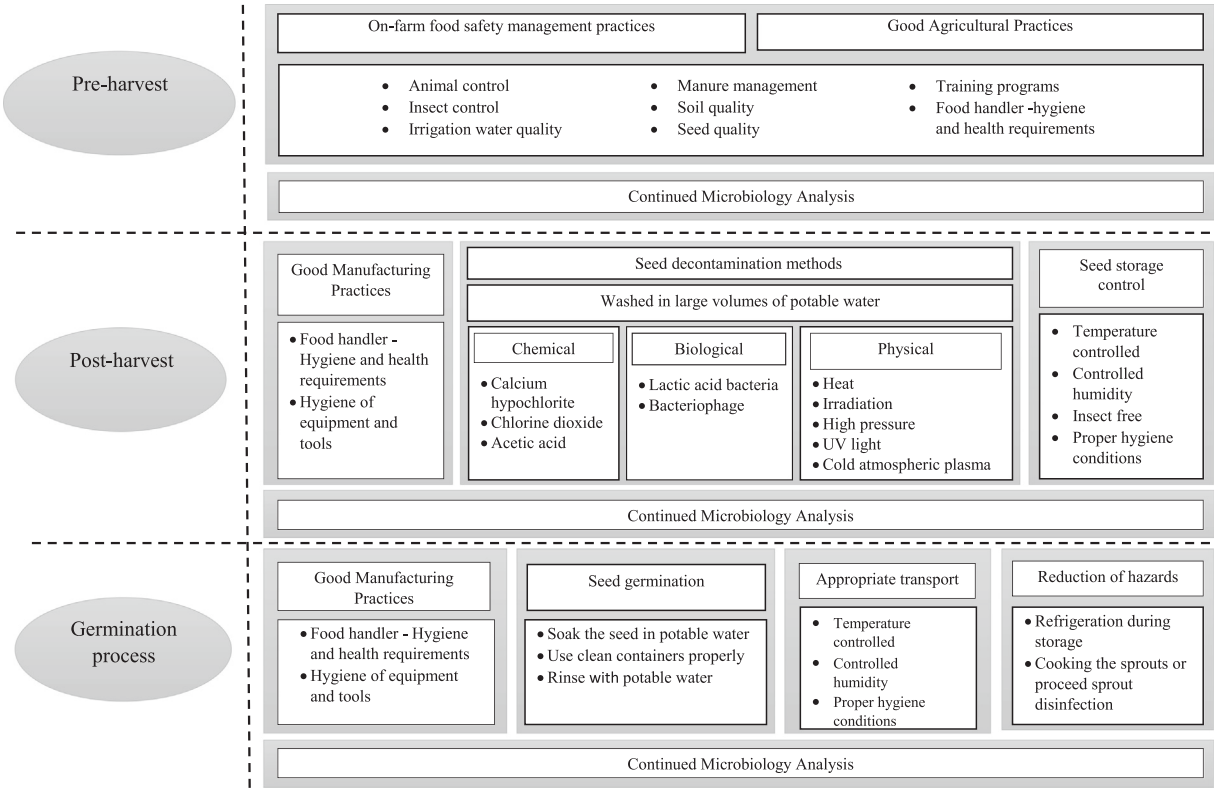

Fig. 1. Safety measures to reduce seed and sprout contamination. The safety measures were divided into three control steps: pre-harvest, post-harvest and during the germination process. In the pre-harvest stage, on-farm food safety management practices and good agricultural practices must be implemented. The post-harvest phase included good manufacturing practices, seed decontamination procedures and seed storage control. In the seed germination stage, good manufacturing practices, adequate procedures for production and transport of sprouts, as well as measures to reduce hazards before consumption need to be rigorously performed. Microbiological analyses should be carried out throughout the sprout production process, including spent irrigation water testing.

consumption in the same way as fresh vegetables by immersion in 200 ppm sodium hypochlorite solution and subsequent rinsing (NACMCF, 1999; Nagar et al., 2016).

