Innovative Food Science and Emerging Technologies 45 (2018) 161–168

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Inactivation of *Aspergillus carbonarius* and *Aspergillus flavus* in malting barley by pulsed light and impact on germination capacity and microstructure

María Helga Zenklusen[a,1], María Bernarda Coronel[a,c,1], María Águeda Castro[d], Stella Maris Alzamora[a,c,*], Héctor Horacio Lucas González[b,c,*]

[a] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Industrias, Intendente Güiraldes 2160, C1428EGA Buenos Aires, Argentina
[b] Universidad de Buenos Aires, Facultad de Ingeniería, Departamento de Ingeniería Química, Intendente Güiraldes 2160, C1428EGA Buenos Aires, Argentina
[c] CONICET, Buenos Aires, Argentina
[d] Expert in Applied Plant Anatomy, Argentina

ARTICLE INFO

Keywords:
Malting barley
Aspergillus
Inactivation
Pulsed light
Germination capacity
Caryopsis structure

ABSTRACT

The impact of pulsed light (PL) fluence on reduction of *Aspergillus carbonarius* and *Aspergillus flavus* conidia in barley grains (a_w 0.60 and 0.98), germinability and structure was studied. Treatments were done for 5–75 s at 5 and 10 cm distance from the flash lamp (fluence: 11.0–165.8 J/cm^2 at 5 cm, and 6.0–89.6 J/cm^2 at 10 cm). Maximum reduction in fungal population was 1.2–1.7 log cycles up to 5–15 s (fluence: 6.0–18.0 J/cm^2). Acceptable germinability values were obtained up to 25 s treatment at 10 cm distance. Light microscopy study supported changes in germinability. Most of the structure elements of the caryopsis were drastically altered with high fluence values, while embryo viability was maintained with doses up to 29.9 J/cm^2. PL up to 18.0 J/cm^2 could be a nonchemical option to reduce moulds in barley with minor impact on germinability.

Industrial relevance text: The reduction of fungal load in malting barley will contribute to improve the production process and the quality of beer. The application of intense light pulses up to 18.0 J/cm^2 (15 s, 10 cm from the lamp quartz window) could be a potentially suitable nonchemical (residue-free) option to reduce fungal presence in barley grains with minor impact on structure and germinability.

1. Introduction

The main use of barley in Argentina, Brazil, Chile, Uruguay and Mexico is the obtainment of malt for beer. Production of barley in these countries is increasing because of the rise in beer consumption in the continent and worldwide. In particular, Argentina is in the top ten barley producing countries, with over 4.2 million metric tons for 2015/16 (Ministerio de Agroindustria de la República Argentina, 2016). The Argentinean regulation for the commercialisation of barley destined for malting (i.e., malting barley) establishes requirements regarding germinative capacity, protein content, moisture content, size, percent of damaged grains and presence of heterogeneous particles in the bulk (Ministerio de Agricultura, Ganadería y Pesca de la República Argentina - Servicio Nacional de Sanidad y Calidad Agroalimentaria, 2013).

There are five stages in the process of converting barley into malt: barley grading and cleaning, steeping (≈ 24–52 h), germination (≈ 96 h), kilning (≈ 24 h) and malt cleaning and grading (FAO, 2009). The processing cycle is completed in approximately nine days. Steeping is the start of the active malting process. Steep water is added to cover the grain, and grain moisture content increases from around 12% to between 40 and 45%, allowing the beginning of germination. Afterwards, partially germinated grains are dried to roughly 4–5% moisture in a kiln. The process of malting leads to the production of amylase enzymes which convert starches in the grain into simple sugars for yeasts to metabolize them during beer fermentation. In a modern pneumatic malt house, the grain is alternatively submerged and then drained for two or three cycles to achieve the target grain moisture content (Lewis & Young, 2001). From the stated above, germinative capacity of the grains is essential in the malting process.

Fungal and mycotoxin contamination in barley grains and malt is a problem for the malting and brewing industries, with an impact on the safety and quality of malt and beer (Wolf-Hall, 2007). Barley and/or other brewing adjuncts such as corn or wheat may become contaminated by moulds and mycotoxins while in the field and during storage; moreover contamination can increase during malting where nutrients, moisture and temperature enable microbial growth

* Corresponding authors at: Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
E-mail addresses: macastro681@gmail.com (M.Á. Castro), alzamora@di.fcen.uba.ar (S.M. Alzamora), glucasmeister@gmail.com (H.H.L. González).
[1] Both authors share the first place.

http://dx.doi.org/10.1016/j.ifset.2017.09.003
Received 5 May 2017; Received in revised form 26 July 2017; Accepted 11 September 2017
Available online 13 September 2017

(Gonzalez Pereyra, Rosa, Dalcero, & Cavaglieri, 2011; Molto, Samar, Resnik, Martínez, & Pacin, 2000). It has been demonstrated that fungal growth is favoured during air rests and germination and it has also been detected during the first step of kilning (Oliveira, Mauch, Jacob, Waters, & Arendt, 2012). Molto et al. (2000) studied the presence of trichothecenes in 50 samples of Argentinean beer and found low concentrations of DON in 44% of the samples. Gonzalez Pereyra et al. (2011) evaluated the mycobiota and mycotoxin contamination in different malted barley types and brands as well as in the brewer's grain collected from a major brewery in Córdoba province in Argentina. They isolated important mycotoxin producer species: *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp.. All samples were contaminated with fumonisin B_1, while 18% of brewer's grain samples were contaminated with aflatoxin B_1. Wolf-Hall (2007) reported that *Fusarium* mould is still capable of growth and mycotoxin production during steeping, germination and kilning and placed particular emphasis on the necessity of reducing the amount of mould growth during malting.

