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Survey of the microbiological safety of pre-packaged ready-to-eat nuts, seeds and dried fruit (15NS1)

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SUMMARY

This survey investigated the microbiological safety of ready-to-eat, pre-packaged nuts, seeds and dried fruit available for retail sale in Ireland. In total, 982 food samples were taken. Samples were tested for *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC),[1] *Salmonella* and *Escherichia coli*.

Salmonella was detected in 0.2% (2/890) of samples; these were *S*. Mikawasima in ground almonds from the United States and *S*. Elomrane in nigella seeds from India imported to Ireland via a wholesaler in the United Kingdom. *L. monocytogenes* was below the limit of enumeration (<10 cfu/g) for all samples tested (n=886). The maximum legal limit for *L*. *monocytogenes* is 100 cfu/g.

Of the 821 samples tested for the presence of STEC using the CEN ISO/TS 13136 method (which targets the major STEC virulence genes, *stx* and *eae*), all were negative for STEC. In addition, out of 889 samples tested for *E. coli*, 887 were below the limit of enumeration (<10 cfu/g), one was at the 10 cfu/g limit and one was at 30 cfu/g, considered satisfactory and borderline, respectively. The sample containing borderline levels of *E. coli* was walnuts.

This survey was carried out from 31 October 2015 to 1 December 2015 inclusive, as increased seasonal consumption of these products takes place during these months.

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INTRODUCTION

Nuts, seeds and dried fruit have low water activity that will prevent pathogens from growing, but still allows them to survive. Because nuts, seeds and dried fruits are frequently eaten as ready-to-eat snacks, or used as ingredients in other ready-to-eat foods (e.g. peanut butter, tahini, breakfast cereals, pesto, chocolate bars, protein/health bars), contamination from pathogens, even in low numbers, has the potential to cause illness. Nuts, seeds and dried coconut have been implicated in various salmonellosis outbreaks, and one outbreak of *E. coli* O157 infection has been linked to hazelnuts (see Appendix 2). A number of studies have found nuts, seeds and dried fruit to be contaminated with food-borne pathogens (see Appendix 3).

[1] Shiga toxin-producing *Escherichia coli* (STEC) are synonymous with Verocytotoxigenic *Escherichia coli* (VTEC). Similarly, *stx* genes are synonymous with *vtx* genes. For the purposes of this report, the terms STEC and *stx* have been used throughout the document.
[2] (i) Public Health Laboratory, Limerick; (ii) Public Health Laboratory, Sligo University Hospital, Sligo; (iii) Public Health Laboratory, Waterford Regional Hospital, Waterford; (iv) Public Analyst's Laboratory, Sir Patrick Dun's Hospital, Dublin; (v) Public Health Microbiology Laboratory, St Finbarr's Hospital, Cork; (vi) Public Health Microbiology Laboratory, Cherry Orchard Hospital, Dublin; and (vii) Public Health Microbiology Laboratory, University Hospital Galway.

Prevalence of pathogens

During 2015, the European Commission's Rapid Alert System for Food and Feed (RASFF) issued 70 notifications for pathogens in nuts, nut products and seeds.[3] In total, 69 notifications were for *Salmonella* in sesame seeds (64), nigella seeds (1), melon seeds (1), tahini (1), pine nut kernels (1) and roasted chopped hazelnuts (1); there was also one notification for *L. monocytogenes* in sesame pasta. Eighty-eight per cent (61/69) of the *Salmonella* notifications in 2015 came from nuts and seeds that originated from India (see Appendix 1). Since 2002, the FSAI has issued six food alerts relating to *Salmonella* in sesame seeds (FSAI, 2009b; FSAI, 2009c; FSAI, 2010a; FSAI, 2010b; FSAI, 2016a; FSAI, 2016b) and one alert due to *Salmonella* in peanuts (FSAI, 2009a).

Nuts, seeds and dried fruit can become contaminated with pathogens during growth, harvesting or processing. A previous survey of nuts and seeds, conducted under the Irish National Microbiological Monitoring and Surveillance Programme, detected *Salmonella* in 0.3% (3/891) of samples: two sesame seed samples (the subject of two of the Irish food alerts referred to above) and one sample of melon (egusi) seeds[4] (FSAI, 2012). The survey also found that 0.3% (3/891) of samples contained unsatisfactory levels of *E. coli*, with two poppy seed samples and one pumpkin seed sample being above the 100 cfu/g *E. coli* limit recommended in FSAI Guidance Note No. 3, *Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 2)*. A similar study in the United Kingdom (UK) detected *Salmonella* in 0.6% of samples (23/3,735), over half of which were sesame seeds (Willis *et al*., 2009). Other studies have also found that although the prevalence of *Salmonella* in nuts and seeds is low, almonds, pistachios and seeds (particularly sesame seeds) were the types most frequently contaminated (see Appendix 3). There are few published studies on the prevalence of pathogens in dried fruit. In a UK survey of ready-to-eat foods, none of the 555 samples of dried fruit analysed contained *Salmonella*, nor did any samples contain *L. monocytogenes* at ≥100 cfu/g, *Staphylococcus aureus* at $\geq 10^4$ cfu/g, or *Bacillus cereus* at $\geq 10^5$ cfu/g (Meldrum *et al*., 2006).

Only small quantities of some pathogens such as *Salmonella* need to be consumed in order to cause human illness. Investigations of outbreaks involving sesame seed products and almonds found that *Salmonella* was present in the implicated foods in very small quantities (Unicomb *et al*., 2005; Danyluk *et al*., 2007; Paine *et al*., 2014).

