

2018

Current and Future Technologies for Microbiological Decontamination of Cereal Grains

Agatha Los

Technological University Dublin

Dana Ziuzina

Technological University Dublin, dana.ziuzina@tudublin.ie

Paula Bourke

Technological University Dublin, paula.bourke@tudublin.ie

Follow this and additional works at: <https://arrow.tudublin.ie/scschbioart>



Part of the [Food Science Commons](#)

Recommended Citation

Los, A., Ziuzina, D. & Bourke, P. (2018). Current and future technologies for microbiological decontamination of cereal grains. *Journal of Food Science*, 83(6), pp. 1484-149. doi:10.1111/1750-3841.14181

This Article is brought to you for free and open access by the School of Biological Sciences at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact arrow.admin@tudublin.ie, aisling.coyne@tudublin.ie.



This work is licensed under a [Creative Commons Attribution-NonCommercial-Share Alike 4.0 License](#)

1 **Current and Future Technologies for Microbiological Decontamination of Cereal Grains**

2 Agata Los^a, Dana Ziuzina^a, Paula Bourke^{a*}

3 ^a*Food and Health Research Centre, School of Food Science and Environmental Health, Dublin*

4 *Institute of Technology, Dublin 1, Ireland*

5

6 *Corresponding author:

7 E-mail: paula.bourke@dit.ie

8

9 **Word count of text: 9,304 words**

10 **Short version of title:** Decontamination methods of cereal grains

11 **Choice of journal/section:**

12 *Journal of Food Science* sections:

13 • Concise Reviews and Hypotheses in Food Science

14 **ABSTRACT**

15 Cereal grains are the most important staple foods for mankind worldwide. The constantly
16 increasing annual production and yield is matched by demand for cereals, which is expected
17 to increase drastically along with the global population growth. A critical food safety and
18 quality issue is to minimize the microbiological contamination of grains as it affects cereals
19 both quantitatively and qualitatively. Microorganisms present in cereals can affect the safety,
20 quality and functional properties of grains. Some molds have the potential to produce harmful
21 mycotoxins and pose a serious health risk for consumers. Therefore, it is essential to reduce
22 cereal grain contamination to the minimum to ensure safety both for human and animal
23 consumption. Current production of cereals relies heavily on pesticides input, however,
24 numerous harmful effects on human health and on the environment highlight the need for more
25 sustainable pest management and agricultural methods. This review evaluates microbiological
26 risks, as well as currently used and potential technologies for microbiological decontamination
27 of cereal grains.

28

29 **Keywords:** cereal grains, microflora, microbial inactivation, decontamination, food safety

30 **1. Introduction**

31 Cereals are one of the most important agricultural products in the world, both as human foods
32 and as the main constituent of animal feed. Development of agriculture in prehistoric times was
33 heavily associated with domestication of cereal grains and since their first cultivation most
34 civilizations have become dependent upon cereals for the majority of its food supply (Cordain,
35 1999). Cereal grains are the most commonly consumed food group worldwide and they are
36 grown on about 60% of the cultivated land in the world (Harlan 1992, Koehler & Wieser,
37 2013). In order to meet the requirements of a growing world population, worldwide production
38 and yield of cereals has been increased for the last 50 years (Fig. 1.) (**Food and Agriculture**
39 **Organization [FAO]**, 2017). Major types of cereal grains include maize, rice, wheat, barley,
40 sorghum, millet, oats, and rye (Fig. 2.) (FAO, 2017).

41 Worldwide significance and extensive use of cereal grains and their products makes cereals
42 preservation and decontamination one of the most important food safety issues. Contamination
43 of stored grain with insects and microorganisms is a major concern of the grain industry as it
44 affects the grains both quantitatively and qualitatively (Yadav, Anand, Sharma & Gupta, 2014).
45 Microorganisms present in cereals constitute a principle control point since their development
46 may affect the safety, quality and properties of the grains. Some molds can potentially produce
47 harmful mycotoxins and pose a serious health risk for consumers (Laca, Mousia, Díaz, Webb,
48 & Pandiella, 2006). Losses of cereal grains during storage are estimated between 5 and 30%
49 due to molds and mycotoxins, 5% for insects and 2% for rodents, with an average yield loss of
50 1% for developed and 10-30% for developing countries (Rajendran, 2002). It is essential
51 to reduce cereal grain contamination to the minimum and ensure safety both for human and
52 animal consumption.

53 Currently, industrial production of cereals relies heavily on chemical input of pesticides, which
54 brings high economic benefits, minimizes labor input and improves yield and quality of

55 agricultural products. However, pesticides can be harmful also to non-target organisms and
56 have negative effects on human health and the environment. Resistance to pesticides in most
57 major pest species is also increasing. In 2009, the Directive on the sustainable use of pesticides
58 was adopted by the EU and its overall objective is “to achieve the sustainable use of pesticides
59 by reducing the risks and impacts of pesticides use on human health and environment and
60 promoting the use of integrated pest management and other non-chemical alternatives
61 to pesticides” (European Commission [EC], 2009). Within the next decade, the new
62 regulations will drastically reduce the number of active substances permitted in crop
63 production, which drives the research to develop new disruptive technologies that are
64 environmentally and societally acceptable control methods for cereal grains preservation. This
65 review compares currently used technologies with novel and potential methods for microbial
66 decontamination of cereal grains.

67 **2. Microbial challenges associated with cereal grains**

68 **2.1 The sources of microbial contamination of cereals**

69 Microbial contamination of cereal grains occurs during crop growth, harvesting and post-
70 harvest drying and storage (Magan & Aldred, 2006) and it derives from several sources,
71 including air, dust, water, soil, insects, birds and rodents feces as well as contaminated
72 equipment and unsanitary handling (Fig. 3). The type of microbial contamination varies
73 according to the growing region and is heavily influenced by environmental conditions such as
74 drought, rainfall, temperature and sunlight, as well as unsanitary handling, harvesting and
75 processing equipment, and poor storage conditions (Nierop, 2006; Bullerman & Bianchini,
76 2009). High rainfall just before harvest is a factor inducing extensive colonization of the grain
77 ears by *Alternaria* spp., causing black fungi discoloration, that can be observable both on
78 the surface of the kernels and as beneath the pericarp (Kosiak, Torp, Skjerve, & Andersen,
79 2004). Doohan, Brennan, & Cooke (2003) investigated the influence of climatic factors on

80 *Fusarium* species pathogenic to cereal grains and found that they differ significantly in their
81 climatic distribution as well as in the optimum climatic conditions required for their
82 persistence.

83 **2.2 The field microflora**

84 The field microflora consists of microorganisms that occur on or in grains until the time of
85 harvest and depends on the conditions under which the crops were grown. The kernels are
86 numerically dominated by bacteria, with yeasts as the next most abundant component.
87 The number of filamentous fungi increases during the later stage of ripening (Noots, Delcour
88 & Michiels, 1999; Flannigan, 2003; Nierop, 2006).

89 **2.2.1 Bacteria**

90 Levels of cereal grains contamination with bacterial pathogens are usually very low and
91 although contamination with species such as *Salmonella*, *Escherichia coli* and *Bacillus cereus*
92 can occur, bacteria associated with cereals are generally non-pathogenic. The most often they
93 belong to the families *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae* and
94 *Bacillaceae* (Laca et al., 2006; Hocking, 2003). Some species of enteric bacteria that are found
95 on cereal grains are plant saprophytes and their presence is not related to fecal contamination
96 (Harris, Shebuski, Danyluk, Palumbo, & Beuchat, 2013). Gram-negative bacteria numerically
97 dominate the microflora of pre-harvest barley, with *Erwinia herbicola* (now: *Pantoea*
98 *agglomerans*) and *Xanthomonas campestris* as predominant bacterial species (Noots et al.,
99 1999; Flannigan, 1996). Numerous bacteria belonging to *Streptomyces* genus were recently
100 found on barley and spring wheat grains. The authors also reported the presence of antimycin
101 A toxin-producing strains in barley, which is the first report of antimycin A in a food substance
102 (Rasmus-sahari, Mikkola, Andersson, Jestoi, & Salkinoja-salonen, 2016).

103 2.2.2 Filamentous fungi and yeasts

104 The fungi growing on crops have been traditionally divided into two groups – “field” and
105 “storage” fungi (Pitt & Hocking, 2009). The main difference between these groups is the time
106 at which they invade the grains and growth conditions, however, the distinction between field
107 and storage fungi is not absolute. It was found that although some field fungi invade the grains
108 on the field, they are still able to grow in storage conditions. Similarly, some fungi commonly
109 classified as storage fungi may invade the grains at earlier stages (Christensen & Meronuck,
110 1986).

111 Field fungi, including species such as *Alternaria*, *Cladosporium*, *Fusarium*, and
112 *Helminthosporium*, invade grain in the field at high relative humidities (90 to 100%) when
113 the grain is high in moisture (18 to 30%) (i.e., at high a_w) (Bullerman & Bianchini, 2009)
114 Christensen & Meronuck, 1986). Significant increase in a number of infections with *Fusarium*
115 species is often observed when ripening is performed during wet periods. Also, phyllosphere
116 fungi are responsible for pre-harvest fungal contamination (Magan & Aldred, 2006).

117 As high moisture content is required for field fungi, conditions during the storage are not
118 favorable for their growth, however, some fungi including *Penicillium* and *Fusarium* species
119 and various species of yeasts are able to invade seeds before harvest and continue to grow
120 during storage (Christensen & Meronuck, 1986). Although most *Penicillium* species are
121 xerophiles and they usually are considered to be storage fungi, at certain conditions they can
122 also attack the grains before harvest – it was found that *P. oxalicum* can infect pre-harvest
123 maize due to insect damage or wounding (Pitt & Hocking, 2009).

124 Similarly, *A. flavus*, usually regarded as a storage fungus, was found in freshly harvested maize
125 (Lillehoj et al., 1976). Insect damage to cobs and fungal invasion down the silks are the main
126 factors increasing the risk of *A. flavus* contamination of corn (Lillehoj et al., 1980;
127 Williams et al., 2006).