Several postharvest decontamination treatments aimed at the reduction of fungal load in barley grains have been explored, before or during processing, such as the application of ozone, hot water, hydrogen peroxide, electron- beam irradiation and biopreservatives (Dodd et al., 2011; Kottapalli & Wolf-Hall, 2008; Kottapalli, Wolf-Hall, & Schwarz, 2005, 2006; Laitila, Alakomi, Raaska, Mattila-Sandholm, & Haikara, 2002; Rouse & Van Sinderen, 2008). Pulsed light (PL) is an emerging technique that has been approved by the FDA in 1996 for the decontamination of food and food surfaces. It involves the use of intense and short-duration (1 μs–0.1 s) pulses of broad spectrum light comprising ultraviolet, visible and near infrared irradiation. Power is magnified by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths of thousandths of a second) (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007). The significant microbial reduction in very short treatment times, the limited energy cost, its great flexibility, the low environmental impact, the lack of residual compounds, and the ability to be incorporated in processing lines are some of the major benefits claimed for this technique (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010). The effectiveness of PL treatment highly depends on many critical factors related to the sample and to the equipment, among them the number of pulses, the discharge voltage, the distance between target and flash lamp, the food composition, and the sample thickness (Kramer, Wunderlich, & Muranyi, 2017; Wambura & Verghese, 2011). Three coexisting modes of action of PL have been proposed to explain the lethal effects on microorganisms: the DNA damage by thymine dimmer formation (photochemical effect), the localized overheating of cells (photothermal effect) and the structural damage caused by the high power peak (photophysical effect) (Krishnamurthy, Demirci, & Irudayaraj, 2007; Wang, MacGregor, Anderson, & Woolsey, 2005; Wekhof, 2000). The relevance of each effect would depend on the PL fluence and the target microorganism (Gómez-López et al., 2007). PL processing has found its application in inactivating microbial populations on various food surfaces: alfalfa seeds (Sharma & Demirci, 2003), cut apples (Gómez, Salvatori, García-Loredo, & Alzamora, 2012), corn meal (Jun, Irudayaraj, Demirci, & Geiser, 2003), raw salmon fillets (Ozer & Demirci, 2006), raspberries and strawberries (Bialka, Demirci, & Puri, 2008), infant foods (Choi, Cheigh, Jeong, Shin, & Chung, 2010), and ham (Wambura & Verghese, 2011). However, very limited data are available concerning the potential use of PL to decontaminate grains. Only one study performed by Aron Maftei, Ramos-Villarroel, Nicolau, Martín-Belloso, and Soliva-Fortuny (2014) evaluated the feasibility of reducing naturally occurring moulds on wheat grains. These authors found a 3–4 log cycles reduction in the mould population when the energy received by the grains was 51.2 J/g. The treatments implied the loss of germination capacity in 14–15% of the wheat seeds.

This work aimed to evaluate the effect of PL fluence on: 1- the reduction of conidia of *Aspergillus carbonarius* and *Aspergillus flavus* inoculated on barley grains, and 2- the grains' germination capacity. In order to evaluate the suitability of PL as a treatment to inactivate moulds before grains enter the beer production process or after the steeping step, grains with low and high moisture contents were examined in some experiments. In addition, microstructure changes induced in the barley caryopsis by PL and their correlation with the germination capacity were determined.

2. Materials and methods

2.1. Sample preparation

Malting barley (*Hordeum vulgare*, Scarlett variety) from the locality of Lobos (Buenos Aires province, Argentina) was used for the experiments. Barley grains (length: 8.4 ± 0.4 mm; width: 4.2 ± 0.4 mm) with low and high moisture contents (MC) were tested: dry grains, corresponding to barley as obtained (MC: 9.4 ± 0.2 wet basis, wb; a_w 0.60 ± 0.02) and wetted grains (MC range: 41.8 ± 1.2–48.1 ± 0.4, wb; a_w 0.98 ± 0.02), which were prepared by immersing the kernels in water for 48 h at 4 ± 1 °C. Wetting was done with the objective of simulating the steeping step of malting process in beer production.

Moisture content was measured by drying approximately 2 g of grains in a vacuum oven (Gallenkamp, United Kingdom) at 65 °C over calcium chloride as desiccant until constant weight (AOAC International, 2005). Water activity (a_w) was measured at 20 °C by an AquaLab CX-2 water activity meter (Decagon Devices Inc., WA, USA) calibrated with saturated aqueous solutions (Resnik, Favetto, Chirife, & Ferro-Fontan, 1984). Measurements were done by quintuplicate and the average was reported.

2.2. Pulsed light equipment and treatments

The RS-3000B Steripulse-XL system (Xenon Corporation, USA), which produces polychromatic radiation in the wavelength range of 200 to 1100 nm (pulsed rate: 3 pulses/s; pulse width: 360 μs), was used as the pulsed light generator. The system consisted of an RC-747 power/control module, a treatment chamber that housed a xenon flash lamp (non-toxic, mercury free) and an air cooling system attached to the lamp housing to avoid lamp overheating during operation. The different PL fluences were obtained by altering the number of applied pulses and/or the distance between the samples and the quartz window of the lamp. Fluence measurements were taken by a pyroelectric head model ED500 (Gentec Electro-Optics, Canada) connected to an oscilloscope model TDS 2014 (Tektronix, Beaverton, USA), with an aperture cover of 20.3 cm^2. Barley grains (10 ± 0.05 g) were spread on a Petri dish (9 cm diameter) on a single layer. In this way, the surface of the dish was completely covered.

Grains were exposed to PL during 5, 25, 50, and 75 s, at two distances from the quartz window: 5 cm or 10 cm. The doses received by samples at 5 cm for each treatment time were 11.0, 55.3, 110.6, and 165.8 J/cm^2, respectively; whereas the doses at 10 cm were 6.0, 29.9, 59.7, and 89.6 J/cm^2, respectively. To minimize variations in PL dose, Petri dishes containing barley were placed within a uniform area of the radiation field (directly below the lamp and around the central point). The treatments were static (no rotation of the grains was done during treatments) and therefore radiation was received on the upper surface of the grains facing the PL lamp.

2.3. Measurement of the temperature in the grains' surface

Temperature of dry and wet grains during PL treatment was measured with an infrared thermometer (model GM-1150, Benetech, Guangdong, China) (accuracy: ± 1.5 °C, repeatability: 1%, distance:-spot ratio: 20:1). The thermometer was equipped with a laser, whose beam was used for pointing the spots of the grains to be measured. The

emissivity was set at 0.93 (for measurements of hot food, as indicated in the instruction manual). Temperature measurement was done immediately after different treatment times in five grains of Petri dishes located at 5 or 10 cm vertical distance from the quartz window.

2.4. Mould strains and grain inoculation

The studied moulds were *Aspergillus flavus* RC 390, and *Aspergillus carbonarius* RC 131. *A. carbonarius* was selected because of its dark pigmentation that would provide high resistance to UV radiation. The strains are deposited in the collection of the Industry Department, School of Exact and Natural Sciences of the University of Buenos Aires.

Culture medium for conidia production for both strains had the following composition: 30 g agar and 10 g milled barley per litre of water. Culture medium (100 mL) was placed in 500 mL flasks and sterilised (15 min, 120 °C, 1 atm). Moulds isolates were cultured and incubated at 30 ± 1 °C for 15 days to induce conidia production.

Prior to inoculation, the amount of dry and wet grains needed for the PL treatments was disinfected by immersion in commercial sodium hypochlorite (5% v/v) for one minute, and rinsed twice in sterile water. Afterwards, sanitized grains were placed in the flasks containing the cultures of *A. flavus* or *A. carbonarius*. Flasks were shaken vigorously for 5 min and let stand for 30 min.