Microbiological analyses of both seeds and sprouts are recommended to evaluate if the procedures performed at each stage are being effective in reducing the bacterial load of sprouts. It should be noted that, during the industrial germination process, spent irrigation water testing for pathogenic bacteria has been proposed as an alternative strategy to analyze a large number of sprout samples (EFSA, 2011). According to Fu et al. (2001), spent irrigation water has some advantages as an analytical sample. Water is easier to collect than sprouts because it is collected as runoff from the drum rotation, and it is generally easier to analyze than sprouts because there is no need for homogenization. Finally, it can provide a more representative sample of the batch of sprouts than individual sprout samples (Fu et al., 2001).

3.2. Seed decontamination for sprout production

Appropriate seed decontamination treatments must inactivate pathogenic microorganisms while preserving the viability, germination and vigor of seeds (NACMCF, 1999). A complete elimination of bacterial contamination by seed disinfection treatments in sprout production is very difficult to achieve (EFSA, 2011). According to European Food Safety Authority (EFSA, 2011), there is no decontamination method that ensures the elimination of pathogens in all types of seeds without affecting the germination process. Seed decontamination should be optimized for each type of seed (EFSA, 2011). Therefore, seed disinfection should be complemented with other preventive strategies, such as good agricultural practices (cleaning, storage, and handling of seeds), good manufacturing practices, and continued risk analysis (Escamilla et al., 2019), as discussed above.

The seeds of different plant species differ in susceptibility to antimicrobial agents, which determines their ability to germinate and grow after treatment (NACMCF, 1999). Therefore, many studies have evaluated different methods of seed disinfection (chemical, biological and physical methods), as well as the impact of these processes on seed germination capacity. First of all, seeds should be washed in large volumes of potable water to remove dirt and increase the effectiveness of decontamination treatment (ESFA, 2011).

3.2.1. Chemical methods

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) recommends that pathogens be reduced by 5 log cycles of CFU in seeds and, for this purpose, producers of seedlings can use one or more methods of sanitization. It is important to highlight that a 5 log cycle reduction may not reduce a pathogen count to zero. Considering a pathogen count of 10^5 CFU/g, with a 5 log cycle reduction by a sanitization process, the concentration of this microorganism would reduce to 1 CFU/g. According to NACMCF, seed disinfection with 20,000 ppm calcium hypochlorite, prior to germination, appears to be effective in reducing outbreaks of foodborne diseases (NACMCF, 1999). However, a review of the literature showed that this disinfection method causes, in most cases, a reduction of only 2.5 log cycles of CFU (Montville and Schaffner, 2004). In some cases, pathogens were reduced in microbiological tests, but when the enrichment steps of the analysis were performed, the bacteria were recovered (Montville and Schaffner, 2004). The use of high concentrations of chlorine can result in dangerous fumes and cause skin irritation, as well as form potentially dangerous organochlorine compounds after seed treatment (Weissinger and Beuchat, 2000). Therefore, identification of other chemical disinfectants is desirable. A study by Weissinger and Beuchat (2000) reported that solutions containing 8% hydrogen peroxide, 1% calcium hydroxide,

and 1% calcinated calcium were able to reduce the amount of *Salmonella* by 3.2, 2.8, and 2.9 log10 CFU/g, respectively, without significantly reducing the germination percentage of alfalfa seeds.

Another study by Rajkowski and Ashurst (2009) showed that using 1% peroxyacetic acid sanitizer achieved a consistent reduction of *Salmonella* or *E. coli* O157:H7 in artificially contaminated alfalfa seeds.

Finally, it should be noted that the use of chemical disinfectants can be viewed by some consumers as contradictory to the marketing of sprouts as 'natural' or 'healthy' foods (Sikin et al., 2013).

