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ORIGINAL RESEARCH PAPER

ATTEMPTS TO USE PULSED LIGHT AS AN EMERGING TECHNOLOGY FOR INACTIVATION OF MOULD NATURALLY PRESENT ON RYE

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Pulsed light technology was used to inactivate moulds, naturally present on rye. The experiments were performed on samples containing 3.5·104 CFU/g and 4.3·103 CFU/g. Treatments of different duration (5, 10, 15, 20, 30, and 40 pulses) at intensity of 0.4 J·cm-2 per pulse were applied and mould inactivation was evaluated. Besides confirming the utilisation of pulsed light as decontamination method for cereals, this work contributes with new information regarding the effects of the spectral range of pulsed light, proving that the whole UV range of the spectrum accounts for the lethal effect against moulds. This research supports pulsed light as emerging technology in food preservation.

Keywords: pulsed light, UV radiation, rye, moulds

Introduction

Cereals are an important economic commodity worldwide, and a major source of energy, protein, B vitamins and minerals for the world population. Although considered to be a "minor cereal", due to the fact that people do not consume it as frequently as they eat wheat, corn and rice (Berti *et al.*, 2005), rye is a major crop in Russia, Poland, Germany and the Scandinavian countries, where it is the main bread grain (FAO, 2002). It is also used to produce crisp bread and alcohol, and as animal feed (Kent and Evers, 1994). A concerning fact related to rye and cereals, due to their global importance in the diet, is their susceptibility to be invaded by moulds and, in certain climatic conditions, to be contaminated by mycotoxins: secondary toxic metabolites produced by moulds that can persist from the crops to the final products (Molinié *et al*., 2005). In spite of many years of research and the introduction of good agricultural practices (GAP) in the food production, and good manufacturing practices (GMP) in the storage and distribution chain, mycotoxins

continue to be a problem (Duarte *et al*., 2010). A correct storage of the grains, after harvest, is important to prevent mould spoilage, grain germination and pest infestation. Minimal nutritional changes will take place if grains with appropriate moisture content (12-14%) are stored for only a few months, but if the grains are held with a higher amount of moisture, their quality can deteriorate because of starch degradation by grain and microbial amylases (McKevith, 2004) and by mycotoxin accumulation.

With increasing consumer concerns over the potential health hazards of mycotoxin and having in view the necessity to replace the thermal and chemical decontamination of cereals with other methods, non-thermal and non-chemical control methods for mould development are becoming more and more important in our days.

As an emerging non-thermal technology involving the application of very short high-intensity pulses of broad spectrum light (100–1100 nm), pulsed light (PL) could be used for decontamination of moulds occurring on grains.

PL studies have been reviewed recently in terms of inactivating food-related spoilage organisms and potential microbial pathogens (Elmnasser et al., 2007; Gómez-López *et al.*, 2007; Oms-Oliu *et al.*, 2008) and to extend the shelf life of baked goods, chilled shrimp and fish, eggs (Dunn *et al*., 1995, 1996), grain (Jun *et. al.*, 2003). Other products that may benefit from PL treatments include chicken wings, hot dogs and cottage cheese. For most applications, a few pulsed light flashes within a fraction of a second will yield high levels of microbial inactivation (López-Gómez *et al*., 2009).

The aim of this work was to evaluate the inactivation efficacy of PL on moulds naturally occurring on rye grains and to further understand the factors responsible for the inactivation of moulds, including the identification of the spectral regions associated with the lethal effect.

Materials and methods

Rye

Two rye samples with different initial mould load were used in the experiments, one with $3.5 \cdot 10^4$ CFU/g and one with $4.3 \cdot 10^3$ CFU/g. Physical characteristics of the rye grains are shown in Table 1. The pH was determined using a pH-meter (Crison Instruments S. A. Barcelona, Spain) in a homogenate of seeds, the water activity with an AQUALAB CX-2 apparatus (Decagon, Pullman, WA, USA) and the initial water content by drying duplicate samples of approximately 5 g at 103°C for 2 h (ISO 712:2010).

Table 1. Physico-chemical characteristics of rye

Rye sample origin	Mould load, (CFU/g)	pH	a_w	Water content, %
Romania	$3.5 \cdot 10^4$	6.37 ± 0.06	0.530 ± 0.003	12.04 ± 0.02
Spain	$4.3 \cdot 10^3$	6.68 ± 0.02	0.601 ± 0.001	12.58 ± 0.03

Rye grains were aseptically packed in bags (10 g per bag) made of 64 μm thick transparent polypropylene film with a permeability to oxygen of 110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} at 23°C and 0% RH (Tecnopack SRL, Mortara, Italy). Inside the bag, grains were displayed in a monolayer, which was exposed to pulsed light on both sides.

