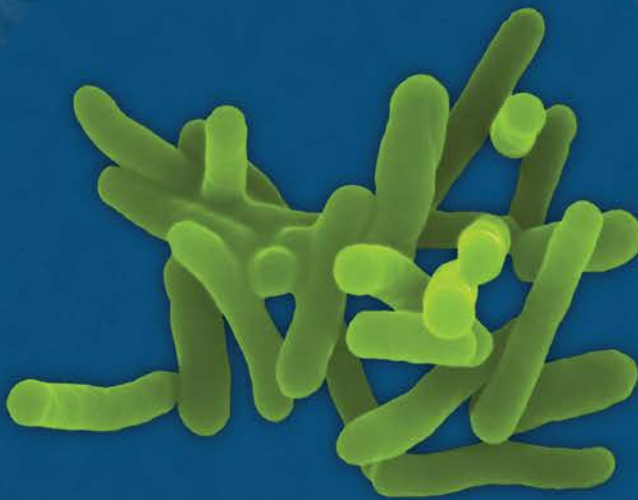




Prevention and control of microbiological hazards in fresh fruits and vegetables — Part 3: Sprout

Meeting report



43

MICROBIOLOGICAL RISK
ASSESSMENT SERIES

Prevention and control of microbiological hazards in fresh fruits and vegetables – Part 3: Sprout

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Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting. The interests declared were not considered by FAO and WHO to present any conflict in light of the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting. All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

Abbreviations and acronyms

CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CCPs	critical control points
CFIA	Canadian Food Inspection Agency
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
GAPs	good agricultural practices
GHPs	good hygiene practices
HACCP	hazard analysis and critical control point
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LMICs	low- and middle-income countries
RTE	ready-to-eat
STEC	Shiga toxin-producing <i>Escherichia coli</i>
WHO	World Health Organization

Executive summary

BACKGROUND AND OBJECTIVE

In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the Codex Alimentarius Committee (CAC) approved new work at its 42nd Session on the development of guidelines for the control of Shiga toxin-producing *Escherichia coli* (STEC) in leafy vegetables and in sprouts (FAO and WHO, 2018).

To support the work of the CCFH and to update and expand the information available in “Microbiological Hazards in Fresh Leafy Vegetables and Herbs” (MRA14, FAO and WHO, 2008), FAO and WHO are convening a series of expert meetings on preventing and controlling microbiological hazards in fresh fruits and vegetables.

In September 2021, the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) meeting on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables reviewed relevant measures for the control of microbiological hazards from primary production to point-of-sale in fresh, ready-to-eat (RTE) and minimally processed fruits and vegetables, including leafy vegetables (FAO and WHO, 2021b).

A sprout expert meeting was held in November 2021 that reconvened a subset of the JEMRA Expert Committee to collect, review and discuss relevant measures for the control of microbiological hazards in sprouts, from the production of seed for sprouting, to the harvesting and packing of sprouts and to point-of-sale.

A final meeting on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables to collect, review and discuss relevant commodity-specific interventions for all other fresh fruits and vegetables from primary production to point-of-sale was held in July 2022.

SCOPE

Sprouts have different food safety concerns from other fresh fruits and vegetables because the conditions under which sprouts are produced (time, temperature, humidity, pH and nutrients) are ideal for foodborne pathogen growth. Outbreak investigations have demonstrated that foodborne pathogens found on sprouts most likely originate from the seed, but the contamination could also be attributed to the production environment.

This report covers prevention and control measures specific to the primary production and handling of seed for sprouting, the production of sprouts and hygienic practices applicable to retail and food services. Recommendations for proper record-keeping and the establishment of product traceability programmes that facilitate the identification and investigation of contaminated seed and sprouts in the event of an illness outbreak or product recall are also included.

Microbiological hazards and control measures related to shoots, cress and microgreens where the growth stage is longer and the seeds or roots are not kept in the final product are not covered in this report. Home sprouting or consumer interventions (e.g. cooking) are not covered either.

MICROBIOLOGICAL HAZARDS IN SPROUTS

Sprouts represent a unique food safety challenge because the proliferation of bacterial pathogens, if present, is enhanced due to the high humidity and the ideal sprouting temperature. For this reason, the Expert Committee identified foodborne bacterial pathogens of concern, including Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp., and *Listeria monocytogenes* and specifically focused on interventions against bacterial foodborne pathogens. While the seed for sprouting may be contaminated with viral or parasitic pathogens, viruses and parasites do not increase in numbers during sprout production and few viral or parasitic disease outbreaks have been attributed to sprouts.

PREVENTION AND CONTROL MEASURES FOR SEED PRODUCTION AND HANDLING

When outbreaks have been linked to sprouts, the seed for sprouting was typically identified as the source of contamination. Seed can be produced for use as human food and animal feed and is generally treated as a raw agricultural product. Controlling and/or reducing microbial contamination of seed is difficult, given the diversity of growing and harvesting practices associated with seed production. Bacterial pathogens, if present on the seed, may survive for long periods of time during seed storage. Additionally, there may be difficulties in the traceability of the seed from harvest to sprouting. Nevertheless, interventions aimed at reducing the risk from seed-borne contamination should focus on controlling contamination from animal and human activities, ensuring proper use and application of manure, biosolids, other natural fertilizers and using agricultural water that is fit for purpose. Equipment used to grow, harvest and transport seed should be designed to enable effective cleaning and sanitation, which should be conducted regularly.

Measures should be taken during seed processing, conditioning, storage and transportation to reduce the risk linked to microbial contamination due to improper handling or exposure to extraneous material. Seed treatments represent an approach to reducing microbial contamination.

- **Animal and human activities**
 - > Grazing of domestic animals should not occur in fields while crops are actively being grown for seed production.
 - > Wild animals should be excluded from the production area to the extent possible.
- **Manure, biosolids and other natural fertilizers**
 - > Manure, biosolids, and other natural fertilizers are potential sources of bacterial pathogens.
 - > To reduce the risk linked to seed contamination, only adequately treated or composted manure/biosolids should be utilized during seed production.
 - > In general, the time intervals between application of manure/compost/biosolids and planting and harvest of seed should be maximized, as bacterial pathogens die off over time. A pre-harvest interval of 60 days from application is considered to be the minimum duration.
- **Agricultural water**
 - > Fit-for-purpose water for irrigation as well as other applications should be used to avoid the introduction of pathogens into seed.
 - > The application method and timing of irrigation will also impact the risk.
- **Equipment associated with growing and harvesting**
 - > Equipment should be designed and maintained to minimize soil intake and seed damage and to prevent the introduction of pathogens into seed.
 - > Equipment should be cleaned and sanitized prior to harvest.
- **Seed handling**
 - > Seed may become contaminated during harvesting, threshing and drying.
 - > Control of moisture content will decrease microbial growth and pathogen viability.
- **Storage and transport**
 - > Seed can become contaminated during storage and transportation due to unsanitary conditions or improper handling.
 - > Temperature and humidity should be controlled and appropriate

hygiene conditions implemented, including the cleaning and sanitation of equipment used to transport the seed.

- > Animal and insect controls should be implemented.
- **Seed treatment**
 - > Treatment of seed to reduce the presence of pathogens is a potential critical control point.
 - > Seed treatment can be challenging due to the low water activity of the seed, and the need to preserve the viability of the seed and its ability to germinate.
- **Microbiological testing of seed**
 - > The likelihood of detecting the presence of pathogens in seed is extremely low, due to the heterogeneous distribution and low numbers of the pathogens contaminating the seed.

PREVENTION AND CONTROL MEASURES FOR SPROUT PRODUCTION

Preventive and control measures need to be put in place to avoid water, workers, the production environment, growth media or seed from serving as the source of contamination or as a vehicle for cross-contamination.

The production process should be based on a Hazard Analysis and Critical Control Point (HACCP) system, where all the steps are well-documented and potential critical control points (e.g. decontamination of the seed) can be identified and controlled. If a problem is identified, a critical revision of all the steps should be performed.

- **Water**
 - > Water needs to be fit-for-purpose.
 - > The microbiological quality of water used in production and processing of sprouts should be maintained and monitored during the production and/or processing day, particularly if the same water is used in contact with large quantities of product.
- **Workers**
 - > Personal health and hygiene measures need to be implemented to avoid workers becoming a vector of contamination for sprouts.

- **Production environment**
 - > Sprout producers must take measures to control contamination that may arise from equipment, food and non-food contact surfaces, air and stagnant water. Proper storage, handling and disposal of waste and effective pest control will minimize the risks linked to sprout contamination.
 - > Proper facility design (e.g. differentiation between areas and zones) and operation flow to prevent raw material from coming into contact with the final product will reduce the risks linked to cross-contamination.
 - > Environmental monitoring is important to identify sources of contamination, particularly for *L. monocytogenes*, which may become established in the sprout production environment.
- **Soil/growth media**
 - > Natural fertilizers of animal origin need to be treated and handled so as to minimize the risk of sprout contamination.
- **Seed**
 - > Seed should be sourced from producers or distributors that follow good agricultural practices (GAPs) and good hygiene practices (GHPs) during the production, storage, distribution and sale of the seed.
 - > When seed arrives at a sprout operation, it should be inspected for physical damage and signs of contamination. Once received, it should be stored and handled in a manner that will avoid damage, prevent growth of microorganisms and protect it from pests and other sources of contamination.
 - > Seed treatment:
 - > Due to the difficulty of obtaining seed that can be guaranteed as pathogen free, the decontamination of seed prior to the sprouting process is recommended to reduce the risk of foodborne illness.
 - > Many decontamination treatments are available, including physical and chemical treatments. The effectiveness of treatments is highly variable between published studies and has rarely been validated under industrial conditions, which is a limitation for the extrapolation of results to industrial applications.
- **Microbial testing**
 - > Microbial testing can be done at many different stages of the sprout production. Spent sprout irrigation water has been identified as an appropriate target for microbial testing.

- > Microbial testing should be considered a verification that the seed used for sprouting and the production process does not contribute to sprout contamination. It will enable early detection of contaminated production batches, thus preventing their entrance into the marketplace.

PREVENTION AND CONTROL MEASURES DURING DISTRIBUTION AND POINT-OF-SALE

- Potential for bacterial growth and contamination can occur during transport, distribution and at point-of-sale due to improper handling, poor personal hygiene, contamination through commingling with raw commodities, animals or animal products, and exposure to unsanitary surfaces or water.
- Mitigation strategies include the training of operators and retailers, the use of clean, enclosed, refrigerated transport vehicles, a clean and sanitary point-of-sale environment, and fit-for-purpose water for cleaning, sanitizing and cooling.
- Sprouts should be kept at refrigeration temperature that will minimize microbial growth for the intended shelf-life of the product. The temperature of storage areas and transport vehicles should be monitored.
- For in-restaurant sprouting, interventions recommended for sprout operations should be considered, including seed sourcing programmes, seed treatment (if appropriate), the sampling and testing of spent sprout irrigation water (samples to be tested by contract labs) as well as cleaning and sanitizing food contact surfaces.

RECORDS AND TRACEABILITY

- Seed producers and suppliers should have a system to effectively identify seed lots, trace their associated production sites and agricultural inputs and allow for the physical retrieval of the seed in the event of a suspected hazard.
- Sprout operations should ensure that records and traceability programmes are in place to effectively respond to health risk situations.

TRAINING

- All personnel involved in the production and handling of seed for sprouting or sprouts across the supply chain should receive training on the principles of food hygiene and food safety as well as personal health and hygiene requirements.
- Seed producers, handlers, distributors and processors should be aware of GAPs and GHPs and of their role and responsibility in protecting seed intended for sprouting from contamination.
- Interventions designed to reduce microbiological hazards in sprouts can be highly technical and difficult to implement. Specific training related to seed sourcing and storage, seed treatment, sampling and microbial testing, cleaning and sanitizing and record-keeping are required to ensure successful implementation.
- It is important to develop a network of experts and technical support to enable the dissemination of accurate and complete information on safe production and handling of sprouts.



Introduction

1.1 BACKGROUND

Fresh fruits and vegetables are an important part of a healthy diet and are protective against many chronic health conditions, yet fresh fruits and vegetables are increasingly being implicated in food safety incidents involving microbiological hazards around the globe. Fresh produce contaminated with foodborne pathogens (e.g. bacteria, viruses, protozoa, helminths) has resulted in numerous outbreaks of foodborne illness and trade disruptions.

The Codex Alimentarius Commission (CAC) initially developed the “Code of Hygienic Practice for Fresh Fruits and Vegetables” in 2003, then later revised it in 2010 following a JEMRA meeting, held in 2008, to address the microbiological hazards associated with leafy vegetables and herbs (MRA14) (FAO and WHO, 2008). In addition, several commodity specific annexes were added to the code of practice in 2012, 2013, and 2017 (FAO and WHO, 2017).

Subsequently, in 2018, FAO and WHO published the report “Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring” (MRA31), in which fresh fruits and vegetables were identified as important sources of STEC infection (FAO and WHO, 2018). In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the CAC approved new work at its 42nd Session on the development of guidelines for the control of STEC in leafy greens and in sprouts. More recently, in October 2020, a JEMRA meeting on *Listeria monocytogenes* in ready-to-eat (RTE) foods noted increased reports of listeriosis linked to fresh and minimally processed fruits and vegetables (FAO and WHO, 2022).

To meet the request of the CCFH and to update and expand the information available in the previous report on “Microbiological Hazards in Fresh Leafy Vegetables and Herbs” (MRA14), FAO and WHO held a series of JEMRA meetings from July 2021 to June 2022 on preventing and controlling microbiological hazards in fresh fruits and vegetables. The goal of these expert meetings was to gather recent data, evidence and scientific opinions on the topic.

1.2 OBJECTIVES

The purpose of the JEMRA meeting on sprouts was to collect, review and discuss relevant measures for the control of microbiological hazards in sprouts, from the primary production of seed, to the growing of sprouts and to point-of-sale.

The scope of the meeting included aspects of seed production, processing and procurement, sprout propagation (hydroponically, in substrate and soil) and distribution, point-of-sale, record-keeping and traceability. Emphasis was placed on the identification and evaluation of preventive measures to reduce foodborne illnesses associated with sprouts, taking into consideration their effectiveness and practicalities.

The regulatory expectations and limitations of individual countries were not the focus of the meeting. It is understood that individual country regulations may not align with the definitions or subsections, but it is expected that the information presented will still be useful and can advance the understanding of hazards and risk mitigation.

The objectives of the meeting included:

- identifying and characterizing the microbiological hazards associated with sprouts, including pathogens of concern and potential sources/routes of contamination;
- reviewing mitigation/intervention measures being used at different points along the sprout supply chain and assessing their effectiveness in reducing microbiological hazards;
- reviewing publicly available literature, guidelines from competent authorities and industry associations (e.g. compliance guidelines, codes of practice) to assess the current state of knowledge on controlling microbiological hazards in sprouts; and
- responding to specific questions posed by the CCFH (Annex 1).

1.3 SCOPE

Reports of foodborne illness associated with raw and lightly cooked sprouts have raised concerns among public health agencies and consumers about the safety of these products.

Microbial pathogens associated with sprouts include Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp., *Listeria monocytogenes*, and other pathogens. Sprouts have different food safety concerns from other fresh fruits and vegetables because the conditions that favour sprout production (time, temperature, humidity, pH, and nutrients) are also ideal for foodborne pathogen growth. Outbreak investigations have indicated that the foodborne pathogens found on sprouts most likely originate from the seed, but the contamination can also be attributed to the production environment.

Seed is commonly identified as the primary source of microbial contamination in sprouts. Crops for seed production are grown in typical agricultural environments and potential sources of contamination in the field include water, improperly managed animal manure, contact with wild animals and inadequate worker hygiene. The risk of sprout contamination can be reduced through the implementation of preventive measures used on farms to avoid contamination of seed. Good agricultural practices (GAPs) should be applied at all stages for seed destined for sprout production, including planting, growing, harvesting, cleaning, storage and transportation.

Poor hygienic practices and an insanitary production environment could also lead to sprout contamination. Preventive and control measures should be in place during sprout operations to prevent water, workers, production environment, growth media or seed from serving as the source of contamination or as a vehicle for cross-contamination.

Seed treatments represent one approach to reducing microbial contamination. There is currently no treatment available that can guarantee pathogen-free seed. Research is needed to find and validate effective decontamination treatments that provide sufficient pathogen reduction without affecting the germination rate of the seed.

Microbial testing can be performed at many stages of sprout production. Testing will enable the early detection of contaminated production batches. It is also a verification that seed used for sprouting and the production process does not contribute to sprout contamination. This report covers prevention and control measures specific to the primary production and handling of seed for sprouting, the production of sprouts, and hygienic practices applicable to retail and

food services. Recommendations for proper record-keeping and the establishment of product traceability programmes that facilitate the identification and investigation of contaminated seed and sprouts in the event of foodborne illness outbreak or product recall are also included.

1.4 DEFINITIONS

Cress – grown in substrate and true leaves are developed. The shoots and the leaves are cut during harvest and the final product does not include the seed and roots.

Growth media – material that acts as a substrate during growth of the sprout.

Microgreens – plants reach a later stage of growth than sprouts, typically associated with the emergence of true leaves. Can be grown in soil or substrate and are harvested above the soil or substrate line. Include both shoots and cress.

Seed distributor – a person responsible for the distribution (handling, storage and transportation) of seed to sprout producers and who may deal with one or more seed producers or also be a seed producer.

Seed producer – a person responsible for the management of activities associated with the primary production of seed, including post-harvest practices.

Shoots – grown hydroponically and true leaves are developed. The shoots and the leaves are cut during harvest and the final product does not include the seed and roots.

Spent sprout irrigation water – water that has been in contact with sprouts during the sprouting process.

Sprouts – sprouted seed or beans harvested when the cotyledons (or seed leaves) are still un- or under-developed and true leaves have not begun to emerge. They can be grown in water, soil or substrate, and can be harvested with or without the root (cut sprouts).

Sprout producer – a person responsible for the management of activities associated with the production of sprouts.

Traceability – means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into food and feed, through every stage of production, processing and distribution.



Overview of sprout production and supply chain

2.1 SEED PRODUCTION

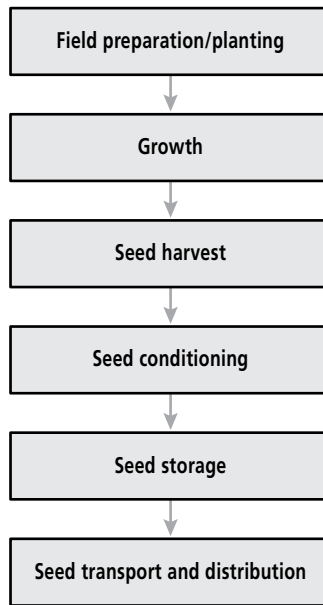
Seed for sprouting is mostly grown in agricultural environments. Specific production practices vary depending on the type of crop and seed used. Some crops are grown exclusively for seed while others are grown for both forage and seed. Only a small proportion of harvested seed is used for sprouting. In many cases, seed purchased by sprout producers is not usually grown specifically for this purpose. Seed production generally consists of the steps shown in **Figure 1**.

2.1.1 Field preparation/planting

Practices to establish a highly productive seed crop include proper site selection, removal of soil limitations with tillage and fertilization, good seeding technique and timing, effective weed management, proper irrigation and proper timing of harvest (Putnam *et al.*, 2012).

The optimal growing environment differs between seed crops. Alfalfa seed production favours deep, well-drained soils, heavier clay or loam soils over lighter, sandy soils (Mueller, 2008). Mung bean production prefers sandy, loam soils with good internal drainage. The field is tilled to remove weeds and to prepare a seedbed that will provide good seed-soil contact. The soil should be smooth, firm and free from clods and debris for optimal seed placement (Oplinger, 1990; Wells, Holen and Sheaffer, 2018). Nutrients are essential for optimal crop growth and seed production. The amount of fertilizer needed is determined by soil or tissue tests (NACMCF, 1999).

FIGURE 1 Typical seed production process



Source: Authors' elaboration for this report.

Planting can be done manually or mechanically using drills, seeders or other planting equipment. For some seed types (e.g. broccoli), seeding is started in a greenhouse or nursery bed, followed by transplantation after several weeks.

2.1.2 Growth

Properly timed agricultural practices are key to successful seed production. Seed yield and quality can be impacted by factors such as pollination, irrigation, insects and weed controls (NACMCE, 1999).

Pollination requirements for seed crops vary among different species and depend on whether a plant is self-pollinating or requires cross-pollination to facilitate the seed set. Insects (e.g. honeybees) are commonly used pollinators.

Irrigation is needed for field preparation, the promotion of the seed set and the prevention of plant stress that may reduce seed yield. Most irrigation systems are surface systems, e.g. flood, furrow or sprinkler irrigation. Timely irrigation scheduling is essential. For alfalfa production, irrigation starts in the winter to provide half of the water requirement for the year. Deciding when to stop irrigating a field for the season is also important. It is a fine balance, as sufficient water is

required for the seed to mature, but the moisture must be removed from the soil prior to desiccation for harvest (NACMCF, 1999).

