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Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Introduction

Sprouted seeds are grown from seed and harvested before formation of the first leaves. They are also known as 'bean sprouts' or 'sprouts'. Production can be done with or without a growth medium like compost or soil and generally takes between 4 and 10 days to complete depending on the variety of seed being sprouted.

Sprouts are generally ready-to-eat products although some types like mung bean sprouts are more normally cooked. Because of their ready-to-eat status, sprouts represent a high risk category of food for which control measures are necessary to reduce the risk to human health. The production of sprouts requires high humidity and warm conditions that are also perfect for the growth of bacteria. Pathogenic bacteria (hereafter called pathogens) like *E. coli* O157 (and related verotoxigenic *E. coli*) and *Salmonella* spp. have been associated with outbreaks of human disease when contaminated sprouts have been consumed. The most recent outbreak, centred in Germany in 2011, was caused by *E. coli* O104:H4 and resulted in more than 47 deaths and several thousand infections. The most likely link between food and the infections were sprouts grown from fenugreek seeds exported to Germany from Egypt[1].

Scope

This document addresses the production of ready-to-eat sprouted seeds. Sprouted seeds that are not produced using the control measures in this document should not be considered ready-to-eat and are therefore not addressed in this document. Such products should be labelled as requiring cooking before consumption.

This document outlines specific control measures that should be adopted by producers of ready-to-eat sprouted seeds to reduce the risk of sprouts being contaminated with pathogens. It does not address chemical or physical hazards. These should be addressed by the sprout producer as separate hazards.

This document should be read in conjunction with the Food Safety Authority of Ireland's (FSAI) Code of Practice No. 4: Food Safety in the Fresh Produce Supply Chain[2] (available as a free web download).

[1] European Food Safety Agency Technical Report (2011) Tracing seeds, in particular fenugreek (Trigonella foenum-graecum) seeds, in relation to the Shiga toxin-producing E. coli (STEC) O104:H4 2011 Outbreaks in Germany and France http://www.efsa.europa.eu/en/supporting/pub/176e.htm

[2] FSAI (2001) Code of Practice No. 4: Food Safety in the Fresh Produce Supply Chain http://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=1206

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

Main Food Safety Requirements for Sprout Producers

It is important that producers of sprouts understand that they have the primary obligation under the General Food Law Regulation (Regulation (EC) No. 178/2002)[3] to only place safe food on the market. Regulation (EC) No. 852/2004[4] on the hygiene of foodstuffs also requires primary producers to ensure that primary products (*sprouts are primary products*) are protected against contamination. Sprout producers are also required to comply with Commission Regulation (EC) No. 2073/2005[5] on microbiological criteria for foodstuffs. There are other provisions for sprout producers in the legislation mentioned above and other food legislation and it is important that food businesses familiarise themselves with these.

Routes of Sprout Contamination

Most outbreaks of human disease due to the consumption of sprouts have been attributed to contamination of the seeds used for sprouting. The moist, warm conditions of sprouting can allow small numbers of pathogens present on seeds to multiply by several orders of magnitude during the sprouting period. However, other potential contamination routes exist in the production process. These include contaminated irrigation water and wash water, contaminated growth media such as compost (when used), contaminated production equipment or infected food handlers. These routes of contamination must be controlled by good agricultural practice and good hygiene practice.

Pathogen Control Measures

In addition to implementing the specific control measures specified in this document, sprout producers should:

- **Adhere to all of the relevant good agricultural practices and good hygiene practices outlined in the FSAI's Code of Practice No. 4: Food Safety in the Fresh Produce Supply Chain[2]**
  [Note: This document and Commission Regulation No. 2073 of 2005 supersede the microbiological guidelines in Code of Practice No. 4]
- **Ensure food handlers are trained in food hygiene practices and that the exclusion from work guidelines for infected food handlers issued by the Health Protection Surveillance Centre[6] are followed**
- **If sprouts are being grown in a solid growth medium like soil or compost, that medium should preferably be sterile or as a minimum not have been in contact with animal or human waste**