3.2.2. Biological methods

The use of protective cultures, particularly lactic acid bacteria (LAB), has been proposed for minimally processed products, but its application has been limited industrially. Considering that LAB are capable of growing in the germination environment, competitive inhibition could provide a method to control pathogens throughout the germination process (Wilderdyke et al., 2004). A study conducted by Wilderdyke et al. (2004) aimed to isolate, identify, and screen LAB for use as competitive inhibitors toward foodborne pathogens in germinated alfalfa seeds. The results of the study showed that the isolated LAB cultures presented antimicrobial properties *in vitro*; however, further studies are needed to determine if these cultures will inhibit pathogens *in situ* (in germinated alfalfa seeds).

Bacteriophage have also been proposed for the control of bacterial pathogens on produce. According to Fong et al. (2017), the bacteriophage treatment of produce is an underdeveloped, an emerging subject of interest and is currently not widely used in industry. The authors performed a study to evaluate the use of four lytic bacteriophages infecting *Salmonella* to determine the suitability of their use for biocontrol in the production of alfalfa sprouts. The results revealed that the four phages had desirable characteristics to be used for biocontrol. Nevertheless, further studies should be carried out to optimize this treatment as well as to evaluate its efficacy in other varieties of sprouts (Fong et al., 2017). Considering the need for alternative methods for inactivation of *Salmonella* in seed sprouts, another study was undertaken to characterize *Salmonella* phages and to assess the potential of these phage isolates to control *Salmonella in vitro* and in experimentally contaminated seed sprouts. The authors concluded that bacteriophage-based treatment has a potential to be developed as an alternative strategy for control of *Salmonella* spp. in sprouts. However, the present study shows that not all combinations of phage types were effective in controlling bacterial contamination (Kocharunchitt et al., 2009).

3.2.3. Physical methods

Another review of the literature reported that physical treatments, especially at high pressure, produced better disinfection results than those obtained by 20,000 ppm of calcium hypochlorite treatment (Ding et al., 2013). Chemical and biological treatments are limited to surface inactivation, while physical methods offer better penetration, to increase the impact on internalized or sheltered microorganisms and may be more readily adapted for commercialization (Sikin et al., 2013). Physical methods have been developed with the aim of reducing the microbial load without impairing seed germination ability such as heat, high pressure, irradiation, UV light, and cold atmospheric pressure plasma (Bari et al., 2010; Hertwig et al., 2018; Neetoo et al., 2009). It should be noted that combination of chemical and physical treatments has also shown to be an effective strategy, *e.g.*, the use of hot water combined with chlorine treatment, as well as the use of ClO2 associated with drying and dry heating.

3.2.3.1. Heat. According to Bari et al. (2010), many mung bean sprout growers in Japan, Europe and the United States use hot water treatment machines for seed disinfection. Use of hot water in addition to chlorine treatment could reduce the concentration of chlorine needed. Hot water treatment has the advantage of softening hard seeds that are occasionally present in seed lots, improving germination. The authors evaluated the hot water treatment to eliminate *E. coli* and *Salmonella* in mung beans seeds. Bacterial load on the seeds was approximately 10^5 to 10^6 CFU/g. The study used a quantitative methodology of analysis and the detection limit was $\leq$2 log CFU/g. The results were that hot water treatment at 85 °C for 40 s followed by soaking in cold water for 30 s and soaking in chlorinated water (2000 ppm) for 2 h, reduced pathogens to undetectable levels (Bari et al., 2010).

Weiss and Hammes (2005) studied the effect of hot water treatment at various time/temperature regimes to design a sprout decontamination process. Alfalfa, mung bean and radish seeds were inoculated by immersion with more than 10^7 CFU/g of enterobacteria (*Salmonella* and *E. coli*), dried and stored at 2 °C. The thermal treatment of contaminated mung bean (2–20 min for 55–80 °C), radish and alfalfa seeds 0.5–8 min (53–64 °C) reduced all pathogens by more than 5 log CFU/g. The authors concluded that for sprouters operating on a small scale, the results of the study provide an effective, simple and inexpensive method to reduce the probability of pathogen presence.

Bang et al. (2011) found that sequential treatments with ClO_2 (500 g/ml, 5 min), drying (45 °C, 23% RH, 24 h), and dry heating (70 °C, 23% RH, 48 h) eliminated *E. coli* O157:H7 in radish seeds and, consequently, in the sprouts produced from them, without impacting the germination rate (Bang et al., 2011).