In order to measure the concentration of conidia, 10 g of inoculated barley were placed in a flask containing 90 mL peptone water - 0.05% Tween 80 (Biopack, Buenos Aires, Argentina). The flask was shaken for 5 min and the number of conidia in the liquid was assessed by the use of a haemocytometer, and confirmed by YGC (Yeast Extract Glucose Chloramphenicol Agar, Merck KGaA, Darmstadt, Germany) plate counts. No mycelia fragments were observed under microscope during enumeration of conidia. Concentrations of about 5×10^6 conidia/g were obtained.

Ten grams of inoculated barley grains were weighted in glass Petri dishes for every replicate of the PL treatments (2 replicates per treatment). Experimental treatments were carried out in triplicate.

2.5. Mould counts

Survival population of moulds during PL treatment was monitored by colony count technique. Ten grams of treated kernels were aseptically put into Erlenmeyers containing 90 mL peptone water - 0.05% Tween 80. Erlenmeyers were shaken for 5 min. Then, serial dilutions were cultured in surface in YGC by quadruplicate. Plates were incubated at 30 ± 1 °C and colonies were counted every 24 h for five days. Counts were expressed as $\log_{10}$ conidia/g of barley grain. Survival curves were generated from experimental data by plotting the average values of $\log N/N_0$ (where N is the number of conidia/g at a given time and N_0 the initial number of conidia/g) versus time of treatment.

2.6. Germination test

Germination capacity was determined in non-inoculated treated dry and wet grains according to the International Seed Testing Association procedure (ISTA, 2011). Briefly, four replications of 100 barley seeds per PL treatment were placed between two layers of filter paper (Whatman No. 1) wetted with 4 mL water. Seeds were incubated in the dark at 20 °C for seven days. Paper layers were kept moistened during the incubation. After this time, seeds were visually examined and those that exhibited normal germination were counted. Germination capacity was the number of normal seedlings produced from 100 pure seeds expressed as a percentage. The same procedure was performed for 100 untreated dry and wet seeds as controls.

2.7. Grain microstructure analysis by light microscopy

Preparation of samples for light microscopy (LM) was performed according to conventional techniques (Sorrivas, Morales, & Yáñez, 2014). Briefly, dry grains were fixed twice in 3% w/w glutaraldehyde solution and then in 0.1 M potassium phosphate buffer (pH 7.4) overnight at room temperature. Grains were then rinsed three times with distilled water, postfixed in 1.5% w/w OsO_4 solution at room temperature and dehydrated in a graded acetone series prior to being embedded in low viscosity Spurr resin (Sorrivas et al., 2014). Sections (1–2 μm thick) of the Spurr-embedded tissue were cut on a Sorvall MT2-B ultracut microtome and stained with aqueous 1% *w*/w toluidine blue and 1% w/w basic fuchsine solutions and examined in a Axioskope 2 Plus light microscope (Carl Zeiss, Jena, Germany). All reagents were from Merck Química Argentina S.A. (Argentina).

2.8. Statistical analysis

Means values and their standard deviations were calculated for conidia plate counts and germination capacity numbers and tested on their significant differences ($P < 0.05$) using InfoStat software (2015 version, InfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

3.1. Temperature profile in barley grains surface during PL treatments

During prolonged PL exposure, temperature of food samples increases affecting quality in certain products. Heat build-up in PL treatments has been mainly attributed to visible and near-infrared portions of pulsed light spectrum, and sample heating could limit the working PL dose (Gómez et al., 2012). In this study, the temperature in the grain surface increased with the PL fluence (Fig. 1). Overall, the

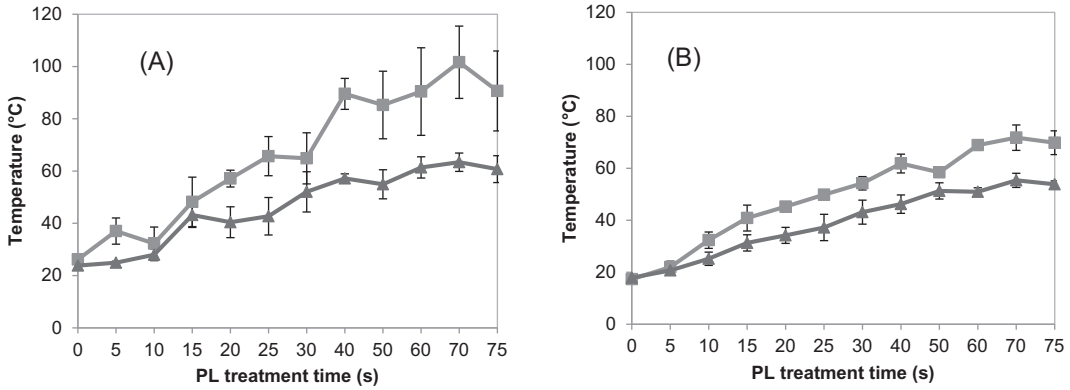

Fig. 1. Temperature increase measured in the surface of barley grains during PL treatment at different distances beneath the lamp. Barley grains irradiated at: 5 cm (A) and 10 cm (B) from the quartz window. Grain moisture content: 9.4 ± 0.2, wb (■) and 45.4 ± 0.1, wb (▲).

temperature change was fast until approximately 40 s (grains at 5 cm) or 50 s (grains at 10 cm) PL treatments, and then diminished. After 75 s of irradiation, temperature values of dry grains located at 5 cm and 10 cm from the quartz window were about 90.7 °C and 69.9 °C, respectively, while in wet grains these values were 60.7 °C and 53.9 °C respectively. PL fluence corresponding to the combination 75 s–5 cm distance (165.8 J/cm^2) in dry grains resulted in many visually detected burnt kernels.

Temperature increase was higher in dry than in wet grains and observed differences in overheating between grains with low and high a_w were higher at high PL fluences. The higher surface temperature in low MC grains could be explained, at least partially, by their lower transmissivity and lower reflection values (that is, higher surface energy absorption) compared with high MC kernels (Staack, Ahrné, Borch, & Knorr, 2008). Many authors reported that PL induced overheating in different food matrices, such as salmon fillets, corn meal, cut apple and other vegetables (Gómez et al., 2012; Gómez-Lopez, Devlieghere, Bonduelle, & Debevere, 2005; Jun et al., 2003; Ozer & Demirci, 2006), but information on whole barley kernels with high and low MC is not available to date.

3.2. Effect of PL treatments on germination capacity of barley grains

To be used in the brewing industry, barley must fulfil the following main criteria: high germination capacity, purity (in the variety), graded grain, and low protein content. The detailed specification of the first requirement establishes a minimum percent germination equal to 97% according to FAO (2009) and 98% according to Ministerio de Agricultura, Ganadería y Pesca de la República Argentina - Servicio Nacional de Sanidad y Calidad Agroalimentaria (2013). Optimal germination performance such as the high vigour and germination capacity or viability of barley at the time of the malting process is the most important quality criterion when selecting or purchasing malting barley (Frančáková, Líšková, Bojnanská, & Mareček, 2012; Møller, 2004). Vigour has been defined as germination percentage after 24 h and viability as that of 72 h (Møller, 2004). In this work, the effect of PL treatment on the beer barley quality was evaluated in terms of germination capacity.

