International Journal of Food Microbiology 155 (2012) 269–272

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

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

Short Communication

Thermal inactivation of *Botrytis cinerea* conidia in synthetic medium and strawberry puree

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ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 20 February 2012
Accepted 25 February 2012
Available online 3 March 2012

Keywords:
Botrytis cinerea
Strawberries
Heat inactivation

ABSTRACT

Botrytis cinerea is one of the most important post-harvest molds that cause quality deterioration of strawberries and other fruits even during refrigeration storage. This research studied the effects of thermal inactivation of *B. cinerea* in synthetic medium and strawberry puree using hot water baths at different temperatures. These media were studied in order to determine if results obtained in a solution with the major components of the fruit (synthetic media), are comparable to the ones obtained in fruit purees. The results demonstrated that *B. cinerea* spores can be inactivated by heat treatments using relatively low temperatures (42–46 °C). Inactivation curves were well described by first order kinetics (R^2 0.91–0.99). *B. cinerea* conidia inoculated in synthetic medium required less time to achieve one log reduction in population than those inoculated in the fruit puree. *D* values were 22, 8.5, 4 and 1.4 min at 42, 44, 46 and 48 °C, respectively, in synthetic medium; while *D* values in strawberry puree were 44.9, 13.8, 4.7 and 1.4 min at 42, 44, 46 and 48 °C, respectively. The *z* values obtained were 4.15 and 5.08 °C for the strawberry puree and synthetic medium respectively, showing higher sensitivity of *B. cinerea* in fruit purees than in the synthetic medium. Thus, a change in the medium composition had a marked difference in the heat inactivation of *B. cinerea* conidia, and the results obtained in synthetic medium are not accurate to describe the behavior of the microorganism in the fruit.

1. Introduction

Strawberries are the second most important cultivated fruit of the berry family worldwide, after grapes, with 3.82 million tons per year (FAO, 2009). As true for all fresh produce, it suffers from various diseases. Gray mold (*Botrytis cinerea*) is one of the most important causes of post-harvest loses of strawberries. For example, this phytopathogenic mold may cause up to 30% to 40% losses of the harvest if no chemical control is applied. In acute infestations, the losses may reach 50–60% with the resulting economic loss of 100% (CSC and CMCC, 2003). Although prevention starts at the pre-harvesting stage, the control is most effective during the post-harvest stages (i.e. during shipment and storage). *B. cinerea* may develop and spread under refrigerated conditions, because conidium germination and mycelia growth may occur at temperatures as low as 0 °C (Dorby and Lichter, 2007; Lahlali et al., 2007). It is necessary to find out conditions to reduce the rate of mold growth. Milkota Gabler et al. (2004) exposed spores of *B. cinerea* to ethanol solutions of up to 30% v/v at 25–50 °C for 30 s. Ethanol was found to have synergistic effect with heat in mold control. At 40 °C, 9.7% ethanol was adequate to inhibit 50% of the germination of *B. cinerea* spores. Judet-Correia et al. (2010) evaluated the combined effect of water activity and temperature on the growth of *B. cinerea* in potato dextrose agar, synthetic grape juice medium and on a grape juice agar. They proposed a mathematic model to determine the optimal growth rate of some molds on grape berries, and even, useful for other fruits, demonstrating that refrigeration alone does not prevent mold growth.

Heating has been recognized as a feasible post-harvest treatment for fruits and vegetables with the potential to delay post-harvest ripening and decay. It is easily applied, leaves no chemical residues, and can reduce the initial population of microorganisms (Armstrong, 1994). Several studies have been reported on possible post-harvest heating treatments in strawberries. Margosan et al. (1994) proposed immersing strawberries in hot ethanol solutions at concentrations of 5% to 20% for control of postharvest fruit decay (against gray mold and black rot). García et al. (1995) studied the use of hydroheating at 45 °C for 15 min to obtain a 3 day shelf-life at 1 °C. Civello et al. (1997) used hot air at 42 or 48 °C for 3 hours to achieve 2 or 3 days of shelf life at 20 °C, respectively; while Vicente et al. (2002) reported 14 days of shelf life at 0 °C after hot air treatment at 45 °C for 3 h. Marquenie et al. (2002) investigated the inactivation of *B. cinerea* spores in a phosphate buffer using a glass capillary tube and a hot water bath at 40, 43, 45 or 48 °C. However, information on the kinetics of thermal inactivation of the fungi in strawberries is scarce. The knowledge of the conditions of inactivation at different temperatures is very important for appropriate design of adequate thermal treatments.