Various studies have used *E. coli* as an indicator of possible faecal contamination in nuts and seeds. However, Danyluk *et al*. (2007) found that *E. coli* levels in almonds did not correlate with the presence or absence of *Salmonella* and concluded that it is not a useful indicator organism for *Salmonella*. Willis *et al*. (2009) also found that the presence of *Salmonella* on edible seeds did not correlate with the presence or unsatisfactory levels of *E. coli*. In that study, only 2 out of 23 samples in which *Salmonella* was detected had an *E. coli* count $\geq 10^2$ cfu/g, while in 17 samples contaminated with *Salmonella, E. coli* was not detected (<3 cfu/g). A previous Irish study also found that unsatisfactory levels of *E. coli* did not correlate with the presence of *Salmonella* (FSAI, 2012). The *E. coli* levels were satisfactory (<20 cfu/g) or acceptable (20–100 cfu/g) in the three samples (out of 891) in which *Salmonella* was detected; conversely, *Salmonella* was not detected in the three samples (out of 891) which had unsatisfactory levels of *E. coli* (≥100 cfu/g).

[3] Source: RASFF portal at https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1
[4] Egusi is typically sold in African stores and consumed cooked in African dishes; however, because the product was not labelled as requiring cooking, it was included in this survey.

Routes of contamination

Nuts, seeds and dried fruit can become contaminated with pathogens from a number of sources, including:

1. Contaminated water used for irrigation or to apply pesticides or fungicides
2. Human or animal sewage used as fertiliser
3. Domestic or wild animal contact with the crop
4. Contaminated water used to wash the crop post-harvest
5. Contaminated equipment used to wash, process or package the crop
6. Contaminated vehicles, crates or storage areas used in distribution
7. Cross-contamination during drying
8. Cross-contamination during food preparation in food businesses or in the home
9. Infected food handlers.

Outbreaks

Outbreaks of salmonellosis, STEC and botulism have all been reported in ready-to-eat nuts, seeds and dried fruit products or in foods that use these products as ingredients. Outbreaks were linked to ingestion of a wide range of contaminated raw or processed products, such as almonds, peanuts, hazelnuts, desiccated coconut, sesame seeds, pine nuts, pistachios, cashews and aniseed (see Appendix 2). More recently, in 2017 an outbreak of *S*. Typhimurium affecting five people in Oregon in the United States of America (USA) was linked to contaminated raw hazelnuts (Oregon Health Authority, 2017). In 2016, the US Centers for Disease Control and Prevention (CDC) reported a multistate outbreak of *S*. Montevideo and *S*. Senftenberg affecting 11 people, 2 of whom were hospitalised, which was linked to pistachios (CDC, 2016); in 2015, the CDC reported a multistate outbreak of *S*. Paratyphi linked to JEM Raw brand sprouted nut butter spreads that affected 10 people (CDC, 2015). In 2016, during the course of an outbreak investigation in Greece, an epidemiological analytical study found an association between infection with a *Salmonella* serotype with the antigenic formula 11:z41:enz15 and sesame seed products. The association was subsequently confirmed when this serotype was isolated from sesame seeds in Germany in October 2016 (EFSA, 2017; EFSA and ECDC, 2017).

Ready-to-eat status

Nuts, seeds and dried fruit are often consumed raw or used as ingredients when preparing ready-to-eat (RTE) foods, meaning they do not receive sufficient heat treatment to kill pathogens, if present. For example, the 2016 *S*. Paratyphi outbreak that affected 13 people in the USA was caused by contamination from using raw nut products as an ingredient to make a range of nut butter spreads (CDC, 2016). Another outbreak involving *S*. Stanley affecting 17 people in the USA was associated with cheese that had been made using raw cashew nuts (CDC, 2014b).

In Ireland, there is an increased range of RTE nuts and dried fruits available for sale during the Halloween and Christmas periods. Isaacs *et al*., 2005, found that the frequency of cases of salmonellosis during an outbreak linked to raw almonds peaked during the Christmas season and then the Easter season, presumably linked to increased consumption of these types of products. This survey therefore was run in autumn and winter of 2015 in order to sample the expanded range of seasonal products available for the Halloween and Christmas markets.

AIM OF SURVEY

The aim of this survey was to investigate the microbiological safety of ready-to-eat (RTE), pre-packaged nuts, seeds and dried fruit sampled from retail establishments in Ireland.

METHOD

Sample collection

Between 31 October and 1 December 2015 (inclusive), environmental health officers (EHOs) collected single and batch samples (n=5) of ready-to-eat nuts, seeds and dried fruit from the following types of retail establishments:

- Supermarkets
- Corner shops
- Greengrocers
- Market stalls
- Health food shops
- Discount retailers
- Manufacturers, packers, distributors and wholesalers.

All samples fell into food categories 08 or 16 as specified in FSAI Guidance Note No. 2 *Guidance Note on the EU Classification of Food*. All samples were fully enclosed in sealed packaging, so that in the event of an unsatisfactory test result, it would be clear that the product was contaminated at the production/packaging stage and not during distribution or retail. Samples were considered ready-to-eat unless they were specifically labelled with the instruction that they must be cooked prior to eating.

Survey questionnaire

EHOs were asked to complete a survey questionnaire for each sample collected (see Appendix 5).

Sample analysis

Samples were analysed for the presence or absence of *Salmonella* spp. using EN/ISO 6579, and for the presence or absence of STEC using CEN/ISO TS 13136. In addition, samples were analysed and enumerated for *L. monocytogenes* using EN/ISO 11290-2, and for *E. coli* using ISO 16649-1 or 16649-2.

Subtyping isolates

Isolates obtained during the study were sent to the relevant reference laboratory for typing.

Interpretation of results

L. monocytogenes results were assessed against the criterion set in Commission Regulation (EC) No 2073/2005 as amended for food category 1.3 “RTE foods unable to support the growth of *Listeria monocytogenes*” (European Commission, 2005):

- ***L. monocytogenes*** – ≤100 cfu/g in all five sample units tested.