The initial percent germination values of untreated dry and wet grains were 99.2 ± 0.5% and 98.7 ± 0.5%, respectively. Changes in germination capacity of dry and wet grains exposed to PL at 5 cm and 10 cm from the quartz window during different time lengths are presented in Fig. 2.

When dry grains were treated at 5 cm, percent germination significantly fell to an inacceptable value (75 ± 2%) after only 25 s PL exposure. When samples were located at 10 cm distance (fluence: 29.9 J/cm^2), there were no significant differences in the percent germination values between untreated dry and wet grains (99.2 ± 0.4% and 98.7 ± 0.5% respectively) and 5 and 25 s PL exposed dry (99.5 ± 0.6% and 97.7 ± 0.9% respectively) and wet (99.5 ± 0.6% and 98.5 ± 0.6% respectively) grains. However, greater PL treatment times significantly impaired seed viability resulting in unacceptable germination levels. For instance, after 75 s PL treatment, percent germination values were 54 ± 17% and 46 ± 9% for dry and wet kernels respectively. When considering differences among treated kernels, germination capacity was significantly different between consecutive treatment times for samples with the same moisture content ($P < 0.05$), except between 25 s and 50 s PL exposed dry grains ($P = 0.3070$). For equal treatments on kernels with different moisture contents, no significant differences were observed for percent germinability values ($P = 0.6325$).

According to the Home-Grown Cereals Authority, the maximum temperature in the grain should not exceed 50 °C to maintain germination capacity of barley seed during hot air drying (HGCA (Home-Grown Cereals Authority)., 2002). In this work, it was observed that at 10 cm distance temperature reached the following average values on the surface of dry grains: 22.0, 49.8, 58.5, and 69.9 °C for treatment times of 5, 25, 50, and 75 s, respectively. In the case of wet grains, average temperature values were 20.7, 37.2, 5.31, and 53.9 °C for the mentioned periods of time, respectively. As observed, the values of temperature on the surface of grains were not above 50 °C for 25 s treated kernels which maintained a high germination capacity. When the number of pulses increased (50 and 75 s treatments), the reduction in germination capacity of dry and wet grains subjected to the same PL fluence was inversely linked to the temperature increase. This fact would indicate that temperature could be hardly considered as the main factor influencing the impairment of germinability. In addition, it is worth mentioning that after treatments were completed, temperature decreased very quickly.

3.3. Effect of PL treatments on moulds inactivation

Drastic changes in germination capacity of barley kernels are a limiting aspect for the application of high PL doses. Accordingly, mould response was only evaluated in grain samples located at 10 cm from the quartz window, where the germination capacity was not significantly affected by PL irradiation up to 25 s (fluence: 29.9 J/cm^2). For the purpose of comparison, moulds' response was evaluated during a greater period of time (50 s, fluence: 59.7 J/cm^2).

The semilogarithmic survival curves for *A. carbonarius* and *A. flavus* conidia are shown in Figs. 3 and 4, respectively. The survival curves were non-log-linear: the fungal population steeply decreased (approximately 1.2–1.7 log cycles) up to 5–15 s (fluence: 6.0–18.0 J/cm^2)

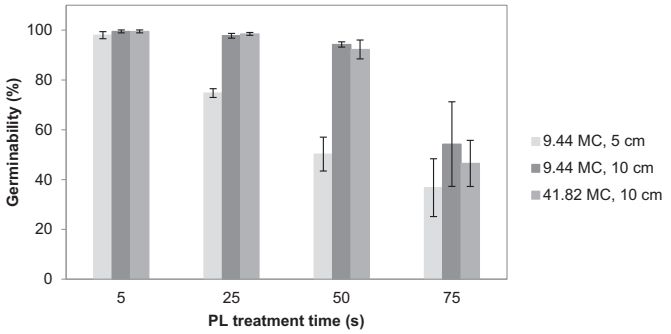

Fig. 2. Germination capacity (average and standard deviation) of barley grains after five PL exposure times at different distances from the quartz window (5 cm and 10 cm). Grain moisture content: 9.4 ± 0.2, wb (dry barley) and 41.8 ± 1.2, wb (wet barley).

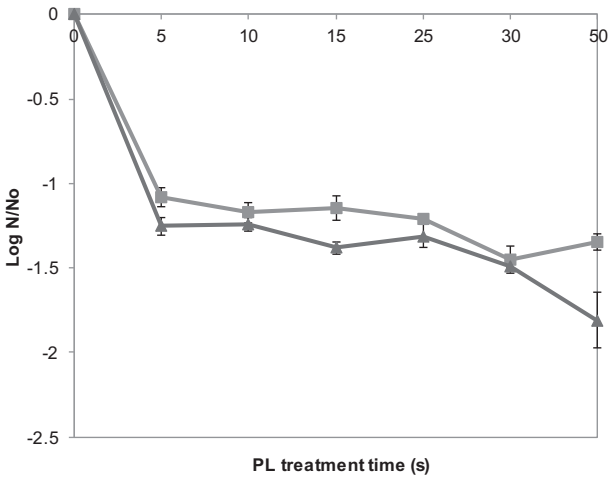

Fig. 3. Inactivation of *Aspergillus carbonarius* conidia inoculated on barley grains and treated by PL at 10 cm distance from the quartz window. Grain moisture content: 9.4 ± 0.2, wb (■) and 47.6 ± 2.3, wb (▲).

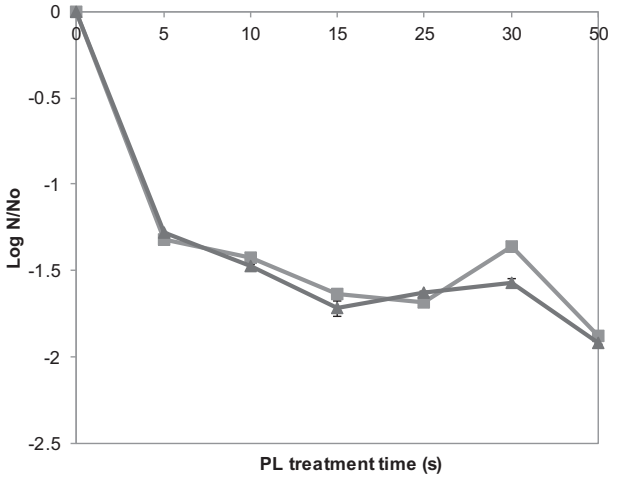

Fig. 4. Inactivation of *Aspergillus flavus* conidia inoculated on barley grains and treated by PL at 10 cm distance from the quartz window. Grain moisture content: 9.4 ± 0.2, wb (■) and 47.6 ± 2.3, wb (▲).

