Lyofast CNBAP inhibits the charge of *Pseudomonas* spp. without repercussions on the sensory characteristics of the finished product.

Abstract

Among the contaminants often found in mozzarella cheese, *Pseudomonas spp*. represent the most frequent one, causing the reduction in shelf life, possible discolouration of the product, and consequent economic losses in dairy companies. A smart strategy to contain *Pseudomonas* spp. contamination during the mozzarella production is the addition of bacterial cultures tested to be able to contain the *Pseudomonas spp*. spreading: a commercial blend of *Carnobacterium spp*. was tested against a *Pseudomonas spp.* cocktail (constituted of strains mostly isolated from mozzarella) directly in mozzarella brine and resulted able to reduce the final *Pseudomonas spp.* count without impairing the sensory panel.

Introduction

The microbial spoilage of fresh cheeses is of great concern for the dairy industry because the changes in sensory characteristics of products reduce the quality, impair the reputation of the cheese manufacturers and may cause serious economic losses for dairy companies (del Olmo, et al., 2018).
mozzarella cheese is the most popular member of the pasta-filata cheese family: this diverse group undergoes a unique stretching process, which takes place in hot water at 65-85 °C. Subsequently, during the production, cheese balls are formed and allowed to cool in a second water bath at 14-15 °C before packaging in brine containing 0.45% salt.
The high moisture content of 50-60%, the pH value around 5.5 (mild acidity) and the low salt content, render Mozzarella cheese susceptible to growth of both spoilage and pathogenic microorganisms (De Candia et al., 2007).
Common contaminants of mozzarella cheese include *Pseudomonas* spp. which represents the main obstacle for extending the shelf-life of this kind of product.
Pseudomonas spp. are ubiquitous and psychrotolerant Gram-negative bacteria, frequently isolated from refrigerated raw milk. Although they are inactivated by pasteurization, *Pseudomonas* spp. can enter the processed products through post-pasteurization contamination from the dairy processing plants via raw materials, soil, and water. *Pseudomonas* spp. can survive and grow in different environments, it may colonize the surfaces of utensils and equipment used in dairy production lines forming biofilms onto the surfaces of dairy processing facilities which help these microorganisms to persist long in the environment.
The defects noted on mozzarella cheese due to their growth are caused by the release of enzymes or pigments, with negative impact on structure, texture and sensory properties of the products.
In order to reduce the initial microbial count, is essential the strict application of good hygiene and good manufacturing practices, but oftenthere is the need of additional strategies to control the growth of *Pseudomonas* spp.
The use of cultures with protective effect represents one potential intervention to enhance the safety of dairy products and to contain the spreading of undesired cultures. Most strains composing the cultures with protective effect belong to the group of lactic acid bacteria (LAB), which have a long history of safe use as starter cultures in food fermentations (Holzapfel et al., 1995). Therefore, the addition of cultures with protective effect to fermented products like cheese does not require additional labeling thus aligning with consumer demand for “clean-label” foods. Furthermore, commercial cultures with protective effect are often screened for desirable properties including

limited effects on the flavor and acidity of foods (Spanu et al., 2018). Among LAB, the genus *Carnobacterium* spp. was deeply studied for its ability to produce bacteriocins (Bhugaloo-Vial et al.,1996, 1999).
Carnobacteria are Gram positive rod shaped lactic acid bacteria (LAB) isolated from different ecological niches. thanks to its ability of producing bacteriocins, the genus *Carnobacterium*, has been used as protective culture to provide durability and safety to various types of foods (cheese, fish and meat products). Among the species included in the genus *Carnobacterium*, two, *C. divergens* and *C. maltaromaticum*, are frequently isolated from natural environment and dairy products.
The objective of this work was to investigate the ability of Lyofast CNBAP (Sacco, Cadorago Italy), a commercial freeze-dried blend of *C. divergens* and *C. maltaromaticum* to control the spread of *Pseudomonas* spp. in mozzarella cheese, artificially contaminated by this spoilage microorganism.