128 The field fungus most frequently present in both barley and wheat kernels is *Alternaria*
129 (Christensen & Meronuck, 1986; Flannigan, 1996). Kosiak et al. (2004) reported that
130 the distribution of field fungi contamination varied significantly in wheat, barley and oats
131 samples of reduced quality with dominant *Fusarium* species as compared to acceptable
132 samples for which *Alternaria* was the most numerous. *Fusarium* species are a common
133 contaminant of various cereal grains. While *F. graminearum*, *F. culmorum*, *F. poae*,
134 *F. avenaceum* and *Microdochium nivale* (formerly known as *F. nivale*) cause diseases of small-
135 grain cereals such as wheat and barley, corn is usually attacked by *F. graminearum*,
136 *F. moniliforme*, *F. proliferatum* and *F. subglutinans* (Doohan et al., 2003).
137 Hill and Lacey (1983) reported that by harvest even 50-85% of barley kernels may be colonized
138 by yeasts, with pink yeasts, such as *Sporobolomyces* and *Rhodotorula*, being predominant
139 (Flannigan, 1996). Other species found on barley are *Hansenula*, *Torulopsis*, *Candida* and
140 *Saccharomyces*, while *Cryptococcus* and *Trichosporon* were isolated from pre-harvest wheat
141 (Flannigan, 1996). Flannigan (1996) suggested that because of the strong resemblance between
142 other components of wheat and barley microfloras, similar yeasts species are likely to be found
143 on both cereals.

144 **2.3 The storage microflora**

145 Modern methods used for harvesting and proper storage practices ensuring the exclusion of
146 water and lack of possibility for birds, insects and rodents to contaminate the grain, are
147 considered sufficient to prevent the microbial growth. However, these conditions are not
148 always met.

149 **2.3.1 Bacteria**

150 Generally, bacteria are not significantly involved in the spoilage of dry grain due to storage
151 conditions unfavorable for their growth. However, it was found that some bacterial pathogens
152 and spore-forming species are able to survive during storage and may contaminate processed

153 products. Lactic acid bacteria present in the raw grain may be carried over through
154 the processing and spoil doughs prepared from flour and cornmeal (Bullerman & Bianchini,
155 2009; Justé et al., 2011). Gram-negative coliforms, pseudomonads and actinomycetes were
156 also found on dry-stored cereals (Hill & Lacey, 1983). Wachowska, Stasiulewicz-Paluch,
157 Glowacka, Mikolajczyk, & Kucharska (2013) reported that the number of bacteria of the genus
158 *Azotobacter* colonizing winter wheat grain was relatively low at harvest, but the counts
159 increased after six months of storage.

160 **2.3.2 Filamentous fungi and yeasts**

161 Spoilage of grains with filamentous fungi during storage occurs usually due to inefficient
162 drying, what favors microbial growth and may result in increased mycotoxins levels (Magan
163 & Aldred, 2006; Harris et al., 2006). If drying is delayed and the moisture content of
164 the harvested grain is suitable, growth of the field fungi, e.g. *Fusarium* spp., may occur
165 (Flannigan, 1996).

166 Storage fungi including species of *Eurotium*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and
167 *Wallemia*, invade stored-grains at low relative humidity's (65 to 90%) and lower moisture
168 contents (14 to 16%) of the grains (Bullerman & Bianchini, 2009). Storage temperature heavily
169 influences the types and rates of microbial spoilage – *Penicillium* species are dominant at
170 cooler temperatures, while *Aspergillus* and *Eurotium* species are more common at ambient
171 temperatures (20-25°C). Species of *Rhizopus* (common bread mold) and *Neurospora* (red bread
172 mold) can also be found on grains at ambient temperatures, however, they are much more
173 common on baking products (Magan & Aldred, 2006).

174 Among *Penicillium* species, *P. verrucosum* is important as contamination with this fungus may
175 result in the production of carcinogenic mycotoxin - ochratoxin a (OTA), especially in cool
176 climates (Lund & Frisvad, 2003). It was shown that *P. verrucosum* attacks wheat and barley
177 only after harvest (Magan & Aldred, 2006; Pitt & Hocking, 2009). In tropical conditions other

178 *Penicillium* species are more common – *P. citrinum* is a fungus commonly detected in rice
179 (Tonon, Marucci, Jerke & Garcia, 2007, Trung, Bailly, Querin, Le Bars & Guerre, 2001; Park,
180 Choi, Hwang & Kim, 2005). In maize, different *Penicillium* species occur depending on
181 the stage of processing – some of them invade only pre-harvest grains (*P. funiculosum*), other
182 are associated only with the stored grain (*P. aurantiogriseum*, *P. viridicatum*), whereas some
183 species, such as *P. citrinum* and *P. oxalicum*, are present all the time (Mislivec and Tuite,
184 1970a, b).

185 Birck, Lorini & Scussel (2005) compared fungal contamination in wheat grain during 180 days
186 of storage. It was observed that *Fusarium* spp. was the most numerous fungus after harvest and
187 after 30 days of storage, however, the counts decreased gradually until the end of the storage
188 period. After 180 days of storage *Aspergillus*, *Fusarium* and *Penicillium* were found in 96.7%,
189 46.7% and 80.0% of wheat samples, respectively. Krnjaja et al. (2015) investigated mycobiota
190 of maize – mycological analyses showed the presence of *Aspergillus*, *Fusarium* and
191 *Penicillium* on both freshly harvested and stored grains, however, the predominant species
192 varied for each stage of processing. Similar observations were noted for maize – samples
193 analyzed immediately after harvest showed predominance species of *Alternaria*, followed by
194 species of *Cladosporium* and *Penicillium*, whereas after 120 days of storage the maize grains
195 mycoflora consisted of various fungi belonging with predominance to *Fusarium*, *Aspergillus*
196 and *Penicillium* genera (Dudoiu, Cristea, Lupu, Popa and Oprea, 2016).

197 Yeasts found on cereal grains during storage are often amylolytic yeasts (Magan & Aldred,
198 2006). Similarly to lactic acid and spore-forming bacteria, yeasts present on cereals may also
199 be carried through into processed products (Bullerman & Bianchini, 2009).

200 **2.4 Distribution of microorganisms within cereal grains**

201 The typical structure of a cereal grain constitutes of three edible parts: the bran which consists
202 of the outer coat (pericarp, testa and aleurone layers), the germ (the embryo) and the starchy

203 endosperm, and an inedible husk that protects the kernel (Fig. 4.) (Dexter & Wood, 1996;
204 Merali et al., 2013). Microbial colonization is generally restricted to the outer layers of cereal
205 grains, i.e. the husk, between the husk and pericarp, and within the pericarp tissue (Briggs,
206 1998). Several studies showed that after debranning – a controlled process in which the outer
207 layers of the grains are removed, cereals are microbiologically purer (Bainotti & Perez, 2000;
208 Laca et al., 2006). However, there are species able to invade the inner part of the grains and
209 penetrate into the endosperm, causing internal infections (Nierop, 2006).

210 Distribution of the microbial populations on various cereal grains has been studied by several
211 authors. Microscopic observations of barley revealed a high number of microorganisms
212 between the husk and pericarp. It was observed that bacteria clustered as randomly distributed
213 micro-colonies with up to 200 cells (Petters, Flannigan & Austin, 1988). Laca et al. (2006)
214 studied distribution of microorganisms within wheat grains and found that most of bacteria and
215 molds were concentrated on the surface of the grain in the pericarp surrounding the endosperm
216 and the germ, therefore, removing some of the outer layers of the grains may be used
217 to substantially reduce the microbial contamination. According to the study, most of
218 the contamination is located in the outer layers, i.e. the first and second pearling fractions,
219 which corresponds to a layer thickness of approximately 30 µm. Colonisation of the grains by
220 *Alternaria* spp. (black fungi discoloration) is observable on the surface of the kernels as well
221 as beneath the pericarp of wheat, barley and oats, and is believed to be a result of rainfall just
222 before harvest (Kosiak et al., 2004). Andersen and Thrane (2006) reported that wheat and
223 barley surface disinfection with sodium hypochlorite removed only 10-15% of *Alternaria* and
224 *Bipolaris*, which indicates that the grains were contaminated beneath the pericarp. A common
225 result of invasion of the germs by *Aspergillus* species, such as *A. restrictus*, *A. glaucus* and
226 *A. candidus*, is germ-damaged wheat (the fungi grow only in the germ) that can often develop
227 without visible sign of moldiness (Christensen & Meronuck, 1986). Bacon and Williamson

228 (1992) investigated the interactions of *F. moniliforme* with corn. Studies based on scanning
229 electron microscopy showed distribution of the fungus mostly over the pericarp, however,
230 contamination of the embryo and endosperm also occurred.

231 **3. Current and potential techniques for control of microbial spoilage of cereal grains**

232 **3.1 Current techniques and their limitations**

233 Current technologies applied to control microbial spoilage of cereals successfully reduce
234 the microbial load, however, they can negatively affect the quality and technological properties
235 of cereals, as well as generate harmful environmental impacts. A brief description of current
236 techniques and their limitations used for cereal grains preservation is summarized in Table 1.

237 **3.1.1 Pesticides**

238 Worldwide cereal production heavily relies on pesticides input, including fungicides,
239 herbicides and insecticides. The primary benefits of pesticides application are crop protection
240 from the damaging influences of pests, higher yields and better quality of cereals. However,
241 pesticides use raises several concerns, related especially to its environmental impacts such as
242 biodiversity reduction, surface and ground water pollution, soil contamination and decrease of
243 fertility, as well as direct harmful impact on humans and other non-target species (Aktar,
244 Sengupta & Chowdhury, 2009; Liu, Pan & Li, 2014). Repeated pesticide use may lead
245 to development of pesticides resistance in pest populations previously susceptible to active
246 agents used (Jess et al., 2014). To reduce pesticides inputs and therefore the risks and impacts
247 on human health and the environment, in 2009, the European Union introduced a strategy (EC,
248 2009) on the sustainable use of pesticides through the use of Integrated Pest Management
249 (IPM), i.e. combining chemical and non-chemical control methods and use alternative
250 approaches such as non-chemical alternatives to reduce the reliability on pesticides (EC, 2009),
251 which is the most effective strategy to prevent the evolution of pesticide resistance.

252 **3.1.2 Drying**

253 Moisture content in the harvested cereal grains is naturally high. Drying is the phase of
254 the post-harvest processing during which the grains are dried until the moisture content level
255 guaranteeing safe storage conditions, i.e. equivalent to $<0.70 a_w$, is reached. A typical moisture
256 content level of properly dried grains is between 10 - 14 percent. Effective drying permits
257 a reduction of losses during storage, as it creates unfavorable conditions for molds growth and
258 proliferation of insects. However, these conditions are not always met. Heat and moisture
259 produced as a result of biological activity and respiration of grains during storage, are major
260 factors influencing spoilage. Damp or warm spots of grain favor fungal growth, which leads
261 to further production of heat and moisture, creating a self-generating process (Magan &
262 Aldred, 2006; Mrema, Gumbe, Chepete & Agullo, 2011).