PL treatment. Then inactivation rate abruptly fell down and curves tended to level off, indicating a persistent residual population even after prolonged treatment times. The occurrence of tailing could be attributed to many phenomena: 1) the phenotypic or genotypic differences within a population which provide enhanced survival for a minority of cells, 2) the inoculated conidia were not subjected to a homogenous treatment because grains were exposed to radiation by only one side; in addition, the microorganisms may not have been evenly distributed on the surface of the grain, and 3) moulds could have been internalized in the grains, or could have been present in grain surface irregularities or injuries, preventing the contact between them and the radiation (Alzamora, Guerrero, Raffellini, Ferrario, & Schenk, 2016; Gómez-López et al., 2007). The flattening of inactivation curves has also been reported for many microorganisms exposed to low or moderate PL doses as in this work: native moulds on wheat grains (Aron Maftei et al., 2014); *Escherichia coli*, *Saccharomyces cerevisiae* and *Listeria innocua* in cut apples (Gómez et al., 2012); *E. coli* O157:H7 on strawberries (Bialka et al., 2008); and *S. cerevisiae* in apple juice (Ferrario, Guerrero, & Alzamora, 2014). In many of these studies, in which a wider PL fluence range was assayed, a greater microorganism population decrease at very high doses was found after the near plateau at moderate doses, indicating actually a sigmoid pattern.

Grain moisture content did not influence inactivation patterns of both moulds. It is well known that low a_w values antagonise the lethal effect of inactivation agents. However, it must be noted that in this study, grains were treated immediately after inoculation and in consequence equilibrium between a_w of conidia and a_w of barley kernel was not reached. Thus, the a_w value of conidia on dry and wet grains during irradiation was practically that of culture medium (≈ a_w 0.98).

Other difference between dry and wet grains that could affect inactivation response was the temperature profile during PL application. PL inactivation should be regarded not only as a multitarget technology but as a multifactor process whose mechanism of action would include the heating effect at large fluences. As seen in Fig. 1, after 25 s PL exposure, temperature in wet and dry grains was 37.2 °C and 49.8 °C respectively; after 50 s PL treatment, temperature increased to 51.3 °C and 58.5 °C for wet and dry grains respectively. This moderate overheating achieved in those short periods of time would not be expected to influence conidia inactivation. Reduction of survival percentage was observed for various moulds in other studies for similar temperatures, but for longer treatment periods. For example, Mohyuddin and Skoropad (1975) heated suspensions of *Aspergillus flavus* conidia in water at temperatures ranging from 25 to 55 °C for 20 min per treatment, and observed that at 35 °C survival was not affected, whereas at 55 °C, survival was 45%. Padwal-Desai, Ghanekar, and Sreenivasan (1976) applied thermal treatments to *A. flavus* conidia suspensions in saline solution or potato-dextrose broth, and found that at 50 °C survival was not affected when exposure lasted up to 75 min. However, at 55 °C and 5 min treatment, conidia population was reduced to around 8% and to 5% after 15 min treatment. Ballestra and Cuq (1998) obtained D-values for thermal treatments on *Aspergillus niger* at 50 °C and 60 °C. Conidia were suspended in Ringer solutions at two a_w (0.99 and 0.90). For each a_w level, D-values were > 200 min and > 300 min at 50 °C, and 1.2 ± 0.6 and 10.1 ± 1.1 at 60 °C.

Small differences were observed in the sensitivity of both moulds to PL treatment (Figs. 3 and 4), with *A. carbonarius* being the most resistant mould. *A. carbonarius* produces single-celled conidia with melanin and aspergilline in their cell walls, which confer tolerance to UV-C (Valero et al., 2007).The photoprotective potential of fungal pigments has been recognized by many authors. Esbelin, Mallea, Ram, and Carlin (2013) investigated the resistance to UV-C and PL of conidia of three *A. niger* strains possessing the same genetic background, but differing in their degree of pigmentation, and concluded that melanin protected pigmented conidia of dark *A. niger* from PL by absorption of considerable amounts of energy.

3.4. Structure alterations of barley caryopsis caused by PL treatments

Structural changes on barley caryopsis induced by PL exposure for 25 s and 75 s were examined using LM (Figs. 5 and 6). Alterations in different degrees were documented. Raw kernel micrograph of the caryopsis distal zone showed from outer to inner a partial view of pericarp, seed teguments, 2–3 layers of aleurone cells somewhat cubical, and the endosperm (Fig. 5A). As a part of embryo, the scutellum was documented; in adjacent position the interface or contact zone and the endosperm were visualized (Fig. 5B). Scutellum parenchyma type cells exhibited thick walls, dense cytoplasm, nucleus and visible lipids and protein bodies. Conspicuous contact zone between scutellum and endosperm was constituted by several (6 or more) layers of tangentially compressed cells. Mentioned cells presented thick and birefringent walls and dense cytoplasm. Endosperm cells of raw grain were elongated in shape and appeared containing numerous amyloplasts, dissimilar in size, and with irregular to oval contours. Amyloplasts were surrounded by the matrix with lipids and protein bodies (Fig. 5B, C).

After 25 s PL treatment, the distal region of caryopsis showed the aleurone and pericarp layers similar to the kernel raw (Fig. 5D). In general, no evident modifications were detected in the scutellum and interface area between scutellum and endosperm (Fig. 5E). Endosperm cells exhibited variable appearance: several were intact, well defined, and similar to the raw kernels, and others appeared altered with undulated and diffused cell walls (Fig. 5F, H). Crowded amyloplasts showed irregular contours (Fig. 5H). Scutellum evidenced preserved cells, some of them in process of mitosis (prophase, metaphase), suggesting the viability of the embryo after 25 s PL exposure (Fig. 5G).

After 75 s PL treatment, analysed specimens showed a heterogeneous response (Fig. 6). A minor part of the samples appeared similar to the raw and 25 s PL exposed caryopsis (Fig. 6D–F). However, the majority of the kernels exhibited severe impact after treatment (Fig. 6A–C). Observations of the caryopsis distal region confirmed the visible destruction of pericarp, aleurone layers, cell walls of endosperm and amyloplasts (Fig. 6A). Likewise scutellum, interface zone and endosperm registered a great impact (Fig. 6B). Scutellum cells, apparently collapsed and with thick birefringent walls, appeared delimiting visible intercellular spaces. Evident collapse was documented in the densely stained interface zone. Impacted endosperm cells were documented with disrupted walls, generating the presence of crowded amyloplasts, irregular in shape (Fig. 6C). Apparently collapsed matrix with lipids and protein bodies appeared entrapped between impacted amyloplasts.

