

Experimental Study of the Bactericidal Function of a Bio-Film for Products Fruit and Vegetables

Peppe Luigi *, Peppe Mario * and Peppe Luca

* Millenium S.r.l.- Via Piemonte, 1 - 04022 Fondi (LT), Italy

This article is distributed under the Creative Commons by-nc-nd Attribution License.
Copyright © 2023 Hikari Ltd.

Abstract

The objective of this experimental study was to verify whether synthetic antibacterial films were capable of manifest a bactericidal power that distinguishes them from the untreated reference films inserted, simulating the real situation that occurs in a common fruit and vegetable product, present on the market field of this protective device may lead to a future use in contact with food of the type of fruit and vegetables and other products.

In the research, therefore, methods and methods were used in a planned way, with recordings of both process followed by the results that led to the development of new models to be able subsequently put on the market in the following years.

As regards the results obtained by the Millenium company, while the codification of knowledge e their diffusion are part of the usual practice in universities and research institutes (although restrictions exist for knowledge arising from contract or partnership work),

in the commercial sphere the results will be protected by secrecy or other means of protection of the intellectual property. However, it is expected that the process and the results will be recorded for use by other researchers and company employees.

Keywords: sustainability, monitoring, bio-film, bactericidal

1. Introduction

In recent years, the Italian system has engaged in an important redevelopment process, aimed at building competitive advantages capable of lasting beyond the short term. The growing integration of world economies and the introduction of the euro, in fact, have made the ability to compete on global markets an essential condition for the very survival of companies, amplifying the importance of factors

that have a positive impact on productivity and competitiveness, such as the quality of human capital, the diffusion of scientific and technological research, innovation, the ability to create complex relationships with other companies and with end markets, even the furthest away. There are several areas of intervention within which to act to stimulate growth of the Italian production system: human capital and innovation.

The internal context of the Company can be considered the environment in which it aims to achieve its objectives. The internal context, therefore, refers to the governance approach, to its contractual relationships with its customers and its stakeholders. The aspects that are taken into consideration concern the culture, the beliefs, values or principles within the organization as well as the complexity of the processes and the organizational structure (human resources and infrastructure). In this sense, some aspects taken into account by the Organization concern:

- intellectual property;
- ethical issues;
- health and safety of workers;
- environmental aspects;
- human resources;
- technological resources and tools

Food packaging is defined as packaging capable of containing food, of any type typology they are, respecting and preserving their characteristics. Its function includes that of isolating the food from foreign bacteria and pollutants and of preventing the deterioration of the food material.

Specifically, natural antibacterial and polymeric packaging include natural antibacterial films and antibacterial polymer films.

Natural antibacterial films consist of naturally produced substances as metabolites, secondary from plant materials, such as flowers, buds, seeds, leaves, wood, fruits, roots, twigs and barks with antifungal, antioxidant and antibacterial action.

For synthetic antibacterial films, nanoparticles used as antimicrobials are used instead (silver nanoparticles, titanium dioxide, zinc oxide, magnesium oxide) or acids sorbic acid and benzoic acid and their salts.

The objective of this experimental study was to verify whether synthetic antibacterial films were in capable of demonstrating a bactericidal power that distinguishes them from untreated reference films inserted on easily biodegradable fruit and vegetable products, simulating the real situation that occurs in a common fruit and vegetable product, present on the market.

Field effectiveness of this protective device may lead to future use a contact with foods such as fruit and vegetables and other products.

2. Materials and methods

Traditional microbiological techniques, used for the detection of microorganisms in samples food and environmental (surfaces intended to come into contact with food, water and air), allow isolation by qualitative or quantitative methods: x Micro-

biological methods quantitative (or enumerative). The enumeration of the number of viable organisms if any present in the sample to be analyzed, is evaluated on the basis of the capacity that each single cell has to form a bacterial colony on Petri dishes containing the solid culture medium. The result is expressed as colony forming units (CFU), related to the unit of measurement used to define the size of the sample analysed: unit of weight (e.g. gram) for foods, unit of surface area (e.g. cm²) for surfaces intended to come into contact with food, unit of volume for water (e.g. ml) and air (e.g. m³) x Qualitative microbiological methods (presence/absence).

They consist in the classification of the sample under examination on the basis of objective evidence of presence or absence of the microorganism sought in the analysis sample. The result in this case is expressed as presence/absence in relation to the unit of measure used to define the size of the sample being analysed. Qualitative microbiological methods are applied for the search for those pathogenic microorganisms often present in low concentrations within the sample, which could hardly be identified using direct counting procedures.

Conceptually, the qualitative methods involve the use of enrichment procedures sample, followed by inoculation on selective culture media and confirmatory testing on colonies suspects (biochemical and serological tests) in order to discriminate the microorganism of interest from closely related microbial forms.

The tests took place on some main products including:

- Pachino tomato (also fresh consumable)
- On persimmons

For the study, the methodology used included 10x10 cm samples of a commercially sold food film with synthetic bactericidal action and a blank (untreated reference film). On these we inoculated the following pathogens:

- *Pseudomonas fluorescens*
- *Escherichia coli*

The analytical method was performed in triplicate, testing three films for each treated film and three films for the untreated reference film.

The test was performed with the support of SEA Tuscia Srl, at room temperature, in a temperature range of 15÷25°C.

For each film, one specimen for each species and one specimen with all the microorganisms together was contaminated.

