INTENSE LIGHT PULSES EFFECT ON FUNGAL BURDEN OF MUSTARD AND BLACK PEPPER

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Abstract: *Mustard seeds and black pepper berries were used as test materials in an experiment that aimed to put the basis of a decontamination procedure for spices and condiments using a non-thermal minimal processing method based on Intense Light Pulses. The method wants to be a better solution to actual decontamination procedures of spices, food ingredients that have to fulfill the safety requirements regarding fungal burden and mycotoxin content. The need for the development of innovative technologies for the production of high quality spices is widely recognized by different authorities involved in food safety.*
The tests were performed on an installation prototype consisting of a vibratory sieve (800 rpm) and IFP 800 flash lamp discharging in Xenon gas. Different energetic densities (0.170 J/cm^2, 0.783 J/cm^2 and 1.393 J/cm^2) and different pulse regimes ($10 \cdot 10^{-3}$ s, $20 \cdot 10^{-3}$ s and $30 \cdot 10^{-3}$ s) were used in order to establish the most suitable regime for fungal decontamination.
The experimental results showed that it is possible to obtain 100% fungal decontamination of mustard seeds by setting appropriate regimes that take into consideration the initial fungal burden, the seeds quantity per sieve squared centimeter, the energetic density and the pulse regime. For black pepper berries, contamination was significantly reduced (1.7 log), but total fungal decontamination was not achieved. Comparatively to mustard seeds, black pepper berries display a similar roughness but a different undulation, the last one being the potential responsible for mould spores protection against light pulses.

Key words: *moulds, decontamination, mustard, black pepper, intense light pulses*

Introduction

Most spices contain a high number of microorganisms among which moulds represent a significant part. Having been dried material from plant origin, spices are commonly heavily contaminated with xerophilic storage moulds [1]. The most frequent fungal contaminants of spices are species from the genera *Aspergillus* and *Penicillium* [2]. Some species that belong to these genera are known as potential producers of different toxic substances such as aflatoxins, ochratoxins and sterigmatocystine, i.e. mycotoxins that exhibit toxic, mutagenic, teratogenic and carcinogenic effects in humans and animals [3].

Mycotoxins synthesized by moulds in spices pose an important health risk in the end-products, being so resistant (degrade at 265^{o}C) that cannot be eliminated during food processing. For example, the contamination of processed meat with aflatoxin was shown to correlate with the addition of spices to fresh meat [4].

To minimize the hygiene and health risks of moulds presence on spices, the European Spice Association (ESA) has set up quality minima (QM), establishing requirements on the minimum quality for spices entering the EU market. In some respects, the quality minima are additional to legislation, in other respects, they further define legal requirements. Thus, ESA stipulates for spices a content of 10^5 yeast and moulds per gram as target and 10^6 yeast and moulds per gram as absolute maximum [5].

Only few conventional technologies, such fumigation and irradiation, are efficient for spices sanitation. However, applications such as the fumigation with ethylene oxide are restricted and even banned by law in the European Union [6], while gamma irradiation of spices (10 kGy) is allowed by the European legislation, but frighten consumers who are afraid of formation of low-molecular-weight volatile or non-volatile radiolysis products that are potentially harmful and may migrate into the food and impair food flavor. Modern technologies as high hydrostatic pressures ranging from 100 MPa to 1000 MPa are not efficient on products with water activities below 0.66 as spices are. As a consequence, there is a need for the development of innovative technologies for the production of high quality spices.

While Lilie and coworkers [7] propose as novel decontamination method of spices a modification of the well-known vacuum-steam-vacuum (VSV) procedures characterized by a rapid evacuation after a short-steam treatment, other solutions should be taken into discussions. In this respect, the present paper aims to put the basis of a new decontamination procedure for spices and condiments using a non-thermal minimal processing method based on Intense Light Pulses.

Materials and Methods

Materials

Two spices were used in the experiments: mustard seeds and black pepper berries.

Mustard seeds packed in 100 g bags by SC ORLANDO IMPORT EXPORT 2001 Ltd., a company that is certified IFS and ISO 9001/2000, were sampled from a local food store. All sampled bags belonged to the same lot (297 014 - LOT 12), but even this, the content of the bags was mixed in order to obtain a homogenous laboratory sample, which was then kept in a sterile plastic bag at room temperature.

Black pepper berries were kindly provided by INTERSERVICES SA, a company that provide insurance related services, case handling and assistance in shipping and trading of goods. A part of these berries were artificially contaminated with spores of *Penicillium camemberti*, in order to obtain a higher fungal contamination.

Equipments

The tests were performed on a prototype of an installation consisting of a vibratory sieve (400 rpm and 800 rpm) driven by an electric motor with eccentric, and an IFP 800 flash lamp discharging in Xenon gas (Figure 1). The distance between the flash lamp and sample was set at 0.10 m.