LM study contributed to support the reported changes in germinability. Exposure to PL during 25 s caused slight modifications in the

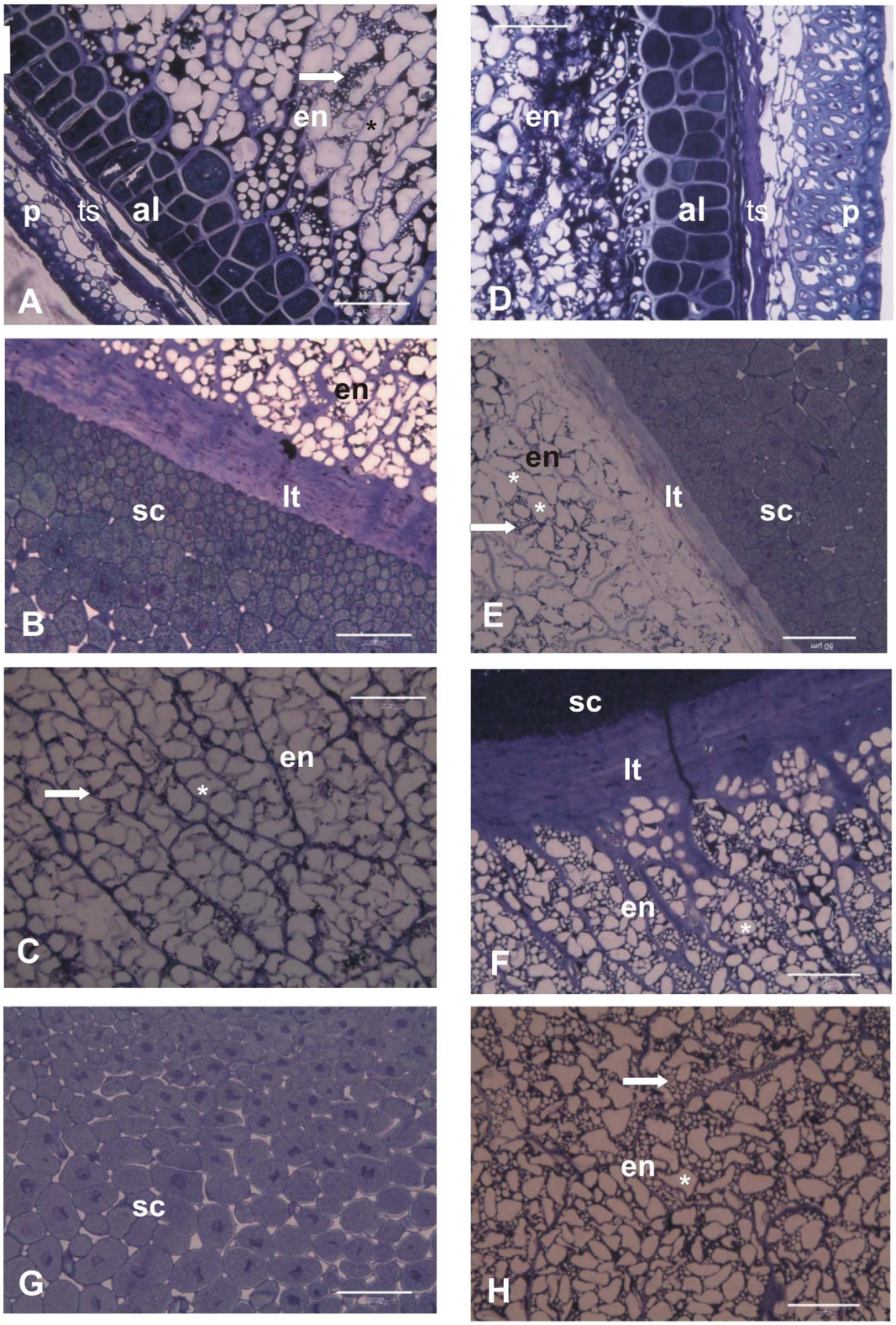

Fig. 5. A–H, light microscopy study. A–C, raw kernels: A, partial view of the caryopsis distal zone; B, scutellum, interface zone and endosperm; C, endosperm (detail). D–H, treated kernels (PL, 25 s): D, partial view of the caryopsis distal zone; *E*-F, scutellum, interface zone and endosperm: E, general aspect; F, detail; H, endosperm, detail. Scales: 50 μm (a = aleurone, arrow = matrix with lipids and protein bodies,* = amyloplast, en = endosperm, it = interface zone, p = pericarp, sc = scutellum, ts = seed teguments).

