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Listeria monocytogenes in Gorgonzola cheese: Study of the behaviour throughout the process and growth prediction during shelf life

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ABSTRACT

As reported on *RASFF*'s portal, in the first 9 months of 2016, a total of 13 "alerts/information for attention" were issued concerning the presence of *Listeria monocytogenes* in mould cheeses throughout Europe. This study analyzes the behaviour of *L. monocytogenes* in Gorgonzola cheese, a typical Italian soft blue-veined cheese, when contaminated at different time points. In the first challenge test, the pasteurized milk was contaminated and the complete cheese manufacture (cheesemaking, ripening) and shelf life was simulated.

After a decrease during the first days of the cheesemaking, the pH remained constant for 35 days (5 weeks) and then it increased rapidly reaching the final values of 6.8 ± 0.02 in the core and 5.8 ± 0.4 on the rind. At the same time, the pathogen concentration decreased (about 2 log CFU/g), although during the last week a rapid pathogen growth was observed after the rise in pH values.

When the cheese was stored at thermal abuse condition (8–12 °C), the pathogen concentration on the rind was 4.8 ± 0.3 log CFU/g and after 66 days (about 9 weeks) no significant difference ($p > 0.05$) was observed; whereas, a growth from 5.4 ± 0.4 to 7.1 ± 0.5 log CFU/g was observed in the core. A second challenge test was performed using three batches of commercial slices of Gorgonzola cheese inoculated by *L. monocytogenes* and stored at 8 °C. The maximum specific growth rates (μ_{max}, 1/h) of *L. monocytogenes* estimated ranged from 0.007 to 0.061. The square root model was used to predict the μ_{max} at others temperature and to establish the time necessary to reach the European critical legal limit of 2 log CFU/g, in different storage scenarios. The predictions obtained in this study can be applied to any time-temperature profile, and in particular to the conditions to which the product is most likely to be subject in normal use, up to its final consumption. This study can be considered a valuable contribution also aimed at supporting the monitoring surveys carried out by officers of the Regional Veterinary Authority.

1. Introduction

The contamination of some varieties of cheeses with *Listeria monocytogenes* is an important problem for consumers' health, as well as for the consequent substantial industrial financial losses (Cocolin et al., 2009; de Cesare et al., 2007; Lomonaco et al., 2009; Rudolf and Scherer, 2001). The presence of *L. monocytogenes* in cheeses can be due to various factors, such as contaminated raw milk, unsatisfactory pasteurization treatment or contamination after the heat treatment (i.e. in ripening rooms or during transportation from the processing plant to ripening facilities) (Lomonaco et al., 2009). Among dairy products, ripened soft cheeses, such as smear or mould surface-ripened, and blue-veined cheeses are known to be the most frequently contaminated (Beckers et al., 1987; Bernini et al., 2013; Griffiths, 1989). As reported by the *Rapid Alert System for Food and Feed* portal (http://ec.europa.eu/food/safety/rasff/index_en.htm), in the first 9 months of 2016, a total of 45 "alerts/information for attention" were issued concerning the presence of *L. monocytogenes* in milk and milk products, 13 of which

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involved mould cheeses (EC, 2010).

When pasteurized milk is used, contamination mainly concerns the rinds, as a consequence of cross-contaminations during handling and contact with the ripening environments (Canillac and Mourey, 1993; Carminati et al., 2000; Frye and Donnelly, 2005; Terplan, 1990). In fact, during cheese ripening, complex microbial communities develop on the surface of some types of cheeses.

At the beginning of the cheesemaking, the lactic acid bacteria grow rapidly and produce acid causing a decrease in pH values. Furthermore, the development of moulds occurs during the first weeks of ripening: yeasts metabolize the lactate completely into CO_2 and H_2O forming alkaline metabolites, such as ammonia, inducing a pH increase on the surface (Bonaïti et al., 2004; van den Tempel and Nielsen, 2000). This deacidification can increase the possibilities of survival and growth of *L. monocytogenes* on the cheese rind.

One of the most well-known blue-veined cheeses is Gorgonzola, a Protected Designation of Origin (PDO) Italian product. Gorgonzola is a mould-ripened cheese characterised by a pH ranging from 4.5 to 6.5 due to the alternative growth of different microrganisms like the lactic acid bacteria and the moulds who acidify or deacidify the cheese as reported above. It is made with pasteurized cow's milk inoculated with *Lactobacillus* and *Streptococcus* starter cultures, together with *Penicillium roqueforti* that develops as an internal blue-green mould (Mucchetti and Neviani, 2006; Gripon and Hubert, 2002). Given the economic importance of Gorgonzola cheese (CPGC, 2014) and its worldwide commercialization, it is of great interest to understand the behaviour of *L. monocytogenes* in these cheeses so as to ensure consumers' safety. In the case of Gorgonzola cheese, the contamination, if present, is limited to the rind, it contains a small number of cells and does not affect the internal portion (Bernini et al., 2013). However, it is not possible to exclude the transferring of the pathogen to the internal portion during cutting and portioning; the dragging is directly proportional to the contamination level of the rinds (Bernini et al., 2016).

