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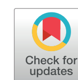
Short communication

Inactivation of mould spores in a model system and on raisins by low-energy electron beam



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ABSTRACT

Low-energy electron beam was investigated as an intervention strategy for the reduction of spores of food-spoiling fungi. Inactivation kinetics of five strains from the genera *Aspergillus*, *Byssochlamys*, *Eurotium* and *Penicillium* were determined. A two-dimensional membrane was used as a model system to exclude matrix effects. Raisins were incorporated into the study as an example of a three-dimensional food surface structure that is regularly being affected by spoilage through moulds. Complete inactivation could be attained on the model system after a maximum dose of 6 kGy, whereas between 1.04 and 1.71 log reductions were achieved on raisins. This novel application of low-energy electron beam was shown to reduce mould spores even on challenging food surfaces, paving the way for implementation in food industry.

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1. Introduction

The consumers demand for fresh, nutritious, and safe products constantly puts pressure on the industries to develop and optimize efficient and effective methods for food processing. Great attention has been brought to non-thermal applications in recent years since they often act less detrimental on the food product than conventional processing techniques. Filamentous fungi lead to great postharvest losses due to outgrowth of fungal spores during food storage (Jeong, Shin, Chu, & Park, 1978). In addition, moulds like *Aspergillus* spp., *Byssochlamys* spp. and *Penicillium* spp. can produce mycotoxins during growth (Calado, Venâncio, & Abrunhosa, 2014). Examples of toxins are aflatoxin, a carcinogen, ochratoxin A (OTA), a nephrotoxin and carcinogen, and patulin, a toxin affecting lungs and liver (Bennett & Klich, 2003). These toxins are very stable and heat-resistant to a certain degree, making them hard to eradicate (Bullerman & Bianchini, 2007). Typically, *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp. are found on grapes (Hewitt, 1988; Hocking, Leong, Kazi, Emmett, & Scott, 2007; Sage, Krivobok, Delbos, Seigle-Murandi, & Creppy, 2002). Mainly OTA produced from *Aspergillus* spp. and *Penicillium* spp. are of concern in dried grape products, the second most important product of viticulture (Hakobyan, Grigoryan, & Trchounian, 2017). The following species were used in our study: *A. niger* is a common contaminant of plant foods including grapes, causing losses due to spoilage and shelf life impairment. Additionally, some strains produce OTA (Schuster, Dunn-Coleman, Frisvad, & van Dijck, 2002). *B. fulva* and *B. nivea* are associated with spoilage of heat treated acidic foods such as pasteurized apple juice and may produce patulin. Thus, conventional hurdles like temperature and pH fail to eradicate these mould spores (Krämer & Prange, 2017). *E. amstelodami* is the sexually reproductive form of *Aspergillus glaucus* and may contribute to intoxication with OTA as well (Bennett & Klich, 2003). *P. roqueforti* is one of the moulds used in food fermentation of blue cheese, but may cause spoilage and intoxication (Krämer & Prange, 2017).

A reasonable approach to minimize food damage due to spoilage and possible risks by mycotoxin production is to reduce the initial load of spores on a product by either preventive or corrective methods (Calado et al., 2014). In this study, low-energy electron

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beam was tested as a possible method to reduce the number of fungal spores on the surface of raisins. Electron beam treatment is an established decontamination strategy for the sanitation of packaging materials and medical devices, but is not yet a routine method for food treatment (Lung, Cheng, Chang, Huang, & Yang, 2015). In the so called electron lamp electrons are generated at the heated cathode, then accelerated in an evacuated electric field to the near speed of light before penetrating a scan window (metal foil) to exit the vacuum and target the sample (Cleland, 2013). On impact they may release secondary electrons in the sample and by this generate cations, therefore ionizing the treated structures. Since almost all energy is consumed by the cleavage of molecules, practically no heat is generated upon irradiation (Sommers, 2012). Depending on the applied potential, electron beam irradiation is divided into high-energy (200 keV-10 MeV) and low-energy electron beam (80 keV-200 keV). The electric potential mainly affects penetration depth of the beam (Pillai, 2008).

Mainly two mechanisms are involved in microbial inactivation by electron beam treatment. Firstly, direct hits of electrons on RNA or DNA may damage these molecules by causing single or double strand breaks (Barkai-Golan & Follett, 2017, pp. 7–28; Miller, 2005b; Pillai, 2008). Secondly, indirect effects on these molecules may occur by radiolysis of water. When water is irradiated it dissociates into different reactive compounds such as hydroxyl radical, hydrogen peroxide and aqueous electrons. The formed reactive oxygen species (ROS), of which H_2O_2 and $OH\cdot$ are the most reactive ones, can interact with DNA and RNA molecules and damage these structures in addition to the direct effects (Diehl, 1995; Miller, 2005a; Skowron et al., 2014). The objectives of this study were to (1) establish inactivation kinetics for different mould spores by electron beam on a filter model system, (2) show inactivation behaviour of the same mould spores on raisins as a food model system and (3) characterize the reduction of native fungal microbiota on raisins by electron beam treatment.