[3] Regulation (EC) No. 178/2002
http://www.fsai.ie/legislation/food_legislation/general_principles_of_food_law.html

[4] Regulation (EC) No. 852/2004
http://www.fsai.ie/legislation/food_legislation/food_hygiene/hygiene_of_foodstuffs.html

[5] Commission Regulation (EC) No. 2073/2005
http://www.fsai.ie/legislation/food_legislation/hygiene_of_foodstuffs/microbiological_criteria.html

[6] HPSC (2004) Preventing Foodborne Disease: A Focus on the Infected Food Handler
http://www.hpsc.ie/hpsc/A-Z/Gastroenteric/FoodborneIllness/Publications/File,871,en.pdf

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

The following pathogen control measures should be implemented by (1) seed suppliers and (2) sprout producers and adapted for their own production processes.

1. Seed Suppliers

1.1. Screening/testing of seed lots for sprouting by seed suppliers

Seeds can be contaminated with pathogens during production and distribution and therefore it is important to ensure that seeds are free from detectable pathogens. Seeds that are supplied for sprouting should be screened for pathogen contamination and handled separately from seeds for other purposes.

The ability to detect pathogens on seeds depends on a combination of the sampling approach and the microbiological testing method used. The larger and more representative the sample is of the seed 'lot' and the more sensitive the test method, the greater the confidence that the seeds in that lot are not contaminated when a negative test result is obtained.

The sprouting process allows for the growth of any pathogens present on the seed. Therefore for those seed suppliers that have the equipment, it is recommended that a sprouting approach is used to test seeds. Alternatively, a direct seed testing protocol can be used but this approach requires more microbiological tests.

1.1.1. Seed suppliers with sprouting test facilities

A seed screening protocol has been published[7] and the following information is based on that approach:

- Take 120 samples of 25g of seeds from across as many bags as possible up to a maximum of 120 bags in the lot
- Put the seed lot on hold pending the results of the screening protocol
- Mix the 120 samples together thoroughly to make a composite 3kg sample of seeds
- Sprout the seeds in non-chlorinated water without any seed disinfection steps
- Aseptically sample the spent irrigation water after 48 hours. One litre of spent irrigation water should be collected in a clean, sterile, pre-labelled container (laboratories will provide these and advice on how to take the sample aseptically). Sample the irrigation water as it leaves the drum or trays during the irrigation cycle. Sampling of water should be representative of the production lot so where there is not a common spent irrigation water collection outlet, separate volumes of water may need to be collected from individual trays or sectors of a drum to make up the one litre. Sub-samples of water should be of equal volumes. Further information on this sampling procedure is available from the USFDA[8]
- Choose a laboratory that uses an appropriate method that is accredited to ISO17025 standards. Methods must be appropriate for testing water samples for verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157[9]) and *Salmonella* spp. (also see the FSAI's factsheet on microbiological testing of foods[10])

[7] International speciality supply
http://sproutnet.com/Research/iss_seed_screening_procedures.htm

[8] USFDA (1999) Guidance for Industry: Sampling And Microbial Testing Of Spent Irrigation Water During Sprout Production
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm120246.htm

[9] Testing for VTEC non-O157 generally involves looking for the presence of virulence genes. The main virulence factors (genes) identified for human pathogenic VTEC are those encoding for verotoxins (*vtx1*, *vtx2*, *vtx2c*) and *eae* (encoding for the attaching and effacing lesion). However, as shown in the German sprout outbreak in 2011 pathogenic VTEC may also be eae negative.

