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Response of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus* to the Thermal Stress Occurring in Model Manufactures of Grana Padano Cheese

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ABSTRACT

The purpose of this research was to investigate the effect of temperature in the technology of production of Grana cheese against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*. According to the technology of production, the cheese curds are cooked at 55°C and then cooled at room temperature (25°C). A curd-cooling model was developed to estimate the temperature variation across the curd during cooling, and the thermal stress was applied to the pathogens according to the model in model-scale productions of Grana cheese artificially contaminated with approximately 10^4 cfu/mL of the selected pathogens. According to the numerical results, the initial temperature inside the cheese is kept at almost the initial value (above 50°C) for at least 4 h during cooling, whereas the crust of the curd cools rapidly to 30°C in the first hour. The best case was that of the core of the cheese where the high temperature was able to efficiently eliminate the contaminating pathogens. Moreover, the worst case was where the external ring of the curd in which a more rapid cooling allowed bacterial survival. Therefore, the thermal stress in the technology of production of Grana cheese can be only partially effective in the control of the selected pathogens. However, the whole technology of production includes other hurdles that can affect the survival of the pathogens and that need to be taken into account as a whole to evaluate the safety of Grana Padano cheese.
(**Key words:** food pathogen, raw milk cheese, Grana Padano cheese, thermal stress)

INTRODUCTION

A great variety of raw milk cheeses are produced through traditional dairy technologies and widespread throughout the world. Cheese quality is greatly influenced by the complex microbial flora from the raw milk, which, together with that arising from the processing environment, contributes to milk acidification, curd production, and ripening, often leading to final products with distinctive flavors and taste (Cogan et al., 1997; Grappin and Beuvier, 1997; Mauriello et al., 2003). However, the untreated milk can be contaminated by pathogenic microorganisms, thus compromising the safety of the cheese and representing a hazard for consumers. In fact, some dairy technologies have been changed after food poisoning outbreaks; for example, in Stilton cheese, a wide intoxication of hypothesized staphylococcal origin occurred in 1989, and as a result, the technology shifted from raw to pasteurized milk (Maguire et al., 1991).

Escherichia coli O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus* are important foodborne pathogens that are widely distributed throughout the environment. They have been associated with severe food poisoning outbreaks and are often found in milk and dairy products (De Valk et al., 2000; Rudol and Sherer, 2001; Araujo et al., 2002; Foschino et al., 2002; Haeghebaert et al., 2003; Conedera et al., 2004). The problem becomes increasingly relevant when raw milk cheeses are considered. Grana Padano cheese is one of the most popular raw milk cheeses; it is produced from semi-skimmed raw milk by using natural whey cultures as starter. After coagulation, the curd is cooked at 54°C under whey, and the cheese mold, of about 40 kg, is then ripened at about 15°C for 14 to 22 mo (Bottazzi, 1993). The core of the curd after draining, due in part to the microbial metabolic activity, is believed to reach a temperature of 55 to 56°C and to stay above 50°C for up to 10 h or more (Neviani et al., 1995; Pellegrino et al., 1997; Giraffa et al., 1998) so that alkaline phosphatase is inactivated in the core of the cheese (Pellegrino et al., 1995). This situation may result in a significant reduction of the microbial load and make the core safe from the presence of viable pathogens. On the other hand, the external part of the cheese cools more quickly

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and is less protected. Therefore, there is a differential distribution of the temperature within the curd that can exert a differential influence on the survival of possible contaminants depending on the considered portion of the dairy matrix.