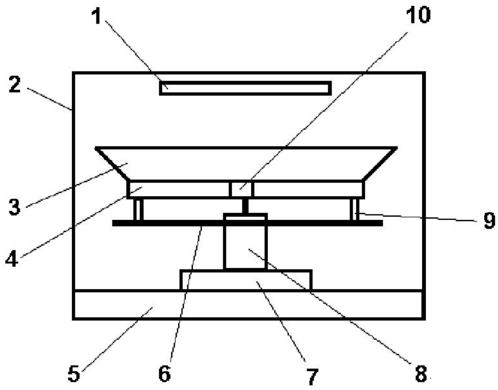

Figure 1. Experimental installation for irradiation of condiments with intense light pulses

1 - flash lamp; 2 - protective enclosure; 3 - tray for seeds; 4 - vibrating platter; 5 - support site; 6 – platter holder (rigid), 7 - engine support (elastic), 8 - electric motor, 9 - platter support (elastic), 10 – eccentric.

Methods

Seeds/berries treatments

Amounts of 20 g of mustard seeds or black pepper berries were aseptically taken, placed on the vibrating sieve and submitted to ILP treatments. The speed of the vibrating sieve was set at 800 rpm, in order to made 1600 flights per minute and to ensure seeds and berries exposure to light pulses on all sides. Different energetic densities (0.170 J/cm^2, 0.783 J/cm^2 and 1.393 J/cm^2) and different pulse regimes ($10\cdot10^{-3}$s, $20\cdot10^{-3}$s and $30\cdot10^{-3}$s) were used in order to establish the most suitable regime for fungal decontamination. Tests were performed in duplicates.

Microbiological analysis

Total fungal counts were performed according to SR ISO 21527-2:2009 (Microbiology of food and fodder. Horizontal method for enumeration of yeasts and molds. Part 2: Colony count technique in products with less than or equal to a_w 0.95). Rose Bengal (Scharlau Chemie, Spain) was used as culture medium. After inoculation, the Petri dishes were incubated at 25°C for 5 days.
The genus of the isolated moulds was determined based on the characteristics of the colonies and the microscopic aspect of the mycelium.

Results and discussion

Mustard seeds and black pepper berries used in these experiments had similar fungal contamination levels (10^3 per gram), and displayed similarities regarding the quality of their microbiota (figure 2 and figure 3), whether considering the presence of *Aspergillus* and *Penicillium* genera as toxicogenic moulds. Representatives of *Penicillium* genus were present in proportion of 26% on mustard seeds and 20% on black pepper berries, while representatives of A*spergillus* genus were present in proportion of 26% on mustard seeds and 79% on black pepper berries.

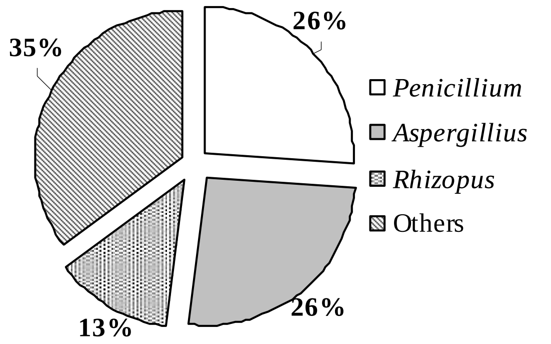

Figure 2. Initial fungal microbiota of mustard seeds

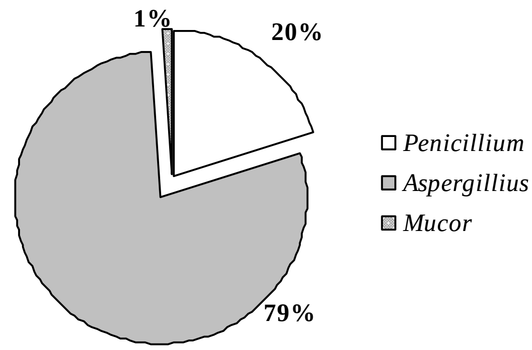

Figure 3. Initial fungal microbiota of black pepper berries

The initial level of the existing fungal microbiota on mustard seeds ($1.1 \cdot 10^3$ CFU$\cdot g^{-1}$) was gradually decreased during ILP treatments. A treatment consisted in applying a certain number of pulses per 10^{-3} second at different tensions. The results obtained for $10 \cdot 10^{-3}$ s and $30 \cdot 10^{-3}$ s at 700 V, 1500 V and 2000 V are presented in figure 4a and figure 4b.
It is noticed that when tension increases, the fungal burden of the seeds decreases.
The decontamination effect is more powerful when a higher number of pulses are applied. At a tension of 2000 V during $10 \cdot 10^{-3}$ s destroyed 90 % of the fungal population, while 30 pulses per 10^{-3} second destroyed 96 % of the fungal population.

