

Research Article

Modelling the Survival of *Escherichia coli* O157:H7 on Raw Portioned Tomatoes, Inoculated with *Aspergillus fumigatus* and *Emericella nidulans*

Daniela Cardillo,¹ Antonio Bevilacqua,¹ Francesca Cibelli,² Clelia Altieri,¹ and Milena Sinigaglia¹

¹ Department of Food Science, Faculty of Agricultural Science, Foggia University, Via Napoli 25, 71100 Foggia, Italy

² Department of Agro-Environmental Science, Chemistry and Crop Protection, Faculty of Agricultural Science, Foggia University, Via Napoli 25, 71100 Foggia, Italy

Correspondence should be addressed to Antonio Bevilacqua, a.bevilacqua@unifg.it

Received 5 March 2009; Revised 2 July 2009; Accepted 23 September 2009

Recommended by Lori Snyder

The metabiotic interactions occurring among two fungi (*Aspergillus fumigatus* and *Emericella nidulans*) and *Escherichia coli* O157:H7 on raw portioned tomatoes were studied. Tomatoes, preinoculated with the moulds and inoculated with the pathogen, were packaged in air and stored at 4, 8 and 12°C for 9 days; pathogen cell number and pH were monitored throughout the storage and the data were modeled using three different equations (Geeraerd, Weibull, and modified Weibull), to assess the shoulder length, the 1-log reduction time, and the death time. Both *A. fumigatus* and *E. nidulans* increased the survival of *E. coli* O157:H7 through the prolongation of the shoulder length; in contrast, the death time was significantly increased. The results of this paper suggested that the metabiotic interactions aspergilli/*E. coli* O157:H7 could be of public concern, as the consumption of tomatoes (or other fruits and vegetables) contaminated both by the moulds and the pathogen is a possible scenario.

Copyright © 2009 Daniela Cardillo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

It is well known that fruits and vegetables can be contaminated with foodborne pathogens, as a result of either preharvest contact with contaminated irrigation water or manure, or postharvest contamination due to incorrect harvesting, washing, minimal processing, distribution, and preparation in foodservice settings or at home [1]. Moulds of the genera *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Rhizoctonia*, and *Rhizopus* and some aspergilli are responsible for postharvest decay of fruits and vegetables [2–4].

The term metabiosis describes the reliance by an organism on another to produce a favourable environment [5]; this can be the removal of oxygen by Gram-negative microflora, allowing the growth of anaerobic microorganisms, like *Clostridium botulinum* [5], or it can be situations where one organism provides nutrients enhancing growth of another [5].

Since 1980s many researchers have shown that a metabiotic interaction could occur between moulds and pathogens, with a benefit for the latter, due to the production of some alkalising compounds [2, 6], and evidence regarding interactions between moulds and bacteria on tomatoes is available [3, 4]. For example, Wade and Beuchat [4] observed that *A. alternata* and *Cladosporium* spp., coinoculated with *Salmonella* into raw ripe tomatoes, increased the pH of pulp, resulting in an enhancement of the rate of growth of the pathogen. Regarding the interaction moulds-psychrotrophic pathogens on minimally processed fruit, Riordan et al. [7] reported that *Glomerella cingulata* enhanced the survival of *Escherichia coli* O157:H7 on ready-to-use apples.

In a previous research, *Fusarium* spp. (*F. avenaceum*, *F. proliferatum* and *F. oxysporum*) were shown to exert a metabiotic effect on *E. coli* O157:H7 on raw portioned tomatoes, but not on *Listeria monocytogenes* [8]. The metabiotic effect, however, was not due to an increase of pH of fruit, as

reported by the literature, but, most likely, to the production of metabolites different from alkalising compounds or due to the release of nutrients as a consequence of a moderate pectinolytic activity [8].

Another topic of great interest in food microbiology is the mathematical modelling, as an important and useful tool to assess survival/growth of microorganisms under investigation. Microbial kinetics can be described by growth curves (modified Gompertz, Baranyi equation, the lag-exponential model, the logistic approach) or survival functions; in this paper we will focus only on the decreasing/survival curves.

As reported elsewhere [9, 10], a viability curve can be described through 9 different functions, as it can show different shapes: a linear, an upward or a downward trend; the log-linear/shoulder model, characterized by an initial shoulder phase (the shoulder has been defined as the time before the beginning of the death of the population) followed by a linear death kinetic; a linear death kinetic followed by a tail (the tail is the residual cell level at the end of the storage); an inactivation trend, showing an initial shoulder and a final tail; finally a biphasic trend, if the inactivation curve can be divided into two strokes, characterized by two different death rates. An exhaustive review of these kinds of models can be found in the paper of Geeraerd et al. [9].