For each film test, 9 10x10 specimens were prepared and placed in 9 sterile 140 mm Petri dishes in contact with the nutrient liquid (mozzarella whey). On the first 3 specimens 50 cfu/ml of *Pseudomonas fluorescens* were inoculated, on the following 3 specimens 10 cfu/ml of *Escherichia coli* were inoculated and on the last three the two microorganisms were inoculated together (50 cfu/ml *Pseudomonas fluorescens*, + 10 cfu/ml *Escherichia coli*) to also monitor any antagonism between the test microorganisms.

As has already been specified previously, the reason for the triplicate test is to confer statistical significance on the data.

The plates are stored at 9°C, a stress temperature higher than refrigeration, for 30 days.

Evaluated that a common mozzarella bought at the supermarket contains 85 ml of whey and is made up of 2 films adjacent to it, to simulate a real situation in the best possible way and therefore the real quantity of whey in contact with the film, was put in contact the film with 42.5 ml of liquid.

4. Results and Conclusion

It can be assumed that *Pseudomonas fluorescens*, inoculated at a concentration of 50 cfu/ml, at time 1 grows exponentially, due to the nutrient rooting medium and due to the incubation temperature of the samples (9°C) which is optimal for its growth. For this reason, the calculation of the abatement for *Pseudomonas fluorescens* was evaluated from T2 onwards.

Escherichia coli, on the other hand, inoculated at the minimum concentration of 10 cfu/g, were detected only after inoculation, about 70% of the 10 inoculated cfu, and only where they were inoculated individually. Together with the second pathogen, already post inoculation they were not detected and were completely inhibited in the treated test film and in the untreated film. *Escherichia coli* were strongly inhibited in the samples where they were inoculated together with *Pseudomonas fluorescens*, probably due to the high concentration of interfering flora. Furthermore, they were also inhibited where they were inoculated individually, probably due to the low quantity of inoculated colonies. The flora present in the sample from the mozzarella whey-like liquid product was always monitored as mesophilic bacterial load and always presented important concentrations. Ultimately, *Escherichia coli*, a gram negative microorganism, grows at a temperature of at least 37°C. One could also assume that the storage temperature did not allow any proliferation of the colonies. The non-proliferation datum could be positive for the treated film, but the fact that no colonies were found even on the REF film demonstrates that the microorganisms died not due to the effectiveness of the films. The *Escherichia coli* data was graphed only for the single inoculum, given that its presence was never detected for the multiple inoculum.

It can be asserted that the test cannot give scientifically valid results for the *Escherichia coli* parameter.

This film does not show a reduction of the microbial flora constituting the food. T2 presented results equivalent to T3. Instead, there was a slight reduction in the interfering flora and *Pseudomonas fluorescens* both in the contamination of the microorganisms individually and simultaneously.

The treated film always presented fluorescence visible to the naked eye.

However, the treated film responded more effectively than the REF film.

In conclusion, it can be stated that the challenge should be remodulated on the concentration of the inoculums, in the specific case of *Escherichia coli*. Furthermore, the interfering flora of the "mozzarella serum" nutrient liquid which becomes a substrate for *Pseudomonas fluorescens* must be evaluated.

References

- [1] Dahlbo, H., et al., Increasing textile circulation-Consequences and requirements, *Sustainable Production and Consumption*, **9** (2017), 44-57.
<https://doi.org/10.1016/j.spc.2016.06.005>
- [2] Monica, P., et al., Cap. 2. *Novel Antimicrobial Agents And Processes For Textile Applications*, R.N. Kumavath, Editor. 2017, INTECH.
<https://doi.org/10.5772/intechopen.68423>
- [3] De Amicis, B., et al. Sostanze Chimiche –Ambiente E Salute Il Reach e alter normative in materia di prodotti chimici. 2017 [cited 2019 11/10]; Available from: www.minambiente.it/sites/default/files/archivio/allegati/reach/reach_bollettino_numero3_ottobre2017_nanomateriali.pdf
- [4] Rai, M., et al., Broadening the spectrum of small-molecule antibacterials by metallic nanoparticles to overcome microbial resistance, *International Journal of Pharmaceutics*, **532** (2017), no. 1, 139-148.
<https://doi.org/10.1016/j.ijpharm.2017.08.127>
- [5] Hartemann, P., et al., Nanosilver: Safety, health and environmental effects and role in antimicrobial resistance, *Materials Today*, **18** (2015), no. 3, 122-123.
<https://doi.org/10.1016/j.mattod.2015.02.014>
- [6] Deshmukh, S.P., et al., Silver nanoparticles as an effective disinfectant: A review, *Materials Science & Engineering C*, **97** (2019), 954-965.
<https://doi.org/10.1016/j.msec.2018.12.102>
- [7] Bhatt, D., et al. Nanotechnology solutions to combat superbugs. 2013 [cited 2019; Available from: <https://www.nanowerk.com/spotlight/spotid=32188.php>
- [8] Rai, M., A. Yadav and A. Gade, Silver nanoparticles as a new generation of antimicrobials, *Biotechnology Advances*, **27** (2009), no. 1, 76-83.
<https://doi.org/10.1016/j.biotechadv.2008.09.002>
- [9] Ferraris, M., et al., Chemical, mechanical and antibacterial properties of silver nanocluster -silica composite coatings obtained by sputtering, *Advanced Engineering Materials*, **12** (2010), B276-B282.
<https://doi.org/10.1002/adem.200980076>
- [10] Schneider, G., Antimicrobial silver nanoparticles – regulatory situation in the European Union, *Materials Today: Proceedings*, **4** (2017), S200-S207.
<https://doi.org/10.1016/j.matpr.2017.09.187>

Received: February 1, 2023; Published: March 1, 2023