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0168-1605/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2012.02.021

The objective of this research was to study the use of hot water to inactivate the conidia of *Botrytis cinerea* inoculated in a strawberry puree and in a synthetic medium (comprised of the principal sugar and organic acid components of the strawberry in a buffered solution), to obtain the thermal death kinetics of the mold.

2. Materials and methods

2.1. Fruits

Physiologically mature strawberries (*Fragaria x ananassa* cv. Festival) were handpicked from an orchard located in Atlixco, Puebla (Mexico) in February 2010. Sample fruits were selected based on the USDA US No. 1 standard grade specifications: strawberries should have a calyx attached, be firm, not over-ripe or undeveloped, being uniform in size, free of decay and damage and with at least 3/4 of its surface showing pink or red color (Mitcham, 2004). The harvested berries were transported to the lab and were stored at −18 °C until use.

2.2. Fungi and inoculum

Botrytis cinerea was donated by Dr. Rosalba Troncoso from the collection of the CIAD (Centro de Investigación y Desarrollo en Alimentos, Hermosillo, Sonora, Mexico). The mold was maintained on potato dextrose agar (PDA, Becton Dickinson, Mexico) at 4 °C. The inoculum was obtained in accordance with the method used by Lichter et al. (2003) in which *B. cinerea* was inoculated on PDA plates and incubated for 12 ± 2 days at 25 °C. To prepare the suspension, spores were harvested using a detergent solution (0.7% NaCl and 0.03% Tween 20), and then filtered through eight layers of sterile gauze into a sterile glass jar. Spores were counted using a Neubauer counting chamber and a microscope, and the concentration was adjusted to obtain 10^5 to 10^6 spores/mL. Fresh inoculum was used for each set of treatment conditions.

2.3. Strawberry puree and synthetic medium

The strawberry sterile puree (SSP) was prepared by blending frozen strawberries in a sterile blender. The puree was stored in a hermetic glass jar, and sterilized at 101 °C for 10 min, as reported by López-Malo (1995). A synthetic medium (SM) formulation was developed in order to study if the inactivation kinetics of the mold inoculated in a system with the major components of a fruit, sugars and acids may be similar to that of the fruit. This medium was obtained by adding to a phosphate buffer (to simulate the buffering substances in the strawberry) the appropriate sugar and organic acid percentages in accordance with the strawberry hybrid 8 composition reported by Kafkas et al. (2007). The formulation included 92.54% water, 4.20% fructose, 2.00% glucose, 0.25% sucrose, 0.70% citric acid, 0.22% malic acid and 0.09% ascorbic acid, and was chosen to present similar pH, titratable acidity (TA) and total soluble solids (TSS) to the strawberry cv. Festival used for the puree in this study; only a minor adjustment was made to the citric acid concentration in order to maintain the pH value of the synthetic medium similar to that of the strawberry puree. The solution was then filter-sterilized through a 0.45 μm membrane and the SM was stored under refrigerated conditions in the hermetically closed sterile glass jar. The pH, °Brix, and water activity (a_w) were measured in both media to ensure that the treatment conditions would be similar.

2.4. Inactivation treatment

Data on thermal death kinetics for *B. cinerea* was achieved by conducting thermal-death-time (TDT) tests. This method requires the inoculation of a known concentration of the microorganism in a small quantity of the system of study forming a suspension. The suspension was heated at different temperatures for different time intervals to generate a death kinetic curve from which parameters for different equations could be derived.