Hereby, we studied the metabiotic interactions occurring between two aspergilli (*Aspergillus fumigatus* and *Emmericella nidulans*) and *E. coli* O157:H7 on tomato slices, through two models, the log/linear shoulder [11] and the Weibull equations, cast in the form of Peleg and Cole [12]; the choice of the models has been done on the basis of trend of the data and the results of a previous paper [8]. Moreover, we propose a reparameterised version of the Weibull function, to assess the death time of the pathogen on tomato slices.

2. Materials and Methods

2.1. Microorganisms. This study focused on two aspergilli species purchased from DSMZ collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), *A. fumigatus* (DSMZ 819) and *E. nidulans* (DSMZ 820), and a foodborne pathogen, *E. coli* O157:H7, isolated from a clinical case and belonging to the Culture Collection of the Department of Food Science (Foggia University).

Fungi were revitalized on Potato Dextrose Agar (PDA) (Oxoid, Milan, Italy), according to the producer instructions, stored on PDA plates at 4°C and monthly transferred.

E. coli O157:H7 was maintained at 4°C on Plate Count Agar slants (PCA) (Oxoid) and revived monthly on the same medium (incubated at 37°C for 24–48 hours); the working cultures were grown at 37°C for 48 hours in Plate Count broth (PCb) (Oxoid) and then diluted in a saline solution (0.9% NaCl) to 10⁶ cfu/mL (working cultures).

2.2. Preparation of Spore Suspension. Fungi were grown on PDA plates, incubated at 25°C for 7 days; then, the plates were washed through a Tween 80 solution (0.05% v/v) (C. Erba, Milan, Italy), as described by Sinigaglia et al. [13].

Spore concentration was evaluated through the spread plate method on PDA plates (incubated at 25°C for 5 days), after diluting the suspension in the saline solution.

Before each assay, spore suspensions were diluted in the Tween 80 solution to ca. 10⁶ spores/mL.

2.3. Samples Preparation. Raw tomatoes, belonging to IGNAZIA F.1 variety, were purchased from local vendors in Foggia (Italy); the tomatoes were processed at the turning stage (i.e., more than 10% of the surface but less than 30% show a definite change in colour from green to tannish-yellow, pink, red, or a combination thereof). Tomatoes, from which the stems had been removed, were washed with an aqueous solution of chlorine (200 ppm) for 30 minutes and then with tap water, air-dried for 30 minutes and cut in four portions of approximately 20 g. The first washing with the aqueous solution of chlorine was performed to inactivate the natural populations of coliform and lactic acid bacteria and avoid a possible interference of these microorganisms with *E. coli* and moulds. The treatment reduced the natural microflora of tomatoes up to the undetectable level.

Then, each portion was inoculated both on the surface and inner section with *A. fumigatus* or *E. nidulans* spores (ca. 10³–10⁴ spores/g on tomato slices) using a sterile swab and packed in plastic bags (nylon/polyethylene, 90 µm) (Tecnovac, San Paolo D'Argon, Bergamo, Italy) by means of S100-Tecnovac equipment. The bags were 170-mm/250-mm long with properties specified by the manufacturer as follows: CO₂ and O₂ permeability of 3.26 × 10⁻¹⁹ and 9.23 × 10⁻¹⁹ mol m/m²/s*Pa, respectively, and water vapour transmission rate of 1.62 × 10⁻¹⁰. Four portions of tomato slices were packed in the bag.

The samples were packaged in air and incubated at 15°C for 7 days. Moulds growth was confirmed periodically by plate counting on PDA, incubated at 25°C for 5 days.

After 7 days, the bags were opened and each tomato portion was inoculated with 200 µl of the working culture of *E. coli* O157:H7 in order to attain an inoculum on tomato slices of ca. 4–5 log cfu/g. The inoculum was applied on the inner section of tomatoes to be retained at the inoculation site and only tomato portions without visible growth of moulds were inoculated with the pathogen.

Then, the samples were packed in high-barrier plastic bags (a tomato slices for each bag) in air and stored at 4, 8 and 12°C for 9 days. Tomato portions, previously stored at 15°C for 7 days without fungi and inoculated with the pathogens and packed in air, were used as controls.