As no legal criteria are set for *Salmonella*, *E. coli* or STEC in nuts, seeds and dried fruit in Commission Regulation (EC) No. 2073/2005, the guideline limits for a satisfactory result for “RTE food placed on the market” as laid out in *Guidance Note No. 3 Revision 2* were used.

- **STEC** – absence in 25 g.
- ***Salmonella*** – absence in 25 g

In the case of ***E. coli***, the guideline limits according to FSAI Guidance Note No. 3 were:

- Satisfactory <20 cfu/g
- Borderline 20 ≤10^2 cfu/g
- Unsatisfactory >10^2 cfu/g

RESULTS AND DISCUSSION

In total, of the 982 total food samples taken in this survey, 34.2% (n=336) were nuts, 27.9% (n=274) were seeds, 26.5% (n=260) were dried fruit, and 11.4% (n=112) were a mixture (Figure 1).

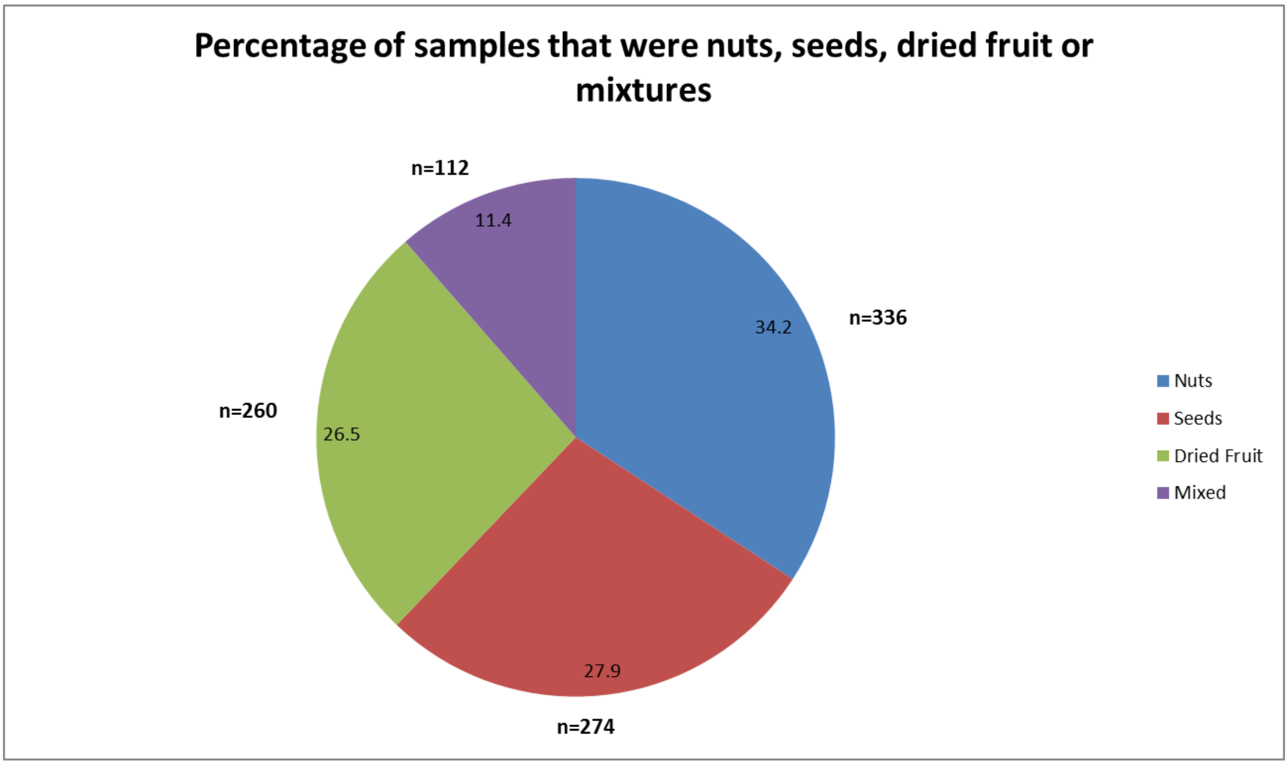

Figure 1: Percentage of samples that were nuts, seeds, dried fruit or mixtures.

Table 1: Breakdown of samples tested by pathogen

Microorganism	No. of food samples tested
Listeria monocytogenes	886 (40 batches; 846 single)[a]
Salmonella	890
Shiga toxin-producing *Escherichia coli* (STEC)	821
Escherichia coli	889

[a]A batch sample comprises n=5 sample units tested for *Listeria monocytogenes, whereas* a single sample is n=1 taken at retail level, e.g. 40 batches x n=5 equals 200 sample units tested.

Microbiological results

The results of the microbiological testing are presented in Table 2.

Table 2: Microbiological results

Parameter	Number of samples	Number (%) satisfactory	Number (%) borderline	Number (%) unsatisfactory
Salmonella spp.	890	888 (99.8)	n/a	2 (0.2)[a]
L. monocytogenes (enumeration)	886[b]	886 (100%)	n/a	0 (0)
STEC	821	821 (100%)	n/a	0 (0)
E. coli (enumeration)	889	888 (99.9%)	1 (0.1%)	0 (0)
Total	**3,471**	**3,468**	**1**	**2**

[a] *S*. Elomrane was detected in nigella seeds and *S. Mikawasima* was detected in ground almonds.
[b] Of the total 886 tested for *Listeria monocytogenes*, 40 were batch samples (n=5) and 846 were single samples (n=1).