The suspensions were placed in TDT cells designed and manufactured by Washington State University (WSU), Pullman WA, USA. The cells are made of aluminum with a cavity of 18 mm in diameter, 4 mm in depth, and a volume of 1.27 mL (Chung et al., 2008). Some of the cells were fitted with a T type thermocouple to record the time temperature history through a data logger system (Digisense DuaLogR 991100-50, Cole-Parmer Instrument Co, USA). Before each treatment, the cells were filled with 0.1 mL of inoculum and 0.9 mL of SMS or SSP, to achieve an initial population of 10^4 to 10^5 spores/mL. The average come up times for SM and SSP were 45 ± 7 and 63 ± 5 s, respectively.

Five of the inoculated cells were dipped in a hot water bath at 42, 44, 46 or 48 ± 0.2 °C; $t = 0$ in each condition was defined as the time to reach steady-state temperature in the cells. This implies that the time required to reach the steady-state temperature was neglected, and the time intervals reported here correspond to the effective time during which samples were held at the target temperature. The cells were taken out at different time intervals and placed in an ice-water bath, after which they were serially diluted and plated. Treatments were done by triplicate, that is, three different sets of cells were treated for each treatment temperature.

The treated suspensions were plated using a sample of 0.1 mL to make the first dilution in 0.1% peptone water, consecutive dilutions were made as deemed necessary depending on the temperature and time interval. Spore survival was assessed by pour plating 1 mL of the dilution in dichloran rose bengal chloramphenicol agar (DRBC, Difco, Mexico), incubating at 25 °C for 5 days and counted.

2.5. Analyses of thermal death kinetics data

First order kinetics was used to describe the inactivation behavior of the *B. cinerea* in the SM and SSP systems. The traditional first order kinetics is described as (USDA, 2005; Peleg, 2006):

$$\mathrm{Log}(N/N_0) = -t/D \tag{1}$$

where N is the microbial population at the time t, N_o is the initial population, t is the time of isothermal treatment, D value is the time needed to achieve one log reduction in the population at a certain temperature under the controlled treatment conditions.

As secondary fitting equation, the used expression was the Bigelow relationship (Eq. (2)), from parameters obtained for the first order kinetics model to calculate the z value (Mafart et al., 2010):

$$\mathrm{Log}\frac{D}{D_{\mathrm{ref}}} = \frac{T_{\mathrm{ref}} - T}{z} \tag{2}$$

where D_{ref} is the time needed to achieve one log reduction in the population at the reference temperature T_{ref}, T is the temperature of the isothermal treatment, T_{ref} is the temperature of reference, and z are the degrees needed to achieve a tenfold change in the D value.

Parameters for fitting equations were calculated using linear regression and the Kaleidagraph software (version 3.51, Synergy Software, Reading PA, USA).

2.6. Physical–chemical parameters

All analyses were performed in triplicates. The total soluble solids (TSS, %) in the strawberry pulp were determined using a hand-held refractometer (Atago, Japan), measuring °Brix of the sample. The titratable acidity (TA) was determined in 3 g of pulp by titration with 0.1 N NaOH, and expressed as percentage of citric acid, following

Table 1
pH, °Brix and a_w of the sterile strawberry puree (SSP) and synthetic medium (SM) used on the thermal death kinetics study of *B. cinerea*.

Medium	pH	°Brix (TSS, %)	a_w
Sterile strawberry puree (SSP)	3.87 ± 0.16^a	9.07 ± 0.12^a	0.985 ± 0.000^a
Synthetic medium (SM)	3.66 ± 0.05^a	6.27 ± 0.12^b	0.986 ± 0.001^a

Means ± standard deviation from three replicates.
Different letter means significant difference between samples, $p<0.05$.

the official method 37.1.37 (AOAC, 1996). pH values were measured by direct immersion of the electrode in the strawberry pulp, using a pre-calibrated pH meter (3310, Jenway Ltd., UK) (Villa-Rojas et al., 2011). The water activity (a_w) of the SM and SSP media was determined Aqualab Device (Aqualab SE, Decagon Inc., Pullman, WA USA) calibrated and operated in accordance with the method described by López-Malo et al. (1994). Table 1 shows the physical–chemical parameters (pH, °Brix and a_w) of the food (strawberry puree) and synthetic medium used in this study (SSP and SM).