2. Materials and methods

2.1. Experimental overview

Spores of five mould strains were used to characterize their inactivation kinetics by electron beam. To exclude any matrix effects a two-dimensional filter membrane was used as a model system. Spores of all mould strains were subsequently inoculated onto raisins to study inactivation on a complex three-dimensional food surface structure. In addition, a sample of naturally contaminated raisins was treated to investigate the reduction of indigenous mould species.

2.2. Mould strains and culture conditions

Aspergillus niger DSMZ 737, *Byssochlamys fulva* DSMZ 1808, *Byssochlamys nivea* DSMZ 1824, *Eurotium amstelodami* DSMZ 62629 and *Penicillium roqueforti* DSMZ 1080 were used in this study. Ascospores of *B. fulva* and *B. nivea* were produced by growing on malt extract agar (Merck, Zug, Switzerland) for 14 days at 30 °C. Those of *E. amstelodami* were gained by incubation on dichloran 18% glycerol agar (Oxoid, Pratteln, Switzerland) for 21 days at 25 °C. Conidiospores of *A. niger* and *P. roqueforti* were grown on yeast glucose chloramphenicol agar (Merck) for 14 days at 25 °C.

2.3. Preparation of spore suspensions

Spores were collected by repeatedly flooding the surface of a culture with 5 mL sterile 0.01% TWEEN 20 solution (Merck). Subsequently, the liquid phase from the plate was transferred to a sterile bottle. The spores were counted using a Fuchs-Rosenthal-chamber. Suspensions were diluted with TWEEN 80 solution (0.5%) (Merck) to obtain approximately 10^6 spores/mL and stored at 4 °C for a maximum of 2 days.

2.4. Filter preparation

Filter funnels (Pall Corporation, Basel, Switzerland) were placed on a vacuum pump (Pall Corporation) and pre wet with 7 mL 0.9% NaCl solution. The liquid was filtered through the membrane (polyethersulfone) for 10–15 s. Then, after stopping the pump, a diluted spore suspension was given onto the membrane and filtered until all liquid had vanished. Prior to filtration, spores were diluted with 10 mL 0.9% NaCl solution (Amresco, Solon, USA) to ensure a homogeneous distribution on the membrane. Filter membranes were removed from the filter funnel system and placed in small 50 mm petri dishes (Greiner bio-one, St. Gallen, Switzerland) with sterile tweezers and dried in a laminar flow for 30 min. Thereafter the membrane was transferred to a 94 mm petri dish (Greiner bio-one) with sterile tweezers. The petri dish was then attached to the electron beam tray with double adhesive tape and the lid was removed for the irradiation treatment. A dried filter with a diluted spore suspension that received an empty-run (passing the electron beam lamp without irradiation) was used as positive control and to determine the inoculation concentration.

2.5. Raisin sample preparation

Turkish raisins (<10 CFU/g) from an industrial processing facility in Switzerland were inoculated with 1 mL mould spore suspensions at a concentration of $1.1–2.9 \times 10^6$ spores per 10 g of raisins. Samples were inoculated in 50 mL centrifuge tubes and then horizontally shaken at 200 rpm at 4 °C overnight. Before treatment the samples were brought to room temperature and dried in sterile petri dishes with slightly opened lid in a laminar flow for 30 min. Naturally contaminated samples (10^4 CFU/g) were brought to room temperature as well and 10 g were placed into sterile petri dishes. Raisins were arranged with sterile tweezers to avoid overlapping.

2.6. Electron beam treatment

The EBLab 200 (Comet AG, Flamatt, Switzerland) was used for all electron beam treatments in these experiments. The device is equipped with a low-energy electron accelerator (80–200 keV) and can be used for doses up to 200 kGy. An energy setting of 180 keV was chosen to ensure the beam was able to penetrate a monolayer of spores, as revealed by mathematical modelling (Comet AG). Dried filters were treated with 1, 2, 3, 4, 5, 6 and 7 kGy at an angle of 90° and a beam distance of 20 mm. Raisins were treated from two sides with flipping steps in between. The doses applied were 2×1, 2×2, 2×4, 2×8, 2×16 and 2×32 kGy. The applied doses were corrected to the real dose Dμ (Helt-Hansen, Miller, & Sharpe, 2005) by means of dosimetry (as mentioned in section 2.8).

2.7. Sample processing and incubation

Filter membranes were directly placed onto the corresponding agar plates (as mentioned in section 2.2) after electron beam treatment. Raisins were incubated in sterile petri dishes (Greiner bio-one) at 30 °C for 24 h to ensure repair of sublethally damaged fungi before placing them in filter blender bags (Grade) and diluting them 1/10 with 0.1% NaCl-peptone solution in a dilumat device (Biomérieux, Geneva, Switzerland). They were then smashed in a SmasherTM blender (Biomérieux) for 2 min. The resulting suspension was then either directly plated or serially

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