263 One of the important limitations of grains drying is a difficulty to achieve a sufficient
264 uniformity of the process as under- or over-dried areas leads to grains with different moisture
265 contents to be found in the same batch (Raghavan, 1993; Magan & Aldred, 2006). Using
266 excessive temperatures damages grains, e.g. cracking and loss of viability, as well as may cause
267 economic losses. For instance, one kilogram of grains at 15 percent moisture content weighs
268 885.4 g at 4 percent moisture content, causing a loss in the value in the market (Yaciuk, 1980;
269 Mrema et al., 2011). Also, it increases the risk of growth of mycotoxin producing molds, which
270 usually colonize only damaged parts of plants (Varga, Kocsube, Peteri, Vagvolgyi, & Toth,
271 2010).

272 **3.1.3 Mechanical debranning**

273 Debranning is an advanced milling process during which the bran layers of a grain are
274 separated from the endosperm and removed by friction and abrasion. This technique can
275 improve the yield and degree of refinement of flour, as well as allowing the production of good-
276 quality milled products from lower quality grains (Dexter & Wood, 1996). Laca et al., (2006)

277 showed that after debranning, grains are microbiologically purer. It was reported that by
278 removal from the surface of 4% of the total weight of the grain, that the total microbial
279 contamination was reduced up to 87%. Due to the complex anatomy of a wheat kernel which
280 has a longitudinal crease that extends to the center of the kernel, complete separation of
281 the bran from starchy endosperm is difficult to achieve in the debranning process (Dexter &
282 Wood, 1996).

283 **3.1.4 Chlorine and hypochlorite**

284 Due to their oxidizing capacity, chlorine-based methods are widely used in the industry for
285 food produce disinfection and microbial control. These techniques are inexpensive and easy
286 to use; however, they bring concerns due to generating toxic by-products as well as off-tastes
287 and odours after the treatment (Richardson et al., 2001; Virto, Manas, Alvarez, Condon, &
288 Raso, 2005). The need to reduce environmental chlorine emissions has led to the consideration
289 of non-chlorinated alternatives.

290 It was found that using chlorine for inactivation of microorganisms on cereal grains was
291 ineffective for highly contaminated products - 0.4% chlorine solution did not inactivate
292 sufficient fungal spores to produce less than 20% contamination when initial contamination
293 levels were greater than 10^4 per gram of barley (Delaquis & Bach, 2012; Andrews, Pardoel,
294 Harun, & Treloar, 1997). Sodium hypochlorite has also been used frequently, however, studies
295 show that this kind of treatment does not completely inactivate fungal spores neither on
296 the surface of corn nor wheat (Sauer & Burroughs, 1986; Sun et al., 2017).

297 **3.1.5 Irradiation**

298 Irradiation in food processing is a process that involves exposing food to a certain amount of
299 ionizing radiation. Three major types of this technology are: (a) gamma-rays generated from
300 the radioactive isotopes of cobalt-60 (^{60}Co) or cesium-137 (^{137}Cs); (b) electron beam
301 processing; (c) X-rays created by electron accelerators. The mechanism of microbial

302 inactivation by irradiation includes direct DNA damage and the production of reactive
303 molecules, such as hydrogen peroxide, hydroxyl radicals and hydrogen atoms. These molecules
304 can damage cellular metabolic pathways inside the cells, promote intracellular oxidation and
305 consequently lead to cell lysis (Farkas, Ehlermann, & Mohácsi-Farkas, 2014; Lung et al.,
306 2015).

307 Irradiation has been successfully used for control of microorganisms on cereals and flours since
308 1950s (comprehensively reviewed by Lorenz & Miller, 1975). The use of 0.5 kGy radiation for
309 the prevention of pest contamination in wheat and flour was approved by the United States
310 Food and Drug Administration (USFDA) in 1963 and the technology has been applied for
311 preservation and decontamination of various crops (Lung et al., 2015).

312 **3.1.6 Ozone**

313 The use of ozone in food processing has become increasingly important since it gained GRAS
314 (Generally Recognized as Safe) status in 1997 (Graham et al., 1997) and four years later it was
315 approved by US Food and Drug Administration (FDA) as a secondary direct food additive and
316 antimicrobial agent for all food types (O'Donnell, Tiwari, Cullen, & Rice, 2012). Ozone (O₃)
317 is the triatomic oxygen formed by addition of a free radical of oxygen to molecular oxygen.
318 When generated from dried air, ozone is a blue gas. Ozone generation from high-purity oxygen
319 leads to formation of a colorless gas (Greene, Guzel-Seydim, & Seydim, 2012). Ozone can be
320 applied in the gaseous or aqueous state. It has been demonstrated that after treatment ozone
321 decomposes into molecular oxygen and hence does not leave hazardous residues on the food
322 product (Graham et al., 1997).

323 Microbial inactivation by ozone have been studied against a wide variety of microorganisms.
324 The bactericidal effect of ozone has been studied against both Gram-positive and Gram-
325 negative bacteria as well as spores and vegetative cells. Microbial inactivation by ozone is
326 a complex process - Victorin, (1992) identified two major mechanisms of ozone destruction of

327 microorganisms: (1) oxidation of sulfhydryl groups and amino acids of enzymes, peptides and
328 proteins to shorter peptides; and (2) oxidation of polyunsaturated fatty acids to acid peroxides.
329 Disruption or disintegration of the cell envelope leads to cell lysis and inactivation of
330 microorganisms (Greene et al., 2012).

331 The use of ozone as a fungicide for decontamination of cereal grains has been investigated in
332 several studies. Kells, Mason, Maier and Woloshuk (2001) used gaseous ozone to reduce
333 the contamination level of *Aspergillus parasiticus* on the kernel surface of corn by 63%. In
334 another study (Allen, Wu, & Doan, 2003), 96% of inactivation was achieved for spores or
335 a mixture of spores and a small number of mycelia on barley after 5 minutes of treatment. It
336 was observed that increases in water activity and temperature of grains enhanced the fungicidal
337 efficacy of ozone. Kottapalli, Wolf-Hall, and Schwarz (2005) reported a significant decrease
338 (24 to 36%) of *Fusarium* survival after 15 min of exposure at either 11 000 or 26 000 ppm
339 ozone concentration in naturally infected malting barley. Wu, Doan, and Cuenca (2006) used
340 gaseous ozone to preserve stored wheat and found that ozone treatment was a very effective
341 method for inactivation of 96.9% of fungal spores associated with wheat. In this study, higher
342 treatment efficacy was achieved when temperature and water activity of wheat were increased,
343 what confirms the results obtained by Allen et al. (2003). Bactericidal effect of ozone was
344 observed by Naito, Okada, and Sakai (1988) – gaseous ozone inactivated up to 3 log units of
345 *Bacillus* spp. and *Micrococcus* spp. on cereal grains, peas, beans and spices. It was also found
346 that the treatment efficacy depends on ozone concentration, relative humidity and treatment
347 temperature. Dodd et al. (2011) investigated the effect of ozonation on malting barley –
348 the treatment did not lead to significant reductions in aerobic plate counts, but it decreased
349 mold and yeast counts by 1.5-log in the final malt. In the study, gaseous ozone did not
350 negatively influence any aspect of malt quality.

351 Ozonated water was also reported to be effective for microbial inactivation of a range of foods,
352 including grains, and can be an alternative to chlorinated water before milling. Dhillon,
353 Wiesenborn, Dhillon, and Wolf-Hall (2010) found that although washing durum wheat grains
354 with ozonated water did not show high antimicrobial efficacy when used alone, it was effective
355 in combination with acetic acid. Similarly, a combination of gaseous ozone, acetic acid, and
356 ozonated water, using a fluidized bed system, was the most effective in reducing microbial
357 counts on durum wheat in another study (Dhillon et al., 2010). In both studies, however, grain
358 moisture content increased after the treatment.

359 **3.2 Future trends for decontamination of cereal grains**

360 Limitations of conventional methods used for inactivation of microorganisms associated with
361 cereals suggest that there is a huge demand for new technologies, which will be rapid and cost
362 effective. An ideal method should reduce microbial loads uniformly on all the treated grains,
363 without formation of toxic, non-target residues and by-products after the treatment. Potential
364 techniques for cereals preservation (Table 2) should not affect their quality as the consumers
365 expect high-quality processed foods with minimal changes in nutritional and sensory
366 properties.

367 **3.2.1 Microwave (MW) treatment**

368 Microwaves are electromagnetic waves with frequency within 300 MHz to 300 GHz
369 (Chandrasekaran, Ramanathan, & Basak, 2013). Microwave inactivation of microorganisms is
370 achieved at temperatures lower than that of conventional pasteurization, however, many studies
371 suggest that microwaves inactivate microbes mainly by a thermal effect, including irreversible
372 heat-denaturation of enzymes, proteins, nucleic acids or other cellular constituents, leading
373 to cell death. a second possible mode of action are non-thermal (“athermal”) effects, caused by
374 the intrinsic nature of microwaves and not related to increase of the temperature during
375 the MW treatment (Heddleson & Doores, 1993). It was found that higher microbial reduction

376 levels of microwave treatment are achieved in presence of other stresses, such as acidic pH or
377 increased temperature (Kozempel, Annous, Cook, Scullen, & Whiting, 1998). Kozempel et al.
378 (1998) emphasizes that efficacy of using microwave energy for microbial inactivation depends
379 on the type of microorganism-food system.

380 The number of studies investigating microwave treatment for inactivation of microorganisms
381 associated with cereal grains is limited. Reddy, Raghavan, Kushalappa, & Paulitz (1998)
382 successfully reduced the seedborne *F. graminearum* infection of wheat to below 7%,
383 maintaining the commercially acceptable seed germination threshold, i.e. 85%. It was observed
384 that seed viability and seedling vigour decreased after the microwave treatment. Microwave
385 energy can also be used for control of stored-grain insects (Vadivambal, Jayas, & White, 2007).