3.2.3.2. High pressure. High pressure technology has been proven effective for eliminating pathogenic microorganisms in seeds (Sikin et al., 2013). The effectiveness of high pressure treatment, in combination with different temperatures to eliminate *E. coli* O157:H7 in artificial contamination alfalfa seeds, have been evaluated (Neetoo et al., 2009). A treatment of 550 MPa for 2 min at 40 °C was highly effective against *E. coli* O157:H7 with minimal impact on seed viability (Neetoo et al., 2009). A study conducted by Neetoo and Chen (2010) evaluated the effectiveness of high hydrostatic pressure to eliminate a 5 log CFU/g load of *Salmonella* and *E. coli* O157:H7 in alfalfa seeds. The results showed that high pressure treatment of 500 MPa for 2 min at 45 °C was able to eliminate a wild-type *Salmonella* strain and *E. coli* O157:H7 without causing any appreciable decrease in seed viability. Peñas et al. (2008) studied the effect of several combinations of time, pressure and temperature applied to mung bean and alfalfa seeds, on germination capacity as well as on reduction of the native microbial load of sprouts developed from treated seeds. The authors concluded that pressure can be used as an efficient tool to improve the safety of mung bean and alfalfa sprouts. However, high pressures were able to inactivate microorganisms, but also reduced the germination percentage of seeds (Peñas et al., 2008). Thus, determining the optimal processing parameters (temperature, time, and magnitude of pressure) is a critical process factors in ensuring the safety of pressure-treated foods (Neetoo and Chen, 2010).

3.2.3.3. Irradiation. The irradiation of sprouts, even at low doses, has been effective for inactivation of pathogens and spoilage bacteria while maintaining consumer acceptability (Sikin et al., 2013). Irradiation holds the most promise for pathogen control and/or extension of the shelf life of sprouts with minimal or no loss of quality (Sikin et al., 2013). Bari et al. (2009) evaluated the use of dry heat and irradiation to eliminate *E. coli* O157:H7 in radish, mung bean, broccoli, and alfalfa seeds, as well as the impact on seed germination. The authors concluded that 17 h of dry heat followed by a 1.0 kGy dose of irradiation completely eliminated *E. coli* O157:H7 in all study seeds. However, this irradiation had a negative impact on the germination of mung beans (Bari et al., 2009).

The viability of broccoli seeds and functional properties, such as ascorbic acid, carotenoid, and total phenol contents, of broccoli sprouts grown from irradiated seeds were evaluated by Waje et al. (2009). The results showed that seed irradiation did not negatively affect the total

phenolic content of sprouts and also showed similar effects on the viability and functional properties of sprouts. In addition, the total carotenoid content of sprouts grown from seeds irradiated at 1 and 3 kGy was not significantly different from that of sprouts grown from the control seeds. However, the ascorbic acid content of broccoli sprouts grown from irradiated seeds decreased as the irradiation dose increased regardless of the radiation source used. The authors concluded that to achieve the recommended 5 log reduction of foodborne pathogens in seeds with minimal effects on the quality of sprouts, low-dose irradiation, in combination with other treatments, may be necessary (Waje et al., 2009).

3.2.3.4. UV light. Sharma and Demirci (2003) investigated the effect of UV light on the inactivation of *E. coli* O157:H7 in alfalfa seeds. Many factors determine the effectiveness of decontamination of alfalfa seeds by UV light pulses, such as distance of the UV strobe, thickness of the seed layer, and treatment time. Owing to the low penetration capacity of UV light, the authors found that an increase in the thickness of the seed layer resulted in a lower reduction of *E. coli* O157:H7. They concluded that pulsed UV light has a promising future in the elimination of pathogens in alfalfa seeds (Sharma and Demirci, 2003). The authors suggested that further studies on voltage input and energy absorbed by the food product, could help in the development of improved treatment methods and models with greater confidence in predicting the inactivation of *E. coli* O157:H7 in alfalfa seeds.