Insecticides must be carefully selected and applied to kill the target insects without harming the bees required for pollination. Weed control will eliminate competition and promote the growth of the seed crop. Mechanical cultivation and herbicides are used to control weeds. In some regions, grazing sheep may be employed in seedling fields to control the weeds (NACMCF, 1999).

2.1.3 Seed harvest

Seed harvest involves cutting (swathing), windrow curing and threshing. For example, alfalfa seed is swathed after two-thirds or three-quarters of the pods have changed from green to dark brown. After cutting, the plants are left in long strips (or windrows) in the field. When seed is mature and adequately dry, it is removed from the pods either by hand or by machine. Harvesting practices expose the seed to dirt and debris and may cause damage to the seed. Harvested seed should be cleaned quickly to remove stems, immature pods, dirt, insect parts and other debris.

2.1.4 Seed conditioning

Seed conditioning eliminates soil, weed seeds and other debris by employing a variety of equipment that separates the seed- and non-seed fractions based on differences in physical characteristics, such as particle size, shape, density and surface texture. Some legume seeds are difficult to germinate because they have a hard seed coat that is less permeable to water. Mechanical scarification or scratching the seed coat are sometimes applied to improve the germination of hard seeds (NACMCF, 1999).

Seed conditioning, shipping and distribution practices often involve mixing multiple lots of seed of different origins, which may complicate traceback and may also present an opportunity for cross-contamination.

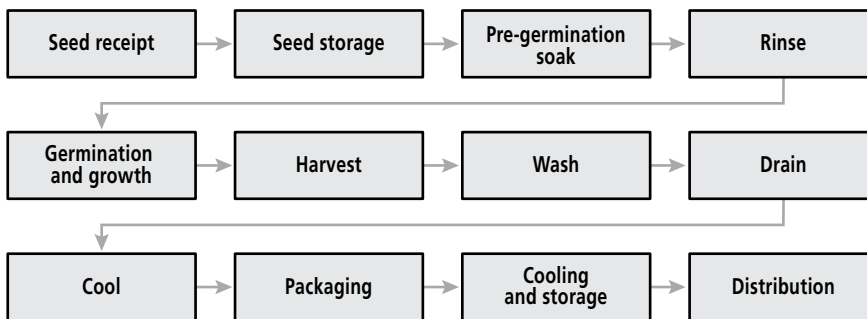
2.1.5 Seed storage and distribution

Seed is commonly warehoused in metal bins until it is bagged. Once bagged, seed needs to be protected from rodents, insects, excess moisture and other contaminants. Proper storage temperature, humidity and seed moisture content are critical to maintain seed longevity and prevent bacteria or mould growth.

2.2 SPROUT PRODUCTION

Sprouts are typically grown in enclosed buildings or greenhouses. They can be grown hydroponically or in soil. During sprout production, the seed is moistened to increase the water content. The water used in sprouting operations is either municipal water or ground water: in any case, water that comes into contact with sprout seed or sprouts should not represent a vector of contamination. The production process broadly follows the steps depicted in **Figure 2**. The specific conditions (e.g. length of pre-germination soak, days of sprouting, irrigation frequency, post-harvest wash, packaging) used may differ depending on the type of seed being sprouted and the size and resources of the operation.

FIGURE 2 Typical sprout production process



Source: Adapted from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1999). NACMCF (National Advisory Committee on Microbiological Criteria for Foods). 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *International Journal of Food Microbiology*, 52(3):123-153. [https://doi.org/10.1016/S0168-1605\(99\)00135-X](https://doi.org/10.1016/S0168-1605(99)00135-X)

2.2.1 Seed receipt

Sprout producers source their seed globally. Established guidelines have recommended that sprout producers only purchase seed that has been grown using good agricultural practices (GAPs) and that is conditioned and stored under sanitary conditions (EC, 2017/C 220/03; FDA, 2017a). The European Union has set regulations requiring sprout producers to have procedures in place to ensure good hygienic production of the seed and the traceability of the production seed lots (EC, 2017/C 220/03).

Seed is received at sprouting operations in bags or sacks (50 lb or 25 kg). The bags are typically tagged with supplier information, seed type, lot code and country of origin. Other documents that may accompany a shipment of seed are: certification of analysis (specifications for purity or percent germination), microbiological testing results and certification of pre-treatment if the seed has been previously treated by the seed supplier.

Bags and seed are inspected upon delivery for physical damage (e.g. holes, tears) and signs of contamination (e.g. insect, bird or rodent droppings, foreign material). Contaminated or potentially contaminated seed should not be used for sprouting.

2.2.2 Seed storage

The storage area for seed should be clean, dry and free of pests and should be separate from areas that store packaging materials, chemicals or finished products. The seed should be handled and stored in a manner that will prevent damage and contamination (e.g. keeping seed away from walls and off the floor to prevent rodent contamination) and facilitate inspection for signs of contamination.

2.2.3 Seed treatment and pre-germination soak

Many sprout producers apply an antimicrobial treatment to seed prior to sprouting to reduce pathogenic microorganisms, if present. Treatment of seed for sprouting is recommended by international guidelines (CFIA, 2008; FAO and WHO, 2017) and is required in certain jurisdictions (e.g. the United States of America). Both chemical (e.g. calcium hypochlorite) and physical (e.g. hot water dip) methods are currently used in sprout operations (FDA, 2017c). The known seed treatment methods based on chemical, physical or biological means or a combination of these have been extensively reviewed (Ding, Fu and Smith, 2013; Sikin, Zoellner and Rizvi, 2013; Yang *et al.*, 2013). All the steps involved in the antimicrobial treatment of seed should be carried out in an area separate from the germination and packaging areas. After treatment, the seed is generally soaked for up to 12 hours in water to soften hulls and improve germination (NACMCF, 1999).

2.2.4 Germination and growth

Sprouts are grown hydroponically, in substrate or in soil. The practices employed for germination, growth, harvest and post-harvest washing vary depending on the operation and the type of sprout grown. Growing units range from rotating drums to bins, beds, trays and buckets. Germination and growing times differ with the type of sprout, the time of year and the germination process used. They can range from 3 to 7 days for alfalfa, 3 to 8 days for mung bean, 6 to 10 days for onions or 3 to 14 days for wheat grass, etc. (NACMCF, 1999).

2.2.4.1 Hydroponically grown sprouts

Green sprouts (e.g. alfalfa, broccoli, clover and radish) are commonly grown in rotating drums. Drums are pre-set for rotation speed, water temperature and air ventilation. The growing sprouts may receive a water spray every 10 to 15 minutes with air blown into the drum to keep the product ventilated.

Some sprout operations germinate the seed initially in rotary drums for 2 to 3 days then transfer it to trays, cups or final packages for further growth. These containers are placed on growing racks for 3 to 5 days. Via this process, sprouts will grow vertically in a more uniform manner and turn greener in colour (NACMCF, 1999).

Mung bean sprouts are generally sprouted in large quantities (25 kg) in deep bins or beds in dark and humid rooms with temperatures between 21 and 30 °C. Irrigation water is typically applied via a moving overhead sprinkler system once every 4 to 6 hours (Hora *et al.*, 2005; NACMCF, 1999).

2.2.4.2 Soil grown sprouts

Soil grown sprouts are commonly planted in plastic trays that contain potting soil or composted soil. The seed is rinsed, then soaked in water and held for 12 to 24 hours to allow for initial germination. The soaked seed is then scattered on top of the soil and levelled out. Water is sprayed over the trays once or twice daily (NACMCF, 1999; SproutPeople, 2022).

2.2.5 Harvest

Sprouts are typically harvested by manually removing them from growing units. Some sprout operations wash sprouts in a water bath or in a flume system to remove hulls and/or to help lower the temperature of the sprouts. After washing, excess water is removed using a centrifuge. Most soil-grown sprouts are harvested at the facility, by cutting them from the trays, and then washed and packaged. Alternatively, the sprout trays are delivered directly to retailers (e.g. juice bars) where sprouts are cut at the point-of-sale. Sprouts are often placed in a cold room or a cooler after harvest to remove the heat generated during the sprouting process before packaging (FDA, 2017a).

2.2.6 Packaging

Sprouts are typically packaged at the growing site, but in some cases, they are transported in bulk to another location to be packaged. Sprouts are commonly packaged in large (e.g. 10 lb) bulk or small (e.g. 4 oz) individual containers for delivery to restaurants or retail markets. Types of packaging used include plastic bags, plastic cups or box-type containers, clam shells and soil trays (NACMCF, 1999).

2.2.7 Storage and distribution

After packaging, sprouts are stored in cold rooms or coolers. Because the sprouts are still respiring, they can generate heat. Rapid cooling is preferred to stop sprout growth and to increase shelf-life. It is important to provide good air circulation for cooling.

The cold chain should be maintained throughout distribution of the finished products. Sprouts are shipped in refrigerated vehicles or with added ice if using trucks with no refrigeration. It is important to maintain the cold chain when preparing the products for loading onto delivery trucks.

Certain jurisdictions have set requirements for the temperature of sprouts received at retail. For example, in the United States of America, sprouts are classified as a “TCS” (Temperature Control for Safety) food and must be maintained at a temperature at or below 5 °C upon arrival at the point-of-sale.



Microbiological hazards in sprouts

3.1 OUTBREAK/SURVEILLANCE DATA

The presence of robust food safety surveillance systems in high-income countries (HICs) enables effective traceback and the epidemiological studies required for source attribution and the identification of foodborne illness outbreaks in which sprouts were the identified vehicle. The lack of such surveillance systems in low- and middle-income countries (LMICs) means that the prevalence of microbial contamination in sprouts or the sources of foodborne illness outbreaks are often not identified. Examples of bacterial contamination rates and prevalence in sprouts sampled from the marketplace and a summary of foodborne illness outbreaks associated with sprouts are provided in Annex 2. The lack of identified sprout-borne outbreaks from LMICs reflects the paucity of data from these countries.

3.2 PATHOGENS OF CONCERN

Sprouts represent a unique food safety challenge because the conditions under which sprouts are produced are ideal for the growth of bacterial foodborne pathogens. For this reason, this document identifies pathogens of concern as bacterial foodborne pathogens, including STEC, *Salmonella* spp. and *L. monocytogenes*, among others, and it specifically focuses on interventions for bacterial foodborne pathogens. While the seed may be contaminated with viral or parasitic pathogens, they do not grow during sprout production. Nevertheless, the presence of viral and parasitic pathogens on sprouts may represent a hazard, should they be present.

3.3 SOURCE AND ROUTE OF CONTAMINATION

The microbial contamination of sprouts is often attributed to the seed (Bazaco *et al.*, 2021; Dechet *et al.*, 2014). Much focus has been placed on the safe production, conditioning, storage and transportation of seed for sprouting to prevent seed contamination. Agricultural practices vary between seed produced for the production of sprouts for human consumption and seed planted for animal feed.

If seed intended for forage, animal grazing or other uses is used for sprouting, sprout producers should be aware of the potential contamination of seed from natural fertilizers or irrigation water. To avoid potential microbial risks, only seed produced using adequate GAPs should be used for the production of sprouts. Under the conditions in which seed is sprouted (time, temperature, humidity, pH, and nutrients), if low levels of microbial contaminants are present on the seed, they can grow to reach high levels. Little information is available for comparing the risk profiles of cut versus uncut sprouts but exudates released from cut sprouts may provide nutrients that favour microbial growth (Brandi and Amundson, 2008).

Poor hygienic practices and insanitary production environments and storage facilities could also lead to sprout contamination. For example, *L. monocytogenes* is widely found in nature and can be introduced into sprout production environments and storage facilities through dust, equipment, raw materials or workers. Once *L. monocytogenes* establishes itself in a sprout operation, it can repeatedly contaminate products and potentially lead to foodborne illness outbreaks. Considering that sprouts are mostly consumed as RTE foods, contamination by asymptomatic carriers of pathogens should also be considered (EFSA, 2011).



4

Prevention and control measures for seed production and handling

4.1 SEED PRODUCTION

Microbial contamination of seed with zoonotic foodborne pathogens may occur during cultivation and harvesting, and during processing, conditioning, storage and transportation. While environmental contamination has been linked to sprout outbreaks, the vast majority of outbreaks attribute the likely source of contamination to the seed (EFSA, 2011). The presence of pathogens in or on seed is amplified by the sprouting process itself. The risk of sprout contamination can be reduced through the implementation of preventive measures used on farms to avoid contamination of the seed. Contamination of the seed may occur at any point in the value chain, during growing, harvesting, milling, sprouting or shipping. Plants for seed production are grown in typical agricultural environments and potential sources of contamination in the field include water, improperly managed animal manure, contact with wild animals and inadequate worker hygiene. Precautions are required at harvesting, as harvesting exposes the seed to debris and dirt and is likely to spread contamination throughout the harvested seed. GAPS should be applied at all stages for seed destined for sprout production, including planting, growing, harvesting, cleaning, storage and transportation (CFIA, 2018; Jin *et al.*, 2019).

Seed can be produced for a number of different purposes, including as human food and animal feed, and is generally treated as a raw agricultural product. There are many agricultural practices that can be used for seed production, depending on the type of seed being produced. It is anticipated that climate change will play an increasing role in outbreaks linked to contaminated seed.

For example, the frequency of extreme weather events including hurricanes, tropical cyclones, tsunamis, monsoons, severe flooding and high winds is expected to increase (Wu *et al.*, 2016; Uyttendaele, Liu and Hofstra, 2015). Additionally, ambient temperature increases are expected to lead to prolonged droughts (Castro-Ibáñez *et al.*, 2015). These events will likely have direct and indirect impacts on seed production. Bacterial concentrations can increase by 25- to 30-fold in agricultural fields following extreme precipitation (Cevallos-Cevallos *et al.*, 2012), and flooding has been linked with an overflow of untreated human sewage (Kenward *et al.*, 2016). As a result, frequent flooding of cropland could lead to increased contamination of seed with foodborne pathogens. Drought also poses direct and indirect threats to seed production. Direct threats may be caused by increasingly desiccated soil, which when coupled with wind events may lead to the carriage of bacterial pathogens on dust particles or dried manure (NSWDPI, 2018) and increased contamination of seed. An indirect consequence of drought is the reduced availability of clean irrigation water, and the use of irrigation water of poor sanitary quality could contaminate seed grown for sprout production, especially in regions already impacted by water quality issues, including LMICs. Finally, climate change has been reported to increase the biotic stress of plants, leading to diseased or weakened food crops and an increased internalization of foodborne pathogens (Garrett *et al.*, 2016) into the roots, leaves and fruits (Critzler and Doyle, 2010) and potentially the seeds of food crops. For additional details on the impact of climate change on the production of fresh produce, please refer to the climate change section of the JEMRA report “Prevention and control of microbiological hazards in fresh fruits and vegetables – general principle” (FAO and WHO, 2021b).

4.1.1 Animal and human activities

As with other fresh produce commodities, wild and domestic animals are a main source of pathogenic zoonotic agents that may lead to contamination in seed. Livestock production and the presence of wildlife in proximity to, or upstream of water sources used to irrigate fresh produce can pose a significant risk of pathogen transfer to produce via aerosols (Dungan, 2010) and fecal deposition (McAllister and Topp, 2012). Additional sources of foodborne pathogens that can impact fresh produce include agricultural runoff or bioaerosols from nearby concentrated animal production operations, overflows from wastewater treatment and septic facilities, infected farmworkers and untreated manure-based natural fertilizers (Bozkurt *et al.*, 2021).

All potential sources of environmental contamination should be identified, assessed and ranked according to risk. For example, primary production should

not be carried out in areas where the potential for environmental contamination due to wild or domestic animal intrusion, manure, dust and contaminated water could lead to the presence of foodborne pathogens in or on seed following harvest. Where possible, seed producers should conduct an analysis of the previous uses of the production sites as well as adjacent sites to identify potential microbial hazards. To the extent possible, steps should be taken to prevent the access of wild animals and insects to the production area. Additionally, grazing of domestic animals should not occur in fields while crops are actively being grown for sprout seed production; this includes animal use to clip back plants to induce seed production (e.g. alfalfa) (CFIA, 2007). Agricultural runoff and dust contamination from concentrated animal feeding operations (CAFOs) and flooding by potentially contaminated water sources should also be prevented (CFIA, 2007).

The scientific literature provides information regarding the routes by which wildlife and domestic animals contaminate fresh produce with foodborne pathogenic microorganisms (Langholz and Jay-Russell, 2013; McAllister and Topp, 2012). The available literature demonstrated that grazing animals are actually helping to spread pathogens, so animals should not be allowed to graze in fields where seed is being produced. Additionally, studies should be conducted to understand wildlife movement patterns and their interactions with human and livestock sources of foodborne pathogens, and how such interactions affect contamination of fresh produce (Langholz and Jay-Russell, 2013), including sprout seed.

4.1.2 Manure, biosolids and other natural fertilizers

The prevention of contamination is particularly important during the production of seed that will be used to produce sprouts for human consumption because of the potential for pathogens to grow during the sprouting process. There are a variety of agricultural inputs that can be used as natural fertilizers, including livestock manure, slurries and biosolids. These natural fertilizers can introduce foodborne pathogens into the seed production environment. Mitigation and intervention methods are the same as for production of other fresh produce commodities and include using only adequately treated or composted manure/biosolids (i.e. they have undergone treatment to reduce pathogens to levels unlikely to result in contamination) (Alegbeye, Singleton and Sant'Ana, 2018; FAO and WHO, 2017).

Physical, chemical or biological treatments such as composting, pasteurization and heat drying have been proven to be effective in reducing the presence of pathogens in manure, sewage sludge and other organic fertilizers. In general, it is recommended not to apply treated natural fertilizers after planting; however, during the production of seed, treated manures can be applied to the soil during the growing period if there is no direct contact with the seed.

If untreated or partially-treated natural fertilizers are used, the time period between the application and the planting and harvesting of seed should be maximized, as bacterial pathogens die off over time (EC, 2017). In most international guidance, a period of 60 days is considered to be the minimum duration. In many countries, there is national legislation in place that establishes the type of treatments required for each specific application of organic amendments. If national guidelines or regulations are available, and include methodologies for assessing vulnerability and risk, selecting appropriate risk mitigation measures and monitoring the treatment process, such guidelines should be followed (FAO and WHO, 2019).

4.1.3 Agricultural water

Irrigation water and other sprays can introduce foodborne pathogens into seed, and the irrigation method will affect the risk of contamination. Water quality management will vary throughout all operations. The quality of water used should be dependent on the stage of seed production (i.e. fit for purpose) (FAO and WHO, 2003). For additional details on the appropriate use of water during the production of fresh produce, please refer to the JEMRA report “Safety and quality of water used in food production and processing” (FAO and WHO, 2019).

4.1.4 Equipment associated with growing and harvesting

Poorly designed, maintained, cleaned and sanitized equipment can introduce foodborne pathogens to seed (EC, 2017). The potential also exists for cross-contamination during harvesting between soil and seed (EC, 2017). Equipment should be designed or adjusted to protect against pest incursion, to minimize soil intake and seed damage, and to allow for easy cleaning and, when necessary, sanitization. Prior to harvest, all debris or soil should be removed from equipment. All equipment should be thoroughly dry cleaned (to minimize the presence of water which could lead to microbial growth) between lots of seed, and sanitized if required. Handling equipment (e.g. augers, conveyors) should be regularly cleaned and inspected (CFIA, 2007).

4.1.5 Tracking the source of contamination

When contamination of seed is detected, approaches should be developed to trace pathogens to their root source. To determine the source of contamination, traceability of seed intended for the production of sprouts for human consumption is essential. Poor traceability of seed may cause delays in the control of the outbreak by the competent authorities (EFSA, 2011).

The introduction of methods such as whole genome sequencing (WGS) and metagenomic sequencing should be used to better understand the originating

sources of foodborne pathogens that contaminate seed, as a first step in developing effective approaches to stop such contamination. Understanding the transmission processes of foodborne pathogens during seed production and the identification of environmental sources of microbial contamination are essential to manage the food safety risks associated with the production of fresh produce (Langholz and Jay-Russell, 2013), including seed.

4.2 SEED HANDLING (PROCESSING AND CONDITIONING)

Seed handling, including harvesting, threshing and drying, can introduce microbial contamination. Great efforts should be made to maintain sanitation in seed drying yards. Exposure of seed to mist, high humidity and fog should be avoided, as controlling moisture content will decrease microbial growth and survival (FAO and WHO, 2017).