2.4. Microbiological Analyses. Tomato portions (20 g) were diluted with 180 mL of the sterile saline solution in a Stomacher bag (Seward, London, UK) and homogenised for 1 minute in a Stomacher Lab Blender 400 (Seward). Serial dilutions of tomatoes homogenates in saline solution were carried out and plated (pour plate method) into Violet Red Bile Agar (VRBA) (Oxoid) incubated at 37°C for 24 hours for the enumeration of *E. coli* O157:H7.

2.5. pH Evaluation. The measurement of pH was performed on a tomato homogenate, prepared by diluting 20 g

of the sample with 180 mL of the saline solution and then homogenised, through a Crison pH meter (Crison, Barcelona, Spain), as reported by Bevilacqua et al. [8, 14].

2.6. Statistical Analyses and Data Modeling. The experiments were performed in duplicate on two different batches, labeled A and B; for each batch the analyses were repeated twice ($n = 4$). Data were analyzed through the one-way analysis of variance (one-way ANOVA) and Tukey's test ($P < .05$) using the software Statistica for Windows, version 6.0 (Statsoft, Tulsa, OK).

Data regarding *E. coli* O157:H7 population were modeled using three different equations:

- (1) the equation of Geeraerd et al. [11]:

$$\log N = \log N_0 - k_{\max} * \frac{t}{\ln(10)} + \log_{10} \left\{ \frac{\exp(k_{\max} * SL)}{[1 + (\exp(k_{\max} * SL) - 1) * \exp(-k_{\max} * t)]} \right\}, \quad (1)$$

where $\log N$ and $\log N_0$ are the population at the time t and the initial cell number, respectively (log cfu/g), k_{\max} is the maximal death rate (log(cfu/g)/day), SL is the shoulder length (i.e., the time before the beginning of the exponential death rate), and t the time (days).

- (2) the model of Weibull, as modified by Peleg and Cole [12]:

$$\log N = \log N_0 - \left(\frac{t}{\delta} \right)^p, \quad (2)$$

where $\log N$ and $\log N_0$ are the population at the time t and the initial cell number, respectively (log cfu/g), δ is the first reduction time (days), that is, the time to attain a 1-log reduction in the population number, and p is the shape parameter, a not-dimensional number which gives some information of the geometrical shape of the curve.

- (3) a modified Weibull equation, cast in the following form:

$$\left(\frac{\log N}{\log N_0} \right) = 1 - \left(\frac{t}{\delta_{\text{stand}}} \right)^p, \quad (3)$$

where δ_{stand} is the death time (days) of the pathogen [8]. This equation reported a standardized measure of cell number as a not-dimensional parameter ($\log N / \log N_0$); it can be >1 (growth) or <1 (death).

For the evaluation of the fitting parameters of the three models, we used all the replicates for each data point (6 data points, replicated 4 times, generated 24 points for data fitting). The significance and the adequacy of the models were evaluated through the regression coefficient and the root mean sum of squared error (RMSSE).

3. Results and Discussion

As reported in the Introduction, this paper aimed to investigate the interactions between two aspergilli and *E. coli* O157:H7; the changes occurring in tomato slices were described through some nonlinear models. In a preliminary phase, we studied the suitability of a negative linear equation; however, this approach described satisfactorily the kinetic of *E. coli* in the control samples, but not the inactivation of the pathogen in tomato slices preinoculated with the moulds ($R^2 < 0.7$), as the population showed an initial shoulder phase. Therefore, some nonlinear functions were used, based also on the results of a previous paper [8].

The choice of a model to describe biological phenomena is a critical step in the field of predictive microbiology; in fact, every model shows both advantages and disadvantages. In particular, the empirical equations cannot be used universally, as each function can describe, or better fit, only an aspect amongst those connected with the changes occurring within a microbiological system. Therefore, an alternative approach could be the use of mechanistic models, derived from some basic principles and based upon biochemical and thermodynamical considerations. An example of this kind of approach is the S/P model, proposed by Van Impe et al. [15], describing the evolution of a population as a function of the exhaustion of a substrate or the production of a toxic end product.

However, this methodology is sometime difficult due to the complex mathematical approach. Therefore, in the past we used two or more empirical functions to fit the same set of data, in order to estimate a different parameter from each equation and try to give a full insight into the system [8, 16]. In this paper, we proposed the use of three different models (the shoulder model [11]; the equation of Weibull, cast in the form of Peleg and Cole [12] and a reparameterized version of the same model [8]).

