2018

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Recommended Citation

Los, A., Ziuzine, 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

Current and Future Technologies for Microbiological Decontamination of Cereal Grains

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Word count of text: 9,304 words

Short version of title: Decontamination methods of cereal grains

Choice of journal/section:

Journal of Food Science sections:

- Concise Reviews and Hypotheses in Food Science

ABSTRACT

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

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

1. Introduction

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

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

Currently, industrial production of cereals relies heavily on chemical input of pesticides, which 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

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

2.2 The field microflora

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

2.2.1 Bacteria

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

2.2.2 Filamentous fungi and yeasts

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

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

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

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

The field fungus most frequently present in both barley and wheat kernels is *Alternaria* (Christensen & Meronuck, 1986; Flannigan, 1996). Kosiak et al. (2004) reported that the distribution of field fungi contamination varied significantly in wheat, barley and oats samples of reduced quality with dominant *Fusarium* species as compared to acceptable samples for which *Alternaria* was the most numerous. *Fusarium* species are a common contaminant of various cereal grains. While *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *Microdochium nivale* (formerly known as *F. nivale*) cause diseases of small-grain cereals such as wheat and barley, corn is usually attacked by *F. graminearum*, *F. moniliforme*, *F. proliferatum* and *F. subglutinans* (Doohan et al., 2003).

Hill and Lacey (1983) reported that by harvest even 50-85% of barley kernels may be colonized by yeasts, with pink yeasts, such as *Sporobolomyces* and *Rhodotorula*, being predominant (Flannigan, 1996). Other species found on barley are *Hansenula*, *Torulopsis*, *Candida* and *Saccharomyces*, while *Cryptococcus* and *Trichosporon* were isolated from pre-harvest wheat (Flannigan, 1996). Flannigan (1996) suggested that because of the strong resemblance between other components of wheat and barley microfloras, similar yeasts species are likely to be found on both cereals.

2.3 The storage microflora

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

2.3.1 Bacteria

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

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

2.3.2 Filamentous fungi and yeasts

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

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

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

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

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

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

2.4 Distribution of microorganisms within cereal grains

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

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

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

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

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

3.1 Current techniques and their limitations

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

3.1.1 Pesticides

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

3.1.2 Drying

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

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

3.1.3 Mechanical debranning

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

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

3.1.4 Chlorine and hypochlorite

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

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

3.1.5 Irradiation

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

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

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

3.1.6 Ozone

The use of ozone in food processing has become increasingly important since it gained GRAS (Generally Recognized as Safe) status in 1997 (Graham et al., 1997) and four years later it was approved by US Food and Drug Administration (FDA) as a secondary direct food additive and antimicrobial agent for all food types (O'Donnell, Tiwari, Cullen, & Rice, 2012). Ozone (O_3) is the triatomic oxygen formed by addition of a free radical of oxygen to molecular oxygen. When generated from dried air, ozone is a blue gas. Ozone generation from high-purity oxygen leads to formation of a colorless gas (Greene, Guzel-Seydim, & Seydim, 2012). Ozone can be applied in the gaseous or aqueous state. It has been demonstrated that after treatment ozone decomposes into molecular oxygen and hence does not leave hazardous residues on the food product (Graham et al., 1997).

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

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

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

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

3.2 Future trends for decontamination of cereal grains

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

3.2.1 Microwave (MW) treatment

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

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

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

3.2.2 Pulsed ultraviolet (UV) light treatment

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

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

3.2.3 Non-thermal (cold) plasma

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

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

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

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

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

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

3.2.4 Organic acids

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

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

Conclusions

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

4. Acknowledgments

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

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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)	· 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	· 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	· not completely suitable for wheat due to the crease on the wheat kernels	Laca et al. (2006); Dexter and Wood (1996)

		▪ 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	▪ 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	▪ 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	▪ the cost of treatment can be relatively high due to complex technology	Greene et al. (2012); Environmental Protection Agency [EPA] (1999)

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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	· 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	· 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); Maftei et al. (2013); Keklik et al. (2012); Keklik et al. (2010)
Non-thermal (cold) plasma	Partially ionized gas consisting of highly	· 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 due to the reduction of the environmental pH	· can increase moisture content and penetrate into the endosperm of grains	Sabillon et al. (2017); Sabillon et al. (2016); Ölmez and Kretzschmar (2009)