Seed for sprouting should be free to the extent possible from foreign matter, including soil, insect fragments, bird and rodent droppings and metal and glass fragments. Conditioning utilizes a variety of equipment to remove soil, weed seeds and other debris from seed, and should be carried out in a hygienic manner employing practices that minimize potential sources of contamination (CFIA, 2007). Care should be taken during seed processing or conditioning to avoid contamination. Processing techniques like scarification of the seed will produce a rough, porous surface in which pathogens can hide and even enter the seed, making the seed decontamination process less effective and more difficult.

Seed conditioning facilities should ensure that the equipment has not previously been used to handle animal products, and such equipment should be thoroughly cleaned and sanitized before cleaning the seed. Any visibly diseased or damaged seed, which could be susceptible to microbial contamination, should not be used for sprout manufacture. Seed intended for sprouting should be segregated from seed to be used as animal feed (e.g. hay production) (CFIA, 2007).

At all times, seed, equipment, storage bins and shipping bags should be protected from rodents and birds with a complete pest control programme that includes monitoring, eradication, cleaning, sanitization and record-keeping. Seed destined for sprouting should be packaged in a hygienic manner in solid bags that are impermeable to contamination during storage and transportation. Contaminated or recycled bags should not be employed. In addition, each package should be labelled with information identifying the source and seed lot. (CFIA, 2007).

There is a lack of information regarding the impact of various seed production steps on microbial food safety. More studies are needed to evaluate the best ways to harvest, thresh and dry seed to reduce the risk of microbial contamination. Additional studies should also investigate the optimal moisture content to reduce microbial contamination and how extraneous matter such as soil can be removed from seed.

4.3 STORAGE AND TRANSPORT

Seed may be stored in various ways, including in bags in traditional warehouses, vertical silos or bins (bulk storage) or horizontally on the floor. Where seed is stored horizontally, there is a requirement for specially constructed floors and proper ventilation. Suitable handling practices and techniques for both domestic as well as industrial storage are required, and safety procedures are therefore mandatory to prevent microbiological contaminations (Galieni *et al.*, 2020).

During seed storage, animal and insect controls should be implemented (EC, 2017/C 220/03). Temperature and humidity should be controlled and appropriate hygiene conditions, including cleaning and sanitation (environmental controls), should be implemented. Storage bins, transport trucks and wagons should be regularly cleaned and sanitized and should be designed to facilitate cleaning and reduce the potential for harbouring extraneous material. Storage containers should be bird and rodent proof or kept in a rodent-free facility. Van der Linden *et al.* (2013) demonstrated the long-term survival of enteric pathogens (*Salmonella enterica* and STEC O157:H7) on seed stored for up to two years but also showed that the pathogens maintain their ability to resuscitate and proliferate on the seedling.

4.4 SEED TREATMENT (BY SEED SUPPLIERS)

Due to the risk associated with foodborne pathogen contamination of seed, it is recommended that the seed be treated prior to sprouting. Ordinarily, seed treatment is performed by the sprout producer, where the sprouting process takes place. However, it could happen that seed is treated by the seed supplier. The same information that applies to the treatment of seed during sprout production applies to seed treatments performed by the seed suppliers. Treatment of seed must be effective at reducing the presence of foodborne pathogens, but not so harsh as to affect the germination of the seed. Similar to other low water activity food products, disinfection of seed can be challenging, especially considering the impact of the treatment on seed viability. The majority of studies on seed treatment were designed to be performed in the sprouting facilities (see Section 5.5). Few published studies have examined the seed treatment conducted by the seed suppliers, although seed

pretreated with a mixture of hydrogen peroxide, peroxyacetic acid and acetic acid is commercially available (ISS, 2022).

4.5 MICROBIOLOGICAL TESTING OF SEED (AT THE FARM OR BY THE SEED SUPPLIER)

Anticipated concentrations and prevalence of foodborne pathogens present on seed are low (e.g. *Salmonella* from seed associated with outbreaks was determined to be 13 MPN/kg to 16 MPN/kg and 20 MPN/kg to 100 MPN/kg of dry seed (Fu *et al.*, 2008; Stewart *et al.*, 2001)). Pathogenic bacteria are distributed heterogeneously in lots of seed (Van Beneden *et al.*, 1999), and may also be heterogeneously distributed in sprouts and in spent irrigation water (Liu and Schaffner, 2007; McEgan, 2008). Detecting low levels of foodborne pathogens in seed prior to sprouting is difficult, as exemplified by a number of outbreaks in which pathogens were not found in the corresponding seed or sprouts linked to outbreaks (Mahon *et al.*, 1997; Watanabe *et al.*, 1999).

Effective testing of each seed lot is recommended due to the sporadic nature of seed contamination (EFSA, 2011). Assuming that pathogens are present in one 25 g sample out of every 1 000 (Montville and Schaffner, 2005), the probability of detecting foodborne pathogens in 160 samples (25 g) of seed, before sprouting, was determined to be 0.1 percent (Montville and Schaffner, 2005); to increase the probability of detection of any pathogen in a seed lot, it is necessary to analyse many samples (Bylund, 2013). For example, according to the European Food Safety Authority (EFSA), if one seed per kilo is contaminated, and contaminated seeds are randomly distributed, then at least three kilos of seed would need to be analysed in order to ensure that there is a 95 percent chance that the contaminated seed is detected (EFSA, 2011). Seed lots should be sampled and tested by the seed supplier rather than the sprout producer to reduce the likelihood of introducing contaminants into the sprout production area (EFSA, 2011).

Given that pathogens are sporadically distributed throughout a lot of seed and that they are likely to be present at low concentrations, the practice of sprouting a sample of seed and analysing the sprouts and/or the spent sprout irrigation water for pathogens has been suggested as a special case of pooling of seed where the sprouting serves as a first pre-enrichment step (EFSA, 2011).

Several methods are used to determine the presence, prevalence and concentration of bacterial pathogens and indicator microorganisms in seed (FDA, 2022a; Fu *et al.*, 2008). Molecular-based methods (polymerase chain reaction (PCR)) can also be used for the detection of bacterial pathogens in seed (Bylund, 2013).

The concentration and prevalence of foodborne pathogens in seed is unknown. This includes variability by seed type and producer. Studies should be conducted to address this knowledge gap, though the challenges associated with testing seed due to low microbial prevalence make such studies difficult to perform.



Prevention and control measures for sprout production

Preventive and control measures need to be put in place to avoid water, workers, production environments, growth media, or seed serving as the source of contamination or as a vehicle of cross-contamination. The production process should be based on an HACCP system, where all the steps are well documented and potential critical control points (e.g. decontamination of seed) can be identified and controlled. If a problem is identified, a critical evaluation of all the steps should be performed. Microbial testing can serve as a verification of the effectiveness of control measures.

5.1 WATER

It has been demonstrated and stated by several organizations, including expert groups from FAO/WHO, that water used along the fresh fruit and vegetable (FFV) supply chain can be a potential source of microbial pathogens in products at consumption (FAO and WHO, 2021a). Water use in sprout production is no exception. The MRA 33 FAO/WHO report (FAO and WHO, 2019) introduced the “fit for purpose” concept, establishing that water should be fit to use for the intended purposes. The main challenge would be to define the requirements for water quality use along the food chain, because it should take into account the purpose of the water use, potential hazards associated with the water use, whether there is any subsequent measure to decrease the potential for contamination further along the food chain and the end use of the food product (e.g. eaten raw).

In the case of sprouts, water is used in several operations during production and processing (e.g. washing, germination, growth, cooling). The water used in sprouting may spread pathogens from contaminated seed to pathogen-free seed within the same sprouting batch or contaminate the sprouting equipment, thereby increasing the total amount of pathogens in the final product (EFSA, 2011). Process water used in washing and cooling can also be a source of cross-contamination of fresh produce (FAO and WHO, 2019). The most important risk factors to be considered include the microbial quality of the water, the stage in the supply chain, how the water is used, the end-use of the crop and the efficacy of risk mitigation measures. Sprouts are frequently eaten raw or only slightly cooked, which implies that there will not be any kill step prior to consumption.

The microbiological quality of the water used in the production and processing of sprouts should be of a quality that does not constitute a hazard to the safety and suitability of the final product. The MRA 33 FAO/WHO report (FAO and WHO, 2019) states that water in contact with fresh produce, which is not usually subjected to an upstream microbial inactivation or reduction treatment, should be of potable quality during all post-harvest use and handling. Recommendations have also been provided by the EFSA, indicating that when sprouts are germinated and grown from seed hydroponically, only potable water should be used during sprouting (EFSA, 2011). If clean water is used, the microbial properties of the water from that source should be analysed based on the risk assessment. The same recommendations are in place for the water used for washing and cooling operations. In this case, if water is used in contact with large quantities of produce or reused in different operations, it should be treated and maintained in good microbiological condition such that no risk to the safety and suitability of food will result from its use. In general, adequate hygienic conditions for processed water have to be maintained using food grade disinfectants at validated concentrations to prevent cross-contamination. The water treatment process should be effectively monitored to maintain potable water quality during the production and/or processing day.

5.2 WORKERS

Workers can carry pathogens on their skin and hands and in their digestive systems or respiratory tracts. They can be a vector for transmitting diseases and causing contamination in sprouts or food contact surfaces. Infected food handlers have been implicated in many illness outbreaks (Greig *et al.*, 2007). An investigation of a cluster of *E. coli* O104:H4 infections linked to a family party where sprouts were served pointed towards transmission via food items contaminated by a food handler (Diercke *et al.*, 2013). The possibility of person-to-person transmission

of *E. coli* O104:H4 has been reported whereby patients could acquire hemolytic uremic syndrome (HUS) by secondary transmission from a person in the household that was infected by consuming contaminated fenugreek sprouts (Aldabe *et al.*, 2011; Kuijper *et al.*, 2011).

A healthy, clean and properly trained workforce that follows good worker health and hygiene practices is critical to ensure that workers do not become a source of sprout contamination. Operations should establish Standard Operating Procedures (SOPs) that address worker training, adequate and appropriately maintained facilities and supplies, as well as company policies on expectations for worker hygiene, illness reporting and exclusion from work guidelines. Operations must ensure that visitors are also aware of and comply with these policies and procedures.

5.2.1 Worker health and hygiene

Persons with symptoms of vomiting, diarrhoea or fever must not perform jobs that require contact with sprouts or food contact surfaces. Open wounds must be covered by dry, tight fitting and impermeable bandages or gloves. Ill persons may be assigned to other tasks but should be restricted from contact with workers who may come into contact with sprouts or food contact surfaces. Employees must be instructed to report infectious illnesses or symptoms of illness to the management (SSA, 2017).

Workers should maintain adequate personal cleanliness and, where appropriate, wear suitable protective clothing and footwear to prevent contamination of sprouts or food contact surfaces. Personal effects (e.g. jewellery, watches, purses, backpacks, clothes) should be removed or covered if they cannot be adequately cleaned and sanitized while handling sprouts.

Workers must wash and dry their hands thoroughly before starting work, before putting on gloves, after using the toilet, upon returning to the workstation after a break or after handling any surfaces or items that could result in contamination of sprouts. If gloves are used, sprout operations should have a policy to ensure proper use of gloves. These include washing hands before putting on gloves, maintaining gloves in an intact and sanitary condition and replacing gloves when sanitary conditions cannot be maintained.

Sprout producers should have procedures in place to ensure that, following an injury, any blood or bodily fluids are removed, all affected surfaces are cleaned and sanitized and all affected products are disposed of (SSA, 2017).

5.2.2 Facilities

Sprout producers should provide adequate washing and toilet facilities so that an appropriate degree of personal hygiene can be maintained. Such facilities should be suitably located. The restrooms should not open onto the sprout production areas. Hand-washing stations should be located at all entrances to the sprouting and packaging areas and should have adequate means for washing and drying hands, including wash basins, clean running water, soap, toilet paper and single use paper towels or an equivalent. Toilet facilities must be equipped with hand-washing stations. The facilities should be appropriately designed to ensure the hygienic removal of waste and should be maintained in sanitary conditions and in good repair (SSA, 2017).

5.3 PRODUCTION ENVIRONMENT

For the hygienic production of sprouts and to minimize the potential for cross-contamination, a proper production environment is important to maintain food safety. Appropriate location and construction of buildings will help protect against sources of external contaminants which may affect the safety of food. The appropriate sanitation in place will help minimize the transfer of microbial contaminants within the facility. Sprout production must be done in a fully enclosed building. The doors of the production area should not be kept open when not in use and the doors should be tight-fitting. The windows in the production area should not be kept open in order to prevent the entry of pests. The zoning of production and non-production areas must be managed to avoid cross-contamination. It is recommended that areas where sprouts are processed or stored are separate from the equipment washing, maintenance and waste areas, laboratories, offices and toilet facilities (FDA, 2017b).

The potential areas/hotspots for contamination must be minimized by proper design, which includes the separation of operations and differentiation between areas in which contamination is likely to occur. Separate storage areas must be identified for the storage of seed, sprouts and chemicals. In addition, the sprout production area and packaging area should be in separate rooms.

One of the critical factors for sprout contamination is stagnant water in the production environment. The construction of floors should be inclined towards trapped drains and the unidirectional flow of the water/waste in the production line should be maintained to avoid any chances of contamination. The stagnation of water on floors may harbour pathogens, particularly *L. monocytogenes*. The accumulation of standing water should be minimized in sprout operation areas where large quantities of water are used during production (SSA, 2017).

In order to minimize the chances of contamination from the non-production area, workers involved in sprout operations should have adequate, readily accessible toilet facilities in close proximity to the production area. Hand-washing stations should be located in both the production and the packaging areas to facilitate their use (FDA, 2017a). The equipment and tools required for production must have a proper design, construction, and workmanship to enable them to be easily cleaned and properly maintained. Inadequate cleaning and sanitizing of the tools and equipment can lead to contamination. Inaccessible or hard-to-clean areas may provide harborage or growth sites for microorganisms. To prevent contamination or cross-contamination due to the equipment used in the production, it is recommended that sprout producers install, store and maintain equipment and tools in such a way as to facilitate cleaning of the equipment and adjacent spaces, protect against contamination and prevent the attraction and harbourage of pests. Sprout producers should store equipment and tools in a fully enclosed building to minimize the potential for contamination (FDA, 2017a).

L. monocytogenes is a pathogen of concern and the target microorganism for environmental monitoring in sprout production (Goulet *et al.*, 2012; Pouillot *et al.*, 2012, 2015). An environmental monitoring plan should be designed to identify *L. monocytogenes* if it is suspected of being present in the production areas. As part of the environmental monitoring plan, sprout producers should also develop a routine sampling plan that includes the frequency, time and date of the sampling and the test microorganism (*Listeria* spp. or *L. monocytogenes*). When the presence of *L. monocytogenes* is detected, additional testing should be done on surfaces and surrounding areas to detect and evaluate the extent of the problem in the production area. The environmental monitoring plan should also include a corrective action plan and details on when and how to implement the corrective actions if the environment for sprout growing, harvesting, packing or holding areas tests positive for *Listeria* spp. or *L. monocytogenes* (FDA, 2017a).

5.4 SOIL/GROWTH MEDIA

Some sprout varieties (e.g. sunflower, peas, buckwheat, daikon) can be grown in water, soil, substrate or other growth media. These soil and growth media may be amended with composted animal manure or wastes and may contain pathogenic bacteria, viruses or parasites (Chen and Jiang, 2017). Foodborne pathogens, such as STEC O157:H7, *Salmonella* spp. and *L. monocytogenes*, present in manure-amended soil, can survive for long periods of time, under a variety of conditions (Chen and Jiang, 2014; Chen, Kim and Jiang, 2018; Gurtler *et al.*, 2018; Jiang *et al.*, 2004). The transmission of STEC O157:H7 from soil amended with

contaminated manure to lettuce plants grown on these soils has been demonstrated (Islam *et al.*, 2004; Solomon, Yaron and Matthews, 2002). STEC O157:H7 entered the lettuce plant through the root system and migrated throughout the edible portion of the plant (Solomon, Yaron and Matthews, 2002).

Natural fertilizers of animal origin must be treated to reduce or eliminate pathogens of public health significance before their application for growing fresh fruits and vegetables (FAO and WHO, 2017; FDA, 2015a). All the information included in section 4.1.2 (Manure, biosolids and other natural fertilizers) is also relevant for sprout production in soil and substrate. A variety of treatment processes and practices are available, including physical (e.g. heat), chemical (e.g. high alkaline pH), and biological (e.g. composting) processes (Chen and Jiang, 2014, 2017; Gurtler *et al.*, 2018). Many factors may affect the reduction and survival of bacterial foodborne pathogens during the composting or heat treatment processes. It is critical that the effectiveness of treatment against pathogens be thoroughly assessed and demonstrated before broad commercial application (Chen and Jiang, 2017). As previously indicated, if national guidelines or regulations are available, and include methodologies for assessing vulnerability and risk, selecting appropriate risk mitigation measures and monitoring the treatment process, such guidelines should be followed (FAO and WHO, 2019).

Sprout producers must implement measures to prevent soil or growth media from becoming a source of contamination or cross-contamination in sprouts, on food contact surfaces or in the production environment. Growers should make sure that the received natural fertilizers of animal origin have been properly treated using a scientifically valid method, and are handled, conveyed and stored in a manner and location that minimizes the risk of contamination.

Activities involving the handling of growth media or the growing and packing of media-grown sprouts should be separate from those involving other sprouts (e.g. hydroponically grown sprouts), either by location or by time. Measures need to be in place to prevent cross-contamination of the growing areas by movements of workers, tools or equipment. All surfaces must be cleaned and sanitized after handling growth media or media-grown sprouts.

5.5 SEED

Seed has been identified as the primary source of contamination in sprout-associated outbreaks of foodborne illness (Bazaco *et al.*, 2021; Dechet *et al.*, 2014). As a raw agricultural product, seed can become contaminated in several ways. The most significant risk factors are associated with the effect of agricultural practices on

seed production, storage and distribution, contaminated irrigation water and/or manure, as well as the presence of dust, soil, birds and rodents in storage facilities. During warehouse storage, seed stored in open containers can be exposed to rodents, birds, faeces of farmyard animals and insect pests which are all potential vectors of contamination (EFSA, 2011).

Seed can be sold directly to sprout producers for sprouting or to seed distributors. The seed is not only used for sprout production, as it is often used for other purposes (e.g. edible seeds, animal feeds, oil production, horticulture) (EFSA, 2011). This is why it is very important to ensure that seed is obtained from producers or distributors that follow GAPs and GHPs during the production, storage, distribution and commercialization of the seed. In most countries, there are codes of practices and guidelines on preventing field contamination during sprout and seed production (EC, 2017; FDA, 2017a). However, imported seed may come from countries where guidelines are not available. For this reason, some countries have established specific certification requirements for sprouts or seed intended for the production of sprouts that are imported from other countries. The certificate attempts to guarantee that the sprouts or seed produced in exporting countries are produced under conditions that comply with the general hygiene provisions for primary production and associated operations set out in the codes of practices. The certificate also implies that consignments of seed for sprouting destined to be exported to these countries may be tested for *Enterobacteriaceae* to verify the hygienic conditions of production prior to exportation. The level of these bacteria in seed cannot exceed 1 000 cfu/g (EC, 2013).

Seed is received in bags. Very often, a bag of seed received at the sprout operation and used for a sprout production batch is a mixture of various lots of seed from different sources. When seed arrives at a sprout operation, it should be inspected for physical damage and signs of contamination. In most cases, seed may be stored for long periods of time (especially in sprout operations with small production volumes) before being sprouted, either in the plant or at some steps of the seed distribution chain (e.g. fenugreek seed from 2009 were sprouted in 2011 (EFSA, 2011)). Once received, seed should be stored and handled in a manner that will avoid damage, prevent the growth of microorganisms and protect it from pests and other sources of contamination.

Due to the difficulty of obtaining seed that can be guaranteed as pathogen free, decontamination of seed prior to the sprouting process is recommended, where appropriate, to reduce the risk of foodborne illness. In the Scientific Opinion published by the EFSA (2011a), there is a good overview of alternative decontamination treatments that can be applied to seed and sprouts. Many seed decontamination treatments, including chemical, biological and physical methods (Ding, Fu and

Smith, 2013; Sikin, Zoellner and Rizvi, 2013) are available. Chemical treatments include calcium hypochlorite, chlorine dioxide, acetic acid, as well as other treatments such as ozone, antimicrobial polymers, plasma-activated water and oxylchloro (Kumar *et al.*, 2006; Machado-Moreira *et al.*, 2021; Mir *et al.*, 2021). Biological methods include the use of antagonistic bacteria (e.g. lactic acid bacteria, *Pseudomonas* spp., *Bacillus* spp.) and bacteriophages (Kimmelshue, Goggi and Cademartiri, 2019; Kocharunchitt, Ross and McNeil, 2008; Ye *et al.*, 2010; Zhang, 2017). Physical approaches include low heat (pasteurization), irradiation, high pressure, UV light and cold atmospheric plasma (Charoux *et al.*, 2020; Miyahira and Antunes, 2021). The effectiveness of treatments is highly variable between published studies (Montville and Schaffner, 2005).