The reason for the choice of these three models relies on the results of a previous research, focusing on the interactions occurring between *Fusarium* spp. and some foodborne pathogens [8]. In that paper we found that no empirical function could describe satisfactorily a complex phenomenon like the metabiosis, as the moulds acted significantly in different steps of the death kinetic of *E. coli* O157:H7, that is, at the beginning by prolonging the no-death phase of the microorganism, by slowing the death rate for the first 3-4 days, or slowing completely the death kinetic of the pathogen.

These three aspects can be described well by three different fitting parameters, that is, the shoulder length [11], the First Reduction Time (the parameter δ of the Weibull equation, cast in the form of Peleg and Cole [12]), and the death time (the parameter δ_{stand} , in the reparameterized version of the Weibull function proposed by Bevilacqua et al. [8]); hereby, these different models were used in combination, as means to describe different elements of the same trend. Finally, the advantage of using three equations for the evaluation of these fitting values stands also on the possibility of deriving for each of them the standard error, that is, their statistical significance.

TABLE 1: Death kinetics of *E. coli* O157:H7 on tomatoes preinoculated with *E. nidulans* DSMZ 820 and *A. fumigatus* DSMZ 819 and packaged in air: fitting parameters of the log linear + shoulder model.

	SL	k_{\max}	$\log N_0$	R^2	RMSSE
<i>E. nidulans</i>					
4°C	7.07 ± 0.49^A	$0.97 \pm 0.25^{A,B}$	4.58 ± 0.05^A	0.964	0.088
8°C	5.02 ± 0.64^B	$0.71 \pm 0.12^{A,B}$	4.61 ± 0.05^A	0.986	0.079
12°C	2.47 ± 0.79^C	0.60 ± 0.07^A	4.67 ± 0.07^A	0.991	0.086
<i>A. fumigatus</i>					
4°C	2.95 ± 0.18^C	0.51 ± 0.10^A	4.75 ± 0.08^A	0.981	0.099
8°C	2.91 ± 0.22^C	0.59 ± 0.12^A	4.75 ± 0.10^A	0.977	0.125
12°C	3.52 ± 0.18^C	$0.76 \pm 0.15^{A,B}$	4.72 ± 0.11^A	0.974	0.159
Control					
4°C	1.09 ± 1.00^D	1.55 ± 0.27^B	4.76 ± 0.52^A	0.959	0.580
8°C	0.29 ± 0.16^D	$1.42 \pm 0.26^{A,B}$	4.78 ± 0.62^A	0.949	0.640
12°C	0.59 ± 0.19^D	$1.46 \pm 0.24^{A,B}$	4.77 ± 0.53^A	0.961	0.558

*SL, shoulder length, is time before the beginning of the death phase of *E. coli* O157:H7 (days); k_{\max} : maximal death rate (log(cfu/g)/day); $\log N_0$: initial cell number of *E. coli* O157:H7 (log cfu/g); R^2 : regression coefficient; RMSSE: root mean sum of squared error.

^{A,B}The letters indicate the significant differences in a column: values with different superscripts are significantly different ($P < .05$) (one-way ANOVA and Tukey's test).

TABLE 2: Fitting parameters of the Weibull function, in its classical [12] and reparameterized forms [8], regarding the death kinetic of *E. coli* O157:H7 on tomato portions preinoculated with *E. nidulans* DSMZ 820 and *A. fumigatus* DSMZ 819.

	p^*	δ	R^2	RMSSE	$\delta_{\text{stand}}^{**}$	R^2	RMSSE
<i>E. nidulans</i>							
4°C	4.48 ± 1.39^A	9.27 ± 0.21^A	0.958	0.088	13.05 ± 1.55^A	0.964	0.019
8°C	$2.37 \pm 0.48^{A,B}$	$8.18 \pm 0.29^{A,B}$	0.984	0.083	15.61 ± 1.82^A	0.984	0.018
12°C	1.43 ± 0.24^B	6.18 ± 0.49^B	0.989	0.093	14.09 ± 2.13^A	0.989	0.020
<i>A. fumigatus</i>							
4°C	1.54 ± 0.33^B	7.35 ± 0.51^B	0.983	0.095	20.09 ± 3.48^B	0.983	0.020
8°C	1.61 ± 0.37^B	6.80 ± 0.59^B	0.979	0.119	17.82 ± 2.92^A	0.979	0.025
12°C	2.02 ± 0.45^B	6.62 ± 0.52^B	0.982	0.134	14.22 ± 2.54^A	0.982	0.028
Control							
4°C	1.11 ± 0.38^B	1.97 ± 0.10^C	0.951	0.632	7.79 ± 1.08^C	0.951	0.136
8°C	0.92 ± 0.31^B	1.42 ± 0.94^C	0.950	0.636	7.52 ± 1.29^C	0.950	0.137
12°C	1.01 ± 0.21^B	1.68 ± 0.94^C	0.958	0.580	7.72 ± 1.10^C	0.958	0.125