386 **3.2.2 Pulsed ultraviolet (UV) light treatment**

387 Pulsed UV light treatment is an emerging non-thermal technology that can be used both for
388 decontamination of foods and food contact surfaces. It involves the use of short-duration, high-
389 power pulses of a broad spectrum of white light from the ultraviolet (UV), which makes 50%
390 of the total spectrum, to the near infrared region (Keklik, Krishnamurthy, & Demirci, 2012).
391 Pulsed UV light is considered to be more efficient in microbial inactivation than continuous
392 UV light, offering a safer and faster decontamination (Krishnamurthy, Tewari, Irudayaraj, &
393 Demirci, 2010). Microbial inactivation by UV light, which can be classified into 4 spectrum
394 regions, is primarily due to DNA structure alternation. UV-C light, with the peak of maximum
395 effectiveness at wavelengths of about 260–265 nm what corresponds with the peak of
396 maximum DNA absorption, is the most effective for inactivating microorganisms. Formation
397 of cyclobutane pyrimidine dimers during UV light treatment leads to mutagenesis and cell
398 death (Gayán, Condón, & Álvarez, 2014). Although the technology is able to kill vegetative
399 cells and bacterial spores, as well as fungal spores and viruses, it has not been applied yet at
400 industrial scale in food processing (Keklik et al., 2012; Ortega – Rivas, 2012).

401 Although it is believed that pulsed UV light is not an adequate technology for cereals due
402 to their rough and uneven surfaces (Oms-oliu, Martín-belloso, & Soliva-fortuny, 2010),
403 the antimicrobial efficacy of this technology against microorganisms occurring on stored cereal
404 grains has been demonstrated. Maftei, Ramos-villaruel, Nicolau, Mart, & Soliva-fortuny
405 (2013) studied the potential of pulsed light technology for the decontamination of naturally
406 occurring molds on wheat grains and achieved a reduction of about 4 log CFU/g, with the seed
407 germination percentage slightly decreased. It was also found that the initial mold load of grains
408 is an important factor for the treatment efficacy.

409 **3.2.3 Non-thermal (cold) plasma**

410 Plasma, considered as a fourth state of matter, is a partially or fully-ionized gas.
411 The terminology “cold” or “nonthermal” describing plasmas refers to the physical parameter.
412 As compared to thermal plasmas generated at high temperatures, cold plasmas are generated at
413 or near room temperature, therefore, mechanism of microbial inactivation does not rely on
414 thermal destruction of microorganisms. As a nonthermal process, cold plasma causes little or
415 no thermal damage to the food product after treatment (Niemira, 2012; Niemira, Boyd, & Sites,
416 2014).

417 Cold plasma can be generated at atmospheric as well as low pressure and consists of UV
418 photons, neutral or excited atoms and molecules, negative and positive ions, free radicals and
419 free electrons. The technique has recently found an extensive range of applications for
420 microbiological decontamination due to chemical and bioactive radicals generated during
421 electrical discharge, including reactive oxygen species (ROS) and reactive nitrogen species
422 (RNS) (Laroussi & Leipold, 2004; Scholtz et al., 2015).

423 Application of cold plasma for decontamination of cereal grains has been studied recently.
424 Selcuk, Oksuz, and Basaran (2008) studied the low pressure cold plasma inactivation of two
425 pathogenic fungi, *Aspergillus* spp. and *Penicillium* spp. artificially inoculated on surface of

426 various seeds, including wheat, barley, rye and corn. Within 15 min of plasma treatment
427 the fungal attachment to seeds was reduced below 1% of initial load. The treatment efficacy
428 was dependent on the initial contamination level of the seeds. In the study, the germination
429 quality of the seeds remained unaffected after the treatment. Filatova et al. (2013) used RF air
430 plasma for treatment of maize, spring wheat and lupinus seeds. The results showed that
431 the treatment reduced both bacterial and fungal contamination of tested seeds, as well as
432 positively influenced their germination. Kordas, Pusz, Czapka, & Kacprzyk (2015)
433 investigated the effect of “packed bed” low temperature plasma on fungi colonizing winter
434 wheat grain. It was found that plasma treatment resulted in the reduction of the number of
435 colonies of fungi on grains, however, the reduction varied heavily for the fungal species
436 examined. Brasoveanu, Nemtanu, Surdu-Bob, Karaca, & Erper (2015) applied glow discharge
437 plasma to barley and corn seeds to reduce the number of seed-borne fungi and found that
438 the fungal loads decreased with the increasing plasma treatment times. After 20 min treatment
439 the initial number of fungi was decreased by 25% for barley seeds. In the same study, treatment
440 of 10 min reduced the fungal load on corn seeds by 40%. In different study, wheat grains
441 artificially contaminated with *Bacillus amyloliquefaciens* endospores, were treated using low
442 pressure circulating fluidized bed reactor. Within 30 s of treatment, the reduction by over two
443 logarithmic units was achieved (Butscher et al., 2015). Butscher, Zimmermann, Schuppler, &
444 Rudolf von Rohr (2016) investigated the inactivation of *Geobacillus stearothermophilus*
445 endospores deposited on either polypropylene substrates or wheat grains. It was observed that
446 endospore inactivation is possible on wheat grains, however, it is much more challenging than
447 the treatment of PP granules, possibly due to a grain anatomy - a rough surface and a deep
448 ventral furrow. Cold plasma effect on various microorganisms on wheat seeds has also been
449 investigated by Zahoranová et al. (2016). Treatment of 120 s reduced the natural microflora of
450 wheat seeds – reduction of 1 log CFU/g was achieved for bacteria, while yeasts and

451 filamentous fungi were completely inactivated. Inactivation levels of wheat seeds artificially
452 contaminated with filamentous fungi (*F. nivale*, *F. culmorum*, *T. roseum*, *A. flavus* and *A.*
453 *clavatus*) were hugely dependent on the fungal type, with *Aspergillus* spp. as the most resistant
454 to the treatment. Dasan, Boyaci, & Mutlu (2017) achieved significant reductions of 5.48 and
455 5.20 log (CFU/g) of *A. flavus* and *A. parasiticus* inoculated on maize after 5 min air plasma
456 treatment, as well as more than 3 log reduction after 3 min of native microbial flora of maize
457 grains.

458 Except of cereal grains, cold plasma has been also used for decontamination of grain-like
459 granular particles (Basaran et al., 2008; Dasan et al., 2017; Dasan, Boyaci, & Mutlu, 2016;
460 Deng et al., 2006; Hertwig et al., 2015a; Hertwig, Reineke, Ehlbeck, Knorr, et al., 2015b;
461 Vleugels et al., 2005) and bacterial contaminants in grain model media (Los, Ziuzina, Boehm,
462 Cullen, & Bourke, 2017).

463 **3.2.4 Organic acids**

464 Organic acids are used as food additives and preservatives due to the reduction of
465 the environmental pH, what prevents food deterioration (Ölmez & Kretzschmar,
466 2009). Addition of organic acids such as propionic, sorbic and acetic acids, as well as their
467 salts, prevent the mold spoilage of bakery products, however, relatively high concentrations
468 are needed due to low efficacy (Magan & Aldred, 2006). Organic acids can also be used for
469 grain preservation. Sabillon, Stratton, Rose, Flores, & Bianchini (2016) evaluated the efficacy
470 of adding organic acids (acetic, citric, lactic, or propionic) or combination of organic acids and
471 NaCl added to tempering water to reduce microbial contamination in hard wheat and noted
472 a significant reduction of microbial load after the treatment. It was reported that
473 the combination lactic acid (5.0%) and NaCl (52%) was the most effective against aerobic plate
474 count (APC) and *Enterobacteriaceae* (Eb), achieving an average reduction of 4.3 and
475 4.7 log CFU/g, respectively. In a further study, the impact of tempering solutions on

476 the functional properties of resulting whole-grain (WGF) and straight-grade flours (SGF) was
477 evaluated and it was found that tested solutions had different effects on the properties of each
478 type of flour. While tempering solutions did not have significant overall effects on pasting or
479 mixing properties of SGF, treatment of WGF resulted in a numeric decrease in several pasting
480 parameters and an increase in bread grittiness, indicating limited penetration of the organic
481 acids into the endosperm of the grain (Sabillon, Bianchini, Stratton, & Rose, 2017).

482 **Conclusions**

483 Potential microbiological risks associated with cereal grains remain a major concern of
484 the grain industry as they may hugely affect the quality and properties of the grains. Current
485 technologies applied for control of microbial spoilage of cereals successfully reduce
486 the microbial load, however, they can negatively affect the quality and technological properties
487 of cereals, as well as generate harmful environmental impacts. In order to overcome
488 the limitations of conventional technologies, recent works have been focused on developing
489 new techniques, such as microwave treatment, pulsed UV light, cold plasma and organic acids,
490 that can be used for microbial decontamination of cereals. Further studies are needed to ensure
491 that these potential technologies could provide an efficient microbial inactivation and rapid,
492 uniform treatment, whilst at the same time do not affect the grains quality. Prevention of
493 contamination with fungi is the most rational and economical approach to reduce the risks
494 associated with the presence of mycotoxins in cereal food and feed products. However, in
495 current production systems even the best agricultural and manufacturing practices cannot fully
496 prevent mycotoxin contamination, therefore, potential technologies will need to be used for
497 degradation and elimination of these toxic metabolites.