The scientific literature contains many examples of studies conducted to evaluate seed decontamination strategies. However, the majority of works aimed at the validation of seed treatment approaches are based on laboratory evaluations of inoculated seed, and it is unclear whether the results of these studies are applicable to naturally contaminated seed and/or use on commercial scales. Additionally, the costs of the treatments are unclear.

The most frequently used decontamination treatments applied to seed before sprouting are chemical sanitizers (e.g. 20 000 ppm free chlorine for 20 minutes). Some authors have also reported that the combination of these treatments with hot water treatments is very effective e.g. 85 °C for 40 seconds followed by soaking in cold chlorine water (2 000 ppm for 30 s) (Bari *et al.*, 2010). It should be considered that, despite considerable efforts, chemical methods of disinfection cannot be relied on as methods capable of ensuring a pathogen-free state for all seed types. There are some disinfection treatments that have been shown to consistently achieve a substantial reduction in pathogen numbers, only managed by combining relatively high concentrations (> 10 000 ppm) and contact times (10 minutes) (Suslow *et al.*, 2002). Measures to prevent the introduction of pathogens in sprout production (including primary production of seed) remain of the utmost importance (EFSA, 2011).

During seed decontamination, producers should ensure that all containers used for microbiological treatment are clean and sanitized prior to use. If liquid decontamination methods are utilized, seed should be agitated well in large volumes of an antimicrobial agent to maximize treatment efficacy. The duration of treatment and the concentration of the antimicrobial agent used should be accurately measured and recorded. Following decontamination, steps should be taken to avoid post-processing contamination or recontamination of treated seed. The antimicrobial agent should always be used according to the manufacturer's instructions, and, as appropriate (depending on the treatment method), following decontamination, seed should be thoroughly rinsed with water of appropriate

quality (ideally potable water or at least clean water). Rinsing should be repeated as necessary to eliminate the antimicrobial agent (FAO and WHO, 2017).

It is important that the impact of the decontamination treatment on the germination rate of the seed be considered. In many cases, before a treatment can be implemented in the industry, it has to be validated for each type of seed variety, as not all seed types will be suitable for the same treatment.

5.6 MICROBIAL TESTING

Currently, the available seed treatment methods are not able to eliminate pathogens in or on seeds. Pathogens that survive seed treatment could grow to high numbers during sprouting. Microbial testing can be a part of the multi-hurdle approach to prevent potentially contaminated sprouts from entering the marketplace. It is also a way to verify that neither the seed used for sprouting nor the production process contributed to the contamination of the sprouts.

5.6.1 Target for testing and sampling plan

Microbial testing can be carried out at various stages throughout sprout production. Samples of incoming seed, in-process sprouts, spent sprout irrigation water, finished products or from the environment (swabs) can all be analysed (EC, 2073/2005).

5.6.1.1 Seed

Although contaminated seed is the likely cause of most reported sprout outbreaks, seed testing has often failed to detect the presence of pathogens due to the low level and sporadic nature of contamination. The European regulation, EC 2073/2005, requires sprout operations to conduct preliminary testing of a representative sample of all batches of seed used for sprouting. A representative sample shall include at least 0.5 % of the weight of the batch of seed in sub-samples of 50 g or be selected based on a structured, statistically equivalent sampling strategy. The regulation also requires that testing be performed by sprouting out the seed samples under normal sprouting conditions. The regulation allows exemption from this requirement either if the sprout operation implements a food safety management system that will reduce the microbiological risk of the seed or if the results from consecutive testing of the sprouts met the microbiological criteria (EC, 2073/2005).

The “Code of Hygienic Practice for Fresh Fruits and Vegetables” (FAO and WHO, 2017) recommended that seed screening be conducted by seed distributors and that the seed sample selected for testing should be sprouted prior to analysis

to increase the likelihood of detecting pathogens, if present. An example of such testing procedures using 3 kg of seed collected from each bag of an entire shipment is available for reference (ISS, 2021).

Screening seed for pathogens by seed suppliers will prevent the use of contaminated seed by consumers who sprout seed at home. Some of the cases linked to the 2011 fenugreek sprout outbreak were due to consumption of self-cultivated sprouts from seed mixtures containing contaminated fenugreek seed (BfR, 2011).

5.6.1.2 Spent sprout irrigation water or in-process sprouts

Testing spent sprout irrigation water or in-process sprouts collected during sprouting increases the likelihood of detecting the pathogens that may be present in seed. It also enables early detection of contamination in the production batch before products enter the marketplace. Testing spent sprout irrigation water is preferred over testing sprouts because water may pick up bacteria as it passes through the production batch, making it easier to collect a representative sample (FDA, 2017a).

Sprout producers should establish a written sampling plan that includes procedures for when, what and how to collect samples. Procedures for holding production batches until negative test results are received (hold and release) should also be in place.

Samples must be collected aseptically by trained personnel to ensure that the sample collection process does not contaminate the sample or the production batch being sampled. The containers, equipment and tools used for sample collection should be sterile (FDA, 2017a).

Samples of sprouts or spent sprout irrigation water should be collected when the level of pathogen, if present, is likely at its highest. Collecting samples as early as 48 hours after the start of sprouting has been recommended, although the optimal time for sample collection may vary depending on the type of sprouts and sprouting practices (CFIA, 2018; FDA, 2017a; SSA, 2017).

The volume of sample collected should be sufficient to be representative of the production batch and for testing target pathogens. Different sample sizes or volumes and sampling frequencies are recommended by different agencies. In the United States of America, the FDA recommends that 1.5 L of spent sprout irrigation water be collected from each production batch of sprouts for testing for STEC O157:H7 and *Salmonella* species. When sampling in-process sprouts, at least 30 sub-samples, 50 g each, are to be collected from multiple locations in the growing unit (FDA, 2017a, 2015a). The Canadian Food Inspection Agency (CFIA) recommends collecting 1 L of spent sprout irrigation water or five samples

of sprouts (200 g each) from each production batch (CFIA, 2018). In Europe, Regulation EC 2073/2005 on microbiological criteria for foodstuffs recommends that sprout producers collect five samples of 200 ml each if spent sprout irrigation water is analysed. Samples must be collected for microbiological analysis at least once a month at the stage where the probability of finding STEC and *Salmonella* spp. is the highest, and in any case not before 48 hours after the start of the sprouting process (EC, 2073/2005).

5.6.1.3 Finished products

The EC 2073/2005 regulation requires that ready-to-eat sprouts must comply with the food safety criteria, i.e. free of *Salmonella* spp. and STEC O157, O26, O111, O103, O145, O104:H4. Five 25-g samples of finished sprouts will need to be analysed for *Salmonella* spp. and another five samples (25 g each) of sprouts be analysed for STEC O157, O26, O111, O103, O145 and O104:H4. If a sprout producer has a sampling plan that includes sampling procedures and sampling points for the spent sprout irrigation water, the sampling requirement for ready-to-eat sprouts may be replaced with the analysis of five samples (200 ml each) of spent sprout irrigation water. The microbiological criterion, in this case, is the absence of *Salmonella* spp. or of STEC in 200 ml of spent sprout irrigation water (EC, 2073/2005).

5.6.2 Testing laboratories

All microbial testing for pathogens should be conducted by a qualified laboratory with the following criteria (CFIA, 2018; SSA, 2017):

- The laboratory is accredited, for example, to the ISO/IEC 17025 standard for the methods specified.
- The laboratory is staffed by personnel with training and experience in analytical microbiology techniques to ensure that tests are performed correctly and that all appropriate safety precautions, including appropriate waste disposal, are followed.
- The laboratory has appropriate resources and is able to demonstrate that a quality management system is followed.
- If microbial analysis is done by the sprout producer, the in-house laboratory must be physically separated from the sprout production facility to prevent cross-contamination. The laboratory facilities, personnel, and quality management system must meet the same criteria required for independent laboratories to ensure that testing is reliable and does not create food safety hazards.

5.6.3 Test methods

The FDA Produce Safety Rule (FDA, 2015a) requires that spent sprout irrigation water (or in-process sprouts) from each production batch be tested for *E. coli* O157:H7 and *Salmonella* spp. using either the methods of analysis specified (FDA, 2015b) or scientifically valid methods that are at least equivalent to the prescribed methods in accuracy, precision and sensitivity. The EC 2073/2005 regulation specifies reference methods for testing of *Salmonella* (EN ISO 6597-1) and STEC (CEN/ISO TS 13136) but states that the use of alternative analytical methods is acceptable when the methods are validated against the reference methods. Alternatively, the methods shall be validated according to internationally accepted protocols and their use authorized by the competent authority (EC, 2005).

Technological advances have resulted in a wide range of rapid methods for the detection of foodborne pathogens (Law *et al.*, 2015; Melo, 2016; Umesha and Manukumar, 2018). Many of these methods are commercially available (Mangal *et al.*, 2016; USDA, 2020). Commercial test kits come with various assay formats and detection principles (Fu *et al.*, 2022). Immunoassay-based tests employ specific antibodies that recognize target pathogens. Molecular assays target specific nucleic acid sequences in microorganisms. The enrichment conditions used vary depending on test kit and target pathogen. They can involve multiple or single steps with times ranging from a few days to as short as 6 hours, making same-day detection possible. Commercial test kits are packaged with ready-to-use supplies and reagents and are often accompanied by automated instruments and software that simplify the analysis and interpretation of test results.

Comparative studies have demonstrated that many commercial test kits, including lateral flow devices, enzyme immunoassays and molecular assays, are able to detect low levels of *Salmonella* or STEC O157:H7 in alfalfa spent sprout irrigation water. Enrichment conditions play a key role in determining the performance of the tests and the success of confirmation (Fu *et al.*, 2022). However, very few test kits have been validated through a formal collaborative study as per internationally accepted protocols for the detection of foodborne pathogens in sprouts or spent sprout irrigation water. None of the currently available test kits are validated against the methods prescribed in the FDA Produce Safety Rule.

The United States Food and Drug Administration (FDA) has determined that several AOAC Official Methods are “scientifically valid” and “at least equivalent to the specified standard methods” (FDA, 2018). The CFIA specifies a number of commercial test kits for detection of *Salmonella* and STEC O157:H7 in sprouts or spent irrigation water samples (CFIA, 2018).

Sprout growers should verify that the methods used by the lab are either the methods specified in the regulation, alternate methods that have been scientifically validated and shown to be at least equivalent to the standard methods or methods that have been authorized by the competent authority.

5.6.4 Corrective actions

Sprout operations should have a written corrective action plan in place for responding to positive test results. Recommended corrective actions include the following (SSA, 2017):

- Discard any sprout production batch that tests positive for pathogens.
- Evaluate other sprout production batches that have contacted tools or equipment shared with contaminated products for potential contamination.
- Thoroughly clean and sanitize anything in the sprout operation that came into contact with the contaminated production batch or its spent irrigation water.
- Discard or return the seed lot used to produce the contaminated sprouts to the supplier to be diverted to non-food use, unless it is proven that the seed lot is not the source of the pathogens found in samples that tested positive.
- Notify seed supplier regarding positive test results so that the supplier may take appropriate actions (e.g. informing other growers that use the same seed lot).
- Perform other actions to prevent recurrence of contamination, e.g. re-evaluate control measures and/or cleaning and sanitizing procedures, retrain employees on proper seed treatment and handling procedures.

5.7 POST-HARVEST STORAGE

Sprouts are generally consumed fresh and, to prevent their growth and retain quality (microbial, and nutritional), they are stored at low temperatures (Świeca and Gawlik-Dziki, 2015). Rapid cooling is essential to accomplish the full storage potential of sprouts. Current industry practice suggests storing finished sprout products at between 0.5 °C to 4 °C. The shelf-life for sprouts under these storage temperatures ranged from 7 days for mung beans and 14 days for other green sprouts (e.g. alfalfa, clover, broccoli) (ISGA, 2022). However, previous studies highlighted that under these conditions, most sprouts may be expected to retain acceptable quality for 5 to 9 days. The shelf-life at 2.5 °C has been described as being less than 5 days, and at 5 °C and at 10 °C it is less than two days (Suslow and Cantwell, 2000). The high respiration rates and perishable nature of sprouts demand distribution and short-term storage at 0 °C at 95 to 100 percent relative humidity (BMT, 2022).

When sprouts are stored, the arrangement of products should allow for good air circulation and rapid cooling. As sprouts are still respiring, they can generate heat, even in a low-temperature room. The storage of sprouts in small containers and good air circulation help prevent “hot spots” in a batch of sprouts that may result due to heat generated by the still living sprouts (FDA, 2017a).

The safety measures to reduce contamination are temperature control, controlled humidity, insect-free and proper hygiene conditions for storage. There is also the need to optimize the storage conditions that allow the chemical composition of the package headspace to be maintained during their shelf-life. The continuity of the cold chain is required as much as possible when staging products to prepare them for loading onto delivery trucks (FDA, 2017a).



6

Prevention and control measures during distribution and at point-of-sale

Distribution covers all the steps from production to the point-of-sale. After production, finished sprouts are usually packed into containers at the sprout growing operation or transported in bulk to another location to be packed before being placed at the point-of-sale or packaged in individual servings for direct sale to consumers or food service establishments. Distribution of sprouts includes activities such as loading and unloading of produce into and out of a transport vehicle; the transport, placement and storage in the receiving facility; and storage (FDA, 2017a). Sprouts are usually stored under refrigerated conditions, and it has been demonstrated that the use of modified atmospheres (5 percent O₂ and 15 percent CO₂) is beneficial for extending the quality of the product (Suslow and Cantwell, 2000). These conditions maintain high relative humidity, and bacterial growth will depend mostly on storage temperature. This is why the problems associated with the distribution and storage of sprouts are mainly linked to the maintenance of the cold chain (EFSA, 2011). Vehicles used to transport sprouts should be refrigerated to avoid temperature increases. However, refrigerated temperatures do not prevent the growth of psychotropic microorganisms, such as *L. monocytogenes*.

6.1 MITIGATION/INTERVENTION MEASURES

The main prevention and control measurements to minimize contamination of sprouts during distribution and at retail and food service establishments are:

- Temperature control: most guidelines recommend a storage temperature as low as possible (< 5 °C) to avoid the growth of microorganisms. Although the survival and growth patterns of *L. monocytogenes* and other microorganisms such as STEC O157:H7 are dependent on the vegetable type, package atmosphere and bacterial strain, it has been demonstrated that reducing the storage temperature from 8 °C to 4 °C reduced the growth of these microorganisms on packaged RTE vegetables (FDA, 2003; Francis and O'Beirne, 2001b).
- Hygiene maintenance: all the facilities, equipment, containers, crates, vehicles and vessels used to transport sprouts and seed should be kept clean and, where possible, disinfected in order to prevent microbiological contamination during transport (EC, 2017).
- Shelf-life means either the time corresponding to the period preceding the “use-by” or the minimum durability (“best before”) date. Date of minimum durability (“best before”) means the date until which the food retains its specific properties when properly stored. This period can therefore be considered as relating to the quality of foods. In the case of foods which, from a microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to human health, the date of minimum durability shall be replaced by the “use-by” date (EFSA, 2020).
- In most countries, sprouts require a “use-by” date. Xylia *et al.* (2021) demonstrated that the expiration date and relevant shelf-life of processed vegetables are important parameters to be considered when post-harvest management is applied to these products, ensuring safety and quality. Therefore, it is highly recommended that consumers do not eat sprouts that are past their use-by date and, during their shelf-life, keep them refrigerated at 5 °C or below and consume them within two days.
- Relevant information that producers should provide to the consumer to ensure the safety of sprouts during the storage, handling and preparation of the product includes: (1) recommended temperature of storage; (2) use-by date; and (3) cooking instructions, which should be included on the label if the product is intended to be consumed as non-RTE.
- To avoid cross-contamination, the food contact surfaces of tools and equipment used in contact with the sprouts should be cleaned and sanitized. This includes such surfaces during transport and storage.

- Use of water misters for maintaining the humidity of unpackaged sprouts at retail: if unpackaged sprouts are displayed for retail, the quality of the sprouts may deteriorate rapidly due to low humidity and wilting of the product. This can be prevented by using misters, which increase the humidity around the product. However, food safety should not be compromised by the use of misters. The temperature should be kept as low as possible, the water used for the misters should be fit-for-purpose, the equipment used for misting should be properly cleaned and maintained and excess water should be drained away from the products.
- In-restaurant sprouting: follow similar interventions recommended for sprout producers, including seed source, seed treatment (if appropriate), and spent sprout irrigation water testing (samples to be tested by contract labs, not in house).
- Consumers have reported food handling practices that could result in cross-contamination in personal kitchens. The lack of appropriate hand washing, as well as the presence of high bacterial counts on sponges and kitchen sinks, cutting boards, and countertops, have been highlighted as critical points to control to avoid cross-contamination.

The goal of prevention and control measures during distribution and at retail and foodservice establishments is to identify the potential sources of contamination throughout these stages of the food chain so that they can be monitored and controlled. The aim of applying such prevention and control measures is to reduce the food safety risks to the public, thus reducing the risk of foodborne outbreaks.

6.2 AVAILABLE DATA

Available data on prevention and control measures during distribution and at retail and food service establishments for sprouts are mostly related to the survival of specific foodborne pathogens (e.g. *L. monocytogenes*, *Salmonella*, STEC) during storage. Several research papers have evaluated the growth rate of *L. monocytogenes* in different types of sprouts (Aytac and Gorris, 1994; Bennik *et al.*, 1999; Francis and O' Beirne, 2001a, 2001b; Lee *et al.*, 2002; Molinos *et al.*, 2005; Thomas *et al.*, 2003; Tian *et al.*, 2012). However, the information is not complete as more data are needed to determine the potential growth at abusive temperatures (> 8 °C).

6.3 UNCERTAINTY AND DATA GAPS

- Data gaps in this area are significant. The experts recognize that there is a lack of data for many relevant aspects, including the fate of foodborne pathogens naturally occurring on seed. The pathogen level and prevalence might vary between different types of seed, but they have not been well-documented.
- The fate of pathogenic bacteria on sprouts during storage at different temperatures, particularly at abusive temperatures, is not documented. Specifically, there is uncertainty regarding the increase in risk due to the differences in growth rates for certain foodborne pathogens between 5 °C and 8 °C.
- Some sprouts may be exposed to some heat during preparation before consumption (e.g. stir-fried). However, the impact of these heat treatments on bacterial pathogens is not known.



Records and traceability

Traceability allows a food to be identified through all stages of production, processing and distribution, thereby allowing rapid reaction in the event of foodborne illness outbreaks (EC, 2013). Based on previous experience in outbreak investigations, it has been demonstrated that the traceability of certain foods of non-animal origin may assist in the removal of unsafe food from the market, thereby protecting consumers. Therefore, traceability can be considered as an efficient tool to ensure food safety because rapid tracing of the commodities concerned in an outbreak is essential to limit the public health impact. In many countries, there is already legislation that provides the general requirements for the traceability of food, which should be established at all stages of production, processing and distribution (EC, 2017/C 220/03).

The traceability of seed intended for production of sprouts for human consumption is of great importance in establishing the microbial quality of the seed and for the implementation of food safety management measures (EFSA, 2011). Seed used for sprouting is very often imported from other countries. In fact, the 2011 *E. coli* O104 outbreak investigation in Germany and France suggested that imported seed in 2009, contaminated prior to leaving the importer, was the most likely cause of the outbreak (EFSA, 2011). It has been suggested that the use of imported seed represents an obstacle for the rapid tracing of the source of the outbreak (EFSA, 2011). This is why some countries have established specific rules for the traceability of sprouts and particularly of seed intended for the production of sprouts. However, it should be taken into account that there may be difficulties in the traceability of sprouts from the point of seed production to point-of-sale and to the consumer.

7.1 MITIGATION/INTERVENTION MEASURES

Sprout and seed producers should ensure that records and recall procedures are in place to effectively respond to health risk situations. Procedures should enable the complete and rapid recall of any implicated seed. The procedures should also assist in providing detailed information for the identification and investigation of any contaminated seed and sprouts.

Several guidance documents are already available where the most relevant measures to ensure traceability have been described (ESSA, 2017; FAO and WHO, 2017; SSA, 2017). The European Sprouted Seeds Association (ESSA) hygiene guideline for the production of sprouts and seed for sprouting published by the European Union (ESSA, 2017) highlights the most important ones.