PL equipment

A XeMaticA-2L System (SteriBeam Systems GmbH, Germany) was used to carry out the Pulsed Light treatments. Pulses were generated by two lamps situated above and below the sample, which was placed on a shelf made of transparent plastic. The radiation spectrum emitted by the lamps ranged in the domain 200 - 1100 nm and 15-20% of it represented UV radiation. Each pulse has a duration of 0.3 ms and a fluence of 0.4 $J \cdot cm^{-2}$.

Two sets of experiments were performed in this study. The first set was designed to reveal the decontamination power of pulsed light against moulds. These experiments were performed with both lamps and rye samples were exposed to 10, 20, 30, 40, 60 and 80 pulses. The second set of treatments was performed in order to evaluate the effect of the different portions of the PL spectrum on moulds inactivation. Thus, two types of UV filters were used: a) a 2 mm thick Pyrex glass filter, cutting off all light below 305 nm allowing wavelengths above 305 nm to pass; b) a Makrolon polycarbonate plastic filter, cutting off all light below 400 nm allowing wavelengths above 400 nm to pass.

These experiments were conducted with 10, 20, 30 and 40, 60 pulses, without filters or with filters mounted in front of the upper lamp, while the action of the bottom lamp was blocked by replacing the transparent plastic shelf with a metallic one. During these treatments, the grain layer received an equal number of pulses on both sides, as result of turning the bag. The energy received by the rye grains, which contributed to mould inactivation, varied from 6.4 J/g for 10 pulses, to 51.2 J/g for 80 pulses (calculations have taken into account the pulse fluence, the number of pulses and the surface of the grain layer formed by the 10 g rye sample within the bag, which was exposed to pulsed light).

Moulds enumeration

A quantity of 10 g rye grains were aseptically transferred to a sterile plastic bag and homogenized for 2 min with 90 mL of 0.1% sterile peptone water using a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of the homogenates were poured in Rose Bengal Chloramphenicol Agar (RBCA Biokar Diagnostics, Beauvais, France) and incubated at 25±1°C for 5 days for mould enumeration (ISO 21527-2/2009). The colonies were counted and the results expressed as Log_{10} CFU/g of rye. Two counts were performed for each sample and the mean of two determinations made in duplicate ± standard deviation was reported.

Statistical analysis

The experimental results were analysed using Statgraphics plus version 5.1. software (Statistical Graphics Co., Rockville, MD, USA). Data were analyzed using multifactor analysis of variance and a Duncan multiple-range test was applied to determine differences among means with a significance level of 0.05.

Results and discussion

Effect of energetic density on mould inactivation

Figure 1 shows the effects of PL on moulds as a function of energetic density (fluence and number of pulses). For a fluence of 0.4 J·cm^{-2}· pulse^{-1}, a maximum reduction of 2 logarithmic cycles was achieved in the case of 80 pulses treatment for the rye samples with $3.5 \cdot 10^4$ CFU/g and of 1.38 logarithmic cycles for the rye samples with $4.3 \cdot 10^3$ CFU/g in the same treatment conditions ($p<0.05$). In terms of mould inactivation efficiency, it is noticed that the lower energy input (6.4 J/g) ensures the death of 92.73% of mould population for the rye samples with $3.5 \cdot 10^4$ CFU/g and 72.49% for the rye samples with $4.3 \cdot 10^3$ CFU/g under the same treatment conditions. For higher energy input (51.2 J/g) the death of mould population was 99.02% and 95.81% for the rye samples with $3.5 \cdot 10^4$ CFU/g and $4.3 \cdot 10^3$ CFU/g, respectively (Figure 2). The results show that by applying the treatment with PL (at small fluence and number of pulses) for inactivation of moulds naturally present on rye, the treatment efficiency is high.

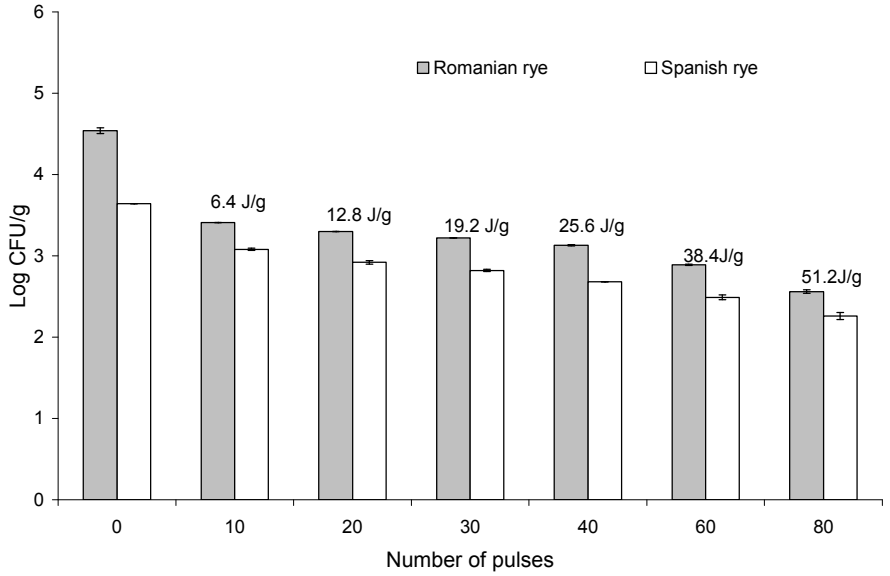

Figure1. Rye moulds inactivation at a different number of pulses (pulse characteristics: duration 0.3 ms, fluence 0.4 J·cm^{-2})