Salmonella

Salmonella was detected in 0.2% (2/890) of samples; this is similar to the 0.3% (3/891) *Salmonella* prevalence found in RTE nuts during a previous national survey on the bacteriological and chemical safety of RTE dried seeds and RTE nuts in 2010 (FSAI, 2012). The unsatisfactory samples consisted of nigella seeds from India imported to Ireland via a UK wholesaler, from which *S.* Elomrane was isolated, and ground almonds from the USA, from which *S.* Mikawasima was isolated. A recall of the contaminated ground almonds still on the market was issued (FSAI, 2015). The contaminated nigella seeds were removed from affected stores, and point of sale notices were erected. The FSAI issued an alert through RASFF[5] to inform other EU member states about the contaminated nigella seeds and to provide the supplier's details to the UK competent authorities, so that they could carry out any follow-up investigations. This emphasised the importance of robust traceability[6] documentation by food business operators (FBOs) as required under Regulation (EC) No 178/2002, as amended (European Commission, 2002).

Out of 70 RASFF notifications issued in 2015 for pathogenic microorganisms in the nuts, nut products and seeds category, 69 (99%) were for *Salmonella* and one (1%) was for *Listeria monocytogenes*, suggesting that *Salmonella* is the primary pathogen of concern for these food matrices. Of the 69 *Salmonella* notifications issued through RASFF in 2015, 88.4% (61/69) came from nuts and seeds that originated from India (see Appendix 4). This indicates that nuts, nut products and seed produce from India may be at higher risk for *Salmonella*

[5] 2015.1420: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationDetail&NOTIF_REFERENCE=2015.1420
[6] See also the FSAI's Guidance Note No. 10 on Product Recall and Traceability (FSAI, 2013)

contamination than these products imported from other regions. Interestingly, the nigella seeds from which *S*. Elomrane was isolated in this survey originated from India. The high levels of contamination found in nuts and seeds from India may be due to contaminated water being used for irrigation during agricultural practices or from cross-contamination during drying. A 2007 report by the Indian Central Pollution Control Board found that India lacked sufficient treatment capacity for its raw sewage and that the discharge of raw sewage into its rivers was the most important source of ground and surface water contamination.

As a matter of routine, clinical, food and veterinary laboratories in Ireland send all *Salmonella* isolates from animals, food, feed and human cases of infection to one of two reference laboratories for typing. This allows the differentiation between strains of the same pathogen and can provide information on sources of contamination or illness, or can help identify diffuse outbreaks of illness that may otherwise go undetected. No human cases of *S*. Elomrane or *S*. Mikawasima were reported by the National Salmonella, Shigella and Listeria Reference Laboratory (NSSLRL) in 2015. Interestingly, according to NSSLRL data and RASFF alerts, this is the first time an *S.* Elomrane isolate has been isolated from official control sampling in Ireland. However, an Irish *S*. Mikawasima was isolated in 2013 from a human faecal clinical sample.

Although no isolates of *S*. *Elomrane* or *S*. Mikawasima were recovered from human cases in Ireland during 2015, these serovars are capable of causing human gastrointestinal illness. This suggests that the contaminated nigella seeds and ground almonds were either not consumed, or illness was not reported.

S. Elomrane is a rare serovar in Ireland and Europe. This serovar has been previously associated with lizard species, which might suggest that the nigella seeds were possibly contaminated with *Salmonella* in India, their country of origin. *S.* Mikawasima is a relatively uncommon serovar within Europe. In 2013, following an outbreak of 75 cases in the UK, an outbreak assessment on *S*. Mikawasima by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) found a gradual increase in the trend of *S*. Mikawasima human infections within Europe since 2009. However, the assessment was unable to determine a common causal link for the rising numbers of cases across Europe. This increasing trend in human *S*. Mikawasima cases raises the possibility of some continuous low level or intermittent source of *S*. Mikawasima contamination in the food chain. The assessment also showed that between 2007 and 2012 the average number of *S.* Mikawasima cases was higher each year for the months of August to December than for January to July (EFSA and ECDC, 2013). This is particularly interesting given that this survey isolated *S*. Mikawasima from ground almonds during the autumn-winter period; additionally, a study by Isaacs *et al*. 2005 found that the frequency of cases of salmonellosis during an outbreak linked to raw almonds peaked during the Christmas season and then the Easter season, presumably linked to increased seasonal consumption of these types of product. In this survey, the contaminated almonds originated from the USA. Several outbreaks of salmonellosis linked to almonds have been reported in North America, and *Salmonella* contamination of almonds has been reported frequently (see Appendix 3) (CDC, 2004; Isaacs *et* al., 2005; Danyluk *et al*., 2007).

Contamination of RTE nuts, seeds and dried fruit could be from a number of possible sources, such as contact with animal faeces, land spreading, cross-contamination during drying and processing, or the use of contaminated irrigation water. Islam *et al*., 2004 found that *Salmonella* persisted in soils sprayed with contaminated irrigation water for up 231 days. They also found that *Salmonella* could be detected on vegetable crops planted in these soils up to 203 days after they were seeded.

Listeria monocytogenes

Commission Regulation (EC) No 2073/2005, as amended, sets a maximum legal limit of 100 cfu/g *L. monocytogenes* for ready-to-eat foods during their shelf life. This limit is based on the general acceptance that consuming food with levels below 100 cfu/g is of low risk to consumers (European Commission, 1999).

In this survey, *Listeria monocytogenes* counts were below the limit of enumeration (10 cfu/g) for 100% (886/886) of samples.

The full compliance of all nuts, seeds and dried fruit samples tested for *L*. *monocytogenes* with Commission Regulation 2073/2005, as amended, may be due to the low available water (a_w) of the sample matrices. Many of these food matrices would have available water contents of ≤0.92, meaning that they would be unable to support the growth of *L*. *monocytogenes*. This may explain why only 1 out of 70 (1%) total RASFF notifications for pathogenic microorganisms in the nuts, nut products and seeds category in 2015 was for *L*. *monocytogenes.*[3] Blessington *et al.* 2012 reported that *L. monocytogenes* survived for up to 105 days at 23–25 °C on inoculated walnut kernels. In addition, Pardeepinder *et al*. 2014 reported that *Listeria monocytogenes* survived for up to 350 days at 4 °C and over 100 days at 22 °C on raw peanut kernels.