- Sprout producers should only purchase seed from trusted suppliers that have procedures in place to assure good hygienic production of the seed and traceability of the seed lots.
- If possible, when seed for the purpose of sprouting is imported, a consignment of seed should be accompanied by an import certificate during all stages of trade.
- When traders are involved in the supply chain of seed for sprouting, they must also follow the same traceability requirements.
- All the relevant information regarding the seed lot should be provided by the seed supplier to the sprout producer. This information includes:
 - > name of the product including the Latin name (taxonomic name);
 - > identification number or equivalent lot reference;
 - > name of the supplier;
 - > name and address of the recipient (if a forwarder or agent is used: name and address of the agent or forwarder);
 - > date of shipping; and
 - > quantity supplied.
- Seed and sprout producers should have a system to effectively identify a seed lot, trace their associated production sites and agricultural inputs, and allow for the physical retrieval of the seed in the event of a suspected hazard.
- Seed production and distribution practices should be in place to minimize the quantity of seed identified as a single lot and avoid the mixing of multiple lots of seed, which would complicate recalls and provide greater opportunities for cross-contamination.
- The customer or next person in the supply chain should receive all the information relevant to them to handle, store, process, prepare and display the

product safely and correctly. Where appropriate and useful, this information can be included as part of the packaging label.

- Recording and traceability requirements should be followed throughout the entire production process and records should be kept until it can reasonably be assumed that the sprouts have been consumed. Traceability codes or numbers printed on the sprout packaging material may facilitate recalls in the event that food contamination occurs.
- The information may be kept on records and transmitted in an appropriate form, provided it is easily retrievable by the Food business operator (FBO) to whom the seed or sprouts have been supplied. The FBO must also provide the information to the competent authority, upon request, without undue delay. For instance, in the event that a sprout producer detects a positive for a foodborne pathogen in the spent sprout irrigation water, this information should be rapidly transferred to the seed supplier to proceed with recalling all the seed in the implicated lot.
- Once on the market, products should be correctly labelled to facilitate traceability and recall where necessary. Inclusion of the identification or sprout batch code, as well as the name and address of the producer on the packaging label, may facilitate traceability and recall.
- Where a lot of seed has been recalled because of a health hazard, other lots of seed produced under similar conditions (e.g. on the same production sites or with the same agricultural inputs) and which may present a similar hazard should be evaluated for safety. Any lot of seed presenting a similar risk should be recalled. Blends containing potentially contaminated seed should also be recalled.

The main objective of traceability is to make it possible, at any moment during the physical flow of the production process, to know which batch of sprouts originates from which immediate supplier. It is clear that digital traceability represents a good alternative to more traditional handwritten systems. Digital traceability is the process of tracking a product via digital systems, removing the risk of human error. Digital traceability can alleviate and reduce many of the agrifood sector's most pressing risks. The Traditional Internet of Things (IoT) traceability systems provide practical solutions for quality monitoring and traceability of food supply chains (Feng *et al.*, 2020). Recent studies have demonstrated that blockchain is a pioneering technology with great potential for improving traceability performance (Galvez, Mejuto and Simal-Gandara, 2018).

7.2 UNCERTAINTY AND DATA GAPS

There is still a lack of information regarding the benefits that the implementation of digital traceability could provide to this sector. There are very high expectations about the improvements that this new technology can provide to the sector. However, the use of blockchain technology still has some barriers, such as high prices, accessibility and acceptance, which has hindered its implementation in the agrifood sector. However, the use of blockchain-based traceability management seems to be imminent.



Training

Proper training is imperative for personnel involved in the production of safe food. This is particularly important in the production of sprouts, which are produced under conditions that support the proliferation of bacterial pathogens and where hygienic interventions can be complicated and technical. Many outbreaks of foodborne illness have been associated with the consumption of contaminated sprouts, highlighting the need for improved awareness of hygiene and the adoption of hygienic practices along the production chain.

The US Food and Drug Administration has published guidance for the sprout seed industry and provides firms with recommended steps to prevent contamination throughout the production chain of seed for sprouting. The publication *Reducing Microbial Food Safety Hazards in the Production of Seed for Sprouting* (FDA, 2022b) highlights the need to take steps to educate and train personnel who have food safety responsibilities in the principles of food hygiene, food safety and personal health and hygiene. For example, seed producers, handlers and distributors should be aware of GAPS and their role and responsibility in producing and protecting seed intended for sprouting from contamination.

Few training initiatives have focused on the production of sprouts and seed for sprouting. Such training is crucial, and should extend along the entire sprout supply chain, from the production of seed for sprouting, through to the production of sprouts and their handling at point-of-sale. There is clearly a need to create a core curriculum that can be delivered to stakeholders involved in sprout production. It should cover the principles of food hygiene and food safety. Sprout producers should follow GHPs, with training in hygienic production of sprouts – including interventions designed to reduce growth of microbiological hazards. Such training should cover seed sourcing and storage, seed treatment, sampling and microbial

testing, cleaning and sanitizing and record-keeping. Equally important is guidance on personal hygiene and maintaining hygienic working environments.

The Sprout Safety Alliance at the Illinois Institute of Technology (<https://www.ifsh.iit.edu/ssa>) currently delivers a two-day Sprout Grower Training Course with a curriculum that is recognized by the FDA. The core curriculum “Safer Sprout Production for Produce Safety Rule Compliance” (SSA, 2017) is divided into five segments:

- An overview of the course and the provisions in the Produce Safety Rule that are applicable to sprout operations;
- An overview of the different types of food safety hazards associated with sprouts and the importance of their control;
- Maintaining a hygienic production environment, including proper operation construction, water safety, employee health and hygiene, cleaning and sanitizing procedures and verification, and environmental monitoring for *Listeria* in a sprout operation;
- Sprout specific requirements, including seed purchasing, receiving and storage, seed treatment, and spent sprout irrigation water or in-process sprout testing;
- Additional control programmes (e.g. employee training, product labelling, trace and recall procedures, sanitary transportation, allergen controls, and food defence) and record-keeping requirements.

Successful completion of the programme assists producers in understanding the regulatory requirements in the United States of America and implementing best practices for enhancing sprout safety.

References

- Aldabe, B., Delmas, Y., Gault, G., Vendrely, B., Llanas, B., Charron, M. & Castor, C.** 2011. Household transmission of haemolytic syndrome associated with *Escherichia coli* O104:H4, South-Western France, June 2011. *Eurosurveillance*, 16(31): 19934. <https://www.eurosurveillance.org/content/10.2807/ese.16.31.19934-en>
- Alegbeleye, O.O., Singleton, I. & Sant'Ana, A.S.** 2018. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. *Food microbiology*, 73: 177–208. <https://doi.org/10.1016/j.fm.2018.01.003>
- Aytac, S.A. & Gorris, L.G.** 1994. Survival of *Aeromonas hydrophila* and *Listeria monocytogenes* on fresh vegetables stored under moderate vacuum. *World Journal of Microbiology & Biotechnology*, 10(6): 670–72. <https://doi.org/10.1007/BF00327956>
- Bari, M.L., Enomoto, K., Nei, D. & Kawamoto, S.** 2010. Practical evaluation of mung bean seed pasteurization method in Japan. *Journal of Food Protection*, 73(4): 752–757. <https://doi.org/10.4315/0362-028x-73.4.752>
- Bazaco, M.C., Viazis, S., Obenhuber, D.C., Homola, P., Shakir, F. & Fields, A.** 2021. An overview of historic foodborne illness outbreak investigations linked to the consumption of sprouts: 2012–2020. *Food Safety Magazine*, 1 June 2021. Cited 11 June 2022. <https://www.food-safety.com/articles/7202-an-overview-of-historic-foodborne-illness-outbreak-investigations-linked-to-the-consumption-of-sprouts-20122020>
- Bennik, M.H., van Overbeek, W., Smid, E.J. & Gorris, L.G.** 1999. Biopreservation in modified atmosphere stored mung bean sprouts: the use of vegetable-associated bacteriocinogenic lactic acid bacteria to control the growth of *Listeria monocytogenes*. *Letters in Applied Microbiology*, 28(3): 226–232. <https://doi.org/10.1046/j.1365-2672.1999.00497.x>
- BfR (German Federal Institute of Risk Assessment).** 2011. EHEC outbreak 2011: updated analysis as a basis for recommended measures. *BfR opinion No. 049/2011*. Cited 11 June 2022 www.bfr.bund.de/cm/349/ehec-outbreak-2011-updated-analysis-as-a-basis-for-recommended-measures.pdf
- BMT (BMT Netherlands B.V.).** 2022. *Perishables, Products, Bean Sprouts*. Cargo Handbook. Cited 26 July 2022. www.cargohandbook.com/Bean_Sprouts#Cooling_and_Storage
- Bozkurt, H., Lai, Y.W., Jay-Russell, M. & McConchie, R.** 2021. Factsheet – The impact of animals on the risk of foodborne illness in fresh produce. *Fresh Produce Safety Centre, Australia and New Zealand*. Cited 22 June 2022. <https://fpssc-anz.com/wp-content/uploads/2021/10/FPSC-Fact-Sheet-Animal-Impact-on-Produce-Safety-Oct-21.pdf>

- Brandi, M.T. & Amundson, R.** 2008. Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica*. *Applied and Environmental Microbiology*, 74(8): 2298–2306. <https://doi.org/10.1128/AEM.02459-07>
- Bylund, J.** 2013. A sample preparation method for microbiological analysis of seeds. Uppsala Sweden, Swedish University of Agricultural Science. Master's dissertation. Cited 22 June 2022. https://stud.epsilon.slu.se/5989/1/bylund_j_130828.pdf
- Castro-Ibáñez, I., Gil, M.I., Tudela, J.A. & Allende, A.** 2015. Microbial safety considerations of flooding in primary production of leafy greens: a case study. *Food Research International*, 68: 62–69. <https://doi.org/10.1016/j.foodres.2014.05.065>
- Cevallos-Cevallos, J.M., Gu, G., Danyluk, M.D., Dufault, N.S. & Van Bruggen, A.H.C.** 2012. *Salmonella* can reach tomato fruits on plants exposed to aerosols formed by rain. *International Journal of Food Microbiology*, 158: 140–146. <https://doi.org/10.1016/j.ijfoodmicro.2012.07.009>
- CFIA (Canadian Food Inspection Agency).** 2007. Code of Practice for the Hygienic Production of Sprouted Seeds. In: *Archived food guidance - Fresh fruits and vegetables – Food safety*. Cited 14 June 2022. <https://inspection.canada.ca/food-safety-for-industry/archived-food-guidance/fresh-fruits-and-vegetables/food-safety/sprouted-seeds/eng/1413825271044/1413825272091>
- CFIA.** 2018. Preventive controls for the hygienic production of sprouted seeds. In: *Preventative controls for food – Fresh fruits or vegetables*. Ottawa. Cited 11 June 2022. <https://inspection.canada.ca/preventive-controls/fresh-fruits-or-vegetables/sprouted-seeds/eng/1524179755850/1524179758065>
- Charoux, C.M.G., Patange, A., Lamba, S., O'Donnell, C.P., Tiwari, B.K. & Scannell, A.G.M.** 2020. Applications of nonthermal plasma technology on safety and quality of dried food ingredients. *Journal of Applied Microbiology*, 130: 325–340. <https://doi.org/10.1111/jam.14823>
- Chen, Z. & Jiang, X.** 2014. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. *Agriculture*, 4: 129. <https://doi.org/10.3390/agriculture4010001>
- Chen, Z. & Jiang, X.** 2017. Microbiological safety of animal wastes processed by physical heat treatment: an alternative to eliminate human pathogens in biological soil amendments as recommended by the food safety modernization act. *Journal of Food Protection*, 80: 392–405. <https://doi.org/10.4315/0362-028X.JFP-16-181>
- Chen, Z., Kim, J. & Jiang, X.** 2018. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* in animal waste-based composts as influenced by compost type, storage condition and inoculum level. *Journal of Applied Microbiology*, 124: 1311–1323. <https://doi.org/10.1111/jam.13719>

- Critzler, F. & Doyle, M.** 2010. Microbial ecology of foodborne pathogens associated with produce. *Current Opinion in Biotechnology*, 21: 125–130. <https://doi.org/10.1016/j.copbio.2010.01.006>
- Dechet, A.M., Herman, K.M., Chen Parker, C., Taormina, P., Johanson, J., Tauxe, R.V. & Mahon, B.E.** 2014. Outbreaks caused by sprouts, United States, 1998–2010: lessons learned and solutions needed. *Foodborne Pathogens and Disease*, 11(8): 635–644. <https://doi.org/10.1089/fpd.2013.1705>
- Diercke, M., Kirchner, M., Claussen, K., Mayr, E. Strotmann, I., Frangenberg, J. Schiffmann, A., Bettge-Weller, G., Arvand, M. & Uphoff, H.** 2013. Transmission of shiga toxin-producing *Escherichia coli* O104:H4 at a family party possibly due to contamination by a food handler, Germany 2011. *Epidemiology and Infection*, 142: 99–106. <https://doi.org/10.1017/S0950268813000769>
- Ding, H., Fu, T.J., & Smith, M.A.** 2013. Microbial contamination in sprouts: how effective is seed disinfection treatment. *Journal of Food Science*, 78(4): R495–R501. <https://doi.org/10.1111/1750-3841.12064>
- Dungan, R.S.** 2010. Board-Invited Review: fate and transport of bioaerosols associated with livestock operations and manures. *Journal of Animal Science*, 88: 3693–3706. <https://doi.org/10.2527/jas.2010-3094>
- EC (European Commission).** 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs 2073/2005, 2005. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02005R2073-20140601&from=EN>
- EC 2013 Regulation (EU) No 208/2013 of 11 March 2013 on traceability requirements for sprouts and seeds intended for the production of sprouts 208/2013, 2013.** <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02013R0211-20140716&from=RO>
- EC.** 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). *Official Journal of the European Union*, C 163/1, 23/05/2017.
- EFSA (European Food Safety Authority).** 2011. Scientific opinion of the panel of Biological Hazards on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal*, 9(11):2424. <https://doi.org/10.2903/j.efsa.2011.2424>
- EFSA.** 2020. Guidance on date marking and related food information: part 1 (date marking). *EFSA Journal*, 18(12):6306. <https://doi.org/10.2903/j.efsa.2020.6306>

- ESSA (European Sprouted Seeds Association).** 2017. ESSA hygiene guideline for the production of sprouts and seeds for sprouting. *Official Journal of the European Union*, C 220/03: 29–52. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.C_.2017.220.01.0029.01.ENG&toc=OJ%3AC%3A2017%3A220%3ATOC
- FAO & WHO (Food and Agriculture Organization of the United Nations & World Health Organization).** 2008. Microbiological hazards in fresh leafy vegetables and herbs: meeting report. Microbiological Risk Assessment Series No. 14. Rome. FAO. Cited 22 June 2022. <https://www.fao.org/3/i0452e/i0452e.pdf>
- FAO & WHO.** 2017. Codex Alimentarius. Code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003), Annex II: Sprouts. Rome, FAO. Cited 14 June 2022. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXC%2B53-2003%252FCXC_053e.pdf
- FAO & WHO.** 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization and monitoring: report. Microbiological Risk Assessment Series No. 31. Rome. FAO. Cited 22 June 2022. <https://www.fao.org/3/ca0032en/ca0032en.pdf>
- FAO & WHO.** 2019. Safety and quality of water used in food production and processing: meeting report. Microbiological Risk Assessment Series No. 33. Rome. FAO. Cited 22 June 2022. <https://www.fao.org/3/ca6062en/ca6062en.pdf>
- FAO & WHO.** 2021a. Safety and quality of water used with fresh fruits and vegetables. Microbiological Risk Assessment Series No. 37. Rome. FAO. Cited 22 June 2022. <https://www.fao.org/3/cb7678en/cb7678en.pdf>
- FAO & WHO.** 2021b. Summary report of Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the prevention and control of microbiological hazards in fresh fruits and vegetables. Cited 3 November 2022. <https://www.fao.org/3/cb7664en/cb7664en.pdf>
- FAO & WHO.** 2022. *Listeria monocytogenes* in ready-to-eat (RTE) food: attribution, characterization and monitoring: meeting report. Microbiological Risk Assessment Series No. 38. Rome. FAO. Cited 3 November 2022. <https://www.fao.org/documents/card/en/c/cc2400en>
- FDA (United States Food and Drug Administration).** 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. In: *FDA, Science and Research (food), CFSAN Risk & Safety Assessment*. Cited 18 June 2022. <https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-assessment-relative-risk-public-health-foodborne-listeria-monocytogenes-among-selected>

- FDA.** 2015a. Standards for the growing, harvesting, packing, and holding of produce for human consumption, Final rule. *Federal Register*, 80: 74354–74568. Cited 22 June 2022. www.govinfo.gov/content/pkg/FR-2015-11-27/pdf/2015-28159.pdf
- FDA.** 2015b. Testing methodologies for *E. coli* O157:H7 and *Salmonella* species in spent sprout irrigation water (or sprouts). Cited 22 June 2022. www.fda.gov/media/94349/download
- FDA.** 2017a. Compliance with and recommendations for implementation of the standards for the growing, harvesting, packing, and holding of produce for human consumption for sprout operations: Draft guidance for industry. Cited 18 June 2022. www.fda.gov/media/102430/download
- FDA.** 2017b. Control of *Listeria monocytogenes* in ready-to-eat foods: draft guidance for industry. Cited 7 August 2022. www.fda.gov/files/food/published/Draft-Guidance-for-Industry-Control-of-Listeria-monocytogenes-in-Ready-To-Eat-Foods-%28PDF%29.pdf
- FDA.** 2017c. FY 2014 – 2016 Microbiological sampling assignment summary report: Sprouts. Cited 14 June 2022. <https://www.fda.gov/files/food/published/FY-2014-%E2%80%93-2016-Microbiological-Sampling-Assignment-Summary-Report-Sprouts-PDF.pdf>
- FDA.** 2018. Equivalent testing methodologies for *E. coli* O157:H7 and *Salmonella* in spent sprout irrigation water or sprouts samples. Cited 22 June 2022. www.fda.gov/food/laboratory-methods-food/equivalent-testing-methodologies-e-coli-o157h7-and-Salmonella-spent-sprout-irrigation-water-or
- FDA.** 2022a. Bacteriological Analytical Manual (BAM), Chapter 5: *Salmonella*. In: *US FDA, Science & Research, Laboratory Methods*. Cited 27 June 2022. www.fda.gov/food/laboratory-methods-food/bam-chapter-5-Salmonella
- FDA.** 2022b. Reducing microbial food safety hazards in the production of seed for sprouting: guidance for industry. Cited 7 August 2022. <https://www.fda.gov/media/127972/download>
- Feng, H., Wang, X., Duan, Y., Zhang, J. & Zhang, X.** 2020. Applying blockchain technology to improve agri-food traceability: a review of development methods, benefits and challenges. *Journal of Cleaner Production*, 260: 121031. <https://doi.org/10.1016/j.jclepro.2020.121031>
- Francis, G.A. & O'Beirne, D.** 2001a. Effects of acid adaptation on the survival of *Listeria monocytogenes* on modified atmosphere packaged vegetables. *International Journal of Food Science and Technology*, 36: 477–487. <https://doi.org/10.1046/j.1365-2621.2001.00489.x>
- Francis, G.A. & O'Beirne, D.** 2001b. Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Journal of Industrial Microbiology and Biotechnology*, 27(2): 111–116. <https://doi.org/10.1038/sj.jim.7000094>

- Fu, T.-J., Reineke, K.F., Chirtel, S. & VanPelt, O.M.** 2008. Factors influencing the growth of *Salmonella* during sprouting of naturally contaminated alfalfa seeds. *Journal of Food Protection*, 71: 888–896. <https://doi.org/10.4315/0362-028X-71.5.888>
- Fu, T.-J., Maks, N., Shazer, A.G. & Chrysogelos, C.** 2022. Comparison of commercial test kits for detection of *Salmonella* and *E. coli* O157: H7 in alfalfa sprout irrigation water. *Journal of AOAC International*, 105(4): 1092–1114. <https://doi.org/10.1093/jaoacint/qsac008>
- Galieni, A., Falcinelli, B., Stagnari, F., Datti, A. & Benincasa, P.** 2020. Sprouts and microgreens: Trends, opportunities, and horizons for novel research. *Agronomy*, 10(9): 1424–1469. <https://doi.org/10.3390/agronomy10091424>
- Galvez, J.F., Mejuto, J.C. & Simal-Gandara, J.** 2018. Future challenges on the use of blockchain for food traceability analysis. *TrAC Trends in Analytical Chemistry*, 107: 222–232. <https://doi.org/10.1016/j.trac.2018.08.011>
- Garrett, K.A., Nita M., De Wolf E.D., Esker P.D., Gomez-Montano L. & Sparks A.H.** 2016. Plant pathogens as indicators of climate change. In: T.M. Letcher, ed. *Climate change: observed impacts on planet Earth*, pp. 425–437. Amsterdam, Elsevier.
- Goulet, V., Hebert, M., Hedberg, C., Laurent, E., Vaillant, V., De Valk, H. & Desenclos, J. C.** 2012. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clinical Infectious Diseases*, 54(5): 652–660. <https://doi.org/10.1093/cid/cir902>
- Greig, J.D., Todd, E.C.D., Bartleson, C.A. & Michaels, B.S.** 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1. Description of the problem, methods and agents involved. *Journal of Food Protection*, 70: 1752–1761.
- Gurtler, J.B., Doyle, M.P., Erickson, M.C., Jiang, X., Millner, P. & Sharma, M.** 2018. Composting to inactivate foodborne pathogens for crop soil application: a review. *Journal of Food Protection*, 81: 1821–1837. <https://doi.org/10.4315/0362-028x.jfp-18-217>
- Hora, R., Kumar, M., Garcia, L., Schumacher, B., Odumeru, J. & Warriner, K.** 2005. Spatial distribution of *Salmonella*, *Escherichia coli* O157:H7, and other bacterial populations in commercial and laboratory-scale sprouting mung bean beds. *Journal of Food Protection*, 68: 2510–2518. <https://doi.org/10.4315/0362-028x-68.12.2510>
- ISGA (International Sprout Growers Association).** 2022. Current industry guidance for sprouts and microgreen temperatures and shelf-life. In: International Sprout Growers Association. Cited 22 June 2022. <https://isga-sprouts.org/current-industry-guidance-for-sprouts-and-microgreen-temperatures-and-shelf-life>