When investigating the effect of light pulses on naturally-occurring moulds on wheat, a 3.81 log reduction was achieved after exposing wheat with $2.2 \cdot 10^5$ CFU/g

to 40 pulses with a fluence of 0.4 J·cm^{-2} per pulse, and a 3-log reduction was obtained for wheat with 1.5·10^4 CFU/g in the same conditions (Aron-Maftei *et al*., 2010). The above discussion indicates that the energetic density (fluence and number of pulses) is a factor that conditions the efficiency of PL treatment as confirmed in present studies.

In terms of mould inactivation levels, those obtained in this work are comparable to the ones previously obtained by our research team and by other researchers. Jun *et al*. (2003) found a reduction of 4.93 log CFU/g for *Aspergillus niger* spores in corn meal using PL. Sharma and Demirci (2003) obtained for alfalfa seeds an inactivation of 4.80 log CFU/g for the fluence of 5.6 J·cm^{-2} per pulse and 90 pulses, while an inactivation of 1.31 log CFU/g was obtained for the same fluence and 15 pulses.

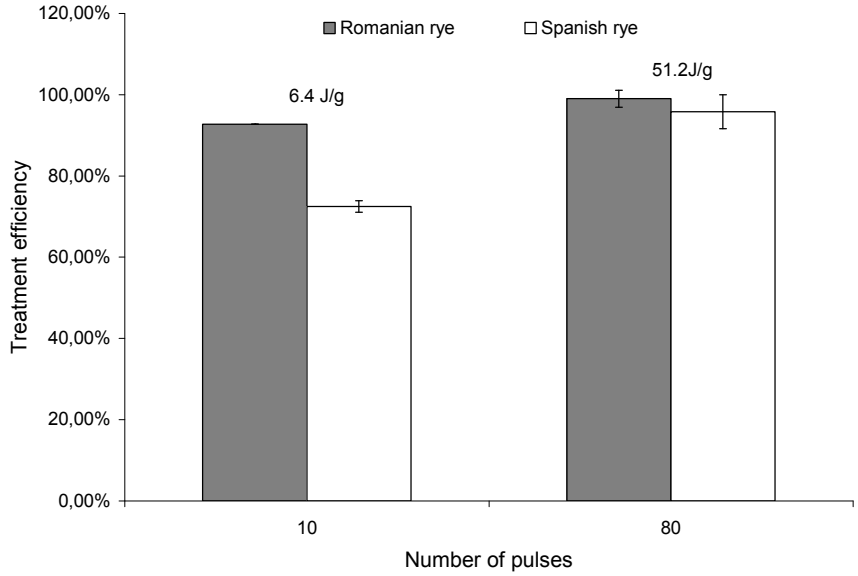

Figure 2. Treatment efficiency at different energy input

Effect of light spectrum on moulds inactivation

The level of moulds inactivation increased progressively along with the number of pulses applied during treatments ($p<0.05$) and the most efficient treatment in the inactivation of rye moulds was the one with full spectrum for both samples. When different components of the radiation emitted by the lamp, such as of UV-C, UV-B, UV-A, VIS and NIR, are removed, the inactivation of moulds on rye was decreased. An inactivation of 1.29 logarithmic cycles for the rye samples with an initial load of 3.5·10^4 CFU/g and 0.59 logarithmic cycles for the rye samples with an initial load of 4.3·10^3 CFU/g were obtained after applying 40 pulses treatment at $\lambda > 305$ nm. This low inactivation level is probably caused by the removal of a small portion of the UV spectrum ($\lambda < 200$ nm). This clearly indicates that the UV range is important in inactivation, as expected, and that even a slight modification of the UV profile in the PL treatment can impact the treatment effect. Thus, when

wavelengths < 400 nm were removed from the radiation spectrum and the rye samples were exposed only to VIS and NIR radiation (λ > 400 nm), the moulds did not receive even sublethal injury (Figure 3a and b).