2.7. Statistical analysis

Results were statistically analyzed using MINITAB ® Release 14 software (Minitab Inc., State College, PA, USA), through a Student *t*-test for the quality parameters; $p = 0.05$ was considered as the significance level.

3. Results and discussion

3.1. Systems characterization

The two strawberry media (SSP and SM) did not show significant difference in pH and a_w, although the TSS of the SSP was higher, probably due to the existence of soluble substances other than sugars in the fruit. Based on the observation made by other authors (McDonough and Hargrove, 1969; Archer et al., 1998), pH, a_w and nature of the soluble solutes are the parameters that may have a significant effect on the microbial response; since those parameters are not significantly different between the strawberry systems, it was assumed that the kinetics parameters should be fairly similar.

3.2. Heat treatment

Fig. 1 displays an example of the temperature profile for the treatment of SSP and SM samples at 42 °C. Figs. 2 and 3 summarize thermal inactivation data, and the fit with the first order kinetics equation for the inactivation of the *B. cinerea* spores in the SM and SSP, respectively. The slopes were steeper with the increase of temperature; that is the rate of inactivation of the spores increased with increases in treatment temperature.

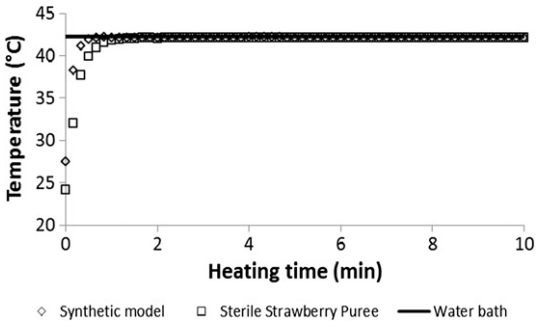

Fig. 1. First 10 min of a heat penetration curve for a synthetic medium (SM, ◊) and a sterile strawberry puree (SSP, □) at a water bath (—) temperature of 42.3 °C for a target temperature of 42.0 ± 0.2 °C.

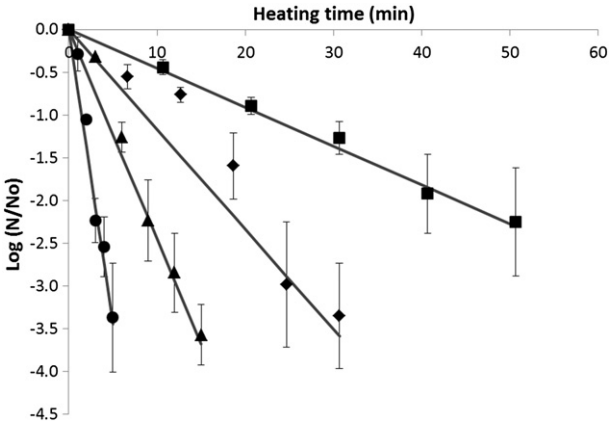

Fig. 2. Heat inactivation kinetics of the *B. cinerea* conidia inoculated on a synthetic medium (SM) at 48 °C (●), 46 °C (▲), 44 °C (♦) and 42 °C (■). Means and standard deviation of three replicates are shown.

The fitted parameters for the first order kinetics are summarized in Table 2. The correlation coefficient (R^2) is an indicator of a well accurate description of the inactivation kinetics of the mold in both strawberry puree and synthetic medium. *D* parameters had a pronounced difference at the lowest temperatures that lessened when the temperature was increased.

The medium composition affected the heat inactivation of *B. cinerea* spores. The thermoresistance of *B. cinerea* in SSP was higher than that in SM, since at 42 °C $D = 44.9$ min for SSP, while $D = 22.0$ min for SM. These differences decreased as the temperature increased. The calculated *D* values at temperatures of 40 and 43 °C for *B. cinerea* in a buffer with pH = 7.2 were reported by Marquenie et al. (2002), with values of 30.0 and 6.8 min, respectively.