Shiga toxin-producing *Escherichia coli*

All 821 STEC samples were negative. All samples were compliant with the absence of STEC in a 25 g sample limit for a satisfactory result as outlined in Guidance Note No. 3 Revision 2. Of note, no RASFF notifications were reported for STEC in nuts, nut products and seeds between January 2010 and April 2018. However, STEC can survive in dry conditions; Hiramatsu *et al.* 2005 reported that 31 of 35 tested STEC strains survived drying on disks with a population of 10^3 to 10^4 cfu/disk. Twelve of these strains survived with populations of 10^3 to 10^4 for 22 to 24 months when stored at 4 °C.

Escherichia coli

In this survey, *E. coli* counts were below the limit of enumeration (10 cfu/g) for 99.8% (887/889) of samples. One bag of poppy seeds was at 10 cfu/g, below the 100 cfu/g unsatisfactory limit outlined in Guidance Note No. 3 Revision 2. One sample of walnuts was borderline at 30 cfu/g; however, this was still below the 100 cfu/g limit outlined in Guidance Note No. 3 Revision 2.

E. coli levels were below the limit of enumeration (10 cfu/g) for both of the *Salmonella* positive samples found in this survey. These findings would agree with previous studies which found that *E*. *coli* levels in seeds or druid fruit did not correlate with the presence of *Salmonella,* thus highlighting that *E*. *coli* is not a useful indicator organism for the presence of this pathogen (Danyluk *et al*., 2004; Willis *et al.*, 2009).

Survey response

In total, for the 982 samples taken in this survey, the total response rate to the survey questionnaire was 70% (n=687), including the single questionnaires submitted for batch samples. 8.9% of samples (n=87/982) had coatings. The most common coating, salt, (n=60) was followed by spices (n=13); these were the most popular coatings used on nuts, seeds and dried fruit products. Examples of other coatings used include chocolate, honey, sugar, sunflower oil, ground cinnamon, sunflower oil and wheat flour.

CONCLUSION

The results of this survey indicated a high level of compliance with legal and guideline microbiological limits for RTE nuts, seeds and dried fruit. The findings were similar to a previous national survey conducted in 2010 (FSAI, 2012). *Salmonella* was detected in 0.2% of samples in this survey and 0.3% in the previous survey. This, along with the RASFF notifications and reported international outbreaks, indicates that *Salmonella* can be found on RTE nuts, seeds and dried fruit.

RECOMMENDATIONS

- FBOs that import RTE nuts, seeds and dried fruit should take all reasonable measures to ensure that their produce is from a reputable supplier who has taken appropriate measures to minimise contamination in the field during harvesting, processing, packaging and distribution.

- FBOs should ensure that their traceability records for nuts, seeds and dried fruit are robust, as per Regulation (EC) No 178/2002, as amended (European Commission, 2002). Following the procedures laid out in the FSAI Guidance Note No. 10 on Product Recall and Traceability (FSAI, 2013) will facilitate rapid control measures to be implemented should a pathogen be detected in a batch of nuts, seeds or dried fruit implicated in an outbreak of illness.

- Extra care should be taken to ensure supplier reputability when importing nuts, seeds and dried fruit from countries which have poorer access to potable water and, as such, pose a higher risk for contamination of their produce.

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APPENDIX 1: 2015 RASFF NOTIFICATIONS FOR MICROBIOLOGICAL CONTAMINATION OF PRE-PACKAGED NUTS AND SEEDS

(Source RASFF portal: https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1)

No.	RASFF type	Notification date	RASFF reference	Notifying country	Subject
1	Alert	15/01/2015	2015.0044	Finland	*Salmonella Enteritidis* (presence /25 g) in organic sesame seeds from Bolivia, packaged in Estonia, via the Netherlands
2	border rejection	21/01/2015	2015.0068	Greece	*Salmonella Bredeney* in sesame seeds from India
3	Alert	23/01/2015	2015.0075	Germany	Salmonella *Barranquilla* in organic tahini from Germany
4	Alert	28/01/2015	2015.0096	Austria	*Salmonella* Johannesburg and *Salmonella Kedougou* (1,13,23 : i : l,w /25 g) in ground melon seeds from the United Kingdom, with raw material from Nigeria, via the Netherlands
5	information for attention	27/04/2015	2015.0525	Finland	*Salmonella Typhimurium* (presence /25 g) in roasted chopped hazelnuts from Turkey
6	information for attention	27/07/2015	2015.0974	Germany	*Salmonella* spp. (presence /25 g) in pine nut kernels from Pakistan
7	Alert	06/08/2015	2015.1018	Netherlands	*Listeria monocytogenes* (presence /25 g) in sesame pasta produced in Turkey, via Belgium
8	Alert	11/11/2015	2015.1420	Ireland	*Salmonella Elomrane* (presence /25 g) in nigella seeds from India, packaged in the United Kingdom
9	border rejection	06/01/2015	2015.AAK	Norway	*Salmonella Anatum* (presence /25 g) in hulled sesame seeds from India
10	border rejection	08/01/2015	2015.AAR	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
11	border rejection	12/01/2015	2015.ABB	Germany	*Salmonella* spp. (presence /25 g) in sesame seeds from India
12	border rejection	13/01/2015	2015.ABI	Greece	*Salmonella Mbandaka* (presence /25 g) in sesame seeds from India
13	border rejection	16/01/2015	2015.ABX	Greece	*Salmonella Senftenberg* (presence /25 g) in sesame seeds from India
14	border rejection	16/01/2015	2015.ABY	Greece	*Salmonella Senftenberg* (presence /25 g) in sesame seeds from India
15	border rejection	16/01/2015	2015.ACB	Cyprus	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
16	border rejection	21/01/2015	2015.ACU	Germany	*Salmonella Agona* (presence /25 g) in sesame seeds from India
17	border rejection	21/01/2015	2015.ADC	United Kingdom	*Salmonella* spp. in sesame seeds from India
18	border rejection	23/01/2015	2015.ADM	Italy	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
19	border rejection	26/01/2015	2015.ADW	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
20	border rejection	29/01/2015	2015.AEN	Italy	*Salmonella* spp. (presence /25 g) in sesame seeds from Nigeria
21	border rejection	04/02/2015	2015.AFQ	Poland	*Salmonella* spp. (presence /25 g) in sesame seeds from India
22	border rejection	09/02/2015	2015.AGF	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India