- Islam, M., Doyle, M.P., Phatak, S.C., Millner, P. & Jiang, X.** 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection*, 67: 1365–1370. <https://doi.org/10.4315/0362-028X-67.7.1365>
- ISS (International Speciality Supply).** 2021. ISS Seed Screening Procedures. In: *International Specialty Supply*. Cited 22 June 2022. <https://sproutnet.com/iss-seed-screening-procedures>
- ISS.** 2022. About sungarden seed. In: *Sungarden Seed*. Cited 14 June 2022. <https://sproutnet.com/about-sungarden-seed>
- Jiang, X., Islam, M., Morgan, J. & Doyle, M.P.** 2004. Fate of *Listeria monocytogenes* in bovine manure-amended soil. *Journal of Food Protection*, 67: 1676–1681.
- Jin, H., Yates, A., Craig, D., Blenman, P. & Crerar, S.A.** 2019. Through-chain Analysis of Microbiological Food Safety Hazards and Control Measures Associated with Production and Supply of Seed Sprouts for Human Consumption – Chapter 7. In: V. Khader, ed. *Current Trends in Food Science, Volume 1*, pp. 57–71. London, B.P. International.
- Kenward, A., Zenes, N., Bronzan, J., Brady, J. & Shah, K.** 2016. Overflow: climate change, heavy rain and sewage. *Climate Central*. Cited 14 June 2022. http://assets.climatecentral.org/pdfs/Overflow_sewagereport_final.pdf
- Kimmelshue, C., Goggi, A. S. & Cademartiri, R.** 2019. The use of biological seed coatings based on bacteriophages and polymers against *Clavibacter michiganensis subsp. nebraskensis* in maize seeds. *Scientific Reports*, 9: 17950. <https://doi.org/10.1038/s41598-019-54068-3>
- Kocharunchitt, C., Ross, T. & McNeil, D.L.** 2008. Use of bacteriophages as biocontrol agents to control *Salmonella* associated with seed sprouts. *International Journal of Food Microbiology*, 128: 453–459. <https://doi.org/10.1016/j.ijfoodmicro.2008.10.014>
- Kuijper, E.J., Soonawala, D., Vermont, C. & van Dissel, J.T.** 2011. Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* O104:H4 in the Netherlands, May 2011. *Eurosurveillance*, 16(25): 19897. Cited 22 June 2022. www.eurosurveillance.org/content/10.2807/ese.16.25.19897-en
- Kumar, M., Hora, R., Kostrzynska, M., Waites, W.M. & Warriner, K.** 2006. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* on mung beans, alfalfa, and other seed types destined for sprout production by using an oxychloro-based sanitizer. *Journal of Food Protection*, 69: 1571–1578.
- Langholz, J.A. & Jay-Russell, M.T.** 2013. Potential Role of Wildlife in Pathogenic Contamination of Fresh Produce. *Human-Wildlife Interactions*, 7(1). <https://doi.org/10.26077/e5gg-r037>

- Law, J.W.F., Ab Mutalib, N.S., Chan, K.G. & Lee, L.H.** 2015. Rapid methods for the detection of foodborne bacterial pathogens: principles, application, advantages and limitations. *Frontiers in Microbiology*, 5: 1–19.
- Lee, S.-Y., Yun, K.-M., Fellman, J. & Kang, D.-H.** 2002. Inhibition of *Salmonella* Typhimurium and *Listeria monocytogenes* in mung bean sprouts by chemical treatment. *Journal of Food Protection*, 65(7): 1088–1092. <https://doi.org/10.4315/0362-028x-65.7.1088>
- Liu, B. & Schaffner, D.W.** 2007. Mathematical modeling and assessment of microbial migration during the sprouting of alfalfa in trays in a nonuniformly contaminated seed batch using *Enterobacter aerogenes* as a surrogate for *Salmonella* Stanley. *Journal of Food Protection*, 70: 2602–2605. <https://doi.org/10.4315/0362-028X-70.11.2602>
- Machado-Moreira, B., Tiwari, B.K., Richards, K.G., Abram, F. & Burgess, C.M.** 2021. Application of plasma activated water for decontamination of alfalfa and mung bean seeds. *Food Microbiology*, 96: 103708. <https://doi.org/10.1016/j.fm.2020.103708>
- Mahon, B.E., Pönkä, A., Hall, W.N., Komatsu, K., Dietrich, S.E., Siitonen, A., Cage, G., Hayes, P.S., Lambert-Fair, M.A., Bean, N.H., Griffin, P.M. & Slutsker, L.** 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. *The Journal of Infectious Diseases*, 175: 876–82. <https://doi.org/10.1086/513985>
- Mangal, M., Bansal, S., Sharma, S.K. & Gupta, R.K.** 2016. Molecular detection of foodborne pathogens: a rapid and accurate answer to food safety. *Critical Reviews in Food Science and Nutrition*, 56: 1568–1584.
- McAllister, T.A. & Topp, E.** 2012. Role of livestock in microbiological contamination of water: Commonly the blame, but not always the source, *Animal Frontiers*, 2: 17–27. <https://doi.org/10.2527/af.2012-0039>
- McEgan, R.** 2008. Spent Mung Bean Irrigation Water Sampling and Screening Methodology. Guelph, Canada. University of Guelph. Master's dissertation. Cited 22 June 2022. <https://atrium.lib.uoguelph.ca/xmlui/handle/10214/20931?show=full>
- Melo, A.M.A., Alexandre, D.L., Furtado, R.F., Borges, M.F., Figueiredo, E.A.T., Biswas, A., Cheng, H. N. & Alves, C.R.** 2016. Electrochemical immunosensors for *Salmonella* detection in food. *Applied Microbiology and Biotechnology*, 100: 5301–5312.
- Mir, S.A., Farooq, S., Shah, M.A., Sofi, S.A., Dar, B.N., Hamdani, A.M. & Khaneghah, A.M.** 2021. An overview of sprouts nutritional properties, pathogens and decontamination technologies, *LWT*, 141: 110900. <https://doi.org/10.1016/j.LWT.2021.110900>

- Miyahira, R.F. & Antunes, A.E.C.** 2021. Bacteriological safety of sprouts: A brief review. *International Journal of Food Microbiology*, 352: 109266. <https://doi.org/10.1016/j.ijfoodmicro.2021.109198>
- Molinos, A.C., Abriouel, H., Ben Omar, N., Valdivia, E., López, R. L., Maqueda, M., Cañamero, M.M. & Gálvez, A.** 2005. Effect of immersion solutions containing enterocin AS-48 on *Listeria monocytogenes* in vegetable foods. *Applied and Environmental Microbiology*, 71(12): 7781–7787. <https://doi.org/10.1128/AEM.71.12.7781-7787.2005>
- Montville, R. & Schaffner, D.** 2005. Monte Carlo simulation of pathogen behavior during the sprout production process. *Applied and Environmental Microbiology*, 71: 746–753. <https://doi.org/10.1128/aem.71.2.746-753.2005>
- Mueller, S.C.** 2008. Producing quality alfalfa seed for the forage industry. In: *University of California Alfalfa & Forages – Archive of proceedings*. Cited 14 June 2022. <https://alfalfa.ucdavis.edu/+symposium/proceedings/2008/08-27.pdf>
- NACMCF (National Advisory Committee on Microbiological Criteria for Foods).** 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *International Journal of Food Microbiology*, 52(3):123–153. [https://doi.org/10.1016/S0168-1605\(99\)00135-X](https://doi.org/10.1016/S0168-1605(99)00135-X)
- NSWDPI (New South Wales Department of Primary Industries).** 2018. *Listeria* Outbreak Investigation. Summary Report for the Melon Industry, October 2018. Cited 14 June 2022. www.foodauthority.nsw.gov.au/sites/default/files/_Documents/foodsafetyandyou/Listeria_outbreak_investigation.pdf
- Oplinger, E.S., Hardman, L.L., Kaminski, A.R., Combs, S.M. & Doll, J.D.** 1990. Mungbean. Alternative Field Crops Manual. In: *Perdue University, Horticulture and landscape architecture*. Cited 14 June 2022. <https://hort.purdue.edu/newcrop/afcm/mungbean.html>
- Page, E.T.** 2018. Trends in food recalls 2004-13, economic and information bulletin, 191. In: *U.S. Department of Agriculture, Economic Research Service*. Cited 24 June 2022. www.ers.usda.gov/webdocs/publications/88497/eib-191.pdf?v=2753.9
- Pouillot, R., Hoelzer, K., Jackson, K.A., Henaoui, O.L., & Silk, B.J.** 2012. Relative risk of listeriosis in Foodborne Diseases Active Surveillance Network (FoodNet) sites according to age, pregnancy, and ethnicity. *Clinical Infectious Diseases*, 54(suppl_5): S405–S410.
- Pouillot, R., Hoelzer, K., Chen, Y., & Dennis, S.B.** 2015. *Listeria monocytogenes* dose response revisited—incorporating adjustments for variability in strain virulence and host susceptibility. *Risk Analysis*, 35(1): 90–108.

- Putnam, D., Mueller, S., Frate, C., Canevari, M., Orloff, S.** 2012. Key practices for alfalfa stand establishment. In: *University of California, Alfalfa & Forages*. Cited 14 June 2022. <https://alfalfa.ucdavis.edu/+symposium/proceedings/2012/12-19.pdf>
- Sikin, A.M., Zoellner, C. & Rizvi, S.S.** 2013. Current intervention strategies for the microbial safety of sprouts. *Journal of Food Protection*, 76(12): 2099–2123. <https://doi.org/10.4315/0362-028X.JFP-12-437>
- Solomon, E.B., Yaron, S. & Matthews, K.R.** 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology*, 68: 397–400.
- SproutPeople.** 2022. The basics of sprouting: planting. In: *SproutPeople, Growing sprouts and microgreens*. Cited 14 June 2022. <https://sproutpeople.org/growing-sprouts/sprouting-basics>
- SSA (Sprout Safety Alliance).** 2017. Safer sprout production for produce safety rule compliance, training curriculum. Cited 23 June 2022. <https://d1vy0qa05cdjr5.cloudfront.net/c6f30ca0-84ae-4613-bec0-5439702d4b9e/FSPCA%20-%20Sprouts/SSA%20curriculum%20V2.3%20-%20For%20PRINT%20watermark%20optimized.pdf>
- Stewart, D.S., Reineke, K.F., Ulaszek, J.M. & Tortorello, M.L.** 2001. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. *Journal of Food Protection*, 64: 618–622. <https://doi.org/10.4315/0362-028X-64.5.618>
- Suslow, T. & Cantwell, M.** 2000. Sprouts, seed: recommendations for maintaining postharvest quality. https://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English/?uid=31&ds=799
- Suslow T.V., Wu, J., Fett, W.F. & Harris, L.J.** 2002. Detection and elimination of *Salmonella* Mbandaka from naturally contaminated alfalfa seed by treatment with heat or calcium hypochlorite *Journal of Food Protection*, 65: 452–458.
- Świeca, M. & Gawlik-Dziki, U.** 2015. Effects of sprouting and postharvest storage under cool temperature conditions on starch content and antioxidant capacity of green pea, lentil and young mung bean sprouts. *Food Chemistry*, 185: 99–105. <https://doi.org/10.1016/j.foodchem.2015.03.108>
- Thomas, J.L., Palumbo, M.S., Farrar, J.A., Farver, T.B. & Cliver, D.O.** 2003. Industry practices and compliance with U.S. Food and Drug Administration guidelines among California sprout firms. *Journal of Food Protection*, 66(7):1253–1259. <https://doi.org/10.4315/0362-028X-66.7.1253>

- Tian, J.Q., Bae, Y.M., Choi, N.Y., Kang, D.H., Heu, S. & Lee, S.Y.** 2012. Survival and growth of foodborne pathogens in minimally processed vegetables at 4 and 15 °C. *Journal of Food Science*, 77(1): M48–M50. <https://doi.org/10.1111/j.1750-3841.2011.02457.x>
- Umesha, S. & Manukumar, H.M.** 2018. Advanced molecular diagnostic techniques for detection of foodborne pathogens: current applications and future challenges. *Critical Reviews in Food Science and Nutrition*, 58: 84–104. <https://doi.org/10.1080/10408398.2015.1126701>
- USDA (United States Department of Agriculture).** 2020. Foodborne pathogen test kits validated by independent organizations (FSIS-GD-2019-0008). In: USDA, FSIS Guidelines. Cited 27 June 2022. <https://www.fsis.usda.gov/guidelines/2019-0008>
- Uyttendaele, M., Liu C. & Hofstra, N.** 2015. Special issue on the impacts of climate change on food safety. *Food Research International*, 68: 1–6. <https://doi.org/10.1016/j.foodres.2014.09.001>
- Van Beneden, C.A., Keene, W.E., Strang, R.A., Werker, D.H., King, A.S., Mahon, B., Hedberg, K., et al.** 1999. Multinational outbreak of *Salmonella* enterica serotype Newport infections due to contaminated alfalfa sprouts. *JAMA*, 281: 158–162. <https://doi.org/10.1001/jama.281.2.158>
- Van der Linden, I., Cottyn, B., Uyttendaele, M., Vlaemynck, G., Maes, M. & Heyndrickx, M.** 2013. Survival of enteric pathogens during butterhead lettuce growth: Crop stage, leaf age, and irrigation. *Foodborne Pathogens and Disease*, 10: 485–491.
- Watanabe, Y., Ozasa, K., Mermin, J.H., Griffin, P.M., Masuda, K., Imashuku, S. & Sawada, T.** 1999. Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. *Emerging Infectious Diseases*, 5: 424–428. <https://doi.org/10.3201/eid0503.990313>
- Wells, M.S., Holen, D. & Sheaffer, C.** 2018. Alfalfa establishment: Steps to maximize yield. In: *University of Minnesota Extension*. Cited 14 June 2022. <https://extension.umn.edu/planting-forages/alfalfa-establishment-steps-maximize-yield#field-preparation-and-seeders-703013>
- Wu, X., Lu Y., Zhou S., Chen L. & Xu B.** 2016. Impact of climate change on human infectious diseases: empirical evidence and human adaptation. *Environment International*, 86: 14–23.
- Xylia, P., Botsaris, G., Skandamis, P. & Tzortzakis, N.** 2021. Expiration date of ready-to-eat salads: effects on microbial load and biochemical attributes. *Foods*, 10(5): 941. <https://doi.org/10.3390/foods10050941>

- Yang, Y., Meier, F., Lo, J.A., Yuan, W., Sze, V.L.P., Chung, H.J. & Yuk, H.G.** 2013. Overview of recent events in the microbiological safety of sprouts and new intervention technologies. *Comprehensive Reviews in Food Science and Food Safety*, 12: 265–280. <https://doi.org/10.1111/1541-4337.12010>
- Ye, J., Kostrzynska, M., Dunfield, K. & Warriner, K.** 2010. Control of *Salmonella* on sprouting mung bean and alfalfa seeds by using a biocontrol preparation based on antagonistic bacteria and lytic bacteriophages. *Journal of Food Protection*, 73: 9–17. <https://doi.org/10.4315/0362-028x-73.1.9>
- Zhang, X.** 2017. Using lytic bacteriophages as a biocontrol intervention to eliminate *Salmonella* in romaine lettuce, mung bean sprouts, mung bean seeds and mung bean germinated sprouts and to prevent shiga-toxigenic *Escherichia coli* biofilm formation on romaine lettuce. Winnipeg, Canada, University of Manitoba. PhD dissertation. Cited 14 June 2022. <https://mspace.lib.umanitoba.ca/xmlui/handle/1993/32781>



Annexes

Response to the Codex Committee on Food Hygiene (CCFH) regarding specific interventions for sprouts

The following questions posed on 27 July 2021 by the CCFH E-Working Group (EWG) for the development of ‘Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts’ were addressed by the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the Prevention and Control of Microbiological Hazards in Sprouts.

Q1. Most control measures in this Annex (the question refers to Annex 2 ‘Fresh leafy vegetables’ of the draft ‘Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts’) are not specific to STEC (and thus information in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* would suffice). JEMRA - Please provide input on control measures that have been studied scientifically with respect to controls for STEC and thus warrant inclusion. (These measures may also control for other pathogens, but we need to know if there is sufficient scientific information related to controls for STEC to warrant including them in this Annex.)

A1. Many potential measures have been scientifically studied with respect to controls for microbiological hazards in sprouts. Based on the experts’ opinions, while much of this research was not carried out with STEC, the conclusions are valid for STEC control as well. Specific experiments using different STEC serotypes are not necessary; there is no evidence to indicate that different STEC serotypes behave differently from one other, or from other enteric pathogens like *Salmonella*, in response to these control measures. The most significant control measures include:

- Ensuring seeds intended for sprouting are produced under Good Agricultural Practices (GAPs).
- Ensuring water that comes into contact with the seed production crop directly is fit-for-purpose.

- Seed treatment to reduce microbiological foodborne pathogens on seed surfaces.
- Sampling and testing of spent sprout irrigation water for bacterial pathogens of concern.
- Maintenance of a sanitary sprout production environment.
- Maintenance of the cold chain at every stage following sprouting.

Q2. It has been suggested that the guidelines address HACCP system principles. Please provide input on whether good hygiene practices (GHPs) or good agricultural practices (GAPs) provide adequate control for STEC in a single step or whether there are applicable CCPs.

A2. In sprout seed production, GHPs or GAPs provide an effective means of establishing farming practices which minimize potential contamination by microbiological hazards, including STEC. Providing guidance to producers on minimizing contamination should be encouraged.

In sprout operations, guidelines that address HACCP system principles, including prerequisite GHPs are appropriate to reduce the risks associated with microbiological hazards, including STEC. While we were unable to identify any CCPs that eliminate microbiological hazards, seed treatment under some circumstances (i.e. chemical or physical treatments where critical limits can be set) can be an appropriate CCP to reduce pathogen contamination on seeds for sprouting, and subsequently reduce public health risk associated with sprouts (Chen *et al.*, 2018).

Q3. Can JEMRA provide advice on the role of testing of water to control STEC during sprout seed production? Is testing for STEC warranted and under what circumstances? What results would indicate a cause for concern? Are there appropriate indicator organisms that could be used in lieu of or in addition to testing for STEC? What would be an acceptable level (or levels of concern)? What should the frequency of water testing be?

A3. JEMRA does not recommend the routine testing of irrigation water for sprout seed production for the presence of STEC. Information on testing and indicator organisms were addressed during a JEMRA meeting on the use and reuse of water in vegetable production (FAO and WHO, 2021a).

Q4. It has been suggested that we include a recommendation for storage under 7 °C. JEMRA, does the science support this as an appropriate temperature for preventing growth of STEC in sprouts? Are there other temperatures combined with time that could apply?

A4. Sprouts should be kept at refrigerated temperatures that will minimize microbial growth for the intended shelf-life of the product. The temperature of storage areas and transport vehicles should be monitored. Currently there are limited studies about the minimal temperatures of growth for STEC and other microbiological hazards for sprouts (Aytac and Gorris, 1994; Tian *et al.*, 2012).

Q5. Question: What is the role of testing sprout seeds and sprouts for STEC and/or indicator organisms (including acceptable levels of organisms or levels of concern and frequency of testing)?