[10] FSAI (2011) Best Practice for Testing Foods when Assessing Compliance with the Microbiological Criteria specified in Commission Regulation (EC) No. 2073/2005
http://www.fsai.ie/compliancewiththemicrobiologicalcriteriaspecifiedincommissionregulationECNo2073/2005.html

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

- From the one litre sample of spent irrigation water, the laboratory should test two 200ml analytical sample units for the presence of verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157) and two 200ml analytical sample units for the presence of *Salmonella* spp.
- If the analytical sample is negative for both of these pathogens, then the seed lot can be released for use with a certificate of analysis. If the analytical sample is positive for any one of these pathogens then the seed lot must not be used for sprouting

1.1.2. Seed suppliers with no sprouting facilities

Contamination rates for *Salmonella* spp. in seeds implicated in outbreaks of human illness have been reported to be as low as 4 cells per 1,000g of seed[11]. Because of this low level of contamination, a sampling protocol and the microbiological test used must be sensitive enough to provide a reasonable level of confidence in the results. The following sampling plan is based on the ICMSF case 15 sampling plan[12]:

- Aseptically take 60 samples of 60g of seeds from each bag in the seed lot up to 60 bags
- Place each sample in a separate clean, sterile, pre-labelled container
- Choose a laboratory that uses an appropriate method that is accredited to ISO17025 standards. Methods must be appropriate for testing dried seed samples for verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157[9]) and *Salmonella* spp. (also see the FSAI's factsheet on microbiological testing of foods[10])
- Test 25g of each of the 60 samples for *E. coli* (VTEC O157 and VTEC non-O157) and 25g of each of the 60 samples for *Salmonella* spp. *(Do not pool samples unless the laboratory has a validated pooling procedure and can guarantee that the test results will be the same as testing 60 individual 25g analytical sample units)*
- If the analytical samples are all negative for both of these pathogens then the seed lot can be released for use with a certificate of analysis. If any one of the analytical sample units is positive for any one of these pathogens then the seed lot must not be used for sprouting

[11] USFDA (1999) Microbiological Safety Evaluations and Recommendations on Sprouted Seed http://www.fda.gov/food/foodsafety/product-specificinformation/fruitsvegetablesjuices/ucm078789.htm

[12] International Commission on Microbiological Specifications for Food (2002) Micro-organisms in foods 7. Kluwer Academic/ Plenum publishers, New York. p164.

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

2. Sprout producers

2.1. Verification of safe seed supply by sprout producers

Seeds can be contaminated with pathogens during production and distribution and therefore it is important to ensure that seeds are free from detectable pathogens. Investigations of outbreaks of salmonellosis that have been traced to the seeds used for sprouting have found that the seeds contain very low levels of pathogens. Seeds should only be purchased for sprouting if they are specifically supplied for that purpose and if they are accompanied by a certificate of analysis confirming that they are free from pathogens such as verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157) and *Salmonella* spp.

- Seed suppliers should be selected on the basis that they operate seed screening protocols that are similar to the protocols outlined in this document.
- Visual checks on bags of seeds during delivery should be carried out to ensure that they are clean and sealed. Seed deliveries should be rejected if they are dirty or the bags are not sealed.
- Occasional verification of the suppliers' certificate of analysis should be carried out using the dry seed testing protocol outlined earlier for 'seed suppliers with no sprouting facilities'. **The protocol outlined earlier for 'seed suppliers with sprouting test facilities' is not recommended for use by sprout producers, unless they can carry out the screening procedure in a separate area from main sprout production using dedicated equipment. If this protocol is done in the normal production area then there is increased potential for contamination of the production area with pathogens which may affect the safety of future sprout production.**

2.2. Seed disinfection by sprout producers

Seeds that have passed the seed screening protocol should be visually checked for physical contamination prior to use. If seeds are free of visual contamination they should then be chemically disinfected before they are used for sprouting.

It should be noted that trials on chemical disinfection of seeds have demonstrated a high degree of variability regarding inactivation of pathogens on seeds. Consequently, it is important that seed screening protocols as outlined above have been adhered to prior to disinfection.

In the USA, the FDA recommends the use of 20,000ppm calcium hypochlorite[11], whilst in Canada, the CFIA recommend 2,000ppm calcium hypochlorite for 15-20 minutes or 6-10% hydrogen peroxide for 10 minutes[13]. A good review of chemical treatments for seeds has been published by Montville and Schaffner in 2004[14]. All disinfectants used must be approved for food use in Ireland by the Department of Agriculture, Fisheries and Food[15].