23	border rejection	11/02/2015	2015.AGT	Greece	*Salmonella Amsterdam* (one out of five samples /25 g) and *Salmonella Mbandaka* (four out of five samples /25 g) in sesame seeds from India
24	border rejection	17/02/2015	2015.AHR	Greece	*Salmonella Fresno* (presence /25 g) in sesame seeds from India
25	border rejection	18/02/2015	2015.AHU	Netherlands	*Salmonella* spp. (presence /25 g) in sesame seeds from India
26	border rejection	19/02/2015	2015.AHZ	Netherlands	*Salmonella* spp. (presence /25 g) in sesame seeds from India
27	border rejection	19/02/2015	2015.AIA	Netherlands	*Salmonella* spp. (presence /25 g) in sesame seeds from India
28	border rejection	26/02/2015	2015.AJB	Spain	*Salmonella* spp. (presence /25 g) in sesame seeds from India
29	border rejection	10/03/2015	2015.AKL	Germany	*Salmonella Mbandaka* (presence /25 g) in sesame seeds from India
30	border rejection	13/03/2015	2015.ALG	Poland	*Salmonella* (presence /25 g) in hulled sesame seeds from India
31	border rejection	30/03/2015	2015.ANW	Germany	*Salmonella* spp. (presence /25 g) in sesame seeds from India
32	border rejection	30/03/2015	2015.ANX	Germany	*Salmonella* spp. in sesame seeds from India
33	border rejection	31/03/2015	2015.AOA	Poland	*Salmonella* spp. (presence /25 g) in sesame seeds from India
34	border rejection	01/04/2015	2015.AOF	Cyprus	*Salmonella* (presence /25 g) in sesame seeds from India
35	border rejection	07/04/2015	2015.APL	Greece	*Salmonella Montevideo* (presence /25 g) in hulled sesame seeds from India
36	border rejection	15/04/2015	2015.AQS	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
37	border rejection	15/04/2015	2015.AQT	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
38	border rejection	16/04/2015	2015.ARD	Lithuania	*Salmonella Senftenberg* (presence /25 g) in hulled sesame seeds from India, via Moldova
39	border rejection	17/04/2015	2015.ARI	Netherlands	*Salmonella* spp. (presence /25 g) in sesame seeds from India
40	border rejection	17/04/2015	2015.ARJ	Netherlands	*Salmonella* spp. (in one out of five samples /25 g) in hulled sesame seeds from India
41	border rejection	17/04/2015	2015.ARP	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
42	border rejection	23/04/2015	2015.AST	Cyprus	*Salmonella* spp. (presence /25 g) in sesame seeds from India
43	border rejection	29/04/2015	2015.ATX	Greece	*Salmonella* Orion (presence /25 g) in sesame seeds from India
44	border rejection	30/04/2015	2015.ATZ	United Kingdom	*Salmonella Kentucky* (in one out of five samples /25 g) in hulled sesame seeds from India
45	border rejection	30/04/2015	2015.AUA	Germany	*Salmonella* spp. (presence) in sesame seeds from India
46	border rejection	30/04/2015	2015.AUC	Greece	*Salmonella Mbandaka* (presence /25 g) and *Salmonella Orion* (presence /25 g) in sesame seeds from India
47	border rejection	05/05/2015	2015.AUL	Italy	*Salmonella* spp. (in two out of five samples /25 g) in hulled sesame seeds from Nicaragua
48	border rejection	05/05/2015	2015.AUN	Poland	*Salmonella* spp. (in three out of five samples /25 g) in hulled sesame seeds from India
49	border rejection	06/05/2015	2015.AUS	Poland	*Salmonella* spp. (in three out of five samples /25 g) in hulled sesame seeds from India

50	border rejection	06/05/2015	2015.AUU	Poland	*Salmonella* spp. (in three out of five samples /25 g) in hulled sesame seeds from India
51	border rejection	11/05/2015	2015.AWA	Germany	*Salmonella* spp. (presence /25 g) in sesame seeds from India
52	border rejection	27/05/2015	2015.AXX	Greece	*Salmonella Ekotedo* and *Salmonella Hongkong* (presence /25 g) in natural sesame seeds from Nigeria
53	border rejection	17/06/2015	2015.BAF	Greece	*Salmonella Tennessee* (presence /25 g) in hulled sesame seeds from India
54	border rejection	17/06/2015	2015.BAG	Greece	*Salmonella* spp. (presence /25 g) in sesame seeds from India
55	border rejection	06/07/2015	2015.BCL	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
56	border rejection	28/07/2015	2015.BFX	Poland	*Salmonella* spp. in hulled sesame seeds from India
57	border rejection	28/07/2015	2015.BFZ	Poland	*Salmonella* spp. (presence /25 g) in hulled sesame seeds from India
58	border rejection	29/07/2015	2015.BGD	Greece	*Salmonella Kristianstad* (presence /25 g) and *Salmonella Montevideo* (presence /25 g) in hulled sesame seeds from India
59	border rejection	29/07/2015	2015.BGE	France	*Salmonella* spp. (in one out of five samples /25 g) in auto dried hulled sesame seeds from India
60	border rejection	03/08/2015	2015.BGP	Poland	*Salmonella* (presence /25 g) in hulled sesame seeds from India
61	border rejection	17/08/2015	2015.BIE	Poland	*Salmonella* (presence /25 g) in hulled sesame seeds from India
62	border rejection	15/09/2015	2015.BLV	Poland	*Salmonella* (presence /25 g) in hulled sesame seeds from India
63	border rejection	22/09/2015	2015.BML	Poland	*Salmonella* (presence /25 g) in hulled sesame seeds from India
64	border rejection	07/10/2015	2015.BOL	Greece	*Salmonella Dallgow* (presence /25 g) in hulled sesame seeds from India
65	border rejection	07/10/2015	2015.BOO	Germany	*Salmonella* (presence /25 g) in sesame seeds from India
66	border rejection	09/10/2015	2015.BOX	Greece	*Salmonella* (presence /25 g) in hulled sesame seeds from India
67	border rejection	12/10/2015	2015.BOY	Greece	*Salmonella* (presence /25 g) in hulled sesame seeds from India
68	border rejection	13/10/2015	2015.BPM	Greece	*Salmonella Orion* (in two out of five samples /25 g) in sesame seeds from India
69	border rejection	22/10/2015	2015.BQV	Germany	*Salmonella* (presence /25 g) in sesame seeds from India
70	border rejection	24/11/2015	2015.BWE	Greece	*Salmonella* (presence /25 g) in hulled sesame seeds from India

APPENDIX 2: OUTBREAKS ASSOCIATED WITH NUTS, SEEDS AND NUT/SEED PRODUCTS

Vehicle	Pathogen	Year	Country	Number of cases	Reference
Nuts					
Almonds (raw, whole)	*Salmonella Enteritidis* PT30	2000–2001	Canada (and USA)	168	(Isaacs *et al*., 2005)
Almonds	*Salmonella Enteritidis*	2003–2004	USA and Canada	29	(CDC, 2004)
Almonds	*Salmonella Enteritidis*	2005–2006	Sweden	15	(Müller *et al*., 2007)
Hazelnuts (in-shell)	*E. coli* O157:H7	2011	USA	8	(Miller *et al*., 2012)
Peanuts (canned)	*Clostridium botulinum*	1987	Taiwan	9	(Chou *et al*., 1988)
Peanuts (in-shell)	*Salmonella Stanley* and *Newport*	2001	Australia, Canada and UK	109	(Kirk *et al*., 2004)
Pine nuts	*Salmonella Enteritidis* and *Typhimurium*	2011	USA	47	(CDC, 2011; Bedard *et al*., 2014)
Pistachios	*Salmonella Montevideo*, *Newport* and *Senftenberg*	2009	USA	Not stated	(CDC, 2009)
Cashew cheese	*Salmonella Stanley*	2014	USA	17	(CDC, 2014b)
Peanut-flavoured snack	*Salmonella Agona*	1994–1995	England, Wales and Israel	107	(Killalea *et al*. 1996; Shohat *et al*., 1996)
Peanut butter	*Salmonella Mbandaka*	1996	Australia	15	(Scheil *et al*., 1998)
Peanut butter	*Salmonella Tennessee*	2006–2007	USA	715	(Sheth *et al*., 2011)
Peanut butter, peanuts paste and roasted peanuts	*Salmonella Typhimurium*	2008–2009	USA	714	(Cavallaro *et al*., 2011)
Peanut butter	*Salmonella Bredeney*	2012	USA	42	(CDC, 2012a)
Peanut butter	*Salmonella Braenderup*	2014	USA	6	(CDC, 2014a)
Seeds					
Aniseed containing herbal teabags	*Salmonella Agona*	2002–2003	Germany	42	(Koch *et al*., 2005)
Sesame seed dessert or sweet (helva/halva)	*Salmonella Typhimurium* DT10	2001	Sweden	27	(de Jong *et al*., 2001)
Sesame seed paste (tahini)	*Salmonella Montevideo*	2002–2003	Australia and New Zealand	68	(Unicomb *et al*., 2005)
Sesame seed paste (tahini)	*Salmonella Bovismorbificans*	2011	USA	23	(CDC, 2012b)
Sesame seed paste (tahini)	*Salmonella Montevideo*, *Maastricht* and *Mbandaka*	2012	New Zealand	27	(Paine *et al*., 2014)
Sesame seed paste (tahini)	*Salmonella Montevideo* and *Mbandaka*	2013	USA	16	(CDC, 2013)
Dried fruit					
Coconut (desiccated)	Salmonella *Typhi*, *Senftenburg*, and possible others	1953	Australia	>50	(Wilson and MacKenzie, 1955; Beauchat *et al*., 2013)
Coconut (desiccated)	*Salmonella Java* PT *Dundee*	1999	UK	18	(Ward *et al*., 1999)