498 **4. Acknowledgments**

499 This work was funded through Science Foundation Ireland (SFI) under Grant Number
500 14/IA/2626.

501 **5. References**

502 Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture:
503 their benefits and hazards. *Interdisciplinary Toxicology*, 2(1), 1–12. doi: 10.2478/v10102-
504 009-0001-7

505 Allen, B., Wu, J., & Doan, H. (2003). Inactivation of fungi associated with barley grain by
506 gaseous ozone. *Journal of Environmental Science and Health. Part. B, Pesticides, Food*
507 *Contaminants, and Agricultural Wastes*, 38(5), 617–630. doi: 10.1081/PFC-120023519

508 Andersen, B., & Thrane, U. (2006). Food-borne fungi in fruit and cereals and their production
509 of mycotoxins. *Advances in Experimental Medicine and Biology*, 571, 137–152. doi:
510 10.1007/0-387-28391-9_8

511 Andrews, S., Pardoel, D., Harun, A., & Treloar, T. (1997). Chlorine inactivation of fungal
512 spores on cereal grains. *International Journal of Food Microbiology*, 35(2), 153-162. doi:
513 10.1016/S0168-1605(96)01214-7

514 Bacon, C. W., & Williamson, J. W. (1992). Interactions of *Fusarium moniliforme*, its
515 metabolites and bacteria with corn. *Mycopathologia*, 117(1-2), 65–71. doi:
516 10.1007/BF00497280

517 Barbosa-Canovas, G. V., Schaffner, D. W., Pierson, M. D. & Zhang, Q. H. (2000), Pulsed Light
518 Technology. *Journal of Food Science*, 65, 82-85. doi:10.1111/j.1750-
519 3841.2000.tb00621.x

520 Basaran, P., Basaran-Akgul, N., & Oksuz, L. (2008). Elimination of *Aspergillus parasiticus*
521 from nut surface with low pressure cold plasma (LPCP) treatment. *Food Microbiology*,
522 25(4), 626–632. doi: 10.1016/j.fm.2007.12.005

523 Birck, N. M. M., Lorini, I., & Scussel, V. M. (2005). Fungus and mycotoxins in wheat grain at
524 post harvest. Paper presented at *International Working Conference on Stored Product*
525 *Protection*, Sao Paolo, Brazil. Passo Fundo, RS, Brazil: Brazilian Post-harvest

526 **Association.**

527 Brasoveanu, M., Nemtanu, M. R., Surdu-Bob, C., Karaca, G., & Erper, I. (2015). Effect of
528 glow discharge plasma on germination and fungal load of some cereal seeds. *Romanian*
529 *Reports in Physics*, 67(2), 617–624.

530 Briggs, D.E. (1998). *Malting and Brewing Science*. London, UK: Blackie Academic &
531 **Professional.**

532 Bullerman, L. B., & Bianchini, A. (2009). Food safety issues and the microbiology of cereals
533 and cereal products. In Heredia, N., Wesley, I., and Garcia, S. (Eds.), *Microbiologically*
534 *Safe Foods* (pp. 315–335). New York, USA: John Wiley & Sons.

535 Butscher, D., Schlup, T., Roth, C., Müller-Fischer, N., Gantenbein-Demarchi, C., & Rudolf
536 Von Rohr, P. (2015). Inactivation of microorganisms on granular materials: Reduction of
537 *Bacillus amyloliquefaciens* endospores on wheat grains in a low pressure plasma
538 circulating fluidized bed reactor. *Journal of Food Engineering*, 159, 48–56. doi:
539 10.1016/j.jfoodeng.2015.03.009

540 Butscher, D., Zimmermann, D., Schuppler, M., & Rudolf von Rohr, P. (2016). Plasma
541 inactivation of bacterial endospores on wheat grains and polymeric model substrates in
542 a dielectric barrier discharge. *Food Control*, 60, 636–645. doi:
543 10.1016/j.foodcont.2015.09.003

544 Chandrasekaran, S., Ramanathan, S., & Basak, T. (2013). Microwave food processing –
545 a review. *Food Research International*, 52(1), 243 - 261.

546 Christensen, C. M. & Meronuck, R. A., (1986). *Quality maintenance in stored grains and*
547 *seeds*. Minneapolis, USA: University of Minnesota Press.

548 Cordain, L. (1999). Cereal grains: humanity’s double-edged sword. *World Review Nutrition*
549 *Diet*, 84, 19–73. doi: 10.1186/1550-2783-10-30

550 Dasan, B. G., Boyaci, I. H., & Mutlu, M. (2017). Nonthermal plasma treatment of *Aspergillus*

551 spp. spores on hazelnuts in an atmospheric pressure fluidized bed plasma. *Journal of Food*
552 *Engineering*, 196, 139–149. doi: 10.1016/j.jfoodeng.2016.09.028

553 Dasan, B. G., Mutlu, M., & Boyaci, I. H. (2016). Decontamination of *Aspergillus flavus* and
554 *Aspergillus parasiticus* spores on hazelnuts via atmospheric pressure fluidized bed plasma
555 reactor. *International Journal of Food Microbiology*, 216, 50–59. doi:
556 10.1016/j.ijfoodmicro.2015.09.006

557 Delaquis, P. & Bach, S. (2012). Resistance and sublethal damage. Produce contamination. In
558 Gomez-Lopez, V. M. (Ed.), *Decontamination of Fresh and Minimally Processed Produce*
559 (pp. 77-86). New Jersey, USA: Wiley-Blackwell Publishing.

560 Deng, X. T., Shi, J. ., & Kong, M. G. (2006). Physical mechanisms of inactivation of *Bacillus*
561 *subtilis* spores using cold atmospheric plasmas. *IEEE Transactions on Plasma Science*,
562 34, 1310–1316.

563 Dexter, J. E., & Wood, P. J. (1996). Recent applications of debranning of wheat before milling.
564 *Trends in Food Science & Technology*, 7(2), 35–41. doi: 10.1016/0924-2244(96)81326-4

565 Dhillon, B., Wiesenborn, D., Dhillon, H., & Wolf-Hall, C. (2010). Development and evaluation
566 of a fluidized bed system for wheat grain disinfection. *Journal of Food Science*, 75(6),
567 E372-8. doi: 10.1111/j.1750-3841.2010.01668.x

568 Dodd, J. G., Vegi, A., Vashisht, A., Tobias, D., Schwarz, P., & Wolf-Hall, C. E. (2011). Effect
569 of ozone treatment on the safety and quality of malting barley. *Journal of Food Protection*,
570 74(12), 2134–2141. doi: 10.4315/0362-028X.JFP-11-193

571 Doohan, F. M., Brennan, J., & Cooke, B. M. (2003). Influence of climatic factors on *Fusarium*
572 *species pathogenic to cereals*. *European Journal of Plant Pathology*, 109(7), 755–768.
573 doi: 10.1023/A:1026090626994

574 Dudoiu, R., Cristea, S., Lupu, C., Popa, D., & Oprea, M. (2016). Microflora associated with
575 maize grains during storage period. *AgroLife Scientific Journal*, 5(1), 63-68.

576 **Environmental Protection Agency.** (1999). Wastewater Technology Fact Sheet - Ozone
577 Disinfection. Retrieved from <https://www3.epa.gov/npdes/pubs/ozon.pdf>

578 **European Commission.** (2009). Directive 2009/128/EC of the European Parliament and of
579 the Council of 21 October 2009 establishing a framework for Community action to
580 achieve the sustainable use of pesticides. *Official Journal*, L309, 71. Retrieved from
581 [http://eur-lex.europa.eu/legal-](http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32009L0128&from=EN)
582 [content/EN/TXT/HTML/?uri=CELEX:32009L0128&from=EN](http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32009L0128&from=EN)

583 Farkas, J., Ehlermann, D. A. E., & Mohácsi-Farkas, C. (2014). Food technologies: food
584 irradiation. *Encyclopedia of Food Safety*, 3, 178–186. doi: 10.1016/B978-0-12-378612-
585 8.00259-6

586 Filatova, I., Azharonok, V., Lushkevich, V., Zhukovsky, A., Gadzhieva, G., & Spasi, K.
587 (2013). Plasma seeds treatment as a promising technique for seed germination
588 improvement. Paper presented at International Conference on Phenomena in Ionized
589 Gases, Granada, Spain.

590 Flannigan, B. (1996). The microflora of barley and malt. In Priest, F. G., & Campbell I. (Eds.),
591 *Brewing Microbiology* (pp. 83-125). doi: 10.1007/978-1-4419-9250-5_4

592 **Food and Agriculture Organization of the United Nations.** (2017). FAOSTAT Database.
593 Retrieved from <http://faostat.fao.org/site/567/default.aspx#ancor/>

594 Gayán, E., Condón, S., & Álvarez, I. (2014). Biological aspects in food preservation by
595 ultraviolet light: a review. *Food and Bioprocess Technology*, 7(1), 1–20. doi:
596 10.1007/s11947-013-1168-7

597 Graham, D.M., Pariza, M.W., Glaze, W.H., Erdman, J.W., Newell, G.W. & Borzelleca, J.F.
598 (1997). Use of ozone for food processing. *Food Technology*, 51(6), 72–6.

599 Greene, A. K., Guzel-Seydim, Z. B. & Seydim, A. C. (2012). Chemical and physical properties
600 of ozone. In O'Donnell, C., Tiwary, B. K., Cullen, P. J. & Rice, R. G. (Eds.) *Ozone in*

601 *Food Processing* (pp. 26–28). UK: Blackwell Publishing Ltd.

602 Harlan, J. R. (1992). *Crops and Man*. Madison, USA: American Society of Agronomy.

603 Harris, L., Shebuski, J., Danyluk, M., Palumbo, M., & Beuchat, L. (2013). Nuts, seeds, and
604 cereals. In Doyle, M., & Buchanan, R. (Eds.), *Food Microbiology* (pp. 203-221).
605 Washington, USA: ASM Press.

606 Heddleson, R. A., & Doores, S. (1993). Factors affecting microwave heating of foods and
607 microwave induced destruction of foodborne pathogens - a review. *Journal of Food*
608 *Protection*, *11*, 1025–1037.

609 Hertwig, C., Reineke, K., Ehlbeck, J., Erdogdu, B., Rauh, C., & Schluter, O. (2015a). Impact
610 of remote plasma treatment on natural microbial load and quality parameters of selected
611 herbs and spices. *Journal of Food Engineering*, *167*, 12–17. doi:
612 10.1016/j.jfoodeng.2014.12.017

613 Hertwig, C., Reineke, K., Ehlbeck, J., Knorr, D., & Schluter, O. (2015b). Decontamination of
614 whole black pepper using different cold atmospheric pressure plasma applications. *Food*
615 *Control*, *55*, 221–229. doi: 10.1016/j.foodcont.2015.03.003

616 Hill, R. A., & Lacey, J. (1983). The microflora of ripening barley grain and the effects of pre-
617 harvest fungicide application. *Annals of Applied Biology*, *102*, 455-465.

618 Hocking, A. D. (2003). Microbiological facts and fictions in grain storage ochratoxin A. In
619 Wright, E. J., Webb, M.C., & Highley, E. (Eds.) *Stored grain in Australia 2003*. Paper
620 presented at *Australian Postharvest Technical Conference, Canberra, Australia*.

621 Jess, S., Kildea, S., Moody, A., Rennick, G., Murchie, A. K., & Cooke, L. R. (2014). European
622 Union policy on pesticides: implications for agriculture in Ireland. *Pest Management*
623 *Science*, *70*(11), 1646-54. doi: 10.1002/ps.3801

624 Justé, A., Malfliet, S., Lenaerts, M., De Cooman, L., Aerts, G., Willems, K. A., & Lievens, B.
625 (2011). Microflora during malting of barley: Overview and impact on malt quality.