The differences in *D* between the tested media and reported data may be attributed to the difference in their composition. The buffer provides a neutral pH with no substances such as sugars or organic acids as a protection, leading to lower *D* values than those for the mold in the strawberry systems. In the strawberry systems used in this study (pH 3.7–3.9), *B. cinerea* had higher *D*, corresponding to a more heat resistant behavior.

z Values obtained from this study also depend on medium characteristics. The *z* values for *B. cinerea* in SSP and SM were 4.15 and 5.08 °C, respectively, showing higher sensitivity of the mold spores to temperature in real foods than in the synthetic medium. Marquenie et al. (2002) reported a *z* value of 4.65 °C for *B. cinerea* in phosphate buffer. The *z* values of *B. cinerea* were smaller than those reported in citrate buffer (pH = 4.0) for inactivation of *Monascus ruber*

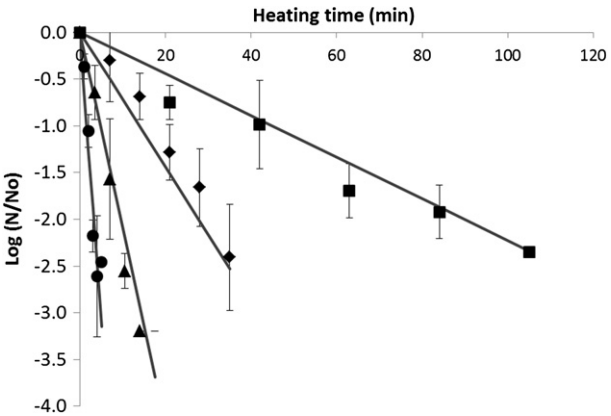

Fig. 3. Heat inactivation kinetics of the *B. cinerea* conidia inoculated on a sterile strawberry puree (SSP) at 48 °C (●), 46 °C (▲), 44 °C (♦) and 42 °C (■). Means and standard deviation of three replicates are shown.

Table 2
Calculated *D* values of the first order kinetics equation for the thermal inactivation of *B. cinerea* conidia in a sterile strawberry puree (SSP), and in a synthetic medium (SM).

Medium	First order kinetics		
	T (°C)	*D* (min)*	R^2
Sterile strawberry puree (SSP)	42	44.9 ± 5.8	0.98
	44	13.8 ± 3.6	0.99
	46	4.7 ± 0.7	0.99
	48	1.7 ± 0.5	0.91
Synthetic medium (SM)	42	22 ± 5.8	0.99
	44	8.5 ± 2.3	0.95
	46	4.1 ± 0.2	0.98
	48	1.4 ± 0.1	0.97

* Means ± standard deviation from three replicates.

ascospores, being 7.9 °C (Panagou et al., 2002) and for *Penicillium digitatum*, $z = 10.64$ °C ($a_w = 0.99$ and pH = 3.0), reported by López-Malo et al. (2005), but closer to those reported for *Aspergillus flavus*, which had $z = 5.85$ °C at $a_w = 0.99$ and pH = 3.0 (López-Malo et al., 2005). Differences between reported *z* values depend on mold species.

Obtained *D* and *z* values are considered valuable, as there are few studies devoted to study the thermal inactivation of molds, such as *Botrytis cinerea*.

4. Conclusion

This study demonstrates that heat treatment at 42–48 °C for a few minutes was effective in inactivating *B. cinerea* in strawberries. Using the data collected from different time–temperature combinations led to selection of appropriate treatment conditions to achieve the desired level of disinfection. The mold in the synthetic medium did not have the same behavior as that inoculated in the fruit puree. Thus, it is recommended to use the results of any studies made on a synthetic medium only as a general guide for treatment development. Such treatments need to be validated by inoculating the target molds on a real foods system or commodity.

Acknowledgments

Authors thank the financial support from CONACyT (Consejo Nacional de Ciencia y Tecnología, Mexico) for the scholarship to author R. Villa-Rojas and project 084859. The authors would also like to thank the help of Mr. Emilio Salinas Vázquez, owner of the orchard in Atlixco, Puebla, and Dr. Hugo S. García (ITV, Veracruz, Mexico) and Dr. Rosalba Troncoso from CIAD, Mexico, for providing the *Botrytis cinerea* strain.

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