Material and methods

Pseudomonas strains and growth conditions

Pseudomonas strains used in this work are listed in Table 1.

Table 1: List of *Pseudomonas* spp. strains used to constitute the "*Pseudomonas cocktail*"

Species	Isolated from
Pseudomonas putida	Mozzarella cheese
Pseudomonas chlororaphis	Mozzarella cheese
Pseudomonas aeruginosa	Collection strain (provided by LGC standard)
Pseudomonas fluorescens	Mozzarella cheese
Pseudomonas fluorescens	Mozzarella cheese
Pseudomonas jessenii	Mozzarella cheese
Pseudomonas koreensis	Mozzarella cheese
Pseudomonas luteola	Water

Most of the strains were isolated directly from mozzarella cheese, one strain from water and one strain was provided by LGC standard. The strains species was confirmed through 16S rRNA sequencing.
Strains were subcultured twice in BHI broth (Thermo Scientific, Oxoid, UK) and incubated at 30°C overnight. Those strains were added in equal volume, creating a pool referred as *Pseudomonas* cocktail, which was used to contaminate Mozzarella brine.

Mozzarella contamination and experimental design

The study was conducted on mozzarella samples provided by a local industrial cheese-making plant. Mozzarella brine was inoculated with 1 x 10^3 CFU/ml of *Pseudomonas* spp. pool previously described (*Pseudomonas* cocktail).
After the inoculum of *Pseudomonas*, the brines were also inoculated with the culture with protective effect Lyofast CNBAP (Sacco, Cadorago, Italy). The freeze-dried powder, was diluted in phosphate-buffer in order to inoculate 1 x 10^6 CFU/ml in the product. Negative control (only contaminated with *Pseudomonas* spp.) was also set up. After the inoculation, all samples were incubate at 4°C for 20 days.

Triplicate samples of each mozzarella were analyzed the day of inoculation (T0), and after 11 and 20 days of storage at 4° C. At each time point, the samples were analyzed for the determination of *Pseudomonas* spp. count.
Mozzarella samples were first homogenized in phosphate buffer solution; the homogenized samples were serially diluted in sterile phosphate buffer, then 100 µl were spread plated in duplicate on Pseudomonas Cetrimide Agar (Thermo Scientific, Oxoid, UK) and incubated at 30 °C for 48 h.