626 *Brewing Science*, 64(3-4), 22–31.

627 Keklik, N. M., Demirci, A., & Puri, V. M. (2010). Decontamination of unpackaged and
628 vacuum-packaged boneless chicken breast with pulsed ultraviolet light. *Poultry Science*,
629 89(3), 570–581. doi: 10.3382/ps.2008-00476

630 Keklik, N. M., Krishnamurthy, K., & Demirci, A. (2012). Microbial decontamination of food
631 by ultraviolet (UV) and pulsed UV light. In Demirci, A. & Ngadi, M.O. (Eds.), *Microbial*
632 *Decontamination in the Food Industry - Novel Methods and Applications* (pp. 344-369).
633 Cambridge, UK: Woodhead Publishing Limited.

634 Kells, S.A., Mason, L.J., Maier, D.E. & Woloshuk, C.P. (2001) Efficacy and fumigation
635 characteristics of ozone in stored maize. *Journal of Stored Products Research*, 37, 371–
636 382.

637 Koehler, P. & Wieser, H., (2013). Chemistry of cereal grains. In Gobetti M, & Gaenzle M.
638 (Eds.), *Handbook of sourdough biotechnology* (pp. 11–45). New York, USA: Springer.

639 Kordas, L., Pusz, W., Czapka, T., & Kacprzyk, R. (2015). The effect of low-temperature
640 plasma on fungus colonization of winter wheat grain and seed quality. *Polish Journal of*
641 *Environmental Studies*, 24(1), 433–438.

642 Kosiak, B., Torp, M., Skjerve, E., & Andersen, B. (2004). *Alternaria* and *Fusarium* in
643 Norwegian grains of reduced quality - A matched pair sample study. *International Journal*
644 *of Food Microbiology*, 93(1), 51–62. doi: 10.1016/j.ijfoodmicro.2003.10.006

645 Kottapalli, B., Wolf-Hall, C.E. & Schwarz, P. (2005) Evaluation of gaseous ozone and
646 hydrogen peroxide treatments for reducing *Fusarium* survival in malting barley. *Journal*
647 *of Food Protection*, 68(6), 1236–40.

648 Kozempel, M., Annous, B., Cook, R., Scullen, O. J., & Whiting, R. (1998). Inactivation of
649 microorganisms with microwaves at reduced temperatures. *Journal of Food Protection*
650 61(5), 582–585.

651 Krishnamurthy, K., Tewari, J.C., Irudayaraj, J. & Demirci, A. (2010). Microscopic and
652 spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and
653 infrared heating. *Food and Bioprocess Technology*, **3**, 93-104. doi: 10.1007/s11947-008-
654 0084-8

655 Krnjaja, V., Lukic, M., Delic, N., Tomic, Z., Mandic, V., Bijelic, Z., & Gogic, M. (2015).
656 Mycobiota and mycotoxins in freshly harvested and stored maize. *Biotechnology in*
657 *Animal Husbandry*, **31**(2), 291–302. doi: 10.2298/BAH1502291K

658 Laca, A., Mousia, Z., Díaz, M., Webb, C., & Pandiella, S. S. (2006). Distribution of microbial
659 contamination within cereal grains. *Journal of Food Engineering*, **72**(4), 332–338. doi:
660 10.1016/j.jfoodeng.2004.12.012

661 Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV
662 radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure.
663 *International Journal of Mass Spectrometry*, **233**(1-3), 81–86. doi:
664 10.1016/j.ijms.2003.11.016

665 Lillehoj, E.B., Kwolek, W.F., Peterson, R.E., Shotwell, O.L. and Hesseltine, C.W. (1976).
666 Aflatoxin contamination, fluorescence, and insect damage in corn infected with
667 *Aspergillus flavus* before harvest. *Cereal Chemistry*, **53**, 505–512.

668 Lillehoj, E.B., Kwolek, W.F., Horner, E.S., Widstrom, N.W., Josephson, L.M., Franz, A.O. &
669 Catalano, E.A. (1980). Aflatoxin contamination of preharvest corn: role of *Aspergillus*
670 *flavus* inoculum and insect damage. *Cereal Chemistry*, **57**, 255–257.

671 Liu, Y., Pan, X., & Li, J. (2015). A 1961 – 2010 record of fertilizer use, pesticide application
672 and cereal yields: a review. *Agronomy for Sustainable Development*, **35**, 83-93. doi:
673 10.1007/s13593-014-0259-9

674 Lorenz, K., & Miller, B. S. (1975). Irradiation of cereal grains and cereal grain products. *CRC*
675 *Critical Reviews in Food Science and Nutrition*, **6**(4), 317–382. doi:

- 676 10.1080/10408397509527195
- 677 Los, A., Ziuzina, D., Boehm, D., Cullen, P. J., & Bourke, P. (2017). The potential of
678 atmospheric air cold plasma for control of bacterial contaminants relevant to cereal grain
679 production. *Innovative Food Science and Emerging Technologies*, *44*, 36-45. doi:
680 10.1016/j.ifset.2017.08.008
- 681 Lund, F., & Frisvad, J. C. (2003). *Penicillium verrucosum* in wheat and barley indicates
682 presence of ochratoxin A. *Journal of Applied Microbiology*, *95*(5), 1117–1123. doi:
683 10.1046/j.1365-2672.2003.02076.x
- 684 Lung, H. M., Cheng, Y. C., Chang, Y. H., Huang, H. W., Yang, B. B., & Wang, C. Y. (2015).
685 Microbial decontamination of food by electron beam irradiation. *Trends in Food Science
686 and Technology*, *44*(1), 66–78. doi: 10.1016/j.tifs.2015.03.005
- 687 Lynch, M. F., Tauxe, R. V. & Hedberg, C. W. (2009). The growing burden of food borne
688 outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology &
689 Infection*, *137*, 307-315.
- 690 Maftai, N. A., Ramos-villarroel, A. Y., Nicolau, A. I., Mart, O., & Soliva-fortuny, R. (2013).
691 Pulsed light inactivation of naturally occurring moulds on wheat grain. *Journal of
692 the Science of Food and Agriculture*, *94*, 721-726. doi: 10.1002/jsfa.6324
- 693 Magan, N. & Aldred, D. (2006). Managing microbial spoilage in cereals and baking products.
694 **In de Blackburn, C. (Ed.), *Food Spoilage Microorganisms* (pp. 194-212). Cambridge, UK:
695 Woodhead Publishing Ltd.**
- 696 Merali, Z., Ho, J. D., Collins, S. R. A., Gall, G. Le, Elliston, A., Käsper, A., & Waldron, K. W.
697 (2013). Characterization of cell wall components of wheat straw following hydrothermal
698 pretreatment and fractionation. *Bioresource Technology*, *131*, 226–234. doi:
699 10.1016/j.biortech.2012.12.023
- 700 Mislivec, P.B. & Tuite, J. (1970a). Species of *Penicillium* occurring in freshly-harvested and

701 in stored dent corn kernels. *Mycologia*, 62, 67–74.

702 Mislivec, P.B. & Tuite, J. (1970b). Temperature and relative humidity requirements of species
703 of *Penicillium* isolated from yellow dent corn kernels. *Mycologia*, 62, 75–88.

704 Mrema, G., Gumbe, L., Chepete, H., & Agullo, J. (2011). Grain crop drying, handling and
705 storage. In *FAO Rural Structures in the tropics: Design and development* (pp. 363-383).
706 ISBN 978-92-5-107047-5. Rome, Italy: FAO.

707 Naito, S., Okada, Y. and Sakai, T. (1988) Changes in microflora of ozone-treated cereals,
708 grains, peas, beans, spices during storage. *Nippon Shokuhin Kogyo Gakkaishi*, 35(2), 69–
709 77.

710 Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science*
711 *and Technology*, 3, 125–142. doi: 10.1146/annurev-food-022811-101132

712 Niemira, B. A., Boyd, G., & Sites, J. (2014). Cold plasma rapid decontamination of food
713 contact surfaces contaminated with *Salmonella* biofilms. *Journal of Food Science*, 79(5),
714 M917-22. doi: 10.1111/1750-3841.12379

715 Nierop, S. Van. (2006). The impact of microorganisms on barley and malt quality - A review.
716 *Journal of the American Society of Brewing Chemists*, 64(2), 69–78. doi: 10.1094/asbcj-
717 64-0069

718 Noots, I., Delcour, J. A, & Michiels, C. W. (1999). From field barley to malt: detection and
719 specification of microbial activity for quality aspects. *Critical Reviews in Microbiology*,
720 25(2), 121–153. doi: 10.1080/10408419991299257

721 Tiwari, B. K., & Rice, R. G. (2012). Regulatory and Legislative Issues. In O'Donnell, C.,
722 Tiwari, B. K., Cullen, P., & Rice, R. G. (Eds.), *Ozone in food processing* (pp. 6). West
723 Sussex, UK: John Wiley & Sons.

724 Oms-oliu, G., Martín-belloso, O., & Soliva-fortuny, R. (2010). Pulsed light treatments for food
725 preservation. A review. *Food and Bioprocess Technology*, 3(1), 13–23. doi:

726 10.1007/s11947-008-0147-x

727 Ölmez, H., & Kretzschmar U. (2009). Potential alternative disinfection methods for organic
728 fresh-cut industry for minimizing water consumption and environmental impact. *LWT-*
729 *Food Science and Technology*, *42*, 686–693.

730 Ortega-Rivas, E. (2012). Pulsed light technology. In: *Non-Thermal Food Engineering*
731 *Operations* (pp. 263-273). New York, USA: Springer Science+Business Media.

732 Park, J.W., Choi, S.Y., Hwang, H.J. & Kim, Y.B. (2005). Fungal mycoflora and mycotoxins
733 in Korean polished rice destined for humans. *International Journal of Food Microbiology*,
734 *103*, 305–314.

735 Petters, H.I., Flannigan, B. & Austin, B. (1988). Quantitative and qualitative studies of
736 the microflora of barley malt production. *Journal of Applied Bacteriology*, *65*, 279.

737 Pitt, J.I., & Hocking, A.D. (2009). *Fresh and Perishable Foods*. In *Fungi and Food Spoilage*
738 (pp. 395-403). New York, USA: Springer Science+Business Media.