* p : shape parameter; δ : first reduction time (days) (time to attain a 1-log reduction in the cell number of *E. coli* O157:H7); R^2 : regression coefficient; RMSSE: root mean sum of squared error.

** δ_{stand} : death time of *E. coli* O157:H7 (days), evaluated through the Weibull function, cast in the standardized form; R^2 : regression coefficient; RMSSE: root mean sum squared error.

^{A,B}The letters indicate the significant differences in a column: values with different superscripts are significantly different ($P < .05$) (one-way ANOVA and Tukey's test).

Table 1 reports the parameters of the log-linear/shoulder model referred to *E. coli* O157:H7, both in the samples preinoculated with *E. nidulans* and *A. fumigatus* and in the controls; the regression coefficients (>0.949) and the RMSSE values underline the suitability of the proposed approach. The preinoculation of tomatoes with *E. nidulans* prolonged significantly the shoulder length of *E. coli* O157:H7 (SL); the metabiotic effect, however, seemed to be enhanced by the refrigeration, as the SL increased with the decrease of the storage temperature and the maximum value (7.07 days) was observed in the sample stored at 4°C. The effect of *E. nidulans* on the maximal death rate (k_{\max}) was not significant ($P > .05$).

As regards the metabiotic effect of *A. fumigatus*, SL values of *E. coli* O157:H7 (Table 1) were ca. 3 days on tomato portions preinoculated with the mould; the one-way ANOVA revealed a significant difference with the values of the controls (0.29–1.29 days), but not amongst the sample stored at different temperatures. As evidenced for *E. nidulans*, the preinoculation of tomato with the mould did not affect the death rate of the pathogen.

The survival of *E. coli* O157:H7 on tomatoes was modeled also through a second function (the Weibull model), to estimate the δ parameter (time to attain a 1-log reduction in the population) (Table 2). Weibull function, reparameterized by Peleg and Cole [12], is a classical survival curve and

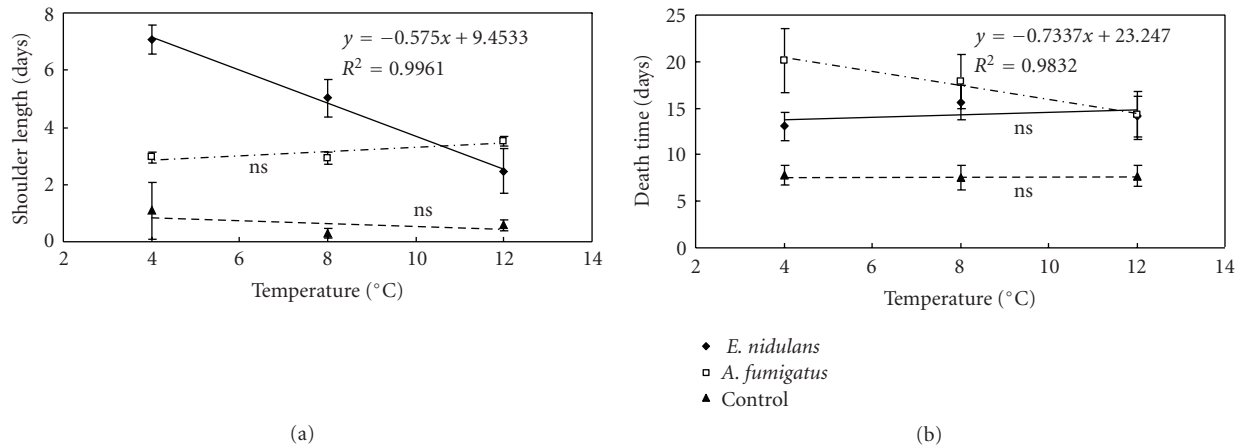


FIGURE 1: Effect of the storage temperature on the shoulder length (a) and death time (b) of *E. coli*, preinoculated with *E. nidulans* and *A. fumigatus*. Bars represent the standard error, whereas the line is the trend stroke obtained through the linear regression (ns, regression not significant).

covers many shapes of the death kinetics, depending on the “shape parameter” (p). A $p > 1$ stands for a downward curve, that is, a death kinetic characterized by a “slow” initial decrease of cell number; on the other hand, a $p < 1$ can be recovered in an upward curve, where the microorganism under investigation undergoes to a drastic decrease in the initial phases, followed by a phase with a reduced death rate. Finally, the Weibull model covers also the classical linear death kinetic (Esty function), when p is 1.