3.2.3.5. Cold atmospheric pressure plasma (CAPP). The application of CAPP is able to reduce the native microbial flora of sprout seeds (Hertwig et al., 2018). However, the mechanisms responsible for microbial inactivation by CAPP have not been elucidated in detail (Hertwig et al., 2018). Waskow et al. (2018) investigated the inactivation of CAPP treatment of artificially inoculated seeds in a diffuse coplanar surface barrier discharge to determine the inactivation efficiency for relevant foodborne pathogens. A maximum logarithmic reduction of 8.8 log after 10 min CAPP treatment was found for seeds with high initial inoculation of the gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*. The highest logarithmic reduction of 5.2 log after a shorter CAPP treatment of 3 min was found for *E. coli*. The germination capacity of the seeds was also evaluated. It was found that the treatment for 120 s resulted in 90% germination. However, after 240 s of the CAPP treatment, 95% of the seeds did not germinate. The authors concluded that the CAPP treatment has the potential to reduce microorganisms on the seed surface while preserving the germination properties of seeds, at least for moderate treatment times (Waskow et al., 2018).

A study was conducted using cold atmospheric plasma, combining the analysis of both its inactivation properties on microorganisms naturally attached to the seed surface and its effects on seed germination. Treatments of 2 and 5 min achieved a reduction of nearly 1 and 2 log cycles, respectively. There was increased seed germination with treatment times of up to 3 min compared to the untreated control (Mitra et al., 2014). The authors concluded that cold atmospheric plasma technology can potentially reduce health risks associated with contaminated seeds; however, the technology has to be optimized for commercial use.

In summary, a review conducted by Sikin et al. (2013), evaluated different decontamination technologies for seeds and sprouts before, during and after germination, and the authors concluded that thermal inactivation of seeds and irradiation of sprouts are the most practical stand-alone microbial safety interventions for sprout production. Although the evaluation of decontamination technologies is still very promising, chlorine remains the most widely used sanitizer (Sikin et al., 2013). The authors pointed out that seed treatment is the most crucial step to obtain a safe final sprout.

4. Conclusions and perspectives

Sprouts are foods frequently involved in foodborne disease outbreaks worldwide. Therefore, many studies have been conducted with the aim of investigating bacterial development in sprouts and ways to prevent it. Implementation of good agricultural practices in the pre-harvest phase and good manufacturing practices in the post-harvest and germination phases is important measures to reduce sprout contamination. In addition, for germination purposes, seeds should be treated with chemical, physical or biological methods to reduce bacterial load and be suitable for human consumption. The recommendation is that at least two different decontamination methods should be used. Sanitization of sprouts before consumption, as with other fresh vegetables that are consumed raw, is also recommended. Microbiological analyses of the entire process, including the water used for germination, should be performed as a way to control bacterial contamination of sprouts.

Finally, the addition of sprouts in food products may be a good strategy to take advantage of their high nutritional value, as long as hygienic-sanitary safety is ensured.

In parallel, there has been an increase in the number of individuals who self-report as vegetarian or vegan, or who are interested in new vegetable foods. It is also worth mentioning the groups of people that adopt a raw food diet (crudivorism), owing to the fact that they depend on the germination of legumes as a way of inhibiting anti-nutritional factors. Crudivorism consists of intake of food that is exclusively raw, and this kind of diet is associated with a reduction of risks of metabolic diseases and colon cancer, but it also can be associated with nutritional deficiencies, especially in children and pregnant women (Souza et al., 2011).

The current COVID-19 pandemic entails an additional risk to be considered in the context of sprout consumption. Even if the SARS-CoV-2 is not proven to be transmitted by food, the complexity of sprout washing can demand a long time of manipulation, which can favor its contamination by a food handler infected by the virus. Further studies should be conducted to help reduce the microbiological risks associated with the consumption of sprouts.

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Declaration of competing interest

The authors declare no conflict of interest.

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