The purpose of this research was to investigate the effect of the temperature in the technology of production of Grana cheese against *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium, and *Staph. aureus*. A curd-cooling model was developed to estimate the temperature variation across the curd during cooling, and thermal stress was applied to the pathogens according to the model in model-scale productions of Grana cheese artificially contaminated with the selected pathogens.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

The following bacterial strains were used in this study: *E. coli* O157:H7 strain 643 (USDA, Washington, DC), *L. monocytogenes* ScottA (from USDA-ARS Eastern Regional Research Center Culture Collection, Wyndmoor, PA), *Salmonella* Typhimurium F01 (from the culture collection of the Department of Food Science, Division of Microbiology, University of Naples Federico II), and *Staph. aureus* ATCC 14458 (American Type Culture Collection, Manassas, VA). The 4 strains were cultured in tryptone soy broth (Oxoid, Garbagnate Milanese, Italy) at 37°C. Each strain was streaked on tryptone soy agar (Oxoid) plates before DNA extraction.

Cooling Model

Cooling history of Grana cheese was analyzed by performing a 3-D computational fluid dynamics simulation. Using a second-order finite volumes code, Navier-Stokes equations written in conservative form for mass continuity:

$$\frac{\partial P}{\partial t} = \nabla\cdot(\rho\mathbf{v}) = 0 \qquad [1]$$

momentum:

$$\frac{\partial(\rho\mathbf{v})}{\partial t} + \nabla\cdot(\rho\mathbf{v}\otimes\mathbf{v}) = -\nabla p + \nabla\cdot(\bar{\bar{\tau}}) + \rho\mathbf{g}, \qquad [2]$$

and energy:

$$\frac{\partial(\rho E)}{\partial t} + \nabla\cdot(\mathbf{v}(\rho E + p)) = \nabla\cdot(k\nabla T + \bar{\bar{\tau}}\cdot\mathbf{v}), \qquad [3]$$

were solved, where E is the total energy

$$E = e + \frac{V^2}{2}$$

$\mathbf{v}$ is the velocity vector, T is the temperature, $e = c_v T$ is the internal energy, p is the static pressure, $\bar{\bar{\tau}}$ is the stress tensor, and $\rho\mathbf{g}$ is the gravitational force.

Geometrical configuration considered an isolated Grana curd inside a plastic mold and positioned over a steel table, with wheel radius, R = 44 cm and height, H = 25 cm.

The model included heat dissipation trough the steel base, the mold containing the cheese, and the natural convection cooling effects in the surrounding room; and consequently, equations 1 to 3 were solved coupled with the heat equations inside the solids (cheese, steel base, and plastic mold)

$$\frac{\partial(\rho c_p T)}{\partial t} = \nabla\cdot(k\nabla T) \qquad [4]$$

where c_p is the specific heat and k is the solid conductivity. Biological heat production was not taken into account.

To speed up convergence, effect of buoyancy in the surrounding air was taken into account by introducing Boussinesq $\rho = \rho_0(1 - \beta(T - T_0))$ approximation to eliminate ρ in the buoyancy term in the momentum equations, being β the air thermal expansion coefficient.

Specific heat for the Grana cheese was chosen as a function of temperature and composition (Thomareis and Hardy, 1985) and set to $c_p = 3.047$ *kJ*/(kg °C), and thermal conductivity (Sweat and Parmelee, 1978) was set to $k = 0.31$ *W/m* °C. The time step Δt was 2 *s* and to capture correctly natural convection effects the simulation required 150,000 control volumes.

Grana Padano Cheese Manufacture

The technology of manufacture of Grana Padano cheese was used to obtain curds of Grana cheese to subject to thermal conditions resembling the hot and the cold spot as suggested by the cooling model. Four separate model-scale productions of Grana cheese were performed in this work using semiskimmed raw milk artificially contaminated with ~10^4 cfu/mL of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium, and *Staph. aureus*, respectively. A uninoculated batch of raw milk was also processed and used as control. Each manufacture was performed twice (n = 2) from 500 mL of milk by addition of natural whey cultures for the production of Grana cheese (inoculum size = 3%) and liquid rennet to obtain coagulation in 20 min. The natural whey cultures where supplied by the Istituto Lattiero Caseario of Lodi and were cultures currently