The use of the Weibull model confirmed that *E. coli* O157:H7 experienced a downward-death kinetic in the samples preinoculated with *E. nidulans*, thus highlighting the presence of a shoulder phase (Table 2); however, this second model was a useful mean to study the effect of the mould at the “beginning of the death kinetic” through the use of the parameter δ . The preinoculation of tomato-slices with *E. nidulans* increased significantly the 1-log reduction time of *E. coli* O157:H7; for example, at 12°C this parameter was increased from 1.68 days in the control to 6.18 days; moreover, the storage temperature exerted a significant effect, as the 1-log reduction time achieved the maximum value (9.27 days) under refrigeration. Finally, the reparameterization of the Weibull equation, as proposed in a previous paper [8], could be considered a useful mean for the evaluation of the death time of the pathogen (δ_{stand} , 13–15.61 days in the samples preinoculated with the mould and ca. 7.5 days in the controls).

As regards the metabiotic effect of *A. fumigatus*, the pre-inoculation of tomato slices with the mould resulted in a prolongation of both the 1-log reduction time (ca. 7 days) and the death time (14.22–20.09 days); focusing on the latter parameter, the storage temperature exerted a significant influence, as the statistical analysis revealed that the death time achieved the maximum value at 4°C.

Figure 1 shows the effect of the storage temperature on the shoulder length and death time of *E. coli* O157:H7. As evidenced by the linear regression, the first parameter was influenced by the storage temperature only in the case of *E. nidulans* and decreased by 0.575 days for an increase of

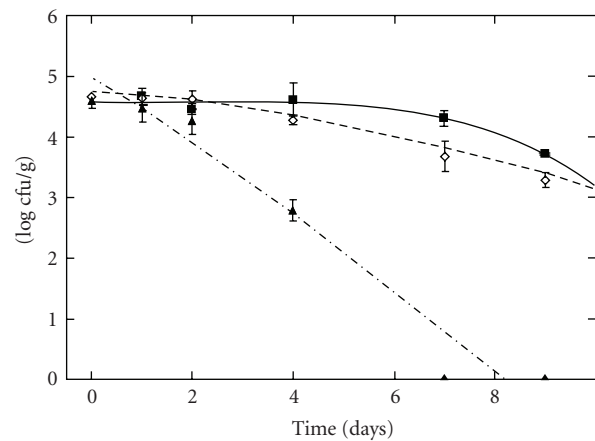


FIGURE 2: Death kinetic of *E. coli* O157:H7 on tomato portions, preinoculated with *E. nidulans* and *A. fumigatus*, stored at 4°C. Data are the mean \pm SD; the lines are the best fit obtained through the classical Weibull equation. ■: samples inoculated with *E. nidulans*; ◇: samples inoculated with *A. fumigatus*; ▲: control.

the temperature of 1°C; moreover, the temperature exerted a significant effect on *E. coli* O157:H7 death time in the samples inoculated with *A. fumigatus*, as it was shortened by 0.734 days for an increase of the storage temperature of 1°C.

As an example, Figure 2 shows the death kinetics experienced by *E. coli* O157:H7 in tomato slices pre-inoculated with *E. nidulans* and *A. fumigatus* and kept at 4°C.

pH values of tomatoes (4.0–4.4) did not undergo any significant changes throughout the storage period, both in the controls and in the samples pre-inoculated with the moulds (data not shown).