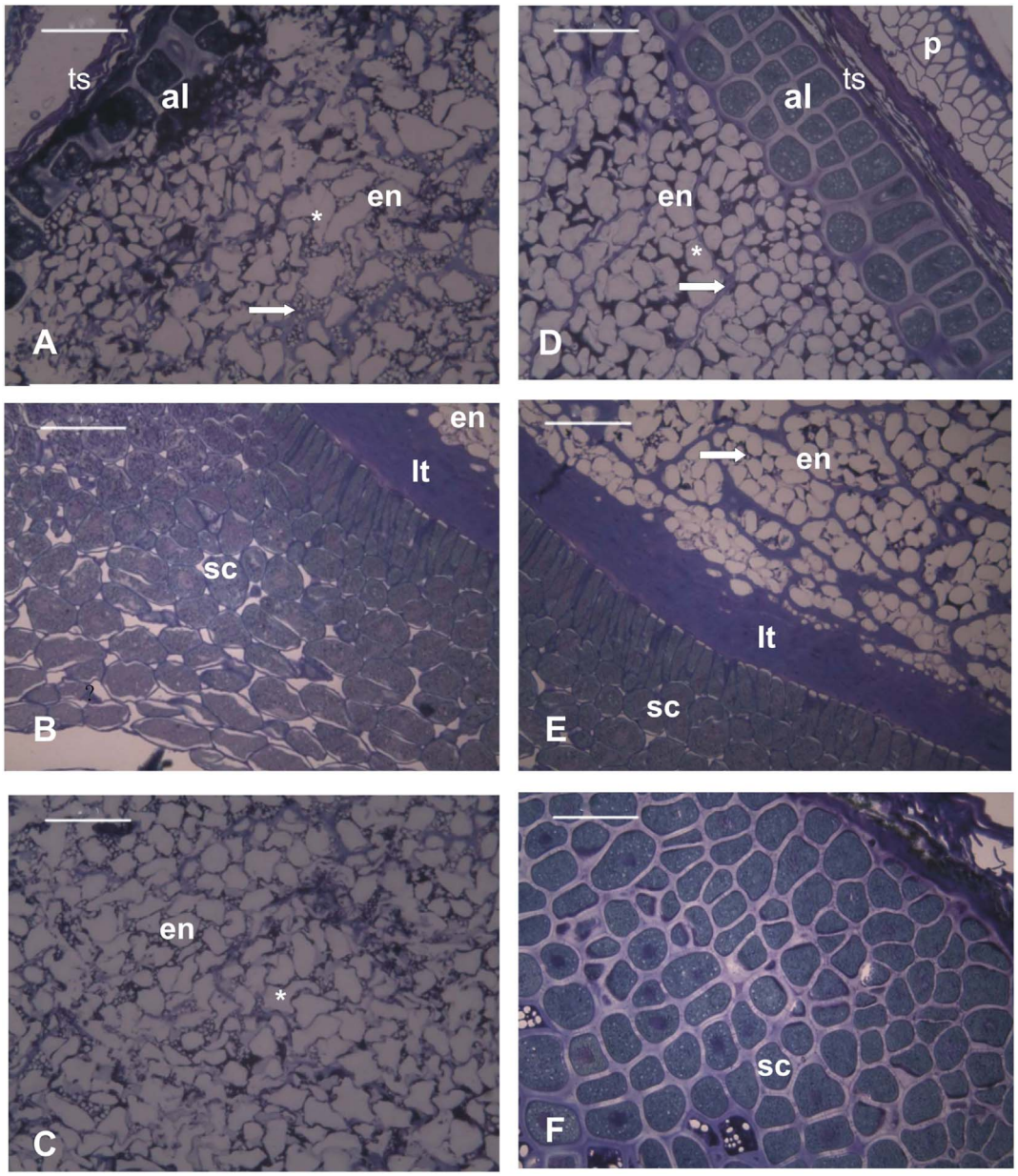

Fig. 6. A–F, light microscopy study of treated kernels (PL, 75 s). A,D, partial view of the caryopsis distal zone; B,E, partial view of scutellum, interface zone, and endosperm; C, impacted endosperm (detail); F, partial view of scutellum cells with birefringent and thick walls, detail. Scales: 50 μm (al = aleurone, arrow = matrix with lipids and protein bodies, * = amiloplast, en = endosperm, it = interface zone, sc = scutellum, ts = seed teguments, p = pericarp).

amylaceous storage tissue or endosperm. Embryo viability was maintained. On the contrary, the caryopsis structure was drastically altered after 75 s PL treatment.

4. Conclusions

A. carbonarius and *A. flavus* inoculated on barley grains were quickly inactivated by 1.2–1.7 log, respectively, when exposed to 15 s PL (fluence: 18.0 J/cm^2). Semilogarithmic survival curves were not influenced by grain moisture content. Germination capacity of both dry and wetted grains was not significantly affected by PL treatment up to 25 s exposure at 10 cm distance from the quartz window (fluence: 29.8 J/cm^2). For exposure times > 25 s, germination capacity reached unacceptable levels. Thus, PL exposure up to this fluence value could be an alternative to reduce *A. carbonarius* and *A. flavus* presence in barley destined to beer elaboration, before or after water uptake in the steeping step of the malting process, with minor loss of germinability. Moreover, survival curves of both moulds exhibited pronounced tailing. Accordingly, the application of prolonged irradiation times did not allow obtaining a much higher microbial reduction: conidia inactivation rate dramatically fell down for fluences > 18 J/cm^2.

Microscopy study allowed explaining changes in germinability. Exposure to PL during 25 s only showed slight modifications in starchy endosperm of dry grains but embryo viability was maintained. On the contrary, most of the structure elements (pericarp, cell walls and amyloplasts of endosperm, aleurone layers and scutellum) of the caryopsis were drastically altered after 75 s PL treatment.

In conclusion, PL irradiation up to 15 s (18.0 J/cm^2) could be a powerful nonchemical (residue-free) option to reduce fungal presence in barley grains with minor impact on structure and germinability. Further studies including rotation or agitation of the grains are currently in course, in order to reach more exposure of mould cells to the PL and improve mould inactivation.

Acknowledgements

The authors would like to acknowledge the financial support from UBA (UBACYT 2014-7 Project 20020130100388BA), CONICET (2013-5 Project 11220120100395) and ANPCyT (2015-8 PICT 1358).

References

Alzamora, S. M., Guerrero, S. N., Raffellini, S., Ferrario, M., & Schenk, M. (2016). Hurdle technology in fruit processing. In S. Pareek (Ed.). *Fresh-cut fruits and vegetables: Technology, physiology, and safety* (pp. 101–138). Boca Raton, FL: CRC Press. Taylor & Francis Group.

AOAC International (2005). AOAC official method 934.06 - moisture in dried fruits. In W. Horwitz, & G. W. Latimer (Eds.). *Official methods of analysis*(18th ed.). Gaithersburg, Maryland: AOAC International.

Aron Maftei, N., Ramos-Villarroel, A. Y., Nicolau, A. I., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Pulsed light inactivation of naturally occurring moulds on wheat grain. *Journal of the Science of Food and Agriculture, 94*(4), 721–726.

Ballestra, P., & Cuq, J.-L. (1998). Influence of pressurized carbon dioxide on the thermal inactivation of bacterial and fungal spores. *LWT - Food Science and Technology, 31*, 84–88.

Bialka, K. L., Demirci, A., & Puri, V. M. (2008). Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering, 85*(3), 444–449.

Choi, M. S., Cheigh, C. I., Jeong, E. A., Shin, J. K., & Chung, M. S. (2010). Nonthermal sterilization of *Listeria* monocytogenes in infant foods by intense pulsed-light treatment. *Journal of Food Engineering, 97*(4), 504–509.

Dodd, J. G., Vegi, A., Vashisht, A., Tobias, D., Schwarz, P., & Wolf-Hall, C. E. (2011). Effect of ozone treatment on the safety and quality of malting barley. *Journal of Food Protection, 74*(12), 2134–2141.

Esbelin, J., Mallea, S., Ram, A. F. J., & Carlin, F. (2013). Role of pigmentation in protecting *Aspergillus niger* conidiospores against pulsed light radiation. *Photochemistry and Photobiology, 89*(3), 758–761.

FAO (2009). *Agribusiness handbook: Barley, malt and beer*. Rome, Italy: FAO (FAO Investment Centre Division and FAO's Rural Division, Ed.).

Ferrario, M., Guerrero, S., & Alzamora, S. M. (2014). Study of pulsed light-induced damage on *Saccharomyces cerevisiae* in apple juice by flow cytometry and transmission electron microscopy. *Food and Bioprocess Technology, 7*(4), 1001–1011.

Frančáková, H., Líšková, M., Bojnanská, T., & Mareček, J. (2012). Germination index as an indicator of malting potential. *Czech Journal of Food Sciences, 30*(4), 377–384.