A5. Routine STEC testing (or any pathogen testing) of sprout seeds is not recommended by the experts. Anticipated concentrations and prevalence of foodborne pathogens present on sprout seeds are low (e.g. *Salmonella* from seeds associated with outbreaks were determined to be 13 MPN/kg to 16 MPN/kg and 20 MPN/kg to 100 MPN/kg of dry seeds (Fu *et al.*, 2008; Stewart *et al.*, 2001)). Pathogenic bacteria are distributed heterogeneously in seed lots (Van Beneden *et al.*, 1999). The probability for detection of foodborne pathogens in sprout seeds is estimated to be 0.1 percent, assuming that pathogens are present in one 25 g sample out of every 1 000 (Montville and Schaffner, 2005). To increase the probability of detecting any pathogen that may be present in a seed lot, it is necessary to analyse a large number of samples. EFSA (2011) gives a theoretical example of this problem. If one seed per kilo is infected, and infected seeds are randomly distributed, then at least three kilos of seeds need to be analysed in order to ensure that there is a 95-percent chance that the infected seed will be identified (EFSA, 2011).

During sprouting, pathogens of concern, if not eliminated by seed treatment, can grow to high numbers. Microbial testing of sprout production batch can contribute to early detection of pathogens and prevent the sale of contaminated sprouts. Research findings (Fu *et al.*, 2001) highlight that spent sprout irrigation water is a good indicator of microbial conditions in sprouts and testing spent sprout irrigation water is an effective method to detect microbial pathogens in a sprout production batch when using an appropriate sampling plan and testing protocol (FDA, 2017). While test methods for the detection of *Salmonella* or *E. coli* O157:H7 in sprouts or spent sprout irrigation water are available, the availability of validated test methods for detection of non-O157:H7 STEC remains limited.

References

- Aytac, S.A. & Gorris, L.G. 1994. Survival of *Aeromonas hydrophila* and *Listeria monocytogenes* on fresh vegetables stored under moderate vacuum. *World Journal of Microbiology and Biotechnology*, 10(6): 670–672. <https://doi.org/10.1007/BF00327956>
- Chen, Z., Kim, J. & Jiang, X. 2018. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* in animal waste-based composts as influenced by compost type, storage condition and inoculum level. *Journal of Applied Microbiology*, 124: 1311–1323. <https://doi.org/10.1111/jam.13719>
- EFSA (European Food Safety Authority). 2011. Scientific opinion of the panel of Biological Hazards on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal*, 9(11): 2424. <https://doi.org/10.2903/j.efsa.2011.2424>
- FAO & WHO. 2021a. Safety and quality of water used with fresh fruits and vegetables. Microbiological Risk Assessment Series No. 37. Rome. FAO. Cited 22 June 2022. <https://www.fao.org/3/cb7678en/cb7678en.pdf>
- FDA (US Food and Drug Administration). 2017. FY 2014 – 2016 Microbiological sampling assignment Summary report: Sprouts. Cited 14 June 2022. www.fda.gov/files/food/published/FY-2014-%E2%80%932016-Microbiological-Sampling-Assignment-Summary-Report-Sprouts-PDF.pdf
- Fu, T-J, Reineke, K.F., Chirtel, S. & VanPelt, O.M. 2008. Factors influencing the growth of *Salmonella* during sprouting of naturally contaminated alfalfa seeds. *Journal of Food Protection*, 71: 888–896. <https://doi.org/10.4315/0362-028x-71.5.888>
- Fu, T., Stewart, D., Reineke, K., Ulaszek, J., Schillessner, J. & Tortorello, M. 2001. Use of spent irrigation water for microbiological analysis of alfalfa sprouts. *Journal of Food Protection*, 64(6): 802–806. <https://doi.org/10.4315/0362-028X-64.6.802>
- Montville, R. & Schaffner, D. 2005. Monte Carlo simulation of pathogen behavior during the sprout production process. *Applied and Environmental Microbiology*, 71: 746–753. <https://doi.org/10.1128/AEM.71.2.746-753.2005>
- Stewart, D.S., Reineke, K.F., Ulaszek, J.M. & Tortorello, M.L. 2001. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. *Journal of Food Protection*, 64: 618–622. <https://doi.org/10.4315/0362-028X-64.5.618>
- Tian, J.Q., Bae, Y.M., Choi, N.Y., Kang, D.H., Heu, S. & Lee, S.Y. 2012. Survival and growth of foodborne pathogens in minimally processed vegetables at 4 and 15 °C. *Journal of Food Science*, 77(1): M48–M50. <https://doi.org/10.1111/j.1750-3841.2011.02457.x>
- Van Beneden, C.A., Keene, W.E., Strang, R.A., Werker, D.H., King, A.S., Mahon, B., Hedberg, K. *et al.* 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. *JAMA*, 281, 158–162. <https://doi.org/10.1001/jama.281.2.158>

Annex 2

Sprout-associated foodborne illness outbreaks and surveillance data

TABLE A2.1 Examples of bacterial contamination rates and their prevalence in sprouts sampled from the marketplace in three WHO regions for which surveillance data were available.

MICROBIAL HAZARD	AMR		EUR		WPR		TOTAL		REFERENCES
	PREVAL WAVG	POS RATE	PREVAL WAVG	POS RATE	PREVAL WAVG	POS RATE	PREVAL WAVG	POS RATE	
<i>Listeria spp.</i>	1%	6/469	0%	0/15	--	--	1%	6/484	Abadias <i>et al.</i> , 2008; FDA 2
<i>Salmonella spp.</i>	0%	0/471	0%	0/15	--	--	0%	0/486	Abadias <i>et al.</i> , 2008; Da Cruz <i>et al.</i> , 2019; FDA 2017
STEC	0%*	0/1383	40%	6/15	--	--	0%	6/1398	Abadias <i>et al.</i> , 2008; CFIA, 2022; FDA 2017
<i>Staphylococcus spp.</i>	0%	0/2	--	--	17%	19/112	0%	19/114	Da Cruz <i>et al.</i> , 2019; Seo, Jang and Moon, 2010
Total	0%	6/2323	13%	6/45	17%	19/112	1%	31/2482	

Source: Authors' elaboration.

* WHO classifications: AMR, Region of the Americas; EUR, European Region; WPR, Western Pacific Region. Preval WAVG, Prevalence as a weighted average; Pos rate, sample positive rate.

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
Jan 2020 – Mar 2020	<i>E. coli</i> O103	51	Clover sprouts	United States of America (multistate)	CDC, 2020
Nov 2019 – Dec 2019	<i>E. coli</i> O103	22	Clover sprouts	United States of America (Iowa)	FDA, 2020
Dec 2017 – Jan 2018	<i>Salmonella</i> Montevideo	10	Sprouts	United States of America (multistate)	CDC, 2018
May – July 2016	<i>Salmonella</i>	30	Alfalfa sprouts	United States of America (multistate)	Beach, 2016
Apr 2016	<i>Salmonella</i> Saintpaul	244	Mung bean sprouts	Australia	Stokes, 2016
Jan 2016	<i>E. coli</i> O157	11	Alfalfa sprouts	United States of America (Minnesota)	CDC, 2016b
Nov 2015 – Jan 2016	<i>Salmonella</i> Muenchen	13	Alfalfa sprouts	United States of America (multistate)	CDC, 2016a
Sep 2014	<i>Salmonella</i> Enteritidis	115	Bean sprouts	United States of America (multistate)	CDC, 2015a
Jun – Aug 2014	<i>Listeria monocytogenes</i>	5	Mung bean sprouts	United States of America (Illinois and Michigan)	CDC, 2015b
May 2014	<i>E. coli</i> O121	19	Raw clover sprouts	United States of America (Washington and Idaho)	CDC, 2014a
Jul 2012	<i>Salmonella</i> Cubana	19	Sprouts, unspecified	United States of America (multistate)	CDC, 2022
Mar 2012	<i>Listeria monocytogenes</i>	6	Sprouts, unspecified	United States of America (multistate)	CDC, 2014b
Dec 2011 – Feb 2012	<i>E. coli</i> O26	29	Raw clover sprouts	United States of America (multistate)	CDC, 2012
Aug 2011	<i>Salmonella</i> Agona	7	Sprouts, unspecified	United States of America (Kansas)	CDC, 2022
Apr – Jul 2011	<i>Salmonella</i> Enteritidis	27	Alfalfa sprouts and spicy sprouts	United States of America (multistate)	CDC, 2011a
May – Jul 2011	<i>E. coli</i> O104:H4	4 075	Fenugreek sprouts	Europe, Canada and United States of America	Buchholz <i>et al.</i> , 2011; CDC 2013
Apr 2011	<i>Salmonella</i> Muenchen	7	Clover sprouts	United States of America (Michigan)	CDC, 2022
Dec 2010 – Jan 2011	<i>Salmonella</i> Newport	9	Clover sprouts	United States of America (Oregon and Washington)	CDC, 2022

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts. (cont.)

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
Dec 2010	<i>Salmonella</i> Cubana	3	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Nov 2010 – Feb 2011	<i>Salmonella</i> serotype I 4,[5],12:i:-	140	Alfalfa sprouts	United States of America (multistate)	CDC, 2011b
Aug - Oct 2010	<i>Salmonella</i> Bareilly	231	Mung bean sprouts	United Kingdom	Cleary <i>et al.</i> , 2010
2010	<i>Salmonella</i> Kottbus	4	Bean sprouts	United Kingdom	EFSA, 2011
Mar – Jun 2010	<i>Salmonella</i> Newport	44	Alfalfa sprouts	United States of America (multistate)	CDC, 2010
Feb 2010	unknown	4	Sprouts, unspecified	United States of America (Colorado)	CDC, 2022
Aug 2009	<i>Salmonella</i> Typhimurium	14	Alfalfa sprouts	United States of America (Michigan)	CDC, 2022
Jun 2009	<i>Salmonella</i> Bovismorbificans	42	Alfalfa sprouts	Finland	Rimhanen-Finne <i>et al.</i> , 2011
Apr – Jul 2009	<i>Salmonella</i> Cubana	20	Onion sprouts and mixed onion/alfalfa sprout	Canada	Garcia and Heredia, 2020
Apr 2009	<i>Salmonella</i> Cubana	2	Sprouts, unspecified	United States of America (Minnesota)	CDC, 2022
Feb – May 2009	<i>Salmonella</i> Saintpaul	256	Alfalfa sprouts	United States of America (multistate)	CDC, 2009a, 2009b
Feb 2009	<i>Salmonella</i> Oranienberg	25	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Sep 2008	<i>E. coli</i> O157:NM	21	Alfalfa sprouts; iceberg lettuce, unspecified	United States of America (Colorado)	CDC, 2022
Jul 2008	<i>Salmonella</i> Typhimurium	24	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Mar 2008	<i>Listeria</i> <i>monocytogenes</i>	20	Sprouts, unspecified	United States of America (multistate)	CDC, 2022
2008	<i>Staph. aureus</i>	42	Bean sprouts	Denmark	EFSA, 2011
Jul – Oct 2007	<i>Salmonella</i> Weltevreden	45	Alfalfa sprouts	Denmark, Norway and Finland	Emberland <i>et al.</i> , 2007

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts. (cont.)

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
Jul - Aug 2007	<i>Salmonella</i> Stanley	44	Alfalfa sprouts	Sweden	Werner <i>et al.</i> , 2007
Apr 2007	<i>Salmonella</i> Mbandaka	15	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
2006	<i>Salmonella</i> Bareilly and Virchow	115	Mung Bean sprouts	Sweden	De Jong, Oberg and Svenungsson, 2007
Feb 2006	<i>Salmonella</i> Braenderup	4	Bean sprouts	United States of America (Oregon)	CDC, 2022
2006	<i>Salmonella</i> Oranienberg	15	Alfalfa sprouts	Australia	OzFoodNet, 2007
Nov 2005	<i>Salmonella</i> Oranienberg	125	Alfalfa sprouts	Australia	ADoH, 2006
Nov 2005	<i>Salmonella</i> Braenderup	2	Mung bean sprouts	United States of America (Massachusetts)	CDC, 2022
Oct - Dec 2005	<i>Salmonella</i> spp.	648	Mung bean sprouts	Canada	Ontario Newsroom, 2005
Apr 2004	<i>E. coli</i> O157:NM	2	Alfalfa sprouts	United States of America (Georgia)	CDC, 2022
Apr 2004	<i>Salmonella</i> Bovismorbificans	35	Alfalfa sprouts	United States of America (Oregon and Washington)	CDC, 2022
Nov 2003	<i>Salmonella</i> Chester	26	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Jul 2003	<i>E. coli</i> O157:NM	13	Alfalfa sprouts	United States of America (Colorado)	Ferguson <i>et al.</i> , 2005
Feb 2003	<i>Salmonella</i> Saintpaul	16	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Feb 2003	<i>E. coli</i> O157:H7	7	Alfalfa sprouts	United States of America (Minnesota)	Ferguson <i>et al.</i> , 2005
Jan 2003	<i>E. coli</i> O157:H7	20	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Jul 2002	<i>E. coli</i> O157:H7	5	Alfalfa sprouts	United States of America (California)	CDC, 2022
2002	<i>Salmonella</i> Abony	13	Mung bean sprouts	Finland	EFSA, 2011
Apr 2001	<i>Salmonella</i>	35	Mung bean sprouts	United States of America (Florida)	CDC, 2022

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts. (cont.)

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
Feb 2001	<i>Salmonella</i> Kottbus	32	Alfalfa sprouts	United States of America (multistate)	CDC, 2022
Jan 2001	<i>Salmonella</i> Enteritidis	22	Mung bean sprouts	United States of America (Hawaii)	CDC, 2022; Mohle-Boetani <i>et al.</i> , 2001
Feb – Mar 2001	<i>Salmonella</i> Enteritidis	84	Mung bean sprouts	Canada	Honish and Nguyen, 2021
Oct 2000	unknown	2	Alfalfa sprouts	United States of America (Florida)	CDC, 2022
Nov 2000	<i>Salmonella</i> Enteritidis phage type 4b	27	Bean sprouts	Netherlands	van Duynhoven <i>et al.</i> , 2002
May 2000	<i>Salmonella enterica</i>	3	Alfalfa sprouts (suspected)	United States of America (Florida)	CDC, 2022
2000	<i>Salmonella</i> Enteritidis	75	Mung bean sprouts	United States of America (multistate)	CDC, 2022
2000	<i>Salmonella</i> Enteritidis	8	Alfalfa sprouts	Canada	Harris <i>et al.</i> , 2003
Aug – Sep 1999	<i>Salmonella</i> Muenchen	157	Alfalfa sprouts	United States of America (multistate)	Proctor <i>et al.</i> , 2001
May 1999	<i>Salmonella</i> Saintpaul	36	Clover sprouts	United States of America (California)	CDC, 2022
1999	<i>Salmonella</i> spp.	34	Alfalfa sprouts	United States of America (multistate)	EFSA, 2011
Jan 1999	<i>Salmonella</i> Mbandaka	83	Alfalfa sprouts	United States of America (multistate)	Gill <i>et al.</i> , 2003
Jan 1999	<i>Salmonella</i> Typhimurium	112	Clover sprouts	United States of America (Colorado)	Brooks <i>et al.</i> , 2001; Winthrop <i>et al.</i> , 2003
Aug – Sep 1999	<i>Salmonella</i> Paratyphi B var java	51	Alfalfa sprouts	Canada	Stratton <i>et al.</i> , 2001
Jun 1998	<i>E. coli</i> O157:NM	8	Alfalfa sprouts	United States of America (California)	CDC, 2022; Mohle-Boetani <i>et al.</i> , 2001
May 1998	<i>Salmonella</i> Havana and Cubana	40	Alfalfa sprouts	United States of America (multistate)	CDC, 2022; Mohle-Boetani <i>et al.</i> , 2001
Jun – Jul 1997	<i>E. coli</i> O157:H7	82	Alfalfa sprouts	United States of America (Michigan and Virginia)	Breuer <i>et al.</i> , 2001; CDC, 1997

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts. (cont.)

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
1997	<i>Salmonella</i> Infantis and Anatum	109	Alfalfa sprouts	United States of America (Kansas and Missouri)	Glynn, Patrick and Wuhib, 1998; Taormina, Beuchat and Slutsker, 1999
Sep 1997 – Jul 1998	<i>Salmonella</i> Senftenberg	60	Alfalfa sprouts	United States of America (California and Nevada)	Mohle-Boetani <i>et al.</i> , 2001
1997	<i>Salmonella</i> Meleagridis	78	Alfalfa sprouts	Canada	Sewell and Farber, 2001; Taormina, Beuchat and Slutsker, 1999
1997	<i>E. coli</i> O157:H7	126	Radish sprouts	Japan	Gutierrez, 1997; Taormina, Beuchat and Slutsker, 1999
May – Jul 1996	<i>Salmonella</i> Meleagridis and Montevideo	500	Alfalfa and clover sprouts	United States of America (California and Nevada)	Mohle-Boetani <i>et al.</i> , 2001
1996	<i>E. coli</i> O157:H7	6 000	Radish sprouts	Japan	Watanabe <i>et al.</i> , 1999
1996	<i>Salmonella</i> Stanley	30	Alfalfa sprouts	United States of America (multistate)	Barrett and Chaos, 1996
1995 –1996	<i>Salmonella</i> Newport	>133	Alfalfa sprouts	United States of America, Canada and Denmark	Taormina, Beuchat and Slutsker, 1999; Van Beneden <i>et al.</i> , 1999; Wegener <i>et al.</i> , 1997
1995	<i>Salmonella</i> Stanley	242	Alfalfa sprouts	Finland and United States of America	Mahon <i>et al.</i> 1997
1994	<i>Salmonella</i> Newport	154	Alfalfa sprouts	Denmark	WHO, 2003
1994	<i>Salmonella</i> Bovismorbificans	595	Alfalfa sprouts	Sweden and Finland	Pönkä <i>et al.</i> , 1995; Puohiniemi, Heiskanen, and Siitonen, 1997; Taormina, Beuchat and Slutsker, 1999:
1992	<i>Salmonella enterica</i> serovar 4, 5, 12:b:-	272	Mung bean sprouts	Finland	Mattila <i>et al.</i> , 1994
Oct 1990	<i>Salmonella</i> Anatum	15	Alfalfa sprouts	United States of America (Washington)	CDC, 1990
Oct 1990	unknown	32	Alfalfa sprouts, cucumber, lettuce	United States of America (Washington)	CDC, 1990
1989	<i>Salmonella</i> Goldcoast	31	Cress sprouts	United Kingdom	Taormina, Beuchat and Slutsker, 1999; Joce <i>et al.</i> , 1990

TABLE A2.2 Foodborne illness outbreaks associated with the consumption of contaminated sprouts. (cont.)