When disinfecting seeds, a fresh disinfection solution should be made up for each batch of seeds to be sprouted and the concentration checked and recorded. The seeds need to be agitated during the full contact period in five times their volume of disinfectant. The contact time should be recorded. Disinfection should be followed by a rinse step in potable water. Strict hygiene must be implemented to prevent recontamination of disinfected seeds. All applicable health and safety requirements for handling chemicals should be adhered to by staff involved in the seed disinfection process.

[13] Canadian Food Inspection Agency (2007) Code of Practice for the Hygienic Production of Sprouted Seeds http://www.inspection.gc.ca/english/fssa/frefra/safsal/sprointe.shtml

[14] Rebecca Montville and Donald Schaffner (2004) Analysis of published sprout seed sanitation studies shows treatments are highly variable. Journal of food protection. Vol 67, No 4, p758-765

[15] For information see http://www.pcs.agriculture.gov.ie/biocides.htm

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

2.3. Monitoring of spent irrigation water by sprout producers after 48 hours

During the growth of sprouts, the humid and warm conditions are ideal for bacterial growth. Consequently, if any pathogens are still present on the seed after disinfection they will grow. The irrigation water will wash some of these pathogens off and therefore testing this water for pathogens at the right time can be used as a means of verifying that the seed screening and seed disinfection steps have worked. Testing can be carried out after 48 hours and results can be returned before release of the sprouts onto the market.

The following protocol is advised where sampling of spent irrigation water is possible:

- Aseptically sample the spent irrigation water after 48 hours. One litre of spent irrigation water should be collected in a clean, sterile, pre-labelled container (laboratories will provide these and advice on how to take the sample aseptically). Sample the irrigation water as it leaves the drum or trays during the irrigation cycle. Sampling of water should be representative of the production lot, so where there is not a common spent irrigation water collection outlet, separate volumes of water may need to be collected from individual trays or sectors of a drum to make up the one litre. Sub-samples of water should be of equal volumes. Further information on this sampling procedure is available from the USFDA[8]
- Choose a laboratory that uses an appropriate method that is accredited to ISO17025 standards. Methods must be appropriate for testing water samples for verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157[9]) and *Salmonella* spp. (also see the FSAI's factsheet on microbiological testing foods[10])
- From the one litre sample of spent irrigation water, the laboratory should test two 200ml analytical sample units for the presence of verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157) and two 200ml analytical sample units for the presence of *Salmonella* spp.
- If any of the analytical samples are positive for either pathogen, the sprouts must not be placed on the market. The sprout producer must investigate the source of the contamination, e.g. contaminated seed, contaminated irrigation or wash water, contaminated growth medium or infected food handler. During this investigation the sprout producer should place the seed lot used for sprouting on hold and notify the seed supplier of the investigation. The production plant and equipment should be thoroughly cleaned and disinfected and the hygiene verified before production of sprouts recommences

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

2.4. Monitoring of sprouts by sprout producers

Commission Regulation (EC) No. 2073/2005[5] on microbiological criteria for foodstuffs requires that ready-to-eat sprouted seeds are free of *Salmonella* spp. and meet the criteria for *Listeria monocytogenes*. The Regulation requires that when testing the acceptability of a batch of sprouted seeds, the sampling plan and test method specified in the legislation must be adhered to. In addition, it is also recommended that finished sprouts are tested for verotoxigenic *E. coli* (VTEC O157 and VTEC non-O157) to ensure that the food is safe in accordance with obligations under the General Food Law Regulation[3].