The EC Regulation 2073 (EC, 2005) states that Food Business Operators (FBO) are required to carry out studies aimed at assessing the growth of *L. monocytogenes* in specific products during shelf life, under reasonably foreseeable conditions of storage and distribution. The mentioned regulation establishes that 2 log CFU/g for Ready To Eat (RTE) foods "able to support the growth of *L. monocytogenes*" is the critical limit that must be satisfied at the end of the shelf life (EC, 2005). The EC and the Codex Alimentarius (1999) endorse the use of predictive microbiology in order to ensure food safety by predicting pathogen dynamics with reference to growth, survival or death. Predicting the behaviour of microorganisms in foods is a challenge carried out by many public and privately employed microbiologists for the benefit of consumers' health and well-being (Havelaar et al., 2010; McMeekin et al., 2006).

In this study, two different challenge tests were carried out with *L. monocytogenes*. The first challenge test was carried out assuming a post-pasteurization contamination of milk, i.e. at the beginning of the Gorgonzola cheese process. The pathogen behaviour was assessed in the core and on the rind both during the cheesemaking and ripening process and during the shelf life of sliced cheese stored at a dynamic temperature (8–12 °C). The second challenge test was carried out assuming a post-processing contamination of cheese, i.e. during portioning of ripened cheese. In this case, slices of commercial Gorgonzola cheese were contaminated to evaluate pathogen behaviour during shelf life at a constant temperature (8 °C).

The objectives were i) to evaluate the effect of physicochemical and microbiological changes on the behaviour of *L. monocytogenes* during the Gorgonzola process in order to increase knowledge concerning the impact of the process on the survival of pathogenic microorganisms, and ii) to calculate the specific growth rate of *L. monocytogenes* in sliced Gorgonzola cheese during shelf life at a constant temperature so as to predict the pathogen behaviour at a dynamic storage temperature in accordance with the *Technical Guidance Document on shelf-life studies for L. monocytogenes in ready-to-eat foods* (EURL, 2014).

2. Materials and methods

2.1. Bacterial cultures

In the first challenge test, three *L. monocytogenes* strains (type strain: ATCC® 19115™, serotype 4b; product isolates: Lm273250, serotype 1/2a, isolated from Gorgonzola cheese and Lm242382/9, serotype unknown, isolated from environmental swab) were used to contaminate the pasteurized milk. The bacterial cultures were prepared following what indicated by Dalzini et al. (2015). Before use, the individual strains were combined in equal volumes in order to obtain a multi-strain cocktail. In the second challenge test, two *L. monocytogenes* bacterial cultures (ATCC® 19115™ and Lm273250) were prepared according to EURL technical document (2014) and used separately to contaminate the sliced cheese. All product isolates, belonging to IZSLER's collection (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy).

2.2. Physicochemical and microbiological evolution during the manufacturing of Gorgonzola cheese: cheesemaking, ripening and shelf life

2.2.1. Milk inoculation

In order to assess the behaviour of *L. monocytogenes* and the changes in physicochemical and microbiological properties during the Gorgonzola cheese process (cheesemaking and ripening), a total of 400 L of milk were used in this experiment. The milk was provided by two different local farms and transported to IZSLER's laboratory where it was pasteurized (63 °C for 30 min). The milk was inoculated once cooled to 32 °C. One batch of 400 L of milk was used in this study: i) 200 L was inoculated with 1% *v/v* of multi-strain cocktail of *L. monocytogenes*, in order to give a final concentration ranging from 4 to 5 log CFU/mL, and was used to manufacture contaminated cheeses (a total of 2 cheeses); ii) 200 L was inoculated (1% v:v) with sterile physiological solution and was used to manufacture control cheeses (a total of 2 cheeses).

2.2.2. Gorgonzola process

The Gorgonzola cheese was manufactured in the pilot plant at the IZSLER's Institute, according to traditional production specifications. To produce the cheese, close collaboration with a local cheese making expert was needed. The milk was inoculated with starter culture (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Penicillum roqueforti*) and cheese rennet (84% ± 5% chymosin and 16% ± 5% pepsin) (Fermostart Integral®, Alce s.r.l., Italy) provided by local manufacturers, and incubated at 21 °C for the acidification and coagulation steps. After 30 min, the coagulum was cut into 2 cm cubes and let stand for 60 min to induce syneresis. The curd was transferred into round moulds (25 cm diameter and 18 cm height), and let stand at 18–20 °C for 72 h for a "stewing phase" in a warm chamber, turning the moulds every 2 h. Later, the cheeses were removed from the mould and the surface was brushed with sea salt. The ripening of the Gorgonzola cheese was carried out for 62 days (about 9 weeks) at 4 ± 2 °C with 90–95% Relative Humidity (RH) in a in a climatic chamber CC2000 model (Piardi, Italia). On day 15 and 25 of ripening, the cheese rind was pierced with needles to facilitate paste aeration and the development of the moulds inoculated.

To evaluate the behaviour of *L. monocytogenes* during shelf life at dynamic temperatures (8–12 °C), the cheese was cut into slices (200 g each), packaged in plastic trays and stored for 66 days. Dynamic temperatures were studied, so as to simulate different stages of the cold chain. Therefore, the packaged cheese was stored at 8 °C for 7 days to simulate the stage from the manufacture to the arrival at the display cabinet, and then at 12 °C for 56 days (8 weeks) to simulate a retail stage and consumers' storage, in accordance with EURL's technical