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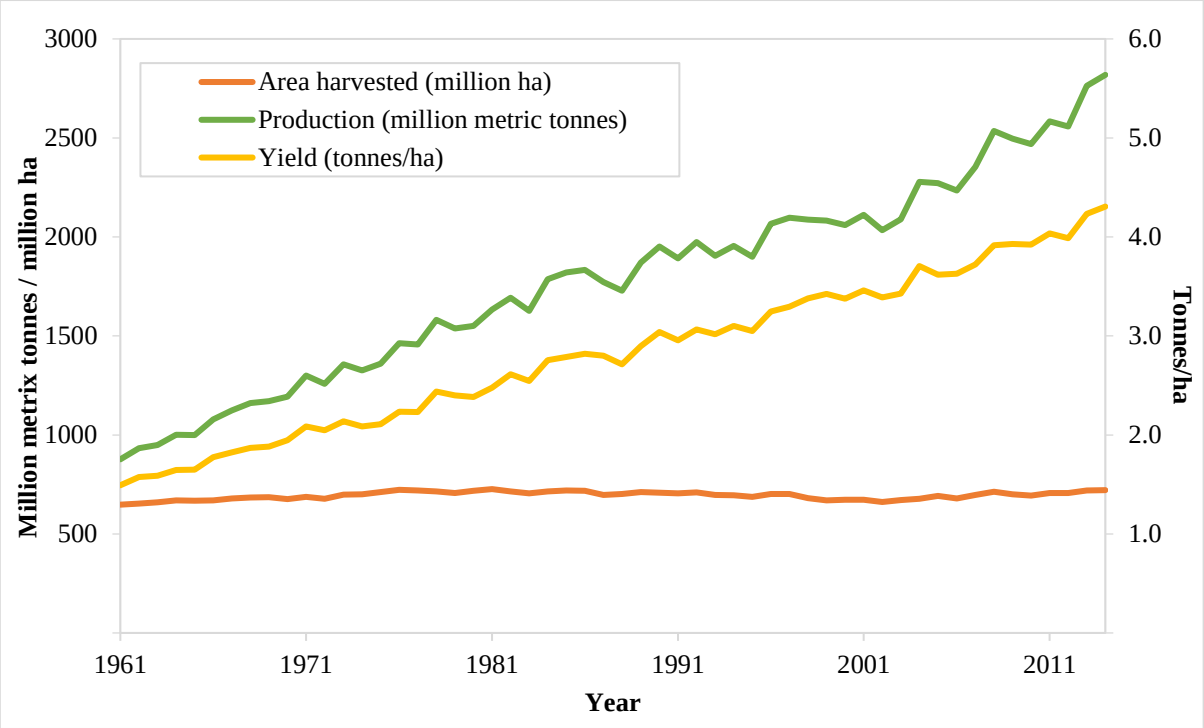

Figure 1-Worldwide production and yields of cereals in 1961 – 2014 (FAO, 2017).

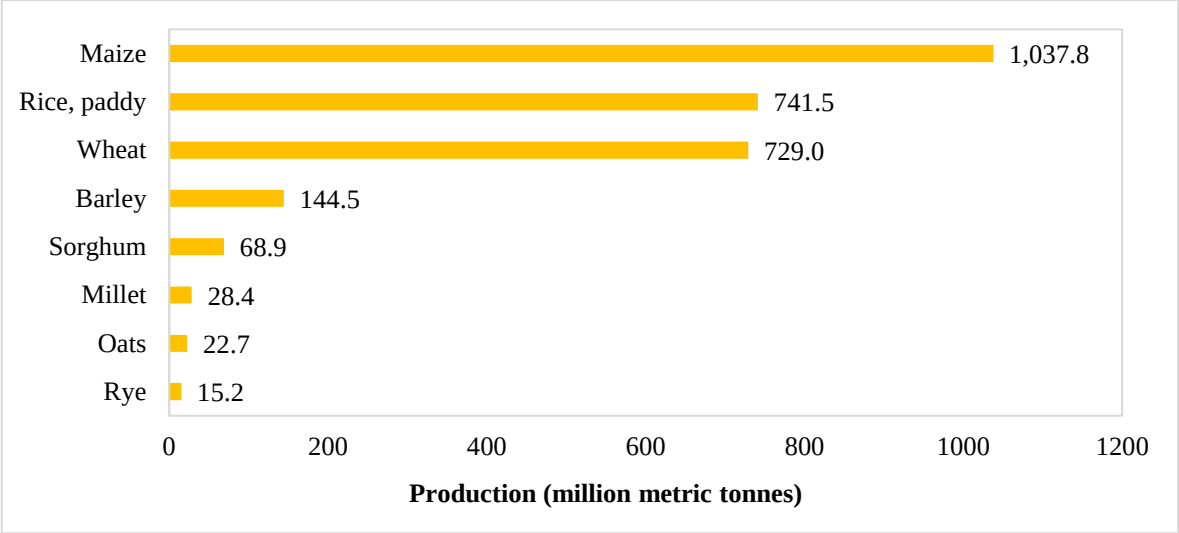

Figure 2-Most commonly cultivated cereal grains in 2014, by type (FAO, 2017).