DATE	CAUSATIVE AGENT	NO. OF ILLNESSES REPORTED	TYPE OF SPROUT IMPLICATED	COUNTRY OF OUTBREAK	REFERENCE
1989	<i>Listeria monocytogenes</i>	1	Alfalfa sprouts	Canada	Farber <i>et al.</i> , 1990
1988	<i>Salmonella</i> Saintpaul, Havana and Muenchen	148	Mung bean sprouts	Sweden	Nguyen-the and Carlin, 1994
1988	<i>Salmonella</i> Saintpaul and Virchow PT34 (7cases)	143	Mung bean sprouts	United Kingdom	O'Mahony <i>et al.</i> , 1990
1982	<i>Yersinia enterocolitica</i>	16	Bean sprouts	United States of America (multistate)	Cover and Aber, 1989
1973	<i>Bacillus cereus</i>	4	Soy, cress, mustard sprouts	United States of America (multistate)	Portnoy, Goepfert and Harmon, 1976

Source: adapted from: **Marler, B.** 2021. Marler Blog. <https://www.marlerblog.com/case-news/barf-blog-a-great-resource-on-why-we-should-reconsider-sprouts/>

References

- Abadias, M., Usall, J., Anguera, M., Solsona, C. & Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, 123(1–2): 121–9. <https://doi.org/10.1016/j.ijfoodmicro.2007.12.013>
- ADoH (Australian Department of Health). 2006. Burden and causes of foodborne disease in Australia: Annual report of the OzFoodNet network, 2005. *Communicable Disease Intelligence (CDI)*, 30(3). Cited 29 June 2022. www1.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3003b.htm
- Barrett, E. & Chaos, C. 1996. An outbreak of salmonellosis associated with eating alfalfa sprouts, Lexington, Virginia. In: *Sproutnet*. Cited 6 July 2022. www.sproutnet.com/Research/an_outbreak_of_salmonellosis.htm
- Beach, C. 2016. Fresh sprouts from Denver linked to 9-state outbreak. *Food Safety News*. 5 August 2016. Cited 24 June 2022. <https://www.foodsafetynews.com/2016/08/fresh-sprouts-from-denver-linked-to-9-state-outbreak/#.WXeKjBTZqf4>
- Breuer, T., Benkel, D.H., Shapiro, R.L., Hall, W.N., Winnett, M.M., Linn, M.J., Neimann, J., *et al.* 2001. A multistate outbreak of *Escherichia coli* O157: H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emerging Infectious Diseases*, 7(6): 977. DOI: 10.3201/eid0706.010609
- Brooks, J.T., Rowe, S.Y., Shillam, P., Heltzel, D.M., Hunter, S.B., Slutsker, L., Hoekstra, R.M. & Luby, S.P. 2001. Salmonella Typhimurium infections transmitted by chlorine-pretreated clover sprout seeds. *American Journal of Epidemiology*, 154(11): 1020–1028. <https://pubmed.ncbi.nlm.nih.gov/11724718/>
- Buchholz, U., Bernard, H., Werber, D., Böhmer, M.M., Renschmidt, C., Wilking, H., Deleré, Y., *et al.* 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *The New England Journal of Medicine*, 365(19): 1763–1770. <https://doi.org/10.1056/NEJMoa1106482>
- CDC (Centers of Disease Control and Prevention). 1997. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts - Michigan and Virginia, June–July 1997. *MMWR*, 46(32): 741–744. Cited 24 June 2022. <https://www.cdc.gov/mmwr/preview/mmwrhtml/00048994.htm>
- CDC. 1990. 1990 Foodborne disease outbreak line listing. In: CDC, Food Safety. Cited 24 June 2022. www.cdc.gov/foodsafety/pdfs/1990_linelist.pdf
- CDC. 2009a. Final update: Multistate outbreak of *Salmonella* Saintpaul infections linked to raw alfalfa sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2006 through 2009 Outbreaks*. Cited 24 June 2022. www.cdc.gov/salmonella/2009/raw-alfalfa-sprouts-5-8-2009.html

- CDC.** 2009b. Outbreak of *Salmonella* serotype Saintpaul infections associated with eating alfalfa sprouts – United States, 2009. *MMWR*, 58(18): 500–503. Cited 24 June 2022. www.cdc.gov/MMWR/preview/MMWRhtml/mm5818a4.htm
- CDC.** 2010. Final Update: Multistate outbreak of human *Salmonella* Newport infections linked to raw alfalfa sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2010 through 2011 Outbreaks*. Cited 24 June 2022. www.cdc.gov/salmonella/2010/newport-alfalfa-sprout-6-29-10.html
- CDC.** 2011a. Final update multistate outbreak of human *Salmonella* Enteritidis infections linked to alfalfa sprouts and spicy sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2010 through 2011 Outbreaks*. Cited 24 June 2022. www.cdc.gov/Salmonella/2011/alfalfa-spicy-sprouts-7-6-2011.html
- CDC.** 2011b. Final update: Multistate outbreak of human *Salmonella* I 4,[5],12:i:-infections linked to alfalfa sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2010 through 2011 Outbreaks*. Cited 24 June 2022. <https://www.cdc.gov/Salmonella/2010/alfalfa-sprouts-2-10-11.html>
- CDC.** 2012. Final update: Multistate outbreak of Shiga toxin-producing *Escherichia coli* O26 infections linked to raw clover sprouts at Jimmy John's restaurants. In: *CDC, E.coli Homepage, Outbreaks, 2012 Outbreaks*. Cited 24 June 2022. www.cdc.gov/ecoli/2012/O26-02-12/index.html
- CDC.** 2013. Outbreak of *Escherichia coli* O104:H4 infections associated with sprout consumption — Europe and North America, May–July 2011. *MMWR*, 62(50); 1029–1031. Cited June 2022. www.cdc.gov/MMWR/preview/MMWRhtml/mm6250a3.htm
- CDC.** 2014a. Final update multistate outbreak of Shiga toxin-producing *Escherichia coli* O121 infections linked to raw clover sprouts. In: *CDC, E. coli Homepage, Outbreaks, 2014 Outbreaks*. Cited 24 June 2022. www.cdc.gov/ecoli/2014/O121-05-14/index.html
- CDC.** 2014b. Surveillance for foodborne disease outbreaks, United States, 2012, Annual report. Atlanta, Georgia: US Department of Health and Human Services. Cited 26 July 2022. www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2012-508c.pdf
- CDC.** 2015a. Final update: Multistate outbreak of *Salmonella* Enteritidis infections linked to bean sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2014 Outbreaks*. Cited 24 June 2022. www.cdc.gov/Salmonella/enteritidis-11-14/index.html
- CDC.** 2015b. Final update: Wholesome Soy Products, Inc. sprouts and investigation of human Listeriosis cases. In: *CDC, Listeria (Listeriosis), Outbreaks*. Cited 24 June 2022. www.cdc.gov/listeria/outbreaks/bean-sprouts-11-14/index.html

- CDC.** 2016a. Final update: Multistate outbreak of *Salmonella* infections linked to alfalfa sprouts from one contaminated seed lot. In: *CDC, Salmonella Homepage, Previous outbreaks, 2016 Outbreaks*. Cited 24 June 2022. www.cdc.gov/Salmonella/muenchen-02-16/index.html
- CDC.** 2016b. Final update: multistate outbreak of Shiga toxin-producing *Escherichia coli* O157 infections linked to alfalfa sprouts produced by Jack & The Green Sprouts. In: *CDC, E. coli Homepage, Outbreaks, 2016 Outbreaks*. Cited 24 June 2022. <https://www.cdc.gov/ecoli/2016/o157-02-16/index.html>
- CDC.** 2018. Final update: Multistate outbreak of *Salmonella* Montevideo Infections linked to raw sprouts. In: *CDC, Salmonella Homepage, Previous outbreaks, 2018 Outbreaks*. Cited 24 June 2022. <https://www.cdc.gov/Salmonella/montevideo-01-18/index.html>
- CDC.** 2020. Final update: Outbreak of *E. coli* infections linked to clover sprouts. In: *CDC, E. coli Homepage, Outbreaks, 2020 Outbreaks*. Cited 24 June 2022. <https://www.cdc.gov/ecoli/2020/o103h2-02-20/index.html>
- CDC.** 2022. National Outbreak Reporting System Dashboard. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC. Cited 29 June 2022. www.cdc.gov/norsdashboard/
- CFIA (Canadian Food Inspection Agency).** 2022. Bacterial pathogens and indicators, viruses and parasites in various food commodities - April 1, 2017 to March 31, 2021. In: *Inspection.gc.ca, Food microbiology - Targeted surveys - Interim report*. Cited 09 August 2022. https://inspection.canada.ca/DAM/DAM-food-aliments/STAGING/text-texte/bact_path_ind_virus_para_food_commodities_1647891775545_eng.pdf
- Cleary, P., Browning, L., Coia, J., Cowden, J., Fox, A., Kearney, J., Lane, C. et al.** 2010. A foodborne outbreak of *Salmonella* Bareilly in the United Kingdom, 2010. *Eurosurveillance*, 15(48): 1–3. <https://doi.org/10.2807/ese.15.48.19732-en>
- Cover, T.L. & Aber, R.C.** 1989. *Yersinia enterocolitica*. *The New England Journal of Medicine*, 321: 16–24. www.nejm.org/doi/pdf/10.1056/NEJM198907063210104
- da Cruz, M.R.G., Leite, Y.J.B. de S., Marques, J.de Lima., Pavelquesi, S.L.S., Oliveira, L.R.de A., da Silva, I.C.R., & Orsi, D.C.** 2019. Microbiological quality of minimally processed vegetables commercialized in Brasilia, DF, Brazil. *Food Science and Technology*, 39: 498–503. <https://doi.org/10.1590/FST.16018>
- de Jong, B., Oberg, J. & Svenungsson, B.** 2007. Outbreak of salmonellosis in a restaurant in Stockholm, Sweden, September - October 2006. *Eurosurveillance*, 12(11): E13–14. <https://doi.org/10.2807/esm.12.11.00749-en>

- EFSA (European Food Safety Authority).** 2011. Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal*, 9(11): 2424. <https://doi.org/10.2903/j.efsa.2011.2424>
- Emberland, K.E., Ethelberg, S., Kuusi, M., Vold, L., Jensvoll, L., Lindstedt, B.A., Nygard, K. et al.** 2007. Outbreak of *Salmonella* Weltevreden infections in Norway, Denmark and Finland associated with alfalfa sprouts, July-October 2007. *Eurosurveillance*, 12(11): E071129.4. <https://doi.org/10.2807/esw.12.48.03321-en>
- Farber, J.M., Carter, A.O., Varughese, P.V., Ashton, F.E. & Ewan, E.P.** 1990. Listeriosis traced to the consumption of alfalfa tablets and soft cheese. *The New England Journal of Medicine*, 322(5): 338. <https://doi.org/10.1056/nejm199002013220519>
- FDA (United States Food and Drug Administration).** 2020. Warning letter: Jimmy John's Franchise, LLC. In: *FDA, Inspections, compliance, enforcement and criminal investigations, Compliance actions and activities, Warning letters*. Cited 24 June 2022. <https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/jimmy-johns-franchise-llc-599962-02212020>
- FDA.** 2017. FY 2014 – 2016 Microbiological sampling assignment Summary report: Sprouts. Cited 3 November 2022. <https://www.fda.gov/files/food/published/FY-2014-%E2%80%932016-Microbiological-Sampling-Assignment-Summary-Report-Sprouts-PDF.pdf>
- Ferguson, D.D., Scheftel, J., Cronquist, A., Smith, K., Woo-Ming, A., Anderson, E., Knutsen, J., De, A. K. & Gershman, K.** 2005. Temporally distinct *Escherichia coli* 0157 outbreaks associated with alfalfa sprouts linked to a common seed source—Colorado and Minnesota, 2003. *Epidemiology and Infection*, 133(3): 439–447. <https://doi.org/10.1017/S0950268804003589>
- Garcia, S. & Heredia, N.** 2020. Production of Microbiologically Safe Fruits and Vegetables, Chapter 2. In: Guadalupe Virginia Nevárez-Moorillón, Arely Prado-Barragán, José Luis Martínez-Hernández, Cristobal Noé Aguilar eds. *Food Microbiology and Biotechnology: Safe and Sustainable Food Production*. Apple Academic Press. Florida, USA. Cited 30 June 2022.
- Gill, C.J., Keene, W.E., Mohle-Boetani, J.C., Farrar, J.A., Waller, P.L., Hahn, C.G. & Cieslak, P.R.** 2003. Alfalfa seed decontamination in *Salmonella* outbreak. *Emerging Infectious Diseases*, 9(4): 474. <https://doi.org/10.3201/eid0904.020519>
- Glynn, M.K., Patrick, S. & Wuhib, T.** 1998. When health food isn't so healthy—an outbreak of *Salmonella* serotypes Anatum and Infantis associated with eating contaminated sprouts, Kansas and Missouri, 1997. In: *47th Annual Epidemic Intelligence Service (EIS) Conference*. April 1998. Atlanta, GA: Centers for Disease Control and Prevention.

- Gutierrez, E.** 1997. Japan prepares as O157 strikes again. *The Lancet*, 349(9059): 1156. [https://doi.org/10.1016/S0140-6736\(05\)63041-3](https://doi.org/10.1016/S0140-6736(05)63041-3)
- Harris, L.J., Farber, J.N., Beuchat, Parish, M.E., Suslow, T.V., Garrett, E.H. & Busta, E.F.** 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, 2(S1): 78–141. <https://doi.org/10.1111/j.1541-4337.2003.tb00031.x>
- Honish, L. & Nguyen, Q.** 2001. Outbreak of *Salmonella* enteritidis phage type 913 gastroenteritis associated with mung bean sprouts--Edmonton, 2001. *Canada Communicable Disease Report*, 27(18): 151. <https://pubmed.ncbi.nlm.nih.gov/11582621>
- Joce, R., O'Sullivan, D.G., Strong, C., Rowe, B., Hall, M.L.M. & Threlfall, E.J.** 1990. A national outbreak of *Salmonella* Gold-Coast. *Canada Communicable Disease Report Rev.*, 4: 3–4.
- Mahon, B.E., Pönkä, A., Hall, W.N., Komatsu, K., Dietrich, S.E., Siitonen, A., Cage, G. et al.** 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. *Journal of Infectious Diseases*, 175(4): 876-882. Cited 24 June 2022. http://jid.oxfordjournals.org/content/175/4/876.full.pdf?origin=publication_detail
- Mattila, L., Leirisalo-Repo, M., Koskimies, S., Granfors, K. & Siitonen, A.** 1994. Reactive arthritis following an outbreak of *Salmonella* infection in Finland. *British Journal of Rheumatology*, 33: 1136–1141. <https://doi.org/10.1093/rheumatology/33.12.1136>
- Marler, B.** 2021. Barf Blog – A great resource on why we should reconsider sprouts. In: *Marler Clark, Marler blog, Case news*. January 4, 2021. Cited 20 June 2022. www.marlerblog.com/case-news/barf-blog-a-great-resource-on-why-we-should-reconsider-sprouts
- Mohle-Boetani, J.C., Farrar, J.A., Werner, S.B., Minassian, D., Bryant, R., Abbott, S., Slutsker, L. & Vugia, D.J.** 2001. *Escherichia coli* O157 and *Salmonella* infections associated with sprouts in California, 1996–1998. *Annals of Internal Medicine*, 135(4): 239-247. <https://doi.org/10.7326/0003-4819-135-4-200108210-00008>
- Nguyen-the, C. & Carlin, F.** 1994. The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34(4): 371–401. <https://doi.org/10.1080/10408399409527668>
- O'Mahony, M., Cowden, J., Smyth, B., Lynch, D., Hall, M., Rowe, B., Teare, R.E. et al.** 1990. An outbreak of *Salmonella* Saint-paul infection associated with beansprouts. *Epidemiology and Infection*, 104(02): 229–235. Cited 24 June 2022. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2271762/pdf/epidinfec00020-0070.pdf>

- Ontario Newsroom.** 2005. Update on *Salmonella* outbreak. In: *Ontario, News Release*. December 14, 2005. Cited 22 June 2022. <https://news.ontario.ca/en/release/90179/update-on-Salmonella-outbreak>
- OzFoodNet.** 2007. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the Ozfoodnet Network, 2006. *Communicable Diseases Intelligence*, 31(4): 345–365. Cited 6 July 2022. <https://search.informit.org/doi/epdf/10.3316/ielapa.505500545067863>
- Pönkä, A., Andersson, Y., Siitonen, A., de Jong, B., Jahkola, M., Haikala, O., Kuhmonen, A., & Pakkala, P.** 1995. *Salmonella* in alfalfa sprouts. *The Lancet*, 345(8947): 462–463. [https://doi.org/10.1016/S0140-6736\(95\)90451-4](https://doi.org/10.1016/S0140-6736(95)90451-4)
- Portnoy, B.L., Goepfert, J.M. & Harmon, S.M.** 1976. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. *American Journal of Epidemiology*, 103(6): 589–594.
- Proctor, M.E., Hamacher, M., Tortorello, M.L., Archer, J.R. & Davis, J.P.** 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. *Journal of Clinical Microbiology*, 39(10): 3461–3465. <https://doi.org/10.1128/JCM.39.10.3461-3465.2001>
- Puohiniemi, R., Heiskanen, T., & Siitonen, A.** 1997. Molecular epidemiology of two international sprout-borne *Salmonella* outbreaks. *Journal of Clinical Microbiology*, 35(10): 2487–2491. <https://doi.org/10.1128/jcm.35.10.2487-2491.1997>
- Rimhanen-Finne, R., Niskanen, T., Lienemann, T., Johansson, T., Sjöman, M., Korhonen, T., Guedes, S. et al.** 2011. A Nationwide Outbreak of *Salmonella* Bovismorbificans Associated with Sprouted Alfalfa Seeds in Finland, 2009. *Zoonoses and Public Health*, 58: 589–596. <https://doi.org/10.1111/j.1863-2378.2011.01408.x>
- Seo, Y.H., Jang, J.H. & Moon, K.D.** 2010. Microbial evaluation of minimally processed vegetables and sprouts produced in Seoul, Korea. *Food Science and Biotechnology*, 19: 1283–1288. <https://doi.org/10.1007/s10068-010-0183-y>
- Sewell, A.M. & Farber, J.M.** 2001. Foodborne outbreaks in Canada linked to produce. *Journal of Food Protection*, 64(11): 1863–1877. <https://doi.org/10.4315/0362-028x-64.11.1863>
- Stratton, J., Stefaniw, L., Grimsrud, K., Werker, D.H., Ellis, A., Ashton, E., Chui, L. et al.** 2001. Outbreak of *Salmonella* paratyphi B var java due to contaminated alfalfa sprouts in Alberta, British Columbia and Saskatchewan. *Canada Communicable Disease Report*, 15; 27(16):133–137; discussion 137–8. <https://pubmed.ncbi.nlm.nih.gov/11523182/>

- Stokes, K.** 2016. Raw bean sprout contamination linked to extra five *Salmonella* cases in SA. *The Advertiser*. 27 April 2016. Adelaide, Australia. Cited 24 June 2022. <https://www.adelaidenow.com.au/news/south-australia/extra-five-cases-in-sa-for-bean-sprout-contamination/news-story/a3579929ba487a9acbcfaa46b58afcb7>
- Taormina, P.J., Beuchat, L.R. & Slutsker, L.** 1999. Infections associated with eating seed sprouts: An international concern. *Emerging Infectious Diseases*, 5(5): 626. Cited 24 June 2022. www.ncbi.nlm.nih.gov/pmc/articles/PMC2627711/pdf/10511518.pdf
- Van Beneden, C.A., Keene, W.E., Strang, R.A., Werker, D.H., King, A.S., Mahon, B., Hedberg, K. et al.** 1999. Multinational outbreak of *Salmonella* enterica serotype Newport infections due to contaminated alfalfa sprouts. *JAMA*, 281(2): 158–162. <https://doi.org/10.1001/jama.281.2.158>
- van Duynhoven, Y.T., Widdowson, M.A., de Jager, C.M., Fernandes, T., Neppelenbroek, S., van den Brandhof, W., Wannet, W.J., van Kooij, J.A., Rietveld, H.J. & van Pelt, W.** 2002. *Salmonella* enterica serotype Enteritidis phage type 4b outbreak associated with bean sprouts. *Emerging Infectious Diseases*, 8(4): 440. doi: 10.3201/eid0804.010213
- Watanabe, Y., Ozasa, K., Mermin, J.H., Griffin, P.M., Masuda, K., Imashuku, S. & Sawada, T.** 1999. Factory outbreak of *Escherichia coli* O157: H7 infection in Japan. *Emerging Infectious Diseases*, 5(3): 424. Cited 24 June 2022. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2640759/pdf/10341179.pdf>
- Wegener, H.C., Baggesen, D.L., Neimann, J. & Nielsen, S.V.** 1997. An outbreak of human salmonellosis in Denmark caused by alfalfa sprouts. In: *Proceedings and abstracts of the International Symposium on Salmonella and Salmonellosis*. 20–22 May 1997. Ploufragan, France. Cited 24 June 2022. [https://om.ku.dk/ledelse-strategi/reaktor/henrik-wegener/?pure=da%2Fpublications%2Fan-outbreak-of-human-salmonellosis-in-denmark-caused-by-alfalfa-sprouts\(77a32e2b-0a59-4881-b516-37c243186f55\).html](https://om.ku.dk/ledelse-strategi/reaktor/henrik-wegener/?pure=da%2Fpublications%2Fan-outbreak-of-human-salmonellosis-in-denmark-caused-by-alfalfa-sprouts(77a32e2b-0a59-4881-b516-37c243186f55).html)
- Werner, S., Boman, K., Einemo, I., Erntell, M., de Jong, B., Lindqvist, A., Löfdahl, M. et al.** 2007. Outbreak of *Salmonella* Stanley in Sweden associated with alfalfa sprouts, July-August 2007. *Euro Surveillance*, 12(10): E071018.2. Cited 29 June 2022. <https://pubmed.ncbi.nlm.nih.gov/17997915>
- Winthrop, K.L., Palumbo, M.S., Farrar, J.A., Mohle-Boetani, J.C., Abbott, S., Beatty, M.E., Inami, G. & Werner, S.B.** 2003. Alfalfa sprouts and *Salmonella* Kottbus infection: a multistate outbreak following inadequate seed disinfection with heat and chlorine. *Journal of Food Protection*, 66(1): 13–17. <http://dx.doi.org/10.4315/0362-028X-66.1.13>

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In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the Codex Alimentarius Committee (CAC) approved new work at its 42nd Session on the development of guidelines for the control of Shiga toxin-producing *Escherichia coli* (STEC) in leafy vegetables and in sprouts.

Sprouts have different food safety concerns from other fresh fruits and vegetables because the conditions under which sprouts are produced (time, temperature, humidity, pH and nutrients) are ideal for foodborne pathogen growth. Outbreak investigations have demonstrated that foodborne pathogens found on sprouts most likely originate from the seed, but the contamination could also be attributed to the production environment.

This report covers prevention and control measures specific to the primary production and handling of seed for sprouting, the production of sprouts and hygienic practices applicable to retail and food services. Recommendations for proper record-keeping and the establishment of product traceability programmes that facilitate the identification and investigation of contaminated seed and sprouts in the event of an illness outbreak or product recall are also included.

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