Nowadays it is generally accepted that acidic fruits could be a potential vehicle of foodborne pathogens. Numerous pathogens, in fact, have been isolated from fruits and vegetables, implicated in some cases of outbreaks in the last years [1]; on the other hand, moulds are overwhelmingly responsible for postharvest diseases of fruits [17]. Another

topic of great concern is the possible metabiotic effects occurring between fungi and foodborne pathogens; they could be regarded as a public health, because the moulds could produce some compounds, like ammonia or amines, that increase the pH and favor bacterial survival [4]. In addition to this activity, known as “alkalinizing effect of the moulds”, it has been suggested that the retention of viability of some pathogens could be enhanced by the production of cellulases and pectinases by phytopathogens; these enzymes cause the release of fluids from plant tissues [18]. The release of cellular fluids as a result of breakdown of the structural integrity of tissues would favor movement of foodborne pathogens from wounded and decayed tissues to otherwise sound tissue on the same or adjacent fruits.

To give an idea of the significance and impact of metabiosis on human health, we could report some data cited in the paper of Wells and Butterfield [19]; these authors reported that a study of healthy and soft-rotted fruits and vegetables in retail markets revealed that the incidence of *Salmonella* that had undergone bacterial soft rot was twice than that of healthy samples.

In a preliminary phase, *A. fumigatus* and *E. nidulans* showed a proteolytic activity on Gelatine Agar (data not published) and increased the pH of Tomato Agar by 0.45–0.56 unit [20]. Compared to the alkalinizing effect of some fusaria (*F. graminearum*, *F. avenaceum*, *F. proliferatum*), the proteolytic activity of the two strains of aspergilli used throughout this research appeared moderate; however, an experiment conducted in a model system (a laboratory medium added with tomato juice) revealed that the increase of the pH of the medium due to the metabolism of a mould (*F. oxysporum*) enhanced significantly the survival of *Salmonella* sp. Elsewhere [8], we studied the metabiotic effect occurring between *F. proliferatum*, *F. avenaceum*, and *F. oxysporum* and *E. coli* O157:H7; the survival of the pathogen was enhanced, without any increase of the pH of the medium, thus suggesting that a metabiotic effect could be due to some metabolites different from alkalinizing compounds and to a moderate pectinolytic activity exerted by moulds. The data hereby reported confirmed this hypothesis, as *E. coli* O157:H7 survival was not related to an increase of the pH, that remained unchanged throughout the running time. What happened into the systems and why we recovered a significant retention of pathogen survival, as a consequence of mould inoculation, could be an interesting matter for further researches, but hereby we can only report the result, without any additional evidence to give an insight into the system.

In addition, what we can say is that the use of three different models highlighted that the pre-inoculation of the moulds seemed to enhance the survival of *E. coli* O157:H7 throughout the first days of storage, as evidenced by the prolongation of the SL and the 1-log reduction time; otherwise, both *E. nidulans* and *A. fumigatus* did not affect the death rate of the pathogen, thus suggesting a kind of transitory protective effect after which the pathogen began to die.

Another interesting result is that the metabiotic effect seemed to be strengthened by the refrigeration; this result,

however, recovered for *E. nidulans* and to some extents for *A. fumigatus* needs to be validated by further studies as this is the first time that a possible effect of the storage temperature on metabiosis has been evidenced.

4. Conclusions

This research showed that a metabiotic interaction could occur between *E. nidulans*/*A. fumigatus* and *E. coli* O157:H7 on minimally processed tomatoes. This effect resulted in a prolongation of the shoulder length and death time of the pathogen, without any significant influence on the death rate, thus suggesting a kind of protective effect exerted by moulds at the beginning of the storage. Moreover, the metabiotic effect appeared to be affected by the storage temperature, as both SL and death time achieved the maximum values under refrigeration.

In conclusion, the results of this paper show that a metabiotic effect exerted by both *E. nidulans* and *A. fumigatus* on *E. coli* O157:H7 could have a strong impact on public health; aspergilli, in fact, are overwhelmingly diffused and the consumption of tomatoes (or other fruits and vegetables) contaminated both by aspergilli and *E. coli* is a possible scenario.