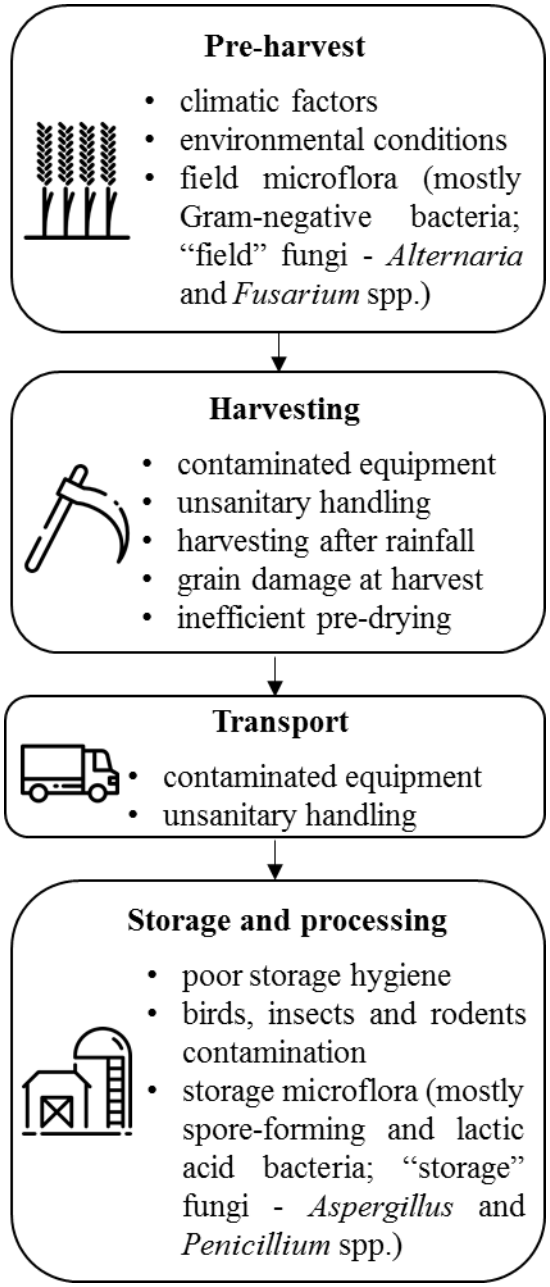

Figure 3-Sources and factors of microbial contamination during cereal grain processing.

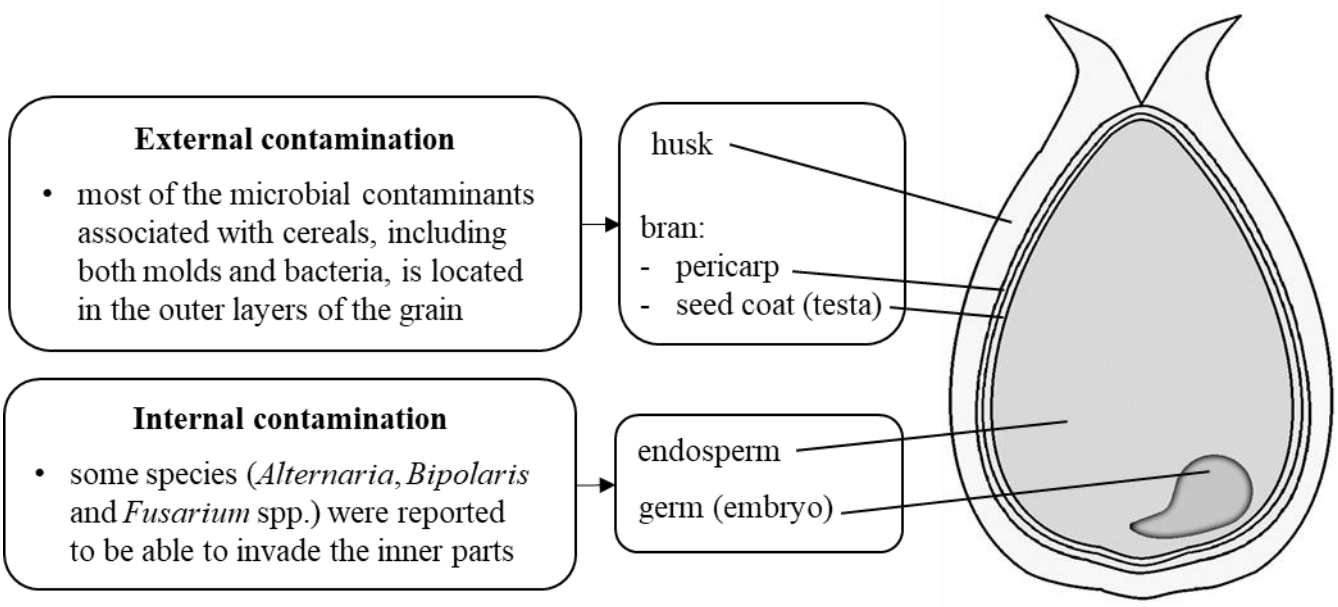

Figure 4-Microbial contamination within a cereal grain.