The following protocol is recommended:

- At the end of the sprouting period aseptically collect 5 samples each composed of 100g sprouts, from different locations in the batch. Each 100g sample unit should be placed directly into individual clean, sterile, pre-labelled containers[16]
- Test 25g of each of the 5 samples for *E. coli* (VTEC O157 and VTEC non-O157[9]) using an appropriate method; test 25g of each of the 5 samples for *Salmonella* spp. using the method specified in ISO6579; and test 25g of each of the 5 samples for *Listeria monocytogenes* using the method specified in ISO11290-1 or ISO11290-2, as appropriate[17]. Alternative methods to the ISO methods can be used providing they meet the requirements of Article 5 of the Regulation. *(Do not pool samples unless the laboratory has a validated pooling procedure and can guarantee that the test results will be the same as testing 5 individual 25g analytical sample units)*
- It is recommended that the testing laboratory is accredited to the ISO17025 standard for the methods specified
- If any of the analytical samples are positive for verotoxigenic *E. coli* or *Salmonella* spp., sprouts on the market must be withdrawn or recalled as appropriate and the competent authorities notified. The sprout producer must investigate the source of the contamination, e.g. contaminated seed, contaminated irrigation or wash water, contaminated growth medium or infected food handler. During this investigation the sprout producer should place the seed lot used for sprouting on hold and notify the seed supplier of the investigation. The production plant and equipment should be thoroughly cleaned and disinfected and the hygiene verified before production of sprouts recommences
- If any of the analytical samples contain *Listeria monocytogenes* above 100cfu/g, then any sprouts on the market must be withdrawn or recalled as appropriate and the competent authorities notified. The sprout producer must investigate the source of the contamination, e.g. contaminated seed or contaminated equipment. During this investigation the sprout producer should place the seed lot used for sprouting on hold and notify the seed supplier of the investigation. The production plant and equipment should be thoroughly cleaned and disinfected and the hygiene verified before production of sprouts recommences
- If any of the analytical samples are positive for *Listeria monocytogenes*, then any sprouts on the market must be withdrawn or recalled, as appropriate, unless the sprout producer has shelf-life studies which demonstrate to the satisfaction of the competent authority that the product will not exceed the limit of 100cfu/g throughout the shelf-life. The sprout producer must investigate the source of the contamination, e.g. contaminated seed or contaminated equipment. During this investigation the sprout producer should place the seed lot used for sprouting on hold and notify the seed supplier of the investigation. The production plant and equipment should be thoroughly cleaned and disinfected and the hygiene verified before production of sprouts recommences

[16] Based on United States Food and Drug Administration 1999
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm120246.htm

[17] Commission Regulation 2073 of 2005 on microbiological criteria for foodstuffs requires that:

- Ready-to-eat foods **unable** to support the growth of *L. monocytogenes* must not exceed the limit of 100 cfu/g throughout the shelf-life of the product. [Note: the Regulation states that products with pH ≤4.4 or aw ≤0.92; products with pH ≤5.0 and aw ≤0.94; and products with a shelf-life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.]
- Ready-to-eat foods **able** to support the growth of *L. monocytogenes* must not exceed the limit of 100 cfu/g throughout the shelf-life of the product. A limit of absence in 25 g shall apply to such products before they have left the immediate control of the sprout producer, where the producer is not able to demonstrate (i.e. through shelf-life studies), to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life of the product.

Guidelines on Safe Production of Ready-to-Eat Sprouted Seeds (Sprouts)

Food Safety AUTHORITY OF IRELAND

Labelling

Sprouted seeds produced using the control measures outlined in this document should be labelled as 'ready-to-eat'. Sprouted seeds that are not produced using these control measures should be labelled 'cook before consumption'.

Traceability and Recall

Articles 18 and 19 of Regulation (EC) No. 178/2002 require all food businesses to have traceability and recall systems in place. Full traceability for lots of seeds and batches of sprouted seeds should be maintained to ensure rapid recall in the event of an incident. Sprout producers should familiarise themselves with the FSAI's Guidance Note No. 10 Food Recall and Traceability (Revision 2)[18].

If a sprout producer finds that a delivery of seeds is contaminated with pathogens or if a batch of sprouts containing pathogens has been placed on the market then they have a legal obligation to inform the competent authorities immediately.

[18] FSAI (2010) Guidance Note No. 10 Product Recall and Traceability (Revision 2) http://www.fsai.ie/gn10productrecallandtraceabilityrevision2.html