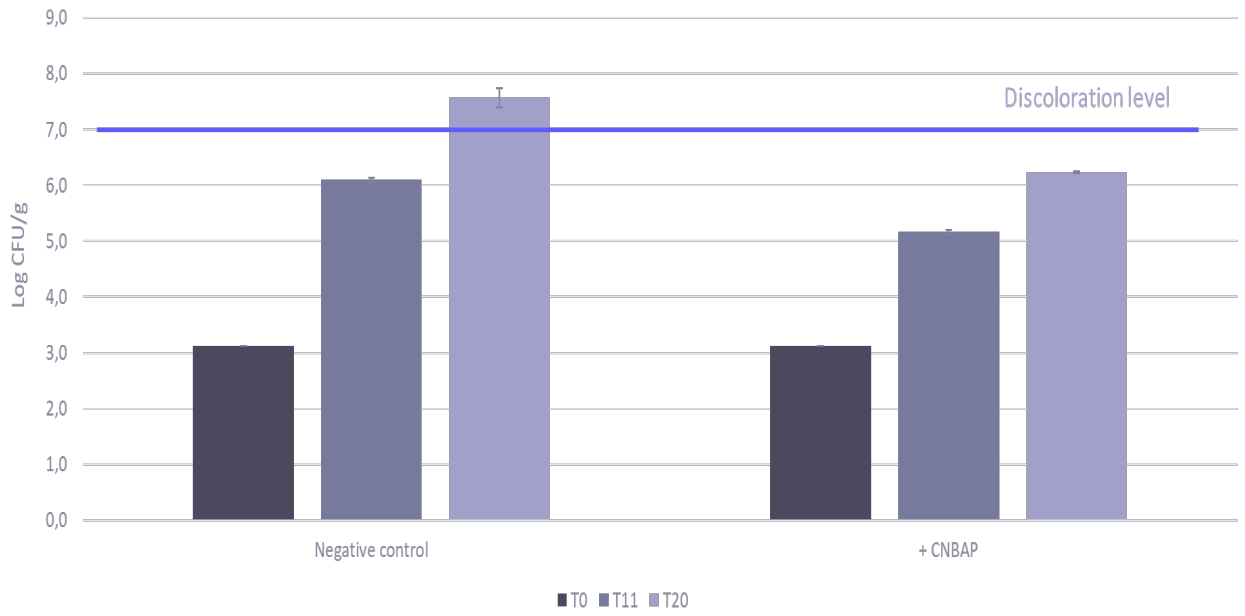
Sensory evaluation

In order to exclude the negative impact of the addition of Lyofast CNBAP in mozzarella, a sensory evaluation was carried out by a trained panel.

Results and Discussions

During the entire shelf life, *Pseudomonas* count was monitored.
Pseudomonas cocktail grew to level as high as 7 log CFU/g after 20 days of refrigerated storage (Figure 1).
The use of the culture with protective effect Lyofast CNBAP was effective in reducing *Pseudomonas* spp concentration, respectively of 0.93 log CFU/g and 1.3 log CFU/g after 11 and 20 days of storage (Figure 1), thus mantaining the contamination level under the "discoloration level" of 7 Log CFU/g (Carminati et al 2019).

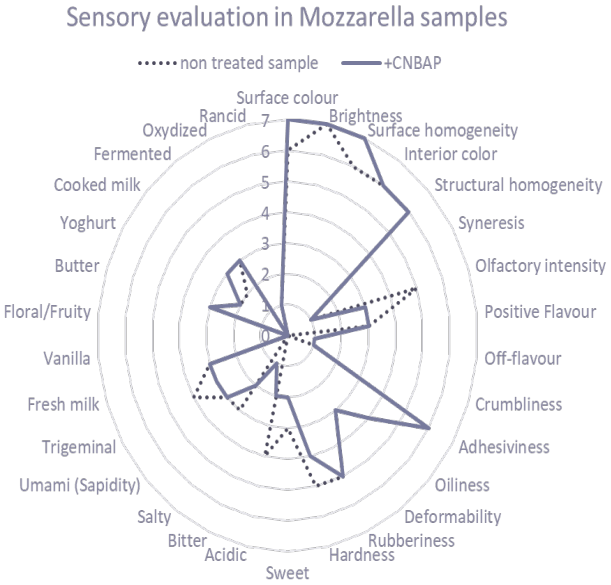
Table 2: Evolution of *Pseudomonas* cocktail growth (log10 CFU/g; x ± SD) in mozzarella during refrigerated storage



Mozzarella is particularly susceptible of secondary contamination from the processing environment and represents an excellent substrate for the growth of psychotropic pathogenic and spoilage microorganisms, since it's not too acid and the brine has a contained NaCl concentration and therefore it is necessary to find a method to protect this substrate.

A possible strategy is the use of microbiological cultures aimed to control the multiplication of contaminants. The study, conducted on "artificially" contaminated Mozzarella, indicated *Carnobacterium* spp as a possible culture with protective effect to be used to contain the growth of *Pseudomonas* spp.
The sensory evaluation of the not contaminated Mozzarella and the Lyofast CNBAP treated one (+ CNBAP) confirmed that the culture with protective effect did not change the aromatic pathway and the texture of the treated samples (Figure 2)

Figure 2: Sensory evaluation of Mozzarella samples.



Conclusions:

Lyofast CNBAP can survive and grow in conditions of pH, NaCl concentration, temperature similarly to the psychotropic spoilage microorganisms: thus, the addition of CNBAP to the Mozzarella brine during the shelf life can limit the growth of *Pseudomonas* spp., thus improving the durability of chilled foods.

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