Gómez, P. L., Salvatori, D. M., García-Loredo, A., & Alzamora, S. M. (2012). Pulsed light treatment of cut apple: Dose effect on color, structure, and microbiological stability. *Food and Bioprocess Technology, 5*(6), 2311–2322.

Gómez-Lopez, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. *International Journal of Food Microbiology, 103*(1), 79–89.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: A review. *Trends in Food Science and Technology, 18*(9), 464–473.

Gonzalez Pereyra, M. L., Rosa, C. A. R., Dalcero, A. M., & Cavaglieri, L. R. (2011). Mycobiota and mycotoxins in malted barley and brewer's spent grain from Argentinean breweries. *Letters in Applied Microbiology, 53*(6), 649–655.

HGCA (Home-Grown Cereals Authority). (2002). Ensuring good germination in malting barley. *Topic Sheet N, 60*.

ISTA (2011). International rules for seed testing 2011. *International seed testing association, 2011*.

Jun, S., Irudayaraj, J., Demirci, A., & Geiser, D. (2003). Pulsed UV-light treatment of corn meal for inactivation of *Aspergillus niger* spores. *International Journal of Food Science and Technology, 38*(8), 883–888.

Kottapalli, B., & Wolf-Hall, C. E. (2008). Effect of hot water treatments on the safety and quality of fusarium-infected malting barley. *International Journal of Food Microbiology, 124*(2), 171–178.

Kottapalli, B., Wolf-Hall, C. E., & Schwarz, P. (2005). Evaluation of gaseous ozone and hydrogen peroxide treatments for reducing *Fusarium* survival in malting barley. *Journal of Food Protection, 68*(6), 1236–1240.

Kottapalli, B., Wolf-Hall, C. E., & Schwarz, P. (2006). Effect of electron-beam irradiation on the safety and quality of fusarium-infected malting barley. *International Journal of Food Microbiology, 110*(3), 224–231.

Kramer, B., Wunderlich, J., & Muranyi, P. (2017). Recent findings in pulsed light disinfection. *Journal of Applied Microbiology, 122*(4), 830–856.

Krishnamurthy, K., Demirci, A., & Irudayaraj, J. M. (2007). Inactivation of *Staphylococcus aureus* in milk using flow-through pulsed UV-light treatment system. *Journal of Food Science, 72*(7), 233–239.

Laitila, A., Alakomi, H.-L., Raaska, L., Mattila-Sandholm, T., & Haikara, A. (2002). Antifungal activities of two lactobacillus plantarum strains against *Fusarium* moulds in vitro and in malting of barley. *Journal of Applied Microbiology, 93*(4), 566–576.

Lewis, M. J., & Young, T. W. (2001). Malting technology: Malt, specialized malts and non-malt adjuncts. *Brewing* (pp. 163–190). Boston, MA: Springer US.

Ministerio de Agricultura, Ganadería y Pesca de la República Argentina- Servicio Nacional de Sanidad y Calidad Agroalimentaria (2013). *Norma V de Calidad y Comercialización de Cebada. Resolución SENASA N° 27 del 10 de diciembre de 2013*.

Ministerio de Agroindustria de la República Argentina (2016). *Informe de cebada. 2016*. Retrieved March 13, 2017, from www.minagri.gob.ar/new/0-0/programas/dma/granos/Informe-de-cebada.pdf (Junio) .

Mohyuddin, M., & Skoropad, W. P. (1975). Radiation-heat synergism for inactivation of conidia of *Aspergillus flavus*. *Radiation Botany, 15*, 185–189.

Møller, B. (2004). Near infrared transmission spectra of barley of malting grade represent a physical-chemical fingerprint of the sample that is able to predict germinative vigour in a multivariate data evaluation model. *Journal of the Institute of Brewing, 110*(1), 18–33.

Molto, G., Samar, M. M., Resnik, S., Martínez, E. J., & Pacin, A. (2000). Occurrence of trichothecenes in Argentinean beer: A preliminary exposure assessment. *Food Additives and Contaminants, 17*(9), 809–813.

Oliveira, P. M., Mauch, A., Jacob, F., Waters, D. M., & Arendt, E. K. (2012). Fundamental study on the influence of *Fusarium* infection on quality and ultrastructure of barley malt. *International Journal of Food Microbiology, 156*(1), 32–43.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology, 3*(1), 13–23.

Ozer, N. P., & Demirci, A. (2006). Inactivation of *Escherichia coli* O157:H7 and *Listeria* monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science and Technology, 41*(4), 354–360.

Padwal-Desai, S. R., Ghanekar, A. S., & Sreenivasan, A. (1976). Studies on *Aspergillus flavus*-I. Factors influencing radiation resistance of non-germinating conidia. *Environmental and Experimental Botany, 16*(1), 45–51.

Resnik, S. L., Favetto, G., Chirife, J., & Ferro-Fontan, C. (1984). A world survey of water activity of selected saturated salt solutions used as standards at 25 °C. *Journal of Food Science, 49*(2), 510–513.

Rouse, S., & Van Sinderen, D. (2008). Bioprotective potential of lactic acid bacteria in malting and brewing. *Journal of Food Protection, 71*(8), 1724–1733.

Sharma, R. R., & Demirci, A. (2003). Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. *International Journal of Food Microbiology, 86*(3), 231–237.

Sorrivas, V., Morales, A., & Yáñez, M. J. Y. (2014). In V. Sorrivas (Ed.). *Principios y práctica de la Microscopía Elecrónica*(1st ed.). Argentina: Bahía Blanca.

Staack, N., Ahrné, L., Borch, E., & Knorr, D. (2008). Effect of infrared heating on quality and microbial decontamination in paprika powder. *Journal of Food Engineering, 86*(1), 17–24.

Valero, A., Begum, M., Leong, S. L., Hocking, A. D., Ramos, A. J., Sanchis, V., & Marín, S. (2007). Effect of germicidal UVC light on fungi isolated from grapes and raisins. *Letters in Applied Microbiology, 45*(3), 238–243.

Wambura, P., & Verghese, M. (2011). Effect of pulsed ultraviolet light on quality of sliced ham. *LWT - Food Science and Technology, 44*(10), 2173–2179.

Wang, T., MacGregor, S. J., Anderson, J. G., & Woolsey, G. A. (2005). Pulsed ultra-violet inactivation spectrum of *Escherichia coli*. *Water Research, 39*(13), 2921–2925.

Wekhof, A. (2000). Disinfection with flash lamps. *PDA Journal of Pharmaceutical Science and Technology, 54*(3), 264–276.

Wolf-Hall, C. E. (2007). Mold and mycotoxin problems encountered during malting and brewing. *International Journal of Food Microbiology, 119*(1–2), 89–94.