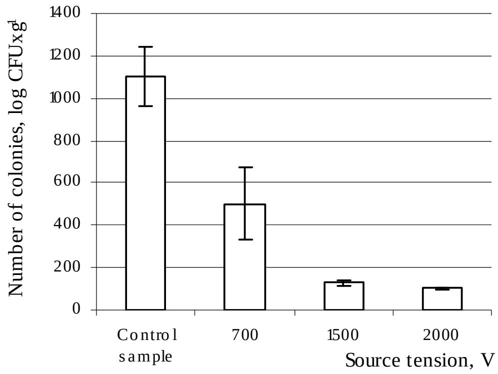

Figure 4a. Evolution of the fungi level on mustard seeds as result of ILP treatments at the regime of impulse $10 \cdot 10^{-3}$s

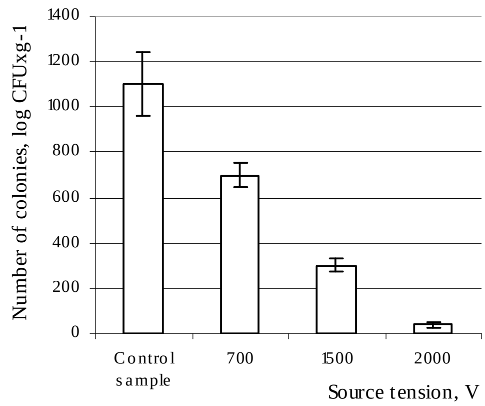

Figure 4b. Evolution of the fungi level on mustard seeds as result of ILP treatments at the regime of impulse $30 \cdot 10^{-3}$s

Total fungal decontamination of mustard seeds is possible to be achieved by setting appropriate ILP regimes.
The initial level of the existing fungal microbiota on black pepper berries ($6.5 \cdot 10^3$ CFU$\cdot g^{-1}$) was not significantly decreased, despite the fact that different ILP treatments were tested and their intensity was gradually increased (700 V / $10 \cdot 10^{-3}$s, 700 V / $30 \cdot 10^{-3}$s, 1500 V / $10 \cdot 10^{-3}$s, 1500 V / $30 \cdot 10^{-3}$s). In all these cases the fungal contamination level was not decreased beside 10^3 CFU$\cdot g^{-1}$, which means that less 1 log decreasing of fungal burden was obtained (figure 5).

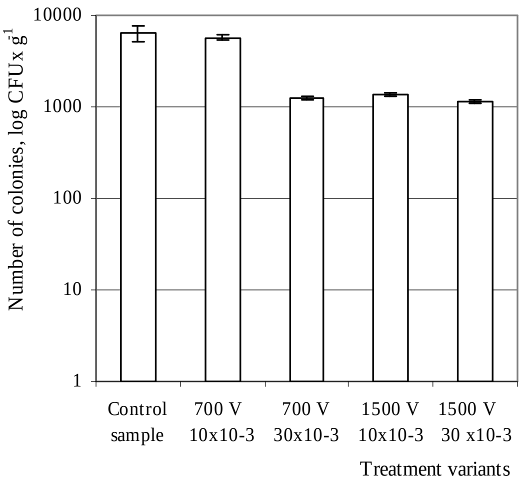

Figure 5. Evolution of the fungi level on naturally contaminated black pepper berries as result of ILP treatments

The experiments were repeated on black pepper berries that were artificially contaminated with *Penicillium camemberti* spores in order to obtain a fungal burden above the limit indicated by ESA (European Spicy Association) as target value (10^5 CFU·g^{-1}). The results of ILP treatments on these artificially contaminated black pepper berries ($8\cdot10^6$ CFU·g^{-1} initial fungal burden) is shown in figure 6. It was necessary a heavy treatment (2000 V / $30\cdot10^{-3}$ s) to reduce the fungal burden with almost 1.7 log.

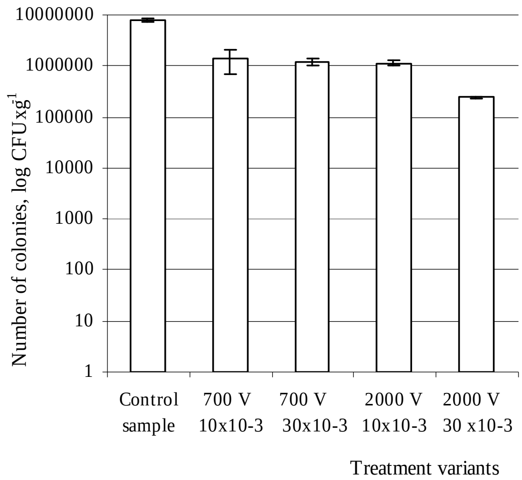

Figure 6. Evolution of the fungi level on artificially contaminated black pepper berries as result of ILP treatments

To reduce the fungal burden of black pepper berries it is necessary to use much intense ILP treatments that in the case of mustard seeds.

It is possible that the surface roughness of the black pepper berries to exert a protection against light pulses for the mould spores present on their surface.

Conclusions

An installation consisting of a vibratory sieve (400 rpm and 800 rpm and an IFP 800 flash lamp discharging in Xenon gas was successfully tested for fungal decontamination of mustard seeds and black pepper berries.

The treatments using intense light pulses succeeded to totally reduce the fungal burden of mustard seeds and to reduce the fungal burden of black pepper berries below the target value indicated by the European Spicy Association.

The study demonstrated that ILP treatments could be a solution to actual decontamination procedures of spices, responding in this way to the need of developing innovative technologies for the production of high quality spices.

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