References

- [1] L. R. Beuchat, “Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables,” *Microbes Infection*, vol. 4, no. 4, pp. 413–423, 2002.
- [2] F. A. Draughon, S. Chen, and J. O. Mundt, “Metabolic association of *Fusarium*, *Alternaria* and *Rhizoctonia* with *Clostridium botulinum* in fresh tomatoes,” *Journal of Food Science*, vol. 53, pp. 120–123, 1988.
- [3] W. N. Wade, R. Vasdinnyei, T. Deak, and L. R. Beuchat, “Proteolytic yeasts isolated from raw, ripe tomatoes and metabiotic association of *Geotrichum candidum* with *Salmonella*,” *International Journal of Food Microbiology*, vol. 86, no. 1–2, pp. 101–111, 2003.
- [4] W. N. Wade and L. R. Beuchat, “Proteolytic fungi isolated from decayed and damaged raw tomatoes and implications associated with changes in pericarp pH favorable for survival and growth of foodborne pathogens,” *Journal of Food Protection*, vol. 66, no. 6, pp. 911–917, 2003.
- [5] L. Gram, L. Ravn, M. Rasch, J. Bartholin Bruhn, A. B. Christensen, and M. Givskov, “Food-spoilage-interactions between food spoilage bacteria,” *International Journal of Food Microbiology*, vol. 78, pp. 79–97, 2002.
- [6] J. O. Mundt, “Effect of mold growth on the pH of tomato juice,” *Journal of Food Protection*, vol. 41, pp. 267–268, 1978.
- [7] D. C. R. Riordan, G. M. Sapers, and B. A. Annous, “The survival of *Escherichia coli* o157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces,” *Journal of Food Protection*, vol. 63, no. 12, pp. 1637–1642, 2000.
- [8] A. Bevilacqua, F. Cibelli, D. Cardillo, C. Altieri, and M. Sinigaglia, “Metabiotic effects of *Fusarium* spp. on *Escherichia coli* O157:H7 and *Listeria monocytogenes* on raw portioned tomatoes,” *Journal of Food Protection*, vol. 71, pp. 1366–1371, 2008.

- [9] A. H. Geeraerd, V. P. Valdramidis, and J. F. Van Impe, "GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves," *International Journal of Food Microbiology*, vol. 102, pp. 95–105, 2005.
- [10] M. R. Corbo, A. Bevilacqua, D. Campaniello, D. D'Amato, B. Speranza, and M. Sinigaglia, "Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches-a review," *International Journal of Food Science and Technology*, vol. 44, no. 2, pp. 223–241, 2009.
- [11] A. H. Geeraerd, C. H. Herremans, and J. F. Van Impe, "Structural model requirements to describe microbial inactivation during a mild heat treatment," *International Journal of Food Microbiology*, vol. 59, pp. 185–209, 2000.
- [12] M. Peleg and M. B. Cole, "Estimating the survival of *Clostridium botulinum* spores during heat treatments," *Journal of Food Protection*, vol. 63, no. 2, pp. 190–195, 2000.
- [13] M. Sinigaglia, M. R. Corbo, and C. Ciccarone, "Influence of temperature, pH and water activity on "in vitro" inhibition of *Penicillium glabrum* (Wehmer) Westling by yeasts," *Microbiological Research*, vol. 153, pp. 137–143, 1998.
- [14] A. Bevilacqua, M. R. Corbo, and M. Sinigaglia, "Combined effects of modified atmosphere and thymol for prolonging the shelf-life of caprese salad," *Journal of Food Protection*, vol. 70, pp. 722–728, 2007.
- [15] J. F. Van Impe, F. Poschet, A. H. Geeraerd, and K. M. Vereecken, "Towards a novel class of predictive microbial growth models," *International Journal of Food Microbiology*, vol. 100, pp. 97–105, 2005.
- [16] C. Altieri, A. Bevilacqua, D. D'Amato, M. A. Del Nobile, and M. Sinigaglia, "Modelling the survival of starter lactic acid bacteria and *Bifidobacterium bifidum* in single and simultaneous cultures," *Food Microbiology*, vol. 25, pp. 729–735, 2008.
- [17] N. F. Sommer, R. J. Fortlage, and D. C. Edwards, "Post-harvest diseases of selected commodities," in *Post-Harvest Technology of Horticultural Crops*, A. A. Kader, Ed., UC Publication no. 3311, pp. 117–160, Division of Agriculture and Natural Resources, University of California, Davis, Calif, USA, 1992.
- [18] J. I. Pitt and A. D. Hocking, *Fungi and Food-Spoilage*, vol. 593, Blackie Academic and Professional, London, UK, 1997.
- [19] J. M. Wells and J. E. Butterfield, "Salmonella contamination with soft rot of fresh fruits and vegetables in the market place," *Plant Disease*, vol. 81, no. 8, pp. 867–872, 1997.
- [20] F. Cibelli, C. Ciccarone, C. Altieri, A. Bevilacqua, and M. Sinigaglia, "Proteolytic activity of molds and their metabiotic association with *Salmonella* in a model system," *Journal of Food Protection*, vol. 71, no. 10, pp. 2129–2132, 2008.