739 Raghavan, G. S. V. (1993). Microwave drying of cereal grains: advantages and limitations.
740 *Postharvest News and Information*, *4*(3), 79–83.

741 Rajendran S. (2002). Postharvest pest losses. In Pimentel, D. (Ed.), *Encyclopedia of pest*
742 *management* (pp. 654). London, UK: CRC Press.

743 Rasimus-sahari, S., Mikkola, R., Andersson, M. A., Jestoi, M., & Salkinoja-salonen, M. (2016).
744 *Streptomyces* strains producing mitochondriotoxic antimycin A found in cereal grains.
745 *International Journal of Food Microbiology*, *218*, 78–85. doi:
746 10.1016/j.ijfoodmicro.2015.11.007

747 Reddy, M. V. B., Raghavan, G. S. V, Kushalappa, A. C., & Paulitz, T. C. (1998). Effect of
748 microwave treatment on quality of wheat seeds infected with *Fusarium graminearum*.
749 *Journal of Agricultural Engineering Research*, *71*, 113–117.

750 Richardson, S. D., Thruston Jr, A. D., Caughran, T. V., Collette, T. W., Patterson, K. S., &

751 **Lykins Jr, W. W.** (1998). Chemical by-products of chlorine and alternative disinfectants.
752 *Food Technology*, **52**, 58–61.

753 Sabillon, L., Bianchini, A., Stratton, J., & Rose, D. J. (2017). Effect of saline organic acid
754 solutions applied during wheat tempering on flour functionality. *Cereal Chemistry*, **94**(3),
755 1–21.

756 Sabillon, L., Stratton, J., Rose, D. J., Flores, R. A., & Bianchini, A. (2016). Reduction in
757 microbial load of wheat by tempering with organic acid and saline solution. *Cereal*
758 *Chemistry*, **93**, 638-646.

759 Sauer, D. B., & Burroughs, R. (1986). Disinfection of seed surfaces with sodium hypochlorite.
760 *Phytopathology*, **76**, 745 – 749.

761 Schlüter, O., Ehlbeck, J., Hertel, C., Habermeyer, M., Roth, A., Engel, K. H., & Eisenbrand,
762 G. (2013). Opinion on the use of plasma processes for treatment of foods. *Molecular*
763 *Nutrition and Food Research*, **57**(5), 920–927. doi: 10.1002/mnfr.201300039

764 Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma - A
765 tool for decontamination and disinfection. *Biotechnology Advances*, **33**(6), 1108–1119.
766 doi: 10.1016/j.biotechadv.2015.01.002

767 Selcuk, M., Oksuz, L., & Basaran, P. (2008). Decontamination of grains and legumes infected
768 with *Aspergillus* spp. and *Penicillium* spp. by cold plasma treatment. *Bioresource*
769 *Technology*, **99**(11), 5104–5109. doi: 10.1016/j.biortech.2007.09.076

770 Sun, C., Zhu, P., Ji, J., Sun, J., Tang, L., Pi, F., & Sun, X. (2017). Role of aqueous chlorine
771 dioxide in controlling the growth of *Fusarium graminearum* and its application on
772 contaminated wheat. *LWT - Food Science and Technology*, **84**, 555–561. doi:
773 10.1016/j.lwt.2017.03.032

774 Tonon, S.A., Marucci, R.S., Jerke, G. & Garcia, A. (1997). Mycoflora of paddy and milled rice
775 produced in the region of Northeastern Argentina and Southern Paraguay. *International*

776 *Journal of Food Microbiology*, **37**, 231–235.

777 Trung, T.S., Bailly, J.D., Querin, A., Le Bars, P. & Guerre, P. (2001). Fungal contamination of
778 rice from south Vietnam, mycotoxinogenesis of selected strains and residues in rice.
779 *Revue de Médecine Vétérinaire*, **152**, 555–560.

780 Vadivambal, R., Jayas, D. S., & White, N. D. G. (2007). Wheat disinfestation using microwave
781 energy. *Journal of Stored Products Research*, **43**(4), 508–514. doi:
782 10.1016/j.jspr.2007.01.007

783 Varga, J., Kocsube, S., Peteri, Z., Vagvolgyi, C., & Toth, B. (2010). Chemical, physical and
784 biological approaches to prevent ochratoxin induced toxicoses in humans and animals.
785 *Toxins*, **2**(7), 1718–1750. doi: 10.3390/toxins2071718

786 Victorin, K. (1992). Review of the genotoxicity of ozone. *Mutation Research*, **277**, 221–38.

787 Virto, R., Manas, P., Alvarez, I., Condon, S., & Raso, J. (2005). Membrane damage and
788 microbial inactivation by chlorine in the absence and presence of a chlorine-demanding
789 substrate. *Applied Environmental Microbiology*, **71**(9), 5022–5028. doi:
790 10.1128/AEM.71.9.5022

791 Vleugels, M., Shama, G., Deng, X. T., Greenacre, E., Brocklehurst, T., & Kong, M. G. (2005).
792 Atmospheric plasma inactivation of biofilm-forming bacteria for food safety control.
793 *IEEE Transactions on Plasma Science*, **33**(2), 824–828. doi: 10.1109/TPS.2005.844524

794 Wachowska, U., Stasiulewicz-Paluch, A. D., Glowacka, K., Mikołajczyk, W., & Kucharska,
795 K. (2013). Response of epiphytes and endophytes isolated from winter wheat grain to
796 biotechnological and fungicidal treatments. *Polish Journal of Environmental Studies*,
797 **22**(1), 267–273.

798 Williams, W.P., Windham, G.L., Buckley, P.M. & Daves, C.A. (2006). Aflatoxin accumulation
799 in corn hybrids infested at different growth stages with southwestern corn borer
800 (Lepidoptera: Crambidae). *Journal of Agricultural and Urban Entomology*, **23**, 97–103.

801 Wu, J., Doan, H., & Cuenca, M. A. (2006). Investigation of gaseous ozone as an anti-fungal
802 fumigant for stored wheat. *Journal of Chemical Technology and Biotechnology*, *81*(7),
803 1288–1293. <http://doi.org/10.1002/jctb.1500>

804 Yaciuk, G. (1980). Agricultural applications of solar energy. *Solar Energy Conversion II,*
805 *Selected Lectures from the 1980 International Symposium on Solar Energy Utilization*
806 *(pp. 337 – 353). Oxford, UK: Pergamon Press.*

807 Yadav, D. N., Anand, T., Sharma, M., & Gupta, R. K. (2014). Microwave technology for
808 disinfection of cereals and pulses: An overview. *Journal of Food Science and*
809 *Technology*, *51*(12), 3568–3576. doi: 10.1007/s13197-012-0912-8

810 Zahoranova, A., Henselova, M., Hudecova, D., Kalinakova, B., Kovacik, D., Medvecká, V., &
811 Cernak, M. (2015). Effect of cold atmospheric pressure plasma on the wheat seedlings
812 vigor and on the inactivation of microorganisms on the seeds surface. *Plasma Chemistry*
813 *and Plasma Processing*, *36*(2), 397–414. doi: 10.1007/s11090-015-9684-z

814

Table 1-Current methods and technologies used for cereal grains preservation.

Method/technology	Description	Limitations	References
Pesticides	Chemicals designed to prevent and control the occurrence of pests causing harm to crops - molds (fungicides), weeds (herbicides) and insects (insecticides)	<ul style="list-style-type: none"> ▪ high environmental impacts ▪ direct negative impact on human health ▪ increasing resistance against pesticides 	Liu et al. (2014); Jess et al. (2014); Aktar et al. (2009)
Drying	Grains are dried to a low moisture content	<ul style="list-style-type: none"> ▪ lack of uniformity of the process ▪ over-drying may damage the grains and cause economic losses as well as increase mycotoxin contamination 	Varga et al. (2010); Magan and Aldred, 2006
Debranning	Process during which the bran layers are removed from the endosperm by friction and abrasion	<ul style="list-style-type: none"> ▪ not completely suitable for wheat due to the crease on the wheat kernels 	Laca et al. (2006); Dexter and Wood (1996)

		<ul style="list-style-type: none"> ▪ whole-grain demand in the market 	
Chlorine and hypochlorite	Due to their oxidizing capacity, chlorine and hypochlorite treatments are one of the most widely used processes for microbial control	<ul style="list-style-type: none"> ▪ low inactivation of fungal spores on cereal grains ▪ generation of toxic by-products after the treatment 	Delaquis and Bach (2012); Virto et al. (2005); Andrews et al. (1995); Sauer and Burroughs (1986)
Irradiation	Exposing food to a certain amount of ionizing radiation	<ul style="list-style-type: none"> ▪ can negatively modify the quality and technological properties of cereals and cereal products 	Lung et al. (2015); Farkas et al. (2014); Lynch et al. (2009)
Ozone	Triatomic oxygen formed by addition of a free radical of oxygen to molecular oxygen	<ul style="list-style-type: none"> ▪ the cost of treatment can be relatively high due to complex technology 	Greene et al. (2012); Environmental Protection Agency [EPA] (1999)

Table 2-Potential methods and technologies for cereal grains preservation.