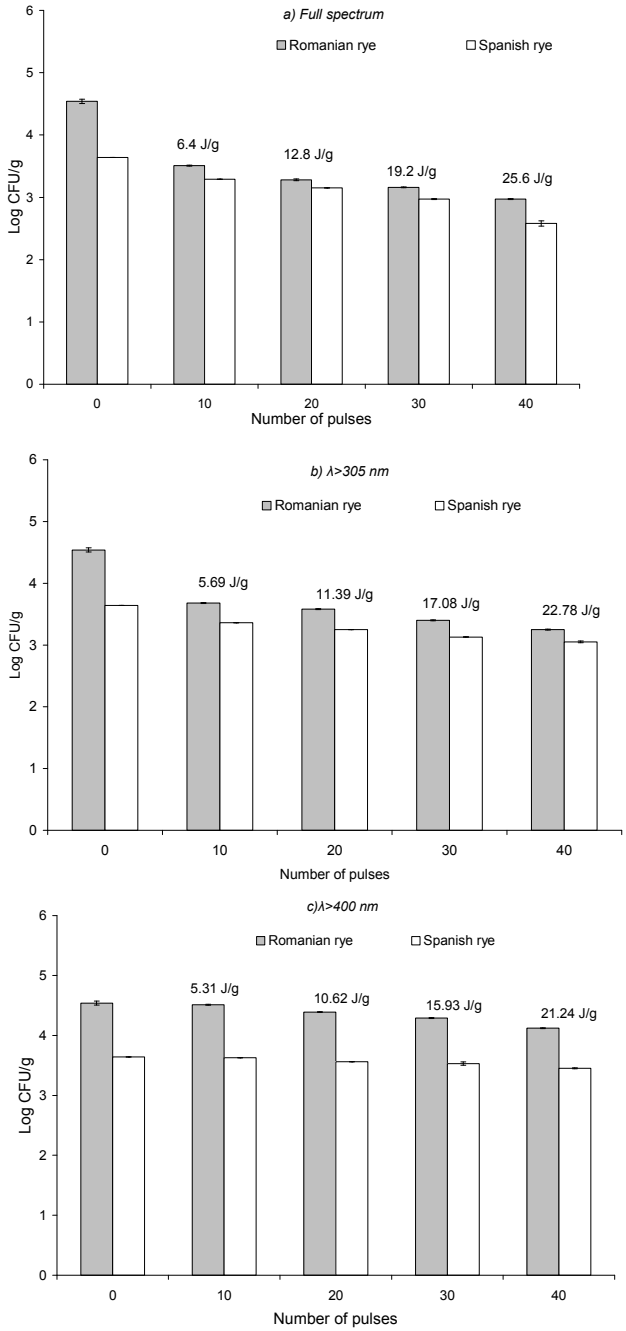

Figure 3. Decontamination of moulds in rye at different wavelengths (pulse characteristics: duration 0.3 ms, fluence of unfiltered light 0.4 J·cm^{-2})

Although the entire UV range seemed to contribute to the inactivation of moulds, the effect of the UV-B and UV-C ranges ($\lambda < 315$ nm) was stronger than the effect of the UV-A range (315 to 400 nm). Wekhof *et al*., (2001) reported that at lower values of the fluence rate, the UV-C contributes 80-90% (or more) to the sterilizing action, while UV-B and UV-A light contributes only 10-20% for *Aspergillus niger* spores. At higher fluence rates from 25 to 33 kw/cm^2, the contribution of UV-B and UV-A to the log reduction rises from 20 to 60% for *A. niger* spores. Fargues *et al.* (1997) reported that the viability of *Paecilomyces fumosoroseus* spores was decreased during prolonged exposure to continuous UV-A wavelengths at energy levels of 10 to 60 J·cm^{-2}, which is several fold higher than the energy levels used in this study. The researchers noted that below 10 J·cm^{-2}, less than 1-log reduction by UV-A radiation was obtained, which is consistent with the findings of the present study. Wekhof *et al*. (2001) noted that to elaborate further on the use of UV-B and UV-A light alone it is necessary to take into account that the UV-C energy also contributes to heating. Its absence has to be compensated both by higher fluencies and fluence rates of UV-B and UV-A light, making their total energy level comparable to that previous carried by all three UV bands together. The slightest modification of the UV radiation spectrum used during the PL treatment has a significant negative impact on the treatments efficiency. The radiation responsible for the death of the mould spores from the rye samples is the UV-C. Therefore this is supplementary evidence to sustain that the death of the mould treated with PL is caused by the UV radiation exposure.

Conclusion

Based on the results obtained from this experiment, the PL treatment seems to be an achievable alternative for the inactivation of the cereal moulds even at the application of a small number of pulses and fluence. It can be also concluded that the inactivation of moulds by PL is caused by exposure to UV light, mostly the UV-B and UV-C portions of the light spectrum, and the NIR or VIS radiation alone has no effect on moulds present on rye, but their presence led to better results regarding moulds inactivation than those obtained only with UV.

Acknowledgements

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