APPENDIX 3: PREVALENCE STUDIES FOR PATHOGENS IN NUTS AND SEEDS

Product type	Pathogen	Percentage positive	Number positive /number tested	Country	Reference
Nuts					
Almond (roasted)	*Salmonella*	0.02	1/60	Australia	Eglezos *et al*., 2008
Almonds (roasted)	*Salmonella*	0	0/83	UK	Little *et al*., 2009
Almonds (raw)	*Salmonella*	0.9	81/9,274	USA	Danyluk *et al*., 2007
Almonds	*Salmonella*	0	0/42	Australia	Eglezos, 2010
	L. monocytogenes	0	0/42		
Almonds	*Salmonella*	0	0/60	Ireland	FSAI, 2012
Brazil nut (roasted)	*Salmonella*	0	0/60	Australia	Eglezos *et al*., 2008
Brazil nuts (roasted)	*Salmonella*	0	0/218	UK	Little *et al*., 2009
Brazil nuts	*Salmonella*	0	0/40	Australia	Eglezos, 2010
	L. monocytogenes	0	0/40		
Brazil nuts	*Salmonella*	0	0/80	Ireland	FSAI, 2012
Cashew (roasted)	*Salmonella*	0	0/100	Australia	(Eglezos *et al*., 2008)
Cashews (roasted)	*Salmonella*	0	0/130	UK	(Little *et al*., 2009)
Cashews	*Salmonella*	0	0/45	Australia	(Eglezos, 2010)
	L. monocytogenes	0	0/45		
Cashew nuts	*Salmonella*	0	0/61	Ireland	(FSAI, 2012)
Hazelnut (roasted)	*Salmonella*	0	0/48	Australia	(Eglezos *et al*., 2008)
Hazel nuts (roasted)	*Salmonella*	0	0/38	UK	(Little *et al*., 2009)
Hazelnuts	*Salmonella*	0	0/51	Australia	(Eglezos, 2010)
	L. monocytogenes	0	0/51		
Hazelnuts	*Salmonella*	0	0/69	Ireland	(FSAI, 2012)
Macadamias (roasted)	*Salmonella*	0	0/14	UK	(Little *et al*., 2009)
Peanut (roasted)	*Salmonella*	0	0/653	Australia	(Eglezos *et al*., 2008)
Peanuts (roasted)	*Salmonella*	0	0/26	UK	(Little *et al*., 2009)
Peanuts	*Salmonella*	0	0/343	Australia	(Eglezos, 2010)
	L. monocytogenes	0	0/343		
Peanuts	*Salmonella*	0	0/39	Ireland	(FSAI, 2012)
Pecan nuts (roasted)	*Salmonella*	0	0/25	UK	(Little *et al*., 2009)
Pistachios (roasted)	*Salmonella*	4.0	1/25	UK	(Little *et al*., 2009)
Pistachios (shelled and unshelled), pistachio flour and pistachio paste	*Salmonella*	68.18	15/22	Italy	(Al-Moghazy *et al*., 2014)
Walnuts	*Salmonella*	0	0/53	Ireland	(FSAI, 2012)
Walnuts (roasted)	*Salmonella*	0	0/74	UK	(Little *et al*., 2009)
Tiger nuts (roasted)	*Salmonella*	0	0/2		
Seeds					
Alfalfa seeds	*Salmonella*	1.7	1/58	UK	(Willis *et al*., 2009)
Hemp seeds	*Salmonella*	0	0/121		
Linseed (flax)	*Salmonella*	0.4	1/284		
Melon (egusi)	*Salmonella*	8.5	4/47		
Poppy seeds	*Salmonella*	0	0/202		
Pumpkin seeds	*Salmonella*	0	0/886		
Pine nuts (roasted)	*Salmonella*	0	0/29	UK	(Little *et al*., 2009)
Pine nuts	*Salmonella*	0	0/38	Ireland	(FSAI, 2012)
Pumpkin seeds	*Salmonella*	0	0/79	Ireland	(FSAI, 2012)
Sesame seeds	*Salmonella*	1.7	13/771	UK	(Willis *et al*., 2009)
Sesame seeds	*Salmonella*	2.63	2/76	Ireland	(FSAI, 2012)
Sunflower seeds	*Salmonella*	0.1	1/976	UK	(Willis *et al*., 2009)
Sunflower seeds	*Salmonella*	0	0/109	Ireland	(FSAI, 2012)

APPENDIX 4: EC RASFF *SALMONELLA* NOTIFICATIONS IN NUTS, NUT PRODUCTS AND SEEDS, BY COUNTRY OF ORIGIN FOR 1/1/2015 TO 31/12/2015[3]

Country of origin	Number of *Salmonella* RASFF notifications	Percentage of total notifications
India	61	88.4
Pakistan	1	1.4
Nigeria	3	4.3
Nicaragua	1	1.4
Turkey	1	1.4
Germany	1	1.4
Bolivia	1	1.4
Total	**69**	**100**

APPENDIX 5: SURVEY QUESTIONNAIRE

1) EHO's name ___________________________________

2) EHO's sample reference number _________________________

3) Laboratory to which sample was sent for analysis___________________________________

4) Laboratory's reference number (see laboratory report) _____________________

5) Was this sample:

Mixed sample ☐ Nuts only ☐ Seeds only ☐ Dried fruit only ☐

6) Tick which nuts, seeds or dried fruit present in sample (tick all that apply and specify any others):

Nuts:

Almonds ☐
Brazil ☐
Cashew ☐
Hazel ☐
Macadamia ☐
Peanuts ☐
Pecans ☐
Pistachios ☐
Walnuts ☐
Other ☐
Specify_____________

Seeds:

Linseed (flax) ☐
Pine nuts ☐
Poppy ☐
Pumpkin ☐
Sesame ☐
Sunflower ☐
Other ☐
Specify________________

Dried fruit:

Apple ☐
Apricot ☐
Banana ☐
Coconut ☐
Cranberries ☐
Currants ☐
Dates ☐
Figs ☐
Mango ☐
Pineapple ☐
Prunes ☐
Raisins ☐
Sultanas ☐
Other ☐
Specify________________

7) Was the sample salted or coated in spices? Yes ☐ or No ☐

8) What type of coating was present? Salt ☐ Spices ☐ Other coating ☐

Specify_________________

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