Method/technology	Description	Limitations	References
Microwave (MW) treatment	Electromagnetic waves with frequency within 300 MHz to 300 GHz; microbial inactivation based mainly on thermal effect	<ul style="list-style-type: none"> seed viability and seedling vigour can be decreased after the treatment higher microbial reduction levels in presence of other stresses, such as acidic pH or increased temperature 	Chandrasekaran et al. (2013); Reddy et al. (1998); Kozempel et al. (1998); Heddleson and Doores (1993)
Pulsed UV light	Short-duration, high-power pulses of a broad spectrum of white light from the UV (50% of the spectrum), to the near infrared region	<ul style="list-style-type: none"> low ability to penetrate grains because of their irregular and complex surface can decrease germination rate of the seeds 	Barbosa-Canovas, Schaffner, Pierson, and Zhang (2000); Maftai et al. (2013); Keklik et al. (2012); Keklik et al. (2010)
Non-thermal (cold) plasma	Partially ionized gas consisting of highly	<ul style="list-style-type: none"> efficiency of the method depends on the specific 	Schlüter et al. (2013); Niemira (2012)

	reactive chemical species	properties of the food product and its surface	
--	---------------------------	--	--

Organic acids	Antimicrobial agents	▪ can increase moisture content and penetrate into the endosperm of grains	Sabillon et al. (2017); Sabillon et al. (2016); Ölmez and Kretzschmar (2009)
---------------	----------------------	--	--

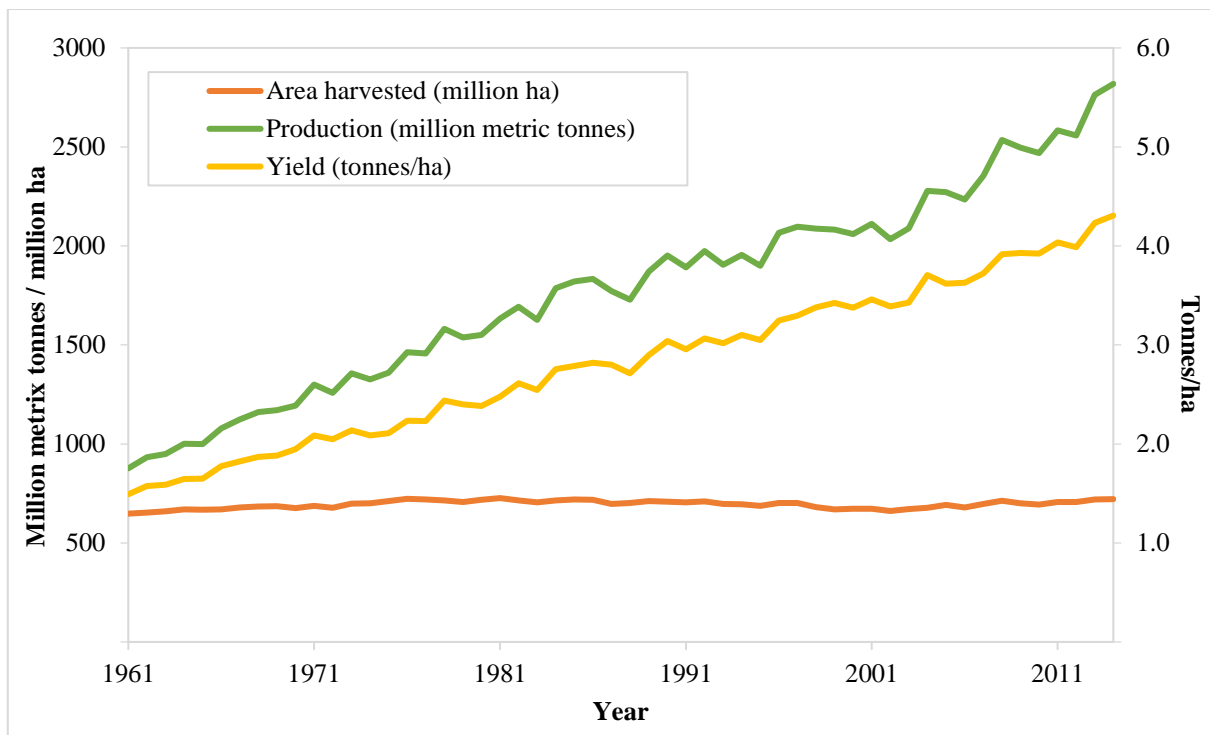


Figure 1-Worldwide production and yields of cereals in 1961 – 2014 (FAO, 2017).

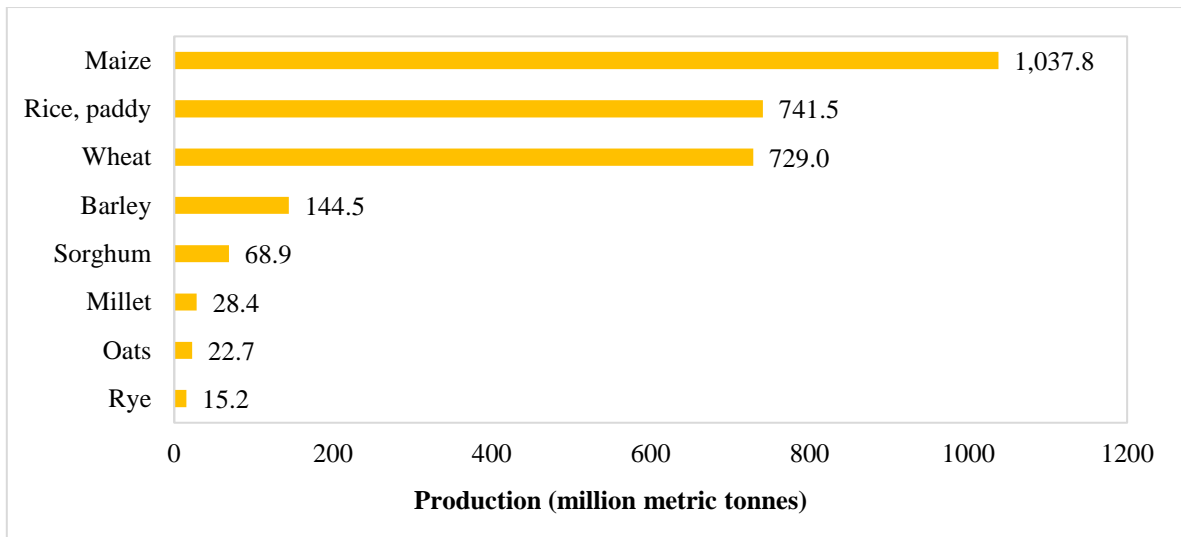


Figure 2-Most commonly cultivated cereal grains in 2014, by type (FAO, 2017).

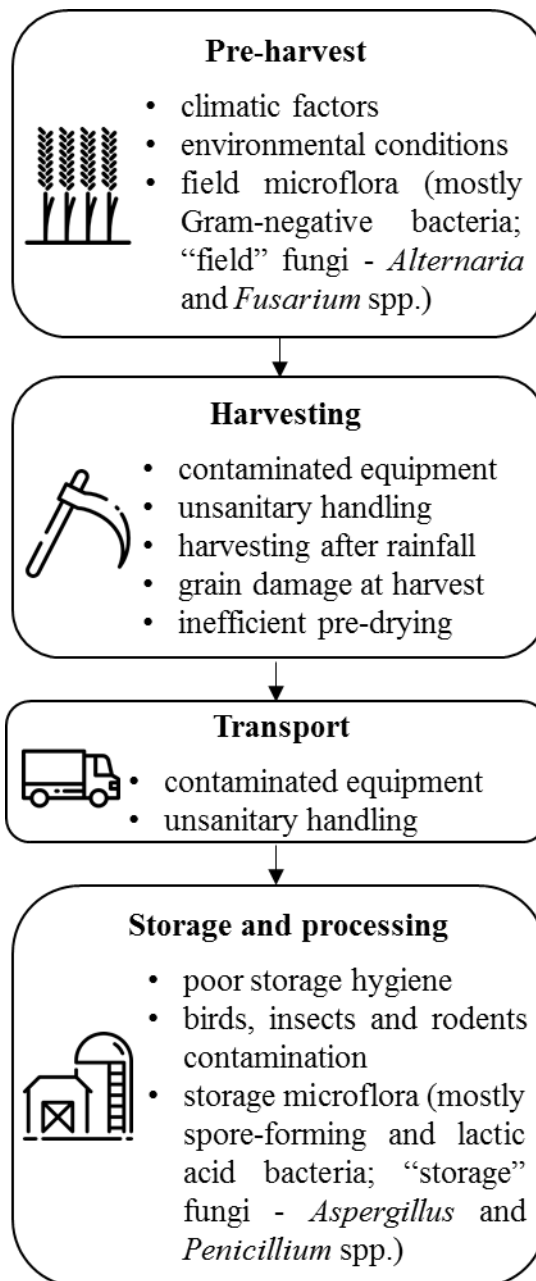


Figure 3-Sources and factors of microbial contamination during cereal grain processing.

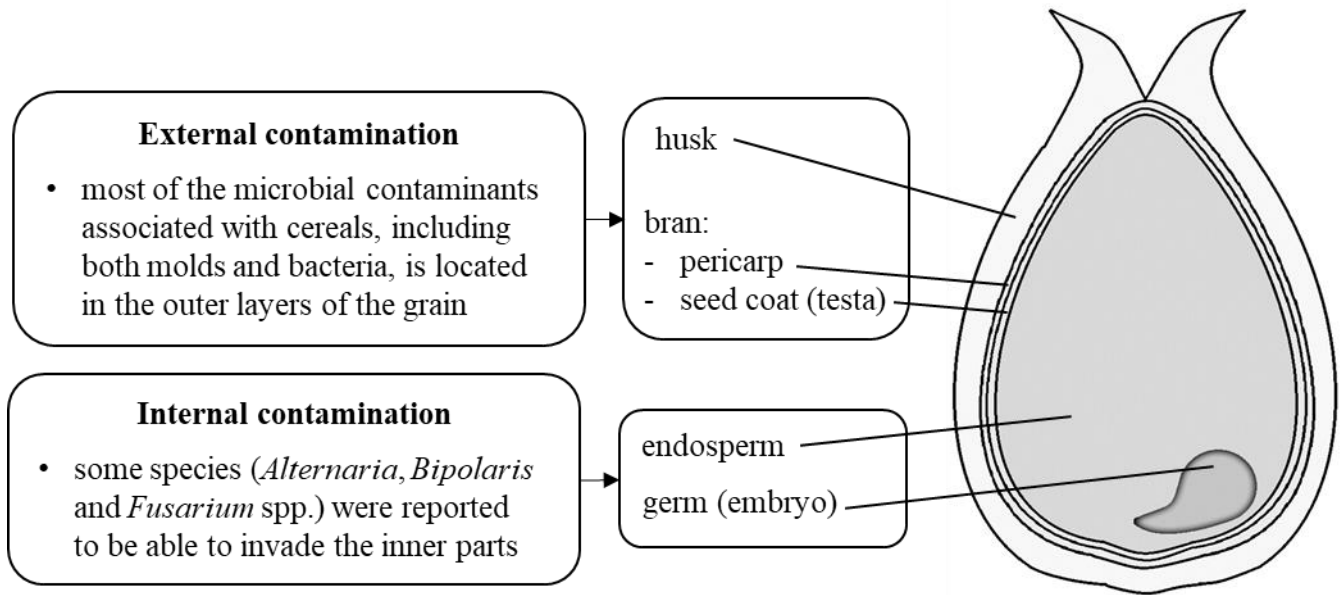


Figure 4-